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Dr. Hasan ONDER

Dr. Ugur SEN

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PREFACE

This volume contains the papers presented at the **International Congress on Domestic Animal Breeding Genetics and Husbandry - 2019 (ICABGEH-19)** held on September 11-13, 2019 in Prague, CZECHIA.

The ICABGEH-19 Congress is organized by the Agricultural Faculty of Ondokuz Mayıs University and Black Sea Publishers, and hosted by Interhotel Ambassador Zlata Husa, Prague, CZECHIA. ICABGEH-19 is an annual International Academic Conference covers main fields of animal science and the third international event of meeting series with participation of very popular invited speakers Prof. Dr. Cathy DWYER (Scotland's Rural College), Prof. Dr. Kaspar BIENEFELD (Humboldt University), Prof. Dr. Adem KAMALAK (Sutcu Imam University) and Prof. Dr. Muhammad KHALID (The Royal Veterinary College). This event has brought together leading researchers, engineers and scientists in the domain of interest from around the world. It also provides opportunities for the delegates to exchange new ideas and application experiences face to face, to establish business or research relations and to find global partners for future collaboration.

Organizing committee is seriously planning, and is already working towards enabling international animal science scientific community to meet the challenges and to move safely and successfully into the advanced information era. To this end, ICABGEH-2019 has been focused on recent developments, as far as research on animal science aiming at protecting the environment and food safety. Thus, ICABGEH-2019 has achieved its main twofold objective: Firstly, presentation of current research works in the field of animal science, and secondly, connecting the animal science community.

Prof. Dr. Hasan ÖNDER,

Congress President

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CAN IMPROVING ANIMAL WELFARE CONTRIBUTE TO SUSTAINABILITY AND PRODUCTIVITY?

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Abstract

Globally we need to feed an increasingly urban and expanding population with a growing demand for meat, milk and eggs, against a background of reducing the carbon footprint of food production. Under these conditions is farm animal welfare a luxury that cannot be sustained? Animal welfare has been characterised in a number of different ways: to include aspects of the animal's biological functioning, ability to live a natural life, and affective state. The oldest conception of animal welfare is the Five Freedoms, which has been adapted to the Five welfare Needs for a suitable environment, a suitable diet, to be able to exhibit normal behaviour patterns, to be with, or apart from, other animals and to be protected from pain, injury, suffering and disease. In lowly productive extensive livestock production systems, animals are often kept in conditions of variable nutrition, and experience high mortality and morbidity from preventable disease. For these systems animal welfare can be improved through actions which will simultaneously improve productivity (e.g. vaccination against disease, education in animal hygiene and management, and provision of improved nutrition, such as improved grassland management). Under these conditions animal welfare improvements are an integral part of improving production efficiency, and can benefit humans and animals simultaneously. Highly productive, intensive systems have increasing control of nutrition and health of the animals, and high productivity but reduce the space and opportunity for animals to express highly motivated behaviour (such as dust-bathing in chickens, or exploration in pigs). Under these conditions productivity gains have been achieved with animal welfare costs. However, these systems often have high inputs, require the use of antibiotics to sustain growth, and may have detrimental impacts on the immune function, fertility and longevity of animals within these systems. Paying attention to the needs of the animal can have both welfare and production efficiency benefits, and animal welfare should be seen as an integral component in improving sustainability of livestock production.

Key words: *animal welfare, sustainability, productivity, behaviour, health*

INTRODUCTION

Global food production is facing unprecedented pressure from multiple demands. An increasing human population is driving a growing requirement for food, but simultaneously having an impact on the amount of agricultural land available to produce food. Impacts of climate change, global warming and associated extreme weather events can devastate harvests, and may reduce the amount of land suitable for agriculture even further. In addition, competing demands to maintain biodiversity and ecosystem services, to produce crops to provide alternative biofuels and the sustainable development goals to eliminate hunger and increase food security have been termed a 'perfect storm' of pressures on food production (Foresight Report, 2011). Where does livestock production fit in these competing demands? On the one hand, livestock produce about a third of human protein sources, and can be an important source of protein in countries with high malnutrition rates. Grazing animals, particularly small ruminants, are able to make use of poor quality forage in environments where it is impossible to grow other foods for human use. However, on the other hand, livestock are a significant contributor to greenhouse gas (GHG) emissions (approximately 10% of GHG of anthropogenic origin come from agriculture), intensive production can reduce biodiversity, and they can be inefficient convertors of plant protein into animal-based proteins. Under these conditions, significant drivers promote a reduction in meat consumption, particularly in the global north and west, and 'sustainable intensification' (or producing more from less land area) of production. Where does animal welfare fit in this picture? Is welfare an expensive luxury that the world can no longer afford, or an integral and essential component of sustainable livestock production? In this paper I will argue for the latter case, and demonstrate how animal welfare must be included in sustainable global food policy.

ANIMAL WELFARE

Animal welfare is about managing animals in a way that gives them what they need, taking into account species-specific requirements. It differs from animal rights, in that it accepts human use of animals, but is concerned about giving the animal the best life possible. It is also different from animal cruelty – animal welfare is concerned about the whole quality of an animal's life, and includes both the absence of suffering and the opportunity for positive welfare. Animal welfare can be seen as concerns falling into three main areas (Fraser, 2008): 1) concerns about the biological functioning of the animal: for example, is it healthy, is it growing normally and reproducing; 2) concerns about the naturalness of the environment in which it is kept: for example, is the animal able to use evolved and adaptive behavioural responses; and 3) concerns about the emotional experiences or feelings of the animal: for example is it fearful or in pain, or relaxed and content (Figure 1).

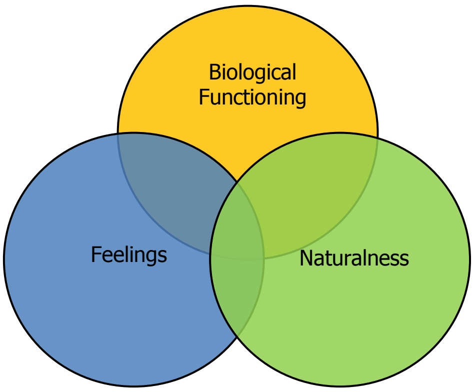

Figure 1. Representation of the three areas of welfare concern (after Fraser, 2008).

Different approaches to characterising animal welfare exist, but the oldest and best known of these is the Five Freedoms (FAWC, 1979), which outlines the main criteria of relevance to animal welfare, namely freedom from hunger and thirst, from discomfort, from pain, injury and disease, from fear and distress, and the freedom to express normal behaviour. Often overlooked, but arguably more important, are the 'provisions' that accompany the freedoms, which outline the main actions that should be taken to ensure good animal welfare. These have since been re-written as welfare needs in UK legislation, which places the onus on the actions taken to care for animals (Animal Welfare Act, 2006). The Freedoms have been criticised, however, and an alternative approach, the Five Domains, has been proposed (Mellor et al., 2009). This model is not vastly different from the Freedoms, but conceptualises welfare as being about four specific domains: nutrition, environment, health, and behaviour, which feed into the fifth domain, mental state. More recently, animal welfare thinking has recognised that these models focus more on the negative parts of animal welfare, and that animal welfare should extend to include positive states, with the goal of giving animals a good life (Yeates and Main, 2008; FAWC, 2009). The Five Domains model has since been extended to include positive characteristics (Mellor and Beausoleil, 2015), and Webster (2016) suggests that freedom of opportunity could perhaps replace the freedom to express normal behaviour. Overall, however, there is broad agreement that animal welfare is about providing an animal with its nutritional, environmental, behavioural and social needs, whilst protecting it from pain, disease and injury. This is described most simply as addressing two questions: is it healthy and does it have what it wants (Dawkins, 2004).

Animal welfare and productivity

Much of the developments in livestock agriculture since the 1940s have been driven by a need to increase productivity. These have involved greater confinement, improvements in nutritional knowledge, genetic selection for productivity traits and improved health care, among others. The relationship between animal welfare and productivity has been theorised to follow an inverted U-curve (McInerney, 2004), where initial activities cause an increase in both productivity and welfare state, before drives to increase productivity will reduce animal welfare (Figure 2). Deciding where on this curve might be an appropriate place to balance desired productivity gains with societally determined acceptable welfare state is an ethical decision for different societies.

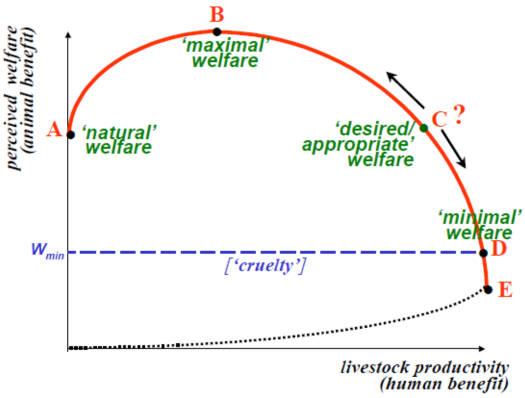

Figure 2. Theoretical representation of the relationship between welfare and livestock productivity (after McInerney, 2004).

For many Low and Middle Income Countries (LMICs), productivity is still very low and livestock may be located somewhere between A (the welfare state of wild animals) and B (the maximal welfare state). For these areas improving animal nutrition, providing better health care and hygiene practices, and a better understanding of animal needs can promote both improved welfare and improved productivity. In these regions, where also the greatest food insecurity occurs, improving animal welfare will be associated with improved productivity and improved human wellbeing. For higher income Western societies, livestock agriculture is much more industrialised, with increasing confinement, often at high stocking densities, and the use of highly managed breeding strategies for narrow breeding goals. This is coupled with high inputs to ensure vaccination schedules, prophylactic treatments, nutritional precision and management complexity. Some of these impacts are of mutual livestock and human benefit (for example, improved nutrition and health care) but many others are neutral or detrimental for animal welfare (for example, loss of ability to express motivated behaviours, frequent social conflict, pain associated with the consequences of breeding for productivity or management activities, loss of choice or opportunity for agency). This suggests a conflict between improving productivity and animal welfare.

However, the impact on the animal in these production systems has also been shown to impact on livestock productivity, suggesting that the theoretical relationship shown in Figure 2 may start to breakdown, or at least impact on profitability as increasing inputs are needed to manage these highly complex systems. For example, a consequence of the selection and confinement of dairy cattle for high productivity has been escalating levels of lameness – studies in UK suggest lameness can be as high as 19-30% (Rutherford et al., 2009; Vee Randall et al., 2019). Management actions to reduce lameness, such as improved hoof care, access to pasture and reducing stocking density, appear to be at the expense of profitability. However, the pain associated with chronic lameness impacts on milk yield, and the financial benefits of increased milk production in animals that have lower lameness can offset any costs in providing better welfare (Barnes et al., 2011). In commercial pig production, confinement of the farrowing sow in a crate is designed to reduce piglet losses from crushing and to make management routines simpler and quicker. However, these levels of extreme confinement prevent the sow from showing highly motivated nest-building behaviour before birth and cause behavioural and physiological indicators of frustration (Jarvis et al., 1997). In addition, the maternal care of the sow is impaired and she is more likely to show aberrant maternal care, such as snapping at piglets and savaging, than sows given the opportunity to express nest-building behaviour (Jarvis et al., 2004). The development of a designed farrowing system, which allows the sow to show evolved and important behaviours, but still affords the piglets some protection, has been shown to have similar piglet survival are confined systems, and greater growth rates to weaning as the sow is more likely to allow the piglets to suckle (Baxter et al., 2015). The improvements in productivity can offset the financial costs of allowing the sow more space at farrowing. These examples demonstrate that productivity does not need to be impaired by improvements in animal welfare, and profitability can be enhanced in higher welfare systems.

For LMICs there are considerable opportunities for improvements in animal welfare that will also achieve improved productivity and these 'win-win' situations should be pursued. For more industrialised countries the consequences of achieving higher and higher productivity are now associated with negative impacts on the animals in these systems, and increasing industrialised animal productivity is also associated with considerable societal concern. This has led to an increase in vegetarianism, and particularly veganism, in these countries, and campaigns to encourage a reduction in meat consumption and greater ethical thinking around animal-based food products (#lessandbetter for example). Whether the pursuit of ever-increasing productivity is sustainable is also an issue, which I will now address.

Animal welfare and sustainability

The examples given above have shown how increasing intensification in a drive for ever greater productivity can sometimes lead to decreases in productivity when animal welfare is compromised. In addition, these systems often require increasingly sophisticated inputs, for example higher quality nutrition to sustain milk production or growth in highly selected animals, provision of antibiotics to prevent disease outbreaks in animal populations kept at high stocking density. If these also lead to food that consumers increasingly do not want to eat then this is not a sustainable food production model. In addition, with pressure to reduce antibiotic use to tackle increasing levels of antimicrobial resistance, the inputs required to sustain production in these systems will need to be reduced. This may lead to reductions in productivity, if animal disease increases, and poorer animal health. However, studies have shown that improving animal welfare may promote better immune function that can decrease the need for antibiotic use. For example, providing enrichment (such as opportunities to play and explore) to growing pigs was associated with an improved clearance of PRSSV RNA in blood, and less histological signs of pneumonia in the lungs (van Dixhoorn et al., 2016).

The production of ruminant livestock, particularly cattle, are often seen as unsustainable as cattle are significant producers of environmental methane, for example 37% of anthropogenic methane is produced by livestock direct emission and manure (Knapp et al., 2014). For this reason, reducing consumption of red meat, and increased consumption of pig and poultry products, are often advocated. However, these are produced in the most confined systems, and where the greatest animal welfare concerns are seen (for example, tail biting in pigs, feather pecking in laying hens, lameness and mobility issues in broilers, hunger in the parent stock in both species etc. D'Eath et al., 2014; D'Eath et al., 2009; Knowles et al., 2008; Nicol et al., 1999). These systems are also greater users of antibiotics than ruminant production (van Boeckel et al., 2015), and in some countries, require the import of plant proteins, such as soya. As described above, these animals are thus fed on foodstuffs that could be consumed directly by humans, compared to ruminants that can utilise plant products that are indigestible by humans, to convert these to proteins that can form human food.

In tackling the requirements to feed an increasing global population in a sustainable way, four types of future food production models have been proposed (Figure 3, Garnett, 2015). Two possibilities directly exclude meat from livestock – suggesting either a very significant increase in veganism such that few animals are kept for human food production, or technological solutions such as the use of alternative meat resources such as in vitro meat production (Hocquette, 2018) or far greater consumption of insect-derived proteins. The other two solutions consider meat of livestock origin is still important as a human protein source. These suggest either a technological solution of increasing confinement and intensification (e.g. use of stacking systems in pig production; Driessen & Korthals, 2012), or a behaviourally driven solution of consuming less meat with this derived from the 'left-overs' or the marginal lands which cannot be farmed for other products. In reality it is likely that no one solution would be universally adopted, and a combination of all options might be the future of food production. However, the impact on animal welfare for these different scenarios is markedly different. For the meat-excluding scenarios the animal welfare impact may be positive, negative or neutral depending on future uses, or not, of animals – for example as environmental managers. For the meat including scenarios, the increasingly intensive production systems are likely to be detrimental to animal welfare, and, as argued above, may in fact require higher and higher inputs such that they are no longer sustainable. Linked to this may be the acceptability for many in society for meat produced in this way, which might make these forms of agriculture unprofitable in the longer term. The 'livestock on leftovers' model may lead to improvements in animal welfare, particularly if management could be designed to combine good welfare and productivity (for example, driving towards B in Figure 2).

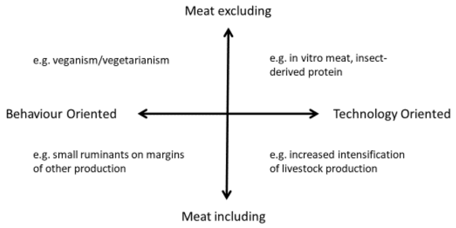

Figure 3. Potential options for food production systems (after Garnett, 2015).

CONCLUSIONS

Economic models of productivity suggest that animal welfare can be improved by the same drivers that lead to improved productivity when production is relatively low, but that welfare will start to decline as productivity increases. However, there are many examples that suggest that a decline in welfare can also limit productivity in very intensive systems. This, coupled with the potentially unsustainably high inputs that are required in these systems, suggest that improving animal welfare is the only viable solution to sustaining livestock productivity in a future food production scenario. I suggest, therefore, that animal welfare cannot be considered as an expensive and expendable luxury, but must be an integral part of livestock production policy and a key driver in achieving global food security.

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STATUS AND PERSPECTIVE OF DISEASE RESISTANCE BREEDING IN THE HONEY BEE

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Abstract

Breeding for disease/Varroa resistance is much more complex than for production traits. Hygienic behaviour against diseased brood is the most important factor of disease resistance and likely to be controlled by a small number of loci, which affect a bee's sensitivity to the stimulus of diseased brood. Disease resistance traits are likely to exceedingly benefit from markers-based models for genetic evaluation.

Key words: *disease resistance, breeding, honey bee*

INTRODUCTION

Approximately 70% of plant species cultivated for human consumption are at least partly dependent on insect pollination, and Apis mellifera is arguably the single most important pollinator species (Klein et al., 2007). Although the worldwide honey bee population increase (FAOSTAT (http://faostat.fao.org), honey bee colony numbers have reportedly declined in some areas with the most likely explanation being a combination of stressors, including synergistic interactions between parasites, pathogens, toxins including pesticides, and other stressors. However, the most serious cause for the periodic colony winter losses of 30% and more are honeybee diseases, especially the ectoparasitc mite Varroa destructor and associated viruses (Genersch et al. 2010). A promi¬sing approach to prevent its spread is to breed for Varroa tolerant honey bees. A trait which has been shown to provide significant resistance against the Varroa mite is hygienic behaviour, a behavioural response of honey bee workers to brood diseases in general. Hygienic behaviour is defined as the ability of honeybee workers to detect and remove pupae that are infected with brood diseases before the causative organism reaches the infectious stage, thereby limiting the spread of infection. Considering these facts, the hygienic behaviour presents a useful mechanism of disease resistance, since almost all serious illnesses of honey bee result from brood diseases. Therefore, hygienic behaviour can be effective not only against Varroa, but also against other brood diseases, which provide a significantly stronger olfactory stimulus by causing more obvious damage to the brood.

RESULTS AND DISCUSSION

Practical Breeding programs

In the past honey bee breeding programs focussed on traits of direct apicultural interest such as honey productivity, gentle temper and low swarming tendency. However, since Varroa destructor began spreading throughout Europe research on mite resistance of honeybees and later breeding program carried out by bee breeder bees started. Starting about 30 years ago, much research focused on the identification of suitable selection characters and a measurement of the within colony Varroa population growth and the hygienic behaviour against freeze-killed or pin-killed brood are now most commonly used as selection traits to breed for Varroa resistance (Büchler et al., 2010). Significant progress has been achieved by the use of BLUP genetic evaluation (Bienefeld et al., 2007) for these traits.

Experimental breeding programs

Uncapping and removing of infested brood are assumed to be important traits within Varroa resistance. Traditionally, selection in honey bee breeding programs is carried out on the basis of colony performance with respect to group traits such as honey production and colony defense. Hygienic behavior of colonies with respect to artificially killed, ill or parasitized brood has a moderate genetic basis but colony level selection may not be very efficient for Varroa resistance breeding because of significant intracolonial variations, caused by the multiple mating of the queen and consequently the composition of several patrilines within colonies. Consequently, we started selecting a Varroa resistance line based on the hygienic behavior towards Varroa parasitize brood cells of individual worker bees (Figure 1) during a 6 d infrared video observation (Bienefeld et al., 2016). Individual, hygienic worker bees, normally infertile, were induced to lay eggs. Unfertilized eggs of worker bees develop in the honeybee into drones, whose sperm can be used for insemination. Consequently, worker bees were use as fathers for the next generation. In those cases in which this procedure was not possible, we used the within sister frequency of hygienic worker bees daughters as a selection objective for the breeding queens. We found that uncapping behaviour towards Varroa infested cells is extremely rare in A. mellifera, but varies (0% to 5%) significantly between colonies. This most promising, direct Varroa resistance trait was found to be genetically determined and we observed a significant genetic progress within our selection line.

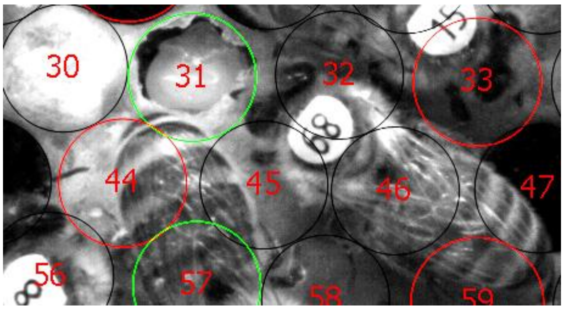

Figure 1. Video screenshot showing a worker bee, which uncapped a Varroa-infested brood cell (marked green). Brood cells marked red are Varroa-free controls.

Genetic markers for Varroa resistance

Genome-wide association studies (GWAS) have evolved into a powerful tool for investigating the genetic architecture of traits in different species. However this approach needs well-defined phenotype of interest and an appropriate control. Our selection line and our long term infrared observation technique provided the conditions for this approach. 22000 worker bees from crossings between our selection line and unselected colonies were monitored with the infrared camera technology and we subjected the 122 top performing hygienic bees and 122 negative controls to a SNP genotyping assay (44K SNP chip), which was specifically developed for the analysis of Varroa resistance traits (Spötter et al., 2012) . After false discovery rate correction of the p-values, six SNP markers had highly significant associations with the trait investigated. Inspection of the genomic regions around these SNPs led to the discovery of putative candidate genes involved in odour reception neuronal sensitivity to external stimuli (Spötter et al., 2016). Further proteomic studies have confirmed these findings (Hu et al. 2016).

Future trends in honey bee breeding: Genomic selection

Traditional breeding programs rely mainly on phenotypes, plus pedigree information. Genomic selection is a new approach for improving quantitative traits that use whole-genome molecular markers. Genomic prediction combines marker data with phenotypic and pedigree data in an attempt to increase the accuracy of breeding and genotypic value prediction. Using next generation sequencing of drones from different A.m. carnica populations, a new 100 000 SNP chip was developed, aiming to initiate genomic selection for traits of Varroa resistance, productivity, and gentleness in honey bees. Due to the lower heritabilities and the time consuming recording of disease resistance, these traits are likely to benefit significantly from genomic selection.

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TRANS-CERVICAL AI IN SHEEP: PROBLEM TO SOLUTION

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Abstract

Within sheep industry, artificial insemination (AI) is a very valuable tool for rapid genetic progress. Besides, AI is performed to maximise the use of superior rams and contain certain contagious diseases within flocks. In the sheep breeding industry three (3) methods of AI are prevalent: vaginal, laparoscopic intrauterine and cervical. Commercially acceptable fertility rates, however, can be achieved by laparoscopic intrauterine insemination using both fresh and cryopreserved semen but with cervical AI using fresh semen only. The most successful method, therefore, seems to be Laparoscopic AI but it is an expensive, invasive surgical procedure and is not considered to be welfare-friendly. A practical solution is, therefore, transcervical intrauterine (TCAI). However, due to the convoluted nature of the cervix, penetration of the inseminating pipette to enable TCAI is rarely achievable.

Key words: *trans-cervical, artificial insemination, sheep, problem, solution*

INTRODUCTION

Studies at the Royal Veterinary Collage (RVC) are focussed at investigating the anatomical and/or technical factors that affect the passage of an insemination pipette through the cervix, particularly the basic mechanism(s) involved in the cervical relaxation. The ultimate aim of research on AI, at the RVC, is to have a greater understanding of the regulation of cervical relaxation in the sheep during the oestrous cycle that may facilitate the development of therapeutic treatments, and develop a method by which semen could be deposited into the uterus through a trans-cervical route.

Preliminary work in our laboratory showed that there is a degree of natural relaxation of the cervix at the time of oestrus and that led us to hypothesise that “the periovulatory changes in oestrogen and progesterone decrease the glycosaminoglycans (GAGs) content of the cervical stroma via a cervical cyclo-oxygenase-2 (COX-2) dependent mechanism, to dilate the cervix at the time of oestrus”.

The results of the studies on ovine cervices collected at different stages of the oestrous cycle (luteal, pre- and post-LH surge) have shown that the cervices collected at the pre-LH surge time of the oestrous cycle have maximum expression of COX-2 mRNA. This expression was seen predominantly at the uterine region and was greatest in the irregular smooth muscle layer of the cervix (Kershaw et al., 2007). Moreover, EP2 mRNA expression was significantly higher in the cervices collected at the time of pre-LH surge compared to other stages of the oestrous cycle (Kershaw-Young et al., 2009). In addition, we also found that almost all the GAGs in cervix were Hyaluronan-like (HA-like), and pre-LH surge cervices contained most HA-like GAG, predominantly at the uterine region and in the luminal epithelium (Kershaw-Young et al., 2009). Pre-LH cervices also had a greater percentage area occupied by collagen compared to smooth muscle particularly at the uterine region (Kershaw et al., 2007). These studies led us to conclude that the major impediment to TCAI in sheep is anatomy of the cervix, in particular the arrangement of cervical rings. The enhanced penetrability of the cervix at the oestrus is not associated with a change in the gross anatomy of the cervix but is instead associated with molecular and structural changes in the cervical ECM. Oestradiol in synergy with FSH increases COX-2 expression in fibroblasts and smooth muscle cells, possibly through an oxytocin-mediated mechanism and the fall in progesterone concentrations may increase the expression of PGE_2 receptor EP_2. The increase in COX-2 leads to increase in PGE_2 synthesis which after binding to its receptors EP_2 and EP_4 initiates cervical relaxation. It is a possibility that activation of EP_2 and EP_4 receptors stimulates adenylate cyclase and cAMP/protein kinase pathway stimulating the relaxation of smooth muscle. In addition, activation of EP4 stimulates the synthesis of GAGs, particularly HA. HA increases the uptake of water into the sheep cervix decreasing relative concentrations of sulphated GAGs like chondroitin sulphate (CS) and dermatan sulphate (DS) that form cross-links with collagen bundles. The reduction in CS and DS concentrations is associated with a reduction in cross-links between collagen bundles and collagen fibres, enabling collagen fibres to move across one another, separate and become disorganised. Separation of collagen bundles and fibres reduces the tensile strength of the sheep cervix and in combination with the relaxation of smooth muscle through the activation of EP2 and EP4 culminates in relaxation of sheep cervix at oestrus.

Considering the increase in HA content of the cervix at the time of oestrus, the main aim of further studies was to investigate the other role(s) that HA may play in the cervical relaxation of the ewe. In this respect, HA content, the expression of its receptor CD44, regulation of HA synthesis by hyaluronan synthases (HAS), and components of the CD44 signalling cascade, including ras-related C3 botulinum toxin substrate 1 (Rac1), actin-related protein 2/3 (Arp 2/3) and Capping protein-Z (Cap Z), along with their hormonal regulation were studied. In addition, it was investigated whether a topical application of HA would affect penetration through the cervix by an inseminating pipette and/or help in achieving the commercially acceptable fertility rates with frozen-thawed semen.

At oestrus, the hormone profile in the ewe is one of higher oestradiol (E2), luteinising hormone (LH) and follicle stimulation Hormone (FSH) and lower progesterone (P4). So using the cervices collected at the luteal, at oestrus (pre-LH surge) and post-LH surge stages of the oestrous cycle, the results of our studies have demonstrated that Pre-LH (at oestrus) surge animals had significantly higher HA content of the cervix compared to the luteal and post-LH groups. Moreover, there were significantly ($P<0.001$) more cervices containing only small HA fragments in the luteal stage, and containing only large HA fragments in the post-LH surge stage of the oestrous cycle. However, there were significantly ($P<0.001$) more cervices containing both small and large HA fragments at the Pre-LH stage of the oestrous cycle (Perry et al., 2010). The HA content of the cervices was increased along with a temporal expression of Hyaluronan synthase-3 (HAS3) that only produces low molecular weight (LMW) HA (Perry et al., 2012). This increase in LMW HA might be responsible for the significant increase in its receptor CD44 in the pre-LH group (Perry et al., (2010)) and the down-stream signalling cascade resulting in an increased expression of Rac1 and ARP2/3 and a decreased expression in CapZ in the cervical tissues. We propose that down-stream signalling cascade is then likely to initiate cellular remodelling through F-actin polymerisation and cellular reorganisation, and result in cervical remodelling. This proposed model of cervical remodelling is confirmed by the results of our further studies which demonstrated that topical application of LMW HA to the cervix does increase cervical penetrability to facilitate the passing of an inseminating pipette in sheep (Perry et al., 2010).

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ENTERIC METHANE AND ITS CONTRIBUTION TO GLOBAL WARMING

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Abstract

The aim of the current study to evaluate the enteric methane production by ruminant animals and its contribution to global warming. Recently pubic concern about enteric methane and its contribution to global warning has considerably increased since CH_4 is the second important greenhouse gases after the CO_2. The global warming potential of CH_4 23 times higher than that of CO_2. Enteric methane comprises 17 and 3.3 % of global CH_4 and greenhouse gases respectively. Even if the contribution of enteric methane to global warming is very small, mitigating the enteric methane emission at farm level or animal level has some practical implications such us decreasing cost of animal production or increasing animal production. So far there is no sustainable mitigation strategies have been developed in ruminant animals without compromising animal production.

Key words: *Enteric methane, Greenhouse gases, Global warming*

INTRODUCTION

Enteric methane is produced during the fermentation of carbohydrate in the rumen. There are a lot factors affecting the enteric methane production by ruminant animals. Recently pubic concern about enteric methane and its contribution to global warning has considerably increased since CH_4 is the second important greenhouse gases after the CO_2. The global warming potential of CH_4 23 times higher than that of CO_2. Enteric methane comprises 17 and 3.3 % of global CH_4 and greenhouse gases respectively (Knapp et al. 2014). Although recently considerable effort has been diverted to mitigate the enteric methane production, so far there is no cheap and practical strategies has been developed in ruminant without compromising animal production. The aim of the current study to evaluate the enteric methane production by ruminant animals and its contribution to global warming.

Enteric Methane Production

Ruminant animal consumes forage and concentrate which are consisted of carbohydrates to meet energy requirement for maintenance and production. The ruminant animal are derived their energy from volatile fatty acids (VFA) produced during the carbohydrate fermentation. The fermentation of carbohydrate not only produces VFA but also produced CO_2 and H_2, which is used by Archea to produce CH_4. The resultant CH_4 during the fermentation is referred to enteric methane. The production of enteric methane has a very important role on the overall fermentation and microbial synthesis of carbohydrate in the rumen. Dissolved H_2 should be removed otherwise the carbohydrate fermentation, microbial growth and microbial protein synthesis decrease by negative feedback mechanisms of dissolved H_2 (McAllister and Newbold, 2008). The enteric methane emission from beef, milk production, buffalos and small ruminant account for 35, 30, 8.7 and 6.7 % of the livestock sector emissions respectively. Although the enteric methane production is inevitable in ruminant animal, the enteric methane production is not desirable due to the contribution of to global warming and energy losses during the fermentation. Therefore recently there are a lot of effort has been diverted to mitigate the enteric methane emissions by ruminant animals. The enteric methane mitigation strategies can be classified in three categories namely dietary, rumen and animal manipulations (Haque 2018).

Physical processing of forages such as chopping, grinding and steam treatment not only improved digestibility but also decreased enteric methane production of ruminant animals. The increase in the concentrate ratio of diet also decreased enteric methane emission by 15 % (Knapp et al. 2014). Supplementation of ruminant diets with fat or fatty acids decreased the enteric methane production (Llonch et al. 2017). Plants containing secondary metabolites such as tannin and saponins have a potential in mitigating of enteric CH_4 emission through antimicrobial properties on bacteria, protozoa and fungi in the rumen (Bodas et al. 2012; Hiristov et al., 2003, Patra and Saxena 2009).

Vaccination of ruminant animals against also reduce the enteric CH_4 emission by 8%(Wrigth et al. 2004). However the long term effect of vaccination still uncertain (Williams et al. 2009). Another CH_4 mitigating strategy is that maintaining high producing animals will increase the total production but CH4 emission per unit of animal product will decrease(Patra 2012; Weisbjerg et al. 2012). However so far there is no sustainable mitigation strategies have been developed in ruminant without compromising animal production.

Contribution of Enteric Methane Emission to Global Warming

Recently incidence of extreme events such as storm, hurricane, tornado, melting of glaciers, downpours, flooding, droughts and wildfire has increased dramatically. Therefore, climate scientists have been trying to find out the causes of these extreme weather events or environmental threat we have been experiencing recently. IPCC (2013) suggested that the increase in incidence of extreme events is thought to be likely associated with global warming. IPCC (2013) also suggested that during the 21st century, the global surface temperature is likely to rise a further 0.3 to 1.7 °C (0.5 to 3.1 °F) in a moderate scenario, or as much as 2.6 to 4.8 °C (4.7 to 8.6 °F) in an extreme scenario, depending on the rate of future greenhouse gas emission and climate effect. Recently pubic concern about enteric methane and its contribution to global warming has considerably increased since CH_4 is the second important greenhouse gases after the CO_2. The global warming potential of CH_4 28 times higher than that of CO_2 (IPCC, 2013). Enteric methane comprises 17 and 3.3 % of global CH_4 and greenhouse gases respectively (Knapp et al. 2014).

CONCLUSION

Even if the contribution of enteric methane to global warming is very small, mitigating the enteric methane emission at farm level or animal level has some practical implications such us decreasing cost of animal production or increasing animal production. So far, there is no sustainable mitigation strategies have been developed in ruminant animals without compromising animal production.

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MOLECULAR DIAGNOSIS OF ONCOGENIC VIRUSES IN POULTRY

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Abstract

Avian Oncogenic viruses are one of the most dangerous pathogenic microorganisms that threaten the poultry industry and cause damage worldwide over a billion dollars annually. It is difficult to detect the diseases caused by these viruses. Furthermore, a vaccine that can provide sterile immunity against these diseases and prevent infection is incomplete. The frequent overlap of lesions caused by avian oncogenic virus infections requires specific laboratory diagnosis. In molecular diagnosis, different methods have been established for detecting avian oncogenic viruses, especially real-time PCR (qPCR) applications. PCR had been a routine technique used in many research and diagnostic laboratories, until the advantages of the qPCR application have emerged, such as enhanced sensitivity, a reduced risk of contamination and the potential for accurate target quantification. In order to be able to struggle more effectively for oncogenic viruses in the future, it is vital to learn more about the host immunity-oncovirus interactions and determine powerful diagnostic techniques. In this review, diagnostic techniques of the qPCR applications for oncogenic viruses are emphasized in poultry.

Key words: *Avian Oncogenic Virus, Molecular Diagnosis, Poultry, Real-time PCR*

EFFECTS OF NEURAL GENE EXPRESSION ON GROOMING BEHAVIOR IN HONEY BEES: A BRIEF REVIEW

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Abstract

Grooming behavior, which is one of the behavioral resistance mechanisms based on the genetic basis in honey bees, is a defense response against parasitic mites, especially Varroa. In recent years, scientists and beekeepers have focused on bee breeding in terms of grooming behavior. Because grooming behavior of honey bees have the potential to protect themselves against Varroa destructor. Before starting the breeding studies, it is the great importance of determine the genes and gene regions which are related to this behavior. In this respect, the right honey bee species or races can be selected and the success rate will increase. In researches, it was found that bees exhibit different grooming behaviors level according to species and races. Therefore, some species and races were found to be more successful than others. Especially in neural, developmental, detoxification and health-related gene expression studies, it has been shown that some gene expression is in direct proportion to the intensity of grooming behavior. While the genes responsible for grooming behavior are not known exactly, studies are underway to solve the genetic mechanism of this behavior. In this study, we reviewed the effects of neural gene expression on grooming behavior which is promising in the ectoparasitic mite Varroa destructor control.

Key words: *gene expression, grooming behavior, honey bee, neural gene, Varroa Destructor*

PROCESSES OF SELECTION AND BREEDING HOLSTEIN BULLS IN THE REPUBLICAN CENTER OF LIVESTOCK BREEDING JSC

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Abstract

National and international experience shows that in dairy cattle breeding the main factor in improving the genetic quality of the bred stock is bulls-producers. Therefore, in modern agricultural programs, lots of attention is paid to the cultivation of young animals genealogical use of producers. In this technological group of animals, an individual assessment of individuals is carried out more accurately than in others, and selection of breeding qualities is intensively conducted. Potential impact of bulls and cows on the improvement of the breed characteristics depend on different herds. For example, one cow in it's life-time can get 7-12 descendants, and bull can get 40-50 thousand heads and more by using artificial insemination. The genetic potential of the population is provided for about 60-80% by the use of bull-leaders. Therefore, cultivation, evaluation and selection of bulls for breeding is essential for the country. But acquisition of bull in some countries is not always advisable.

Key words: *breeding, selection, bull, sexual reflexes, sperm*

INTRODUCTION

Bulls grow up on farms and breeding factories. Technology of cultivation of breeding bulls on the specialized farms in standardized conditions provides the receipt of animals, which are more consistent with the requirements of breeding, than growing by traditional technologies (Alzhan Shamshidin et al., 2017a, 2017b). This is due to the fact that is considered the number and quality of sperm, disposition and behavior, duration of using breed bulls. These indications depend on both individual characteristics and conditions of feeding, keeping, caring and growing animals at an early age (Alzhan Shamshidin et al., 2017, Fedorov 2004, George 1990, Ivanov 1972). Besides that, the effectiveness of the use of producers is determined not only by their breed qualities, but by the quantity and quality of the sperm. Using bulls-leaders promotes the highest rates of further growth of genetic potential, ensuring high productivity of offspring. Therefore, the evaluation and selection of bull producers on reproductive ability, contributes to the improvement of their reproductive qualities and considering as an important moment in the technological process of breeding , as it affects the population of cattle (George 1990, Ivanov 1972, Mussabekov Aidos et al., 2016a).

The aim of the work was to improve the methods of selection of animals and the technological processes of breeding bulls-producers on the basis of using new premixes, mixed fodders and the structure of rations.

MATERIALS AND METHODS

To achieve this goal, a series of scientific and economic experiments were conducted on bulls during the growing period from 1 to 6 months, from 6 to 16 months. The experiments were carried out at Republican Center of livestock breeding JSC "ASYL TULIK" Akmola Region, Kazakhstan. Scientific and economic experiments were carried out by the method of groups and pairs of analogues for 51 heads in each group in accordance with methodological recommendations. The duration of the experiments was 60-120 days. The subject of the study were bulls at the age from 1 to 16 months, and bulls that were waiting for the time of breeding. When developing new premix recipes the content of macro and microelements, vitamins in feeds, and the needs of animals in these nutrients were considered. Instead of imported raw materials, there were introduced composition of mixed fodders from local feeds of protein components (peas, lupins, flax seeds and rapeseed cake). The structure of diets is based on the needs of animals in energy, nutrients and biologically active substances, as well as matching diets breed and live weight of animals [8,9]. Intake accounting for feed was based on the results of the control feeding (1 time per 10 days). The chemical composition of feeds was determined by sampling and analysis. Live weight was accounted by monthly weighting. At the same time, measures were developed to timely identify various developmental abnormalities, subclinical and clinical manifestations of diseases, including male genital organs, as well as preventive treatment and treatment based on the improvement of the feeding system, including the development of premixes, mixed fodders and the structure of diets [6, 10]. Animals were on a common diet and received feed depending on the age: hay - 30-80%, silage - 8-10%, mixed feed - 20-50%. The animals of the experimental group had the same diet, but had an individual adjustment, which was carried out monthly, based on the results obtained in the analysis of the biochemical composition of the blood. Optimization of nutrients provided the inclusion of certain amount of feed premixes. The composition of the premix: Vitamin A - 2000 IU, D - 200 IU, E - 1000 mg, sulfur - 1.2 g, magnesium - 1.5 g, zinc - 5.9 mg, cobalt - 0.2 mg, selenium - 0 , 05 mg, molybdenum - 0.22 mg. During the process of work, changes in the number of cortisol in the blood and the qualitative composition of the sperm were taken in account, also possible changes or remaining the same, depended on the composition of the consumed feeds. For bulls with increased sexual activity and body weight, the number of feed was adjusted individually in accordance with zootechnical requirements [6]. If necessary, in the case of a change in the quality of sperm, the appropriating protein-vitamin-mineral supplements were entered into the diet.

RESULTS AND DISCUSSION

Development of questions of selection and control of a state of health of breeding animals (medical examination) is based on one of necessary conditions of the problems solution of infertility profilactic and intensification of reproduction of cattle, in particular bulls-producers (Ivanov 1972, Mussabekov Aidos et al., 2016b). To carry out such a large and very important work it is necessary to have optimal provision for animals with all the necessary nutritional and biologically active substances, which will make it possible to exclude the influence of all feeding factors in the technological process of breeding animals. In addition, the whole technological process of breeding stock and using bulls-producers is proposed to be carried out in three stages.

The first stage of growth and development of bull-calves (diagnostic) is the designation and evaluation of phenotypic traits, in particular, after recording the animal as a pedigree animal, it is necessary to collect anamnesis, where we know the conditions of feeding , the structure of the feeding ration, the sexual mode , quantitative and qualitative sperm counts, the fertilization of cows, the output of calves in the service area, possible disorders of sexual functions, the conditions for their expression, the violation of sexual reflexes, the previously used treatment and its effectiveness, condition for infectious and invasive diseases, veterinary treatments. After this, clinical study should be general conducted: define the constitution, fatness, temperament, secondary sexual characteristics. In addition, you should learn the animal separate system, musculoskeletal system, pay attention to the condition of the scalp, muscles, joints, hoof, horn. In the study of genital organs, it is necessary to determine the condition of the testes, their appendages, vas deferens, scrotum, prepuce and penis. In addition, and if necessary, quite often prostate and vesicular glands, and also ampoules vas deferens tested. Also in the process of work, getting sperm on an artificial vagina, you should pay attention to the demonstration of sexual reflexes. Recieved sperm should be research visually and microscopically, by studying microbial contamination and compliance with requirements (Mussabekov Aidos et al., 2016b).

Based on the results of the accomplished work, a conclusion is made about the health condition of animals, here with standout healthy with normal sperm production and sick animals with a metabolic disorder and sperm production.

The second stage is based on optimizing the individual needs of animals in nutritional and biologically active substances. For this purpose, in feeding animals there used the process of preventive therapy in protein, carbohydrate, vitamin and mineral insufficiency. After that, if necessary, there carry out veterinary intervention, consisting in corrective and preventive therapy, also therapy carried out at serious disruptions of a metabolism. In addition, veterinary intervention or therapy is applied to clinically healthy animals, but with reduced metabolism (in the acidic state, subclinical course of disease), metabolic disorders, expressed in toxicosis, acidosis, ketonemia, hypoproteinemia, hypoglycemia, disease of ligamentous and bone tissues, also disease musculoskeletal system (Mussabekov A.T. 2016).

The third stage (or preventive) includes activities, directed on cultivation of highly productive young cattle and creation of optimal conditions of keeping and feeding, contributing to maintaining the desired level of spermproducing and observance of the mode of using bulls. For this, it is necessary to optimize the quality of feeding on the basis of the composition of premixes, feed and balance the ration with a gender load in a year (Ivanov 1972). Besides that, the cultivation and breeding of manufacturing bulls should be divided into three stages according to age (Mussabekov A.T. 2016).

Table 1. The degree of manifestation of sexual reflexes

№	The level of raising cortisol in the blood (multiplicity), times		
	every 6 months	every 6 months	every 6 months
10	7 (from 7% to 49 mcg)	6 (5% to 30 mcg)	6.5
16	12 (6% to 72 mcg)	14 (from 4 to 56 ug%)	13.0
18	11 (from 7% to 77 mcg)	12 (from 7% to 84 mcg)	11.5
21	10 (8% to 80 mcg)	14 (from 6% to 84 mcg)	12.0
23	17 (5% to 85 mcg)	18 (from 4 to 72 ug%)	17.5
36	19 (5% to 95 mcg)	17 (5% to 85 mcg)	18.0
28	9 (9 to 81 ug%)	8 (10% to 80 mcg)	7.0
29	10 (9 to 90 ug%)	12 (from 7% to 84 mcg)	11.0
32	8 (10% to 80 mcg)	8 (with 8% to 64 mcg)	8.0
36	18 (5% to 90 mcg)	20 (from 4 to 80 ug%)	19.0
37	15 (6% to 90 mcg)	16 (5% to 80 mcg)	15.5
53	15 (5% to 75 mcg)	17 (from 4 to 68 ug%)	16.0
67	6 (10% to 60 mcg)	8 (with 8% to 64 mcg)	7.5
71	9 (from 11% to 99 mcg)	11 (from 9% to 99 mcg)	10.0
93	5 (8% to 40 mcg)	8 (4% to 32 mcg)	6.5

The first stage refers to the period of growing up of bull-calves to 6 months old and is carried out in breeding factories and special farms. During this period, it is necessary to feed calves in accordance with the scheme of cultivation which adopted by the zootechnical service of breeding factory and special farm. At the same time, animals must be exposed multiple surveys to identify and eliminate bull-calves with inborn anomalies of various origin, and the ones that were received during the growing, also pay special attention to the deformity of growth of the organs of the reproductive system. The second stage is the cultivation of 6-15-month-old bulls, which is carried out on the special farms (Table 1). It is necessary to define degree of demonstrated sexual reflexes of animals, quantity and quality of the received sperm, also reveal bulls with a persistent bridle, hypoplasia, spiral curvature and a fistula of a cavernous body of the penis. Herewith previously evaluate all the phenotypic quality of the animal. The degree of sexual reflexes' evaluation should be demonstrated and determined at an early age, it will reduce the time and cost of cultivation and breeding of bulls. For this purpose, there was developed the method of early evaluation of sexual reflexes, which is based on the determination and comparison of amount of cortisol in the blood of animals. To this end, a method has been developed for an early evaluation of the demonstration of sexual reflexes, which is based on the determination and comparison of the content of cortisol in the blood of animals. The essence of this method is that it’s necessary to take blood samples (10 ml each) from bull-calves at the age of 6-month and stabilize it with heparin. Then for stimulating adrenal cortex, should be prick intramuscularly ACTH at a dose of 50 IU / 100 kg of live weight. The stimulation of the adrenal cortex should be carried out at the same dose 6 months after the first. Consequently, the bull-calves will reach 12 months old.

After 2.5-3.0 hours of ACTH pricking it is required to select a blood sample repeatedly. Then determine the content of cortisol in the obtained blood by radio immunological method. The functional reserves of the adrenal cortex can be considered by the difference in the multiplicity of increase in blood cortisol before injection and after 2.5-3.0 h after injection ACTH.

Research shows that the tendency of saving level immune reactivities in animals at the age of 6 and 12 months is stable and supported all the time. The multiplicity of increase of cortisol in blood is estimated on 100-point scale and indicates a high breeding value of the animal in the future. Five bulls had an increase in cortisol levels – in the range of 5.0 to 8.9 after the introduction of ACTH. They got up to 59 points on the 100-point scale. Total score of the other 10 bull-calves was from 60 and higher points, and the level of cortisol was much higher within of 9.0 to 19.0 times.

The third stage is the arriving and keeping of bulls-producers on the livestock breeding companies and the systematic control of their physiological status (registration with quarantine registration health act, etc.). Results that characterize individual feeding and physiological condition of bulls-producers are entered in a special card on electronic media, and then the "passport of the producer" is made.

After the third stage of breeding bulls cultivation, their evaluation is carried out on the basis of accumulated research data characterizing the quantitative and qualitative composition of the sperm, and also on the basis of cows fertilization results, they are divided into four separate production groups.

The first group consists of bulls with high reproductive ability (70% of the cows get pregnant at the first insemination). Besides that, sexual reflexes are well expressed in these animals, the volume of ejaculate is more than 5 ml, the concentration of spermatozoa in 1 ml should be more than 1 billion pieces, their activity is higher than 8 points, the number of live sperm is 80-95% (Table 2).

Table 2. Evaluation scale breeding productivity points

Reproductive qualities of bulls	Animals groups			
	1	2	3	4
Sexual reflex	Good	Good	Satisfied	Unsatisfied
Assessment scores	20	19	15	10
Ejaculate volume, ml	above 5	5-4	3-2	less than 2
Assessment scores	20	18	16	11
Number ofspermatozoa%	more than 80	80-71	70-50	less than 50
Assessment scores	20	19	18	15
Active sperm ball.	above 8	8-7	6-4	less than 4
Assessment scores	20	17	15	10
Fertility of cows %	above 70	70-51	50-40	less than 40
Assessment scores	20	15	10	5
Comprehensive evaluation of bulls, scores above	90	90-81	80-60	less than 60

The second group includes breeding bulls with a good reproductive ability (fertilization of cows for the first insemination should be 50-70%), the volume of ejaculate - 3-4 ml, the concentration of spermatozoa - 0.4-0.8 billion, activity - 70-80 points, the number of live sperm - at least 70%.

The third group is defined by bulls-producers with reduced reproductive ability. They are characterized by a high percentage of repeated inseminations of cows, frequent refusal to allocate sperm and not to respond to an artificial vagina, produce low-quality ejaculates (ejaculate volume less than 2 ml with a minimum sperm concentration of 0.2-0.5 billion and an activity below 6 points).

The fourth group includes infertile animals. They release little sperm with low concentration and activity, with a large number of pathological forms of spermatozoa. Animals of the third and fourth group are discarded.

CONCLUSIONS

However, the final stage of the technological process of growing young animals and the most effective use of bulls-producers for breeding is the period of definition and quality of their offspring. This period is one of the most complex and lasting. This is due to the fact that the duration of the assessment of bulls-producers on the quality of their offspring under the optimal conditions for the cultivation of young animals and the keeping of breeding animals is 4.5 years and is completed no earlier than 6 years of age. In recent years because of different reasons, there has been a significant reduction in the duration of using bulls-producers production. Now on some state-owned enterprises of the Republic to the beginning the period of estimation of sires on quality of progeny there are only 5 % of the herds. At the age of 6 and older 95% of the bulls retires, although by their nature they have the potential for long-term breeding and productive life, as evidenced by the results of the data analysis, which belongs to Republican Center of livestock breeding JSC "ASYL TULIK" are presented in table 3.

According to the information from table 3 it can be seen that for the future taking and saving of sperm (for productive use) with the existing situation on the breeding remains only 5% of test bulls, which you can use overexposure (waiting time breeding). Therefore, the main task is development of technology for housing, feeding and control mode regimes growing bulls during the period of time of their breeding to accumulate semen after a period of evaluation on the quality of posterity. At the same time it is necessary to develop activities that promote the long-term preservation of breeding qualities of animals with normal constantly identifying bulls, which marked a decrease in the volume of ejaculate, sperm concentration and activity. There is a low survival rate and creaminess of sperms. For these reasons, up to 30% of own sperm is rejected.

ACKNOWLEDGEMENTS

Thus, sexual maturity in bulls occurs at the age of 6-9 months., and physiological maturity-in 16-18 months. Time of sexual and physiological maturity depends on the breed, climate, feeding conditions, the qualitative composition of fodder, care, maintenance, neurohumoral stimuli. Animals reach optimal sexual maturity and are considered highly valuable at the age of 6 years. However at this time Mature and full-aged bulls-producers in the main herd are only 5%.

Plans for breeding and cultivation and especially the checking bulls-producers and the quality of offspring are often violated because of their premature culling due to injuries of the pelvic limb varying degree. In addition, there are diseases of the musculoskeletal system, which reduces their sexual activity and significantly degrade the quality of sperm. In ejaculates increases the number of pathological forms of sperm cells. Also it is established that in bulls of 5-year age deformation is note joints and the emergence of a vast cavern of articular cartilage that does not allow the use of such animals as breeding.

Thus, using new methods is a lot better for breeding bulls cultivation ,also the complex solution of the questions that defines their effective selection, and also realization timely testing or evaluation of bulls' offspring's quality through better feeding and conditions allows the earliest period to reliably evaluate the suitability of breeding animals, or to provide them with the necessary help, for example treatment in extreme cases, to select low-value producers from the main herd. These measures significantly reduce the period of breeding animals while reducing feed costs by 5-8%.

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EVALUATION OF ANTIFREEZE CARBOXYLATED POLY-L-LYSINE AS A REPLACEMENT OF GLYCEROL IN SEMEN EXTENDER FOR BUFFALO SEMEN

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Abstract

Glycerol has long been used as a permeable cryoprotectant for buffalo semen, however it is also reported to have some toxic and contraceptive effects that may be responsible for the often observed poor post thaw quality of buffalo sperm. An alternative to glycerol could be use of Carboxylated Poly-L-Lysine (CPLL) which has both antifreeze and cryoprotectant properties. The main objective of this study was to evaluate Antifreeze CPLL as a replacement of glycerol in semen extender for buffalo semen preservation. CPLL (0.65 mole ratio) was prepared by mixing Poly-L-lysine aqueous solution and succinic anhydride in 30/70 % (PLL/SA) mole ratios and incubated at 50°C for one hour to convert 65% amino groups present in Poly-L-lysine solution to carboxyl groups. Tris-citric egg yolk extender was used and different experimental extenders were prepared as Control (0% CPLL Solution + 7% Glycerol); E1 (1% CPLL Solution + 6% Glycerol); E2 (2% CPLL Solution + 5% Glycerol); E3 (3% CPLL Solution + 4% Glycerol); E4 (4% CPLL Solution +3% Glycerol); E5 (5% CPLL Solution + 2% Glycerol); E6 (6% CPLL Solution + 1% Glycerol) and E7 (7% CPLL Solution + 0% Glycerol). Semen was collected from four Nili-Ravi buffalo bulls (40 ejaculates). Two consecutive ejaculates were collected from each bull for consecutive five weeks (replicates) and cryopreserved in the experimental extenders. Cropreserved semen was evaluated for different parameters. Post-thaw sperm motility, plasma membrane integrity and acrosomal integrity were found to be higher ($P<0.05$) in extenders E1, E2, E3, E4 and E5 compared to E6 and controls. Sperm livability (live/Dead ratio) and viability (Live sperm with intact acrosome) was highest ($P<0.05$) in extender E4 compared to all the other extenders. Sperm DNA integrity was higher ($P<0.05$) in extender E2, E3, E4 and E5 compared to control, E1 and E6 extender. In conclusion, post-thaw buffalo sperm motility, plasma membrane integrity, livability, viability, acrosomal and DNA integrity were improved in the extender containing 4% CPLL and 3% glycerol. Overall, the data obtained suggest that partial replacement of glycerol with CPLL in the Tris-citric egg yolk extender (4% CPLL and 3% glycerol) during buffalo semen cryopreservation improves the post-thaw sperm quality.

Key words: *Glycerol, Buffalo semen.*

CLASSIFICATION OF EGG PHOTOS WITH IMAGE PROCESSING AND ARTIFICIAL NEURAL NETWORKS

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Abstract

The image processing is a method developed to convert the image into a digital form and to perform certain operations, to obtain specific images or to extract some useful information from it. Classification is the most commonly used method for the analysis and interpretation of images obtained as a result of image processing. Although the classification is actually a decision mechanism, the image classification is the assignment of the values to which the pixels correspond to the examined surface. In this study, appropriate network parameters and input-output models are investigated for the effective use of multi-layer feedforward neural networks with image processing. In this study, 960 eggs obtained from white egg color Lohmann chicken were used. Eggs in the dark environment by giving light from the bottom; Photographs were taken for during the 30 days from a distance of 50 cm. Images obtained from the eggs were used as input data set. With each photo matching the different days, the days were formed the target data set. In this study, using RGB values, the model with 10 hidden layers and 70-15-15 network structure achieved 95% success.

Key words: *animal science, eggs, artificial neural network, image processing, classification*

EFFECT OF SPECIES ON CHEMICAL COMPOSITION, DIGESTIBILITY, FEED INTAKE AND RELATIVE FEED VALUE OF CITRUS LEAVES

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Abstract

The aim of the current experiment was to determine the effect of species on the chemical composition, digestibility, feed intake and relative feed value of some citrus leaves. Species had a significant effect on chemical composition, dry matter digestibility (DMD), dry matter intake (DMI) and relative feed value (RFV) of citrus leaves. Crude ash and crude protein contents of citrus leaves ranged from 12.05 to 16.33 % and 11.29 to 15.55 % respectively Crude ash of Citrus reticulate was significantly higher than the other citrus species while crude protein content of Citrus unshiu Marc was significantly higher than the other citrus species. Neutral detergent fiber and acid detergent fiber contents of citrus leaves ranged from 28.20 to 33.72 and 21.31 to 26.64 % respectively. Neutral detergent fiber and acid detergent fiber contents of Citrus paradisi were significantly higher than the other citrus leaves. Dry matter digestibility (DMD) and dry matter intake (DMI) of citrus leaves ranged from 68.22 to 72.29%, 3.56 to 4.26. Dry matter digestibility of leaves of Citrus paradisi was significantly lower than the other citrus leaves. Dry matter intake (DMI) and relative feed value (RFV) of citrus leaves of Citrus paradisi and Citrus sinensis Osb. were significantly lower than the other citrus leaves. As a conclusion, there are considerable variation among citrus leaves in terms of chemical composition, dry matter digestibility, dry matter intake and relative feed value.

Keywords*: Citrus leaves, chemical composition, digestibility, dry matter intake, relative feed value*

INTRODUCTION

Tree and shrub leaves are consumed by ruminant animal to meet their energy, protein and mineral requirement when there are no available feedstuffs during the lean period in most parts of Turkey. Although citrus trees produce considerable amount of leaves during the harvest and pruning periods, the citrus leaves are undervalued because of insufficient knowledge of chemical composition and nutritive value (Karabulut et al. 2007). So far there is limited information about the effect of species on the chemical composition and nutritive value of citrus leaves (Karabulut et al. 2007). Therefore, the aim of the current experiment was to determine the effect of species on the chemical composition, digestibility, feed intake and relative feed value of some citrus leaves.

MATERIALS AND METHODS

Leaves from *Citrus unshiu Marc*, *Citrus paradisi*, *Citrus sinensis*, *Citrus sinensis* Osb., *Citrus reticulate* were collected by hand in December 2017 from Hatay Province in Republic of Turkey and dryied in laboratory. Dry citrus leaves were milled through a 1 mm sieve after drying. Chemical composition of citrus leaves was determined using the methods described by AOAC (1990). Neutral detergent fiber and acid detergent fiber content of citrus leaves were determined with the method described by Van Soest (1991). Dry matter digestibility (DMD), dry matter intake (DMI) and relative feed value (RFV) were estimated as follows (Rohweder et al 1978).

$\% DMD = 88.9 - (0.779 * \%ADF)$

$DMI \% \text{ of } BW = 120 / \%NDF$

$RFV = (\%DMD * \%DMI) / 1.29$

One-way analysis of variance (ANOVA) was used to determine the effect of species on chemical composition, digestibility, feed intake and relative feed value of citrus leaves. Significance between individual means was identified using the Tukey's multiple range tests. Mean differences were considered significant at $p<0.05$

RESULTS AND DISCUSSION

The effects of species on the chemical composition, digestibility, dry matter intake and relative feed value of citrus leaves were given in Table 1. Species had a significant effect on chemical composition, dry matter digestibility (DMD), dry matter intake (DMI) and relative feed value (RFV) of citrus leaves. Crude ash and crude protein contents of citrus leaves ranged from 12.05 to 16.33 % and 11.29 to 15.55 % respectively Crude ash of *Citrus reticulate* was significantly higher than the other citrus species while crude protein content of Citrus unshiu Marc was significantly higher than the other citrus species.

Table 1. The effects of species on the chemical composition, digestibility, dry matter intake and relative feed value of citrus leaves.

	Species						
	C.unshiu	*C.paradisi*	*C.sinensis*	*C.sinensisOsb.*	*C.reticulate*	sem	Sig.
DM	45.66	41.45	40.99	42.64	39.46	2.614	NS
CA	12.05^{b}	15.44^{a}	13.67^{b}	16.64^{b}	16.33^{a}	0.509	***
EE	3.82	3.83	4.11	4.02	4.16	0.320	***
CP	15.55^{a}	12.05^{b}	11.29^{b}	15.28^{a}	12.36^{b}	0.453	***
NDF	28.93^{b}	33.72^{a}	28.20^{b}	31.46^{a}	28.56^{b}	0.719	***
ADF	22.01^{b}	26.54^{a}	23.10^{b}	23.29^{b}	21.31^{b}	0.862	***
DDM	71.75^{a}	68.22^{b}	70.90^{a}	70.76^{a}	72.29^{a}	0.670	***
DMI	4.15^{a}	3.56^{b}	4.26^{a}	3.81^{b}	4.20^{a}	0.099	***
RFV	230.76^{a}	188.33^{c}	234.08^{a}	209.25^{b}	235.68^{a}	5.698	***

^{a,b,c,} Row means with common superscripts do not differ (p>0.05), SEM = standard error mean, NS = not significant, *** = P<0.001, DM = dry matter (%), CA = crude ash (%), EE = ether extract (%), CP = crude protein (%), NDF = neutral detergent fiber (%), ADF = acid detergent fiber (%), DMD = dry matter digestibility, DMI = dry matter intake, RFV = relative feed value.

Neutral detergent fiber and acid detergent fiber contents of citrus leaves ranged from 28.20 to 33.72 and 21.31 to 26.64 % respectively. Neutral detergent fiber and acid detergent fiber contents of *Citrus paradisi* were significantly higher than the other citrus leaves. Dry matter digestibility (DMD) and dry matter intake (DMI) of citrus leaves ranged from 68.22 to 72.29%, 3.56 to 4.26. Dry matter digestibility of leaves of *Citrus paradisi* was significantly lower than the other citrus leaves. Dry matter intake (DMI) and relative feed value of citrus leaves of *Citrus paradisi* and *Citrus sinensis* Osb. were significantly lower than the other citrus leaves.

The chemical composition of citrus leaves is comparable with those reported by Karabulut et al. (2007) who also reported that there is considerable variation in chemical composition. El-Shatnawi and Mohawesh (2000) suggested that ewes require 7-9% CP for maintenance and 10-12% for lactation. It seems to be likely that citrus tree leaves studied in this experiment will meet the CP requirements of ewes for maintenance and lactation since the CP content of citrus tree leaves studied in this experiment higher than those requested for maintenance and lactation of sheep.

CONCLUSIONS

As a conclusion, there are considerable variation among citrus leaves in terms of chemical composition, dry matter digestibility, dry matter intake and relative feed value. Citrus tree leaves studied in this experiment will meet the CP requirements of ewes for maintenance and lactation. However before large implication further in vivo experiments are required.

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DETERMINATION OF POTENTIAL NUTRITIVE VALUE OF LEAVES OF *FICUS CARICA* FOR RUMINANT NUTRITION

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Abstract

The aim of the current experiment was to predict the potential nutritive value of leaves of Ficus carica using the chemical composition and in vitro gas production technique. Leaves of Ficus carica harvested at three different times (June, July and August) in 2018. Maturity had a significant effect on chemical composition, gas production, methane production metabolisable energy and organic matter digestibility of leaves of Ficus carica. The crude ash(CA) contents of leaves of Ficus carica ranged from 15.20 to 20.86% . The CA content of leaves of Ficus carica harvested in June was significantly higher than the others. The crude protein (CP) and ether extract (EE) contents of leaves of Ficus carica ranged from 12.30 to 20.13% and 2.86 to 4.13% respectively. The CP and EE contents of leaves of Ficus carica harvested in June were significantly lower than the others. The neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents of leaves of Ficus carica ranged from 30.43 to 37.36% and 18.26 to 22.93% respectively. The NDF content of leaves of Ficus carica harvested in August was significantly lower than the others. Gas and methane production of leaves of Ficus carica ranged from 23.35 to 36.56 ml and 2.17 to 3.40 ml respectively. The gas production of leaves of Ficus carica harvested in June was significantly higher than the others whereas the methane production of leaves of Ficus carica harvested in August was significantly higher than the others. Metabolisable energy and organic matter digestibility of leaves of Ficus carica ranged from 6.13 to 8.35 MJ/kg DM and 53.95 to 66.31 % respectively. The ME and OMD of leaves of Ficus carica harvested in June was significantly higher than the others. It seems to be likely that leaves of Ficus carica harvested in June and July had moderate and low reduction potential respectively. This preliminary investigation clearly showed that chemical composition, in vitro gas production, methane production, metabolisable energy and organic matter digestibility of leaves of Ficus carica varied with maturity stages. Based on data associated with the chemical analysis, ME and OMD of leaves of Ficus carica have a potential as feedstuffs for ruminant. The leaves of Ficus carica deserved further in vivo investigation in ruminant animals due to anti methanogenic properties of leaves of Ficus carica

Key words: *ficus carica leaves, chemical composition, digestibility, metabolisable energy, organic matter digestibility*

INTRODUCTION

Leaves of tree and shrub in Mediterranean woodlands and shrublands is one of very important component of grazing ruminant animals during lean period of year when there is no available feedstuffs (Kababya et al., 1998; Perevolotsky et al., 1998). Although leaves of *Ficus carica* were consumed by the small ruminant animals, there is limited information about the nutritive value of leaves of *Ficus carica*. Research on chemical composition in combination with *in vitro* gas production technique can help to evaluate the potential nutritive value of previously un-investigated alternative feed sources (Karabulut et al 2006; Karabulut et al 2007; Kamalak et al 2011; Canbolat 2012; Guven 2012; Kaya and Kamalak 2012). Therefore the aim of the current experiment was to predict the potential nutritive value of leaves of *Ficus* *carica* using the chemical composition and in vitro gas production technique.

MATERIALS AND METHODS

Leaves of *Ficus carica* harvested at three different times (June, July and August) in 2018. Leave samples were dried and milled were milled through a 1 mm sieve after drying. Chemical composition of hays was determined using the methods described by AOAC (1990). Neutral detergent fiber and acid detergent fiber content of alfalfa hay were determined with the method described by Van Soest (1991). *In vitro gas* production of leaves was determined for 24 h (Menke et al. 1979). Rumen fluid was obtained from three fistulated sheep fed twice daily with a diet containing alfalfa hay (60%) and concentrate (40%). after incubation. Methane content of gas produced was determined using the infrared methane analyzer (Goel et al., 2008). The metabolisable energy (MJ/kg DM) and organic matter digestibility (OMD) of seeds were calculated using equations of Menke and Steingass (1988) as follows:

$$\text{ME (MJ/kg DM)} = 2.20 + 0.1357\ \text{GP} + 0.057\ \text{CP} + 0.002859\text{EE}^2$$

$$\text{OMD (\%)} = 14.88 + 0.8893\ \text{GP} + 0.448\ \text{CP} + 0.651\text{CA}.$$

One-way analysis of variance (ANOVA) was used to determine the effect of harvesting stage on chemical composition, in vitro gas production, methane production, metabolisable energy and organic matter digestibility of leaves of *Ficus carica*. Significance between individual means was identified using the Tukey's multiple range tests. Mean differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

The effects of species on the chemical composition of leaves of *Ficus carica* is given in Table 1. Harvesting stage had a significant effect on the chemical composition of eaves of *Ficus carica*. The CA contents of leaves of *Ficus carica* ranged from 15.20 to 20.86% . The CA contents of leaves of *Ficus carica* harvested in June was significantly higher than the others. The CP and EE contents of leaves of *Ficus carica* ranged from 12.30 to 20.13% and 2.86 to 4.13% respectively. The CP and EE contents of leaves of *Ficus carica* harvested in June were significantly lower than the others. The NDF and ADF contents of leaves of *Ficus carica* ranged from 30.43 to 37.36% and 18.26 to 22.93% respectively. The NDF content of leaves of *Ficus carica* harvested in August was significantly lower than the others.

Table 1. The effects of harvesting stage on the chemical composition of leaves of *Ficus carica*

	Harvesting Stage			
	June	*July*	*August*	sem
DM	28.90b	29.36b	37.56a	0.946
CA	15.20b	19.23a	20.86a	0.712
CP	20.13a	12.93b	12.30b	0.226
EE	4.13a	2.86b	3.13b	0.304
CT	1.23	1.16	1.23	0.124
NDF	35.86a	37.36a	30.43b	1.260
ADF	21.43	22.93	18.26	1.628

[ab]Row means with common superscripts do not differ ($p>0.05$), sem: Standard error mean, DM: Dry matter(%), CA:Crude ash (%), EE; Ether extract (%), CP: Crude protein (%), ADF: acid detergent fiber(%),NDF: Neutral detergent fiber(%)

The effects of harvesting stage on the gas, methane, metabolisable energy and organic matter digestibility of leaves of *Ficus carica* is given in Table 2.

Gas and methane production of leaves of *Ficus carica* ranged from 23.35 to 36.56 ml and 2.17 to 3.40 ml respectively. The gas production of leaves of *Ficus carica harvested in June was significantly higher than the others whereas the* methane production of leaves of *Ficus carica* harvested in August was significantly higher than the others.

ME and OMD of leaves of *Ficus carica* ranged from 6.13 to 8.35 MJ/kg DM and 53.95 to 66.31 % respectively. ME and OMD of leaves of *Ficus carica* harvested in June was significantly higher than the others.

Table 2. The effects of harvesting stage on the gas, methane, metabolisable energy and organic matter digestibility of leaves of *Ficus carica*

	Harvesting Stage			
	June	*July*	*August*	sem
Gas(ml)	36.56a	23.35c	30.09b	0.946
CH_4(ml)	3.40b	2.17b	4.39a	0.712
CH_4(%)	9.13b	11.55b	14.63a	0.226
ME	8.35a	6.13c	7.01b	0.157
OMD	66.31a	53.95c	60.72b	1.028

[ab]Row means with common superscripts do not differ ($p>0.05$), sem: Standard error mean, ME: Metabolisable energy(MJ/kg DM) , OMD: organic matter digestibility (%)

Lopez et al (2010) suggested that methane percentage of total gas produced after 24 hour fermentation can be used to determine the methane reduction potential of any feedstuffs and the feedstuffs can be classified in three groups, low potential (% methane in gas between >11% and ≤14%), moderate potential (% methane in gas between >6% and <11%), high potential (% methane in gas between >0% and <6%). Therefore it seems to be likely that leaves of *Ficus carica* harvested in June and July had moderate and low reduction potential respectively whereas leaves of *Ficus carica* harvested in August had no reduction potential since percentages of methane of *Ficus carica* harvested in August 14.63 %. However the anti methanogenic potential of leaves of *Ficus carica* should be tested with *in vivo* experiment.

CONCLUSIONS

This preliminary investigation clearly showed that chemical composition, *in vitro* gas production, methane production, metabolisable energy and organic matter digestibility of leaves of *Ficus carica* varied with maturity stages. Based on data associated with the chemical analysis, ME and OMD of leaves of *Ficus carica* have a potential as feedstuffs for ruminant. The leaves of *Ficus carica* deserved further in vivo investigation in ruminant animals due to anti methanogenic properties of leaves of *Ficus carica*.

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Aspergillus niger and *Bacillus subtilis* CHANGES THE NUTRITONAL COMPOSITION OF SWEET CHERRY KERNEL (*Prunus avium* L.)

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Abstract

The effect of Aspergillus niger and Bacillus subtilis on the nutritional composition of sweet cherry kernel (CK) was investigated in this study. Cherry kernels were milled to the size of 2mm and fermented with A. niger and B. subtilis for 7 days. The crude protein (CP), ether extract (EE), ash, crude fiber (CF), neutral detergent fiber (NDF) and acid detergent fiber (ADF) content was determined before and after fermentation. Hemicellulose (HC) was calculated as NDF minus ADF. Both A. niger and B. subtilis increased (P<0.001) CP and ash content, decreased CF (P<0.001), HC (P<0.001) and NFE (P<0.01) content and did not changed EE and ADF content of CK. The NDF content was decreased (P<0.01) by A. niger but was not changed by B. subtilis. Aspergillus niger cause more increase (P<0.001) of CP and ash and higher decrease in CF (P<0.001) and HC (P<0.05) than B. subtilis. These results showed that nutritional composition of CK can be improved by A. niger and B. subtilis through the solid state fermentation. Aspergillus niger shows better performance compared with B. subtilis.

Key words: *sweet cherry kernel, Prunus avium L., solid state fermentation, nutritional enrichment*

INTRODUCTION

Development in animal husbandry with the rapid increase in world population leads to an increase in feed requirement. This demands on animal feed cannot be met with cereal grains, legumes etc. This reason forces the researchers to find new alternative feed sources. Utilization of agricultural residues has been taking interest recently due to billions of tons of waste produced every year (Xie et al., 2016).

Sweet cherry (*Prunus avium* L.) is produced at 2.4 million tonnes annual production in worldwide (FAOSTAT, 2017). Cherry is generally consumed as fresh fruit and also as jam, jelly, stewed fruit and marmalade (Vursavuş et al., 2006). Sweet cherry kernel (CK) contains 13.81% crude protein (CP), 26.28% ether extract (EE), 3.34% ash, 21.65% crude fibre (CF), 27.21% neutral detergent fiber (NDF), 16.24% acid detergent fiber (ADF; Altop, 2019). Although CK having potential to using in animal nutrition, Altop (2019) reported that dietary 1% addition of CK affect feed conversion ratio negatively in broiler chickens, which may be due to high cellulose content or antinutritional components.

Solid state fermentation can improve the nutritional composition of agricultural residues by increasing crude protein and decreasing the structural carbohydrate contents (Dei et al., 2008). *Aspergillus niger* and *Bacillus subtilis* are used as probiotics in animal nutrition and accepted as Generally Recognized as Safe (GRAS) by the US Food and Drug Administration (FDA). Both are preferred microorganisms in the solid state fermentation. *Aspergillus niger* improved the nutritional composition of sweet (Altop, 2019) and sour cherry kernel (Güngör et al., 2017). However, there is not any study comparing the effects of *A. niger* and *B. subtilis* on the nutritional composition of sweet cherry kernel. This study was aimed to investigate the effect of *A. niger* and *B. subtilis* on the nutritional composition of sweet cherry kernel.

MATERIALS AND METHODS

Cherry kernel and microorganisms

Cherry kernels were obtained from a juice factory in Tokat, Turkey. *Aspergillus niger* (ATCC 9142) and *B. subtilis* (ATCC 21556) supplied from American Type Culture Collection (ATCC).

Solid state fermentation

Cherry kernels were grinded to size of 2 mm. After autoclave sterilization at 121 °C for 15 minutes, kernels were enriched with the 1.6 l nutrient salt (glucose:urea:$(NH_4)2SO_4$:peptone: KH_2PO_4:$MgSO_4.7H_2O_4$:2:6:1:4:1) for each 1 kg sample. *A. niger* and *B. subtilis* were inoculated to CK at 10^5 spores/ml and 10^{10} cfu/ml, respectively. Samples were gently mixed to disperse spores and incubated at 28-30 °C for 7 days in plastic containers. Kernels were dried on a bench in the room temperature until reached approximately 90% dry matter after fermentation process.

Chemical composition

The CP, EE, ash, and CF contents of CK were determined according to AOAC (2000). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed as reported by Van Soest et al. (1991). Hemicellulose (HC) was calculated as NDF minus ADF. Nitrogen-free extract (NFE) was estimated on a dry weight basis by subtracting the percentages of CP, EE, CF and ash from 100%.

Statistical analysis

All data were analyzed by one-way ANOVA. The statistical differences between treatments were determined by a Tukey test. Values are presented as the mean and pooled standard error of means (SEM). The level of significance was preset at $P<0.05$.

RESULTS AND DISCUSSION

Nutritional composition of unfermented and fermented CK is given in Table 1. Fermentation increased CP ($P<0.001$) content of CK. *Aspergillus niger* resulted more ($P<0.001$) CP increase than *B. subtilis*. Similarly, Güngör et al. (2017) reported increased crude protein by *A. niger* in sweet and sour cherry kernel. *Aspergillus niger* increased CP of mango kernel (Kayode and Sani, 2008), grape seed (Altop et al., 2018), shea nut (Dei et al., 2008) and palm kernel cake (Iluyemi et al., 2006; Lawal et al., 2010). *Bacillus subtilis* also increased CP content of rapeseed meal (Fazhi et al., 2011), cottonseed meal (Sun et al., 2012), soybean meal (Teng et al., 2012) and mixed feed (Shi et al., 2017). Increase in CP content may be because of the microbial protein produced by *A. niger* and *B. subtilis* (Raimbault, 1998; Shi et al., 2017).

Fermentation did not altered the EE content of CK in this study. Similar results were reported in the studies on sour cherry kernel (Güngör et al., 2017), palm kernel (Iluyemi et al., 2006; Lawal et al., 2010) and grape seed (Altop et al., 2018). However, EE content was increased in the studies of shea nut (Dei et al., 2008) and decreased in mango kernel (Kayode and Sani, 2008), sour cherry kernel (Güngör et al., 2017) and grape seed (Altop et al., 2018).

Ash content was increased ($P<0.001$) by the solid state fermentation. *Aspergillus niger* resulted a higher ($P<0.001$) ash content compared with *B. subtilis*. Similarly, *A. niger* increased ash content of sour cherry kernel (Güngör et al., 2017), shea nut (Dei et al., 2008), mango kernel (Kayode and Sani, 2008) and grape seed (Altop et al., 2018). *Bacillus subtilis* also increased ash content of cottonseed meal (Sun et al., 2012) and mixed feed (Shi et al., 2017). The increase in ash content possibly was relative due to decrease in other nutrients rather than actual increase.

Soluble carbohydrates are preferred by the microorganism to other nutrients for carbon source (Papagianni, 2007). Solid state fermentation decreased ($P<0.01$) the NFE content of CK in this study. Similarly, Güngör et al. (2017) reported a decreased NFE in sour CK by *A. niger*. This result is in line with the studies on shea nut (Dei et al., 2008), mango kernel (Kayode and Sani, 2008) and grape seed (Altop et al., 2018).

Table 1. Nutritional comsposition of unfermented and fermented CK

Composition	CK	CK-B	CK-A	SEM	P
Crude Protein	13.2^c	28.8^b	30.7^a	2.77	***
Ether Extract	27.5	27.4	32.7	1.35	NS
Ash	3.1^c	7.4^b	8.1^a	0.78	***
NFE	35.4^a	20.8^b	16.8^b	3.00	**
Crude Fiber	20.8^a	15.6^b	11.8^c	1.32	***
NDF	25.6^a	23.7^a	19.7^b	0.95	**
ADF	13.7	14.4	12.9	0.33	NS
Hemicellulose	11.9^a	9.3^b	6.8^c	0.76	***

*: P<0.05; ***: P<0.001; NS: Not significant
NFE: nitrogen-free extract, SEM: standart error of means
CK: cherry kernel; CK-B: fermented cherry kernel by *B. subtilis*; CK-A: fermented cherry kernel by *A. niger*

Solid state fermentation decreased the CF ($P<0.001$), and HC ($P<0.001$) but did not changed ADF content. *A. niger* decreased ($P<0.01$) NDF content although *B. subtilis* did not changed NDF content of CK. *Aspergillus* *niger* (Xie et al., 2016) and *B. subtilis* (Ritter et al., 2018) can produce celulase enzyme in the conditions of solid state fermentation, which can be the reason of the degradation and decrease of the structural carbohydrates in this study. Similarly, Güngör et al. (2017) reported decreased NDF and ADF in sour cherry kernel by *A. niger*. *A. niger* also decreased CF, NDF and ADF content of grape seed (Altop et al., 2018), shea nut (Dei et al., 2008) and palm kernel cake (Iluyemi et al., 2006; Lawal et al., 2010). Similarly, *B. subtilis* diminished CF in cottonseed meal (Sun et al., 2012) and decreased NDF in mixed feed (Shi et al., 2017).

CONCLUSIONS

In conclusion, these results showed that solid state fermentation can be used to improve the nutritional composition of cherry kernel and *A. niger* shows better performance compared with *B. subtilis*.

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FERMENTED GRAPE SEED IMPROVES CAECAL MICROFLORA IN BROILER CHICKENS

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Abstract

This study was conducted to determine the effect of unfermented (GS) and fermented grape seed (FGS) on the caecal microflora in broiler chickens. Grape seed was fermented with Aspergillus niger for 7 days. A total of 96 one-d-old Ross 308 female chicks were allocated three treatment groups with 4 replicates and 8 birds for each replicate. The birds fed on a basal diet (control) and basal diet supplemented 5 g/kg either GS or FGS. Fermented grape seed increased (P<0.01) the Lactobacillus spp. count (P<0.01) and decreased (P<0.05) Staphylococcus aureus count in cecum of broiler chickens. The results of this study showed that FGS can be used as a feed additive regulating intestinal microflora in broiler nutrition.

Key words: *intestinal microflora, grape seed, solid-state fermentation, Aspergillus niger*

INTRODUCTION

Grape is one of the world's largest fruit crops. Total production of grapes has reached 74.3 million tonnes annually worldwide (FAOSTAT, 2017). Grape seed is a by-product emerging from the juice extraction by pressing grapes in the fruit juice and wine industry (Viveros et al., 2011). Grape seed may have antimicrobial effect on broiler chickens due to phenolic compounds. Indeed, antimicrobial effect of grape seed has been observed *in vitro* (Rotava et al., 2009) and *in vivo* in rats (Dolara et al., 2005), pigs (Choy et al., 2014) and chickens (Viveros et al., 2011).

In recent years, solid state fermentation has been used to utilize the agricultural residues produced at billions of tonnes every year (Gungor et al., 2017). The biological activity of phenolic compounds in organic materials can be increased by solid state fermentation (Cao et al., 2012). Moreover, fermented feeds have probiotics effect and modulate the intestinal microflora in broiler chickens (Chiang et al., 2010). The effect of dietary GS and FGS on the caecal microflora of broiler chickens was investigated in this study.

MATERIALS AND METHODS

Solid State Fermentation

Grape seeds used in the present study were provided by a juice factory in Turkey. They were divided into 2 lots. One lot was unfermented grape seed and other was fermented using *A. niger*. Grape seeds were milled to a size of 2 mm before being sterilized by autoclaving at 121 °C for 15 min. The nutritional salt (glucose:urea:$(NH_4)_2SO_4$: peptone:KH_2PO_4:$MgSO_4.7H_2O$=4:2:6:1:4:1) were mixed to the substrate to encourage microorganism to grow after sterilizing phase. Prepared *A. niger* spores were added to grape seed and incubated 60 °C for 7 days. Afterwards, samples were dried at room temperature for 6 days in which samples reached approximately 90% dry matter. Three replicates were prepared for each treatment.

Animals and Diets

All experimental procedures were conducted in accordance with the guidelines of Ondokuz Mayis University Local Ethical Committee. A total of 96 one-d-old Ross 308 female broilers were weighed and randomly allocated to 3 treatment groups consisting of 4 replicates of 8 birds each. The temperature of the room maintained at 32 °C for the first 3 days, gradually decreased to 20 °C until the end of the sixth week. A 24 h light:1 h dark regime was provided throughout the experimental period.

Diets were formulated to meet the nutritional requirements of broilers reported by NRC (1994) (Table 1). Dietary treatments were CON (control, basal diet), GS (basal diet supplemented 5 g/kg grape seed) and FGS (basal diet supplemented 5 g/kg fermented grape seed). Feed and fresh water were provided ad libitum throughout the experiment. Nutritional composition of grape seed and fermented grape seed were 10.13% and 29.50% crude protein (CP), 12.50% and 9.82 ether extract (EE), 3.88% and 8.72% ash and 47.43% and 36.25% crude fiber (CF) as reported in our fermentation study (Altop et al., 2018).

Table 1. Ingredient and nutrient composition of broiler diets

Ingredients (%)	Diets (day)			
	0-11	12-21	22-35	36-42
Corn	41.70	44.66	48.71	51.18
Soybean meal	21.15	19.20	12.53	9.48
Full-fat soybean	17.25	18.00	20.43	21.01
Corn gluten	15.00	10.00	10.00	10.00
Sunflower meal	-	-	-	-
Meat and bone meal	-	2.20	2.20	2.24
Chicken meal	-	4.00	4.30	4.30
Dicalcium phosphate	1.75	-	-	-
Vegetable oil	0.83	0.75	1.00	1.00
Marble dust	0.61	-	-	-
NaCl	0.20	0.15	0.15	0.15
DL Methionine	0.36	-	-	-
Liquid methionine	-	0.20	0.18	0.20
L Lysine	0.47	0.08	0.05	0.07
Threonine	0.01	-	-	-
Vitamin and mineral premix[1]	0.30	0.30	0.30	0.30
Vitamin D_3	0.10	0.05	-	-
Sodium bicarbonate	0.17	0.10	0.10	0.10
Anticoccidial	0.05	0.05	0.06	-
Toxin binder	0.05	0.05	-	-
Chemical analysis				
Crude protein	230	220	200	190
Ether extract	60.7	73.2	80.7	82.2
Crude fibre	33.6	34.5	34.4	34.1
Ash	58.1	45.5	42.0	40.2
Calculated analysis				
Metabolizable energy (kcal/kg)	3025	3100	3175	3200
Lysine	15.50	1.31	1.15	1.10
Methionine	0.68	0.51	0.48	0.45
Methionine and cystine	1.12	0.97	0.90	0.87
Threonine	0.98	0.88	0.80	0.76
Tryptophan	0.27	0.27	0.24	0.23
Calcium	1.00	0.87	0.83	0.80
Total P	0.77	0.73	0.71	0.70
Available P	0.50	0.50	0.50	0.48
Na	0.17	0.18	0.18	0.18

[1] provided the following per kilogram of diet: 12 000 IU retinol; 2 400 IU cholecalciferol; 40 mg α-tocopherol; 4 mg menadione; 3 mg thiamine; 6 mg riboflavin; 25 mg nicotinic acid; 10 mg pantothenic acid; 5 mg pyridoxine; 0.03 mg cyanocobalamin; 0.05 mg biotin; 1 mg folic acid; 80 mg Mn; 60 mg Zn; 60 mg Fe; 5 mg Cu; 0.2 mg Co; 1 mg I; 0.15 mg Se, 200 mg choline chloride.

Determination of Caecal Microflora

Each caecal digesta was serially diluted (1:10) to 10−7. Dilutions were plated onto MRS (de Man, Rogosa and Sharpe) agar (110660, Merck® KGaA, Darmstadt, Germany), Slanetz and Bartley agar (Merck® 105262), EMB (Eosin Methylene Blue) agar (Merck® 101347), BAIRD-PARKER Agar (Merck® 105406) and TSC (Tryptose Sulfite Cycloserine) agar (Merck® 111972) to enumerate *Lactobacillus* spp., *Enterococcus* spp., *Escherichia coli*, *Staphylococcus aureus* and *Clostridium perfringens* were enumerated on, respectively.

Statistical analysis

All data were analyzed by one-way analysis of variance (ANOVA) using SPSS statistical software (Ver. 21.0 for windows, SPSS, Inc., Chicago, IL, USA). Significant means were compared by a Tukey test. Differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Grape pomace and grape seed extract increased caecal *Lactobacillus* counts in broiler chickens (Viveros et al., 2011). Although GS did not affect the caecal microflora, *Lactobacillus* spp. count of birds in FGS group was significantly increased ($P<0.01$) compared with CON and GS groups (Table 2). Chiang et al. (2010) reported that fermented feeds has a probiotic effect on broiler chickens. *Aspergillus niger* raised *Lactobacillus* spp. counts in broiler chickens given as a spores or as fermented feed. Zhang et al. (2015) reported that fermented *Ginkgo biloba* leaves increased *Lactobacillus* counts in ileum of broiler chickens. Similarly, Ashayerizadeh et al. (2018) showed that fermented rapeseed meal increased *Lactobacillus* spp. counts in the crop of broilers.

Rotava et al. (2009) reported antimicrobial activity of grape seed extract against *S. aureus* in vitro. Indeed, broilers fed the FGS diet had lower ($P<0.05$) *S. aureus* count in cecum compared with birds from CON group in this study. Ceacal *E. coli*, *Enterococcus* spp. and *C. perfringens* counts were not affected ($P>0.05$) by dietary GS or FGS inclusion. Similarly, Zhang et al. (2015) reported no changed *E. coli* count in ileum and cecum of broiler chickens with dietary fermented *G. biloba* leaves.

Table 2. Effects of dietary grape seed (GS) and fermented grape seed (FGS) on caecal microflora in broiler chickens

	Dietary treatments[1]				
Item (log10 cfu)	CON	GS	FGS	SEM	P
Lactobacillus spp.	7.4^{b}	8.0^{b}	8.8^{a}	0.22	**
E.coli	6.6	7.0	7.8	0.34	NS
Enterococcus spp.	7.0	7.3	7.6	0.15	NS
S. aureus	4.8^{a}	4.3^{ab}	4.0^{b}	0.14	*
C. perfringens	5.9	5.6	6.0	0.15	NS

$^{a-d}$Means within the same row that have no common superscript are significantly different (P < 0.05).

SEM: standart error of mean, *: P <0.05, **: P < 0.01, NS: not significant.

[1] CON: basal diet; GS: basal diet supplemented 5 g/kg grape seed; FGS: basal diet supplemented 5 g/kg fermented grape seed.

CONCLUSIONS

In conclusion, the results of the present study showed that FGS can be used as an intestinal health enhancer with the positive effects on the intestinal microflora in broiler chickens.

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FORAGE DIVERSITIES AND THE USE OF PROTEIN OR ENERGY SUPPLEMENTATION FOR GOAT IN THE TROPIC

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Abstract

Aim of the current research was to identify the diversity of forages used by farmers in Indonesian goat farming system and to evaluate the effects of protein and energy feeding supplementations on nutrients intake and digestibility of goats. Fifteen female Bligon goats were divided into three treatments randomly (n=5): T_0 *(control diet): only forages that commonly given by the local farmers;* T_1*: common local forages + energy concentrates supplement (18.00% crude fiber (CF), 67.5% total digestible nutrient (TDN), and 14.80% crude protein (CP)); and* T_2*: common local forages + protein concentrate supplement (15.5% CF, 65% TDN, and 24.34% CP). Feeding treatments were conducted in eight weeks and preceeded with two weeks total collection period. Parameters observed were: forage diversities, as well as nutrient intake and digestibility in dry matter (DM), organic matter (OM), ether extract (EE), crude fiber (CF), crude protein (CP), non-protein nitrogen (NPN), and total digestible nutrient (TDN). The collected data were statistically analyzed using SPSS Version 16.0 for Windows. The difference between means was separated using Duncan's test. From the study, we observed that there were 40 forage species used in the goat farming system in rural areas in Indonesia, which can be classified into: 5 (five) species of gramineae, 12 (twelve) species of woody plant, 3 (three) species of legumes, 6 (species) of agricultural wastes, and 12 (twelve) species of weeds. Both feeding supplementations (*T_1 *and* T_2*) improved CP intake (*$P<0.05$*), meanwhile DM, OM, CF, EE, NPN intakes did not differ significantly when compared to control group. DM, OM, CF, CP and NPN digestibility were also improved significantly (*$P<0.05$*) in* T_1 *and* T_2*, when compared to control (*T_0*). We may conclude that nutrient quality status in the goat farming system with forage feeding were adequate to meet the nutrients requirement for pregnant Bligon goats, so that supplementation feeding was not compulsory to be conducted in the rural goat farming system in Indonesia.*

Keywords: *Goat, Tropical, Forage-species diversities, Energy-protein supplementations*

INTRODUCTION

Bligon goat is one of well-known small ruminants in Indonesia. This local goat is cross-breeding between kacang goat and Ettawah (Jamnapari) crossbred goat. Bligon has the ability to grow up to 50 – 100 g daily (Sutama and Budiarsana, 2010). Bligon goat also has a good reputation as meat-type with medium body size, high carcass percentage, and is also known to be able to adapt to almost all of Indonesian geographical areas.

Goats in Indonesia, especially in rural areas, are normally kept with traditional methods. Commonly, feeds that were given are forages that grow around the area.

Forage is a feed ingredient derived from plants consisting of leaves mixed with stems, twigs, and flowers, which originate generally from grass plants (*gramineae*), beans (*leguminoceae*), agricultural wastes, or forage from other plants (Hadi *et al*., 2011). Forages that are usually given to the livestock are varies in numbers, types, and qualities, according to the natural and seasonal conditions.

A study of Manik (2015) has reported that intake of DM, OM, EE, CF, and CP were: 790, 710.30, 180, and 80 g/head/day, respectively. Therefore, this study was aimed to determine diversity of the forages or forage species which given to livestock and also nutrient quality in the diets, through the administration of energy sources or protein sources concentrates. The research was expected to provide information and recommendations to the public regarding improving nutritional status of livestock through the fulfillment of the appropriate nutritional requirements in the diets of Bligon Crossbred does at the beginning of pregnancy phase.

MATERIALS AND METHODS

Fifteen pregnant Bligon goats were used in this study. According to the breeding data recorded by the farmers. Ages of the pregnant goats were detected one to three months with a body weight of between 30 and 35 kg.

Feed used in the study were consisted of forages and concentrates. The forages were collected from local areas around the goat pens. The quantity and the common species of forages were determined by the local farmers. Feed concentrate the energy source (CP 14.84%) and protein source (CP 24.34%) was given 500 gram per head daily, according to the treatment group.

Table 1. Concentrate composition

	Group		
	Control	T. I	T. 2
Forages	*Ad libitum*	*Ad libitum*	*Ad libitum*
Concentrate (g)	-		
Wheat pollard	-	101.95	86.3
Soybean husk	-	84	51.38
Soybean Meal	-	32.6	145.87
Dryed cassava	-	116.35	50.14
DDGS	-	59.1	61.65
Premix	-	4.05	0.46
Cassava Leaf	-	101.9	104.18
Total		500	500
Nutrient composition (%)			
Dry Matter		86.25	86.75
Crude Protein		14.84	24.34
Crude Fibre		18.0	15.50
TDN		65.75	62.70

T1: Energy source Supplementation; T2: Protein source Supplementation

The Bligon goats were divided into three treatment groups, namely: T0=forage only basal diet in which the type and quantity were chosen by the farmers (Basal diet) as Control; T1=Basal diet that supplemented with energy source concentrate; T2=Basal diet that supplemented with protein source concentrate. Feed were given in 8 weeks. Data collection, (*in vivo/ feed offered, residu, faecal*) were carried out consecutively for 14 days. than were analyzed for dry matter (DM), organic matter (OM), crude protein (CP), crude fiber (CF), and crude fat or extract ether (EE). The variables observed were nutrient digestion and consumption. The obtained data were statistically analyzed using analyses of variance by Oneway of completely randomized design.

RESULTS AND DISCUSSION

Diversity and chemical composition of forages

Diversity of forage species in the village is obtained by farmers in the surrounding environment. The forage are then grouped into: foliage, leguminoceae, grass, agricultural wastes, and weed groups

Foliage is forage originating from shade trees that are intentionally planted or which grow wild around the maintenance area and are used by farmers to feed goats. These types or species of leaves are the common feed, compared to forages, legumes, agricultural wastes, and weeds. Foliage that commonly given to the animals are sonokeling (*Dalbergia latifolia*) and mahogany (*Swietenia mahagoni*) leaves. Sonokeling is a shelter plant that can still grow well in the dry season and is quite widely known by the community as wood-producing plants. Based on the results of the analysis, it is known that sonokeling leaves contained 39.79% DM, 94.50% OM, and 13.59% CP. These values are similar to the finding of Husna (2016) who reported that sonokeling leaves contained 35.83% DM, 94.70% OM, and 15.66% CP. Result also showed that mahogany leaves contained 38.01% DM, 91.99% OM, and 8.16% CP. Parama (2016) reported that mahogany leaves contained 22 - 37% DM, 88 - 94% OM, and around 10% CP.

Legume plants are favored by ruminants, especially goats. Legumes are also known to have a fairly good nutrient content, especially in the protein content that is higher than that of grass. Kushartono and Iriani (2004) reported that legumes have the highest protein content of all types of forages. Legume plants generally contain CP around 22%, so it is a good decision to feed animals which are commonly only given grass with legumes plants. Kustantinah *et al*. (2013) reported that nutrient content of legume plants is 18.58 - 22.76% CP, 18.47 - 23.25% CF, and 1.33 - 3.02% EE. The cultivated legume plants are also known to improve the processing of agricultural land resources, such as: soil surface protection from erosion and improve soil fertility. Some types of legumes that given during the study were: gliricidia immaculata, leucaena leucocephala, and calliandra calothyrsus.

Some of the given grass are: field grass, king grass, elephant grass, and kolonjono grass. Based on the results of laboratory analysis it was found that grass containing DM from 18.76 - 33.75%, OM around 80.49 – 91.32% and CP 7.32-9.45 %. Agricultural waste is a part of the by-product of agriculture whose main products have been taken. The availability of agricultural waste depends on the harvest period of agricultural crops.

Agricultural wastes are various in kinds, and can be found as straw, plantation by-products, or by-products from the agricultural industries. Agricultural wastes provided by farmers during the research consisted of corn fodder straw and lembayung or long bean straw (*Vigna cylindrica* L.). Long bean straw, in current study, contained 24.57% DM, 88.18% OM, and 14.59% CP. Parama (2016) reported that long bean straw contained 27.55% DM, 89.21% OM, and 12.06% CP. Corn fodder straw (*Zea mays*), in this study, contained 25.70% DM, 91.47% OM, and 7.00% CP.

Weed is a plant that grows wildly in an agricultural environment or land that is not maintained. Plants that are considered as weeds by the community can actually be used as animal feed, supplementing forages, excluding leaves, legumes, grass, and agricultural wastes. The common weed plants that were given to animal, during the study were mambu (*Synedrella nodiflora*), legume (*Calopogonium muconoides*), horse whip (*Stachytarpheta indica*) and imperata (*Imperata cylindrica*).

Nutrient Intake

Results of the statistical analysis showed that there was no significant difference in DM consumption between all three animal groups. Average of the total DM consumption (g/head/day) in each animal group T0, T1, T2 were 1228.54 g/head/day (4.08% LW), 1335.20 g/head/day (3.73% LW), and 1495.82 g/head/day (4.47% LW), respectively.

Nutrient digestibility

Nutrient digestibility of the diets in all animal groups showed that dry matter digestibility (DMD) values of the three animal groups (T0, T1, T2) increased significantly by 71.93, 80.18, and 84.02%, respectively. The subsequent analyses showed that DMD of the diets in T1 and T2 were higher, when compared to control ($P<0.05$). The OMD of the diets in T2 was also higher than that of in control ($P<0.05$), but was not different significantly with T1. The high levels of DMD and OMD in this study might be attributed to high level of nutrients digestibility of concentrated diets in the gastro intestinal tract of ruminant.

Crude fibre digestibility (CFD) obtained in our study were: 74.91% (T0), 79.84% (T1), and 84.08% (T2). Available data showed that dietary supplementation of concentrate tended to increase CFD. Values of CFD in T1 and T2 were higher than that of in P0 ($P<0.05$). CFD value was closely related to the type of given feed. Therefore, T2 group which was the group supplemented with protein source concentrate. The high level of dietary protein will stimulate the growth of ruminal microbes and maximize the activity of gut to optimally digest CF. Another study (Kustantinah *et al.*, 2009) stated that CFD in Bligon goats fed combination of foliage, grass, and agricultural waste in dry season was 22.40%. Parama (2016) reported that kacang goats which supplemented with 50% protein source had the value of CF digestibility 78.06%.

Results showed that the CP digestibility (CPD) values obtained were 66.77% (T0), 75.44% (T1), and 86.09% (T2). Statistical analyses showed that CPD of the T2 group was higher than that of the control (T0), but did not different significantly with the CPD of T1 group. The addition of protein source concentrate in T2 group had a positive effect to improve CPD. Animal feed containing good and sufficient CP provides necessary protein so that the rumen microbes can develop optimally and increase diet digestibility. Our results were in compromise with the findings of Parama (2016) and Hananto (2016) who reported that CPD in the diets of kacang goats who received feed supplemental protein sources by 50% of DM needs was 86.38%.

CONCLUSIONS

Species of forages used by farmers during the study was consisted of leaves, legumes, grass, agricultural wastes, and weeds, covering 41 species of plants in total. The supplementations of energy and protein source for Bligon does in early pregnant phase had not been able to increase overall nutrients consumption and digestibility. Results of this study informed that quality of the local forages given by the farmers to their animals were quite good. No additional feed supplements was needed to improve the performance of early and late pregnant Bligon does.

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EFFECTS OF LIGNOCELLULOSE SUPPLEMENTATION ON GROWTH RATE, ASCITES INCIDENCE AND HETEROPHIL/LYMPHOCYTE RATIO AS A STRESS INDICATOR IN BROILERS

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Abstract

This study was conducted to investigate the effects of lignocellulose supplementation on growth rate, ascites incidence and heterophil/lymphocyte ratio as a stress indicator in broilers. A total of 720 one-day old Ross 308 broiler chickens were randomly assigned to four treatment groups: control (no supplementation), dose 1 (0,5 kg/tonnes), dose 2 (1 kg/tonnes) and dose 3 (2 kg/tonnes) dietary supplementation of lignocellulose. The lignocellulose is a product containing 90% lignin processed at low temperature. Six replicates containing 30 chicks (15 female, 15 male) were used for each dietary group. During experimental period, growth rate of broilers was determined for 1-14 days and 15-35 days. To assess ascites susceptibility, the hearts were removed to obtain heart weight, and right ventricle (RV) and total ventricle (TV) weights to calculate RV:TV ratio as an indicator of ascites. At 35 d of age, serum biochemical parameters (heterophil, lymphocytes, monocytes, eosinophils, basophils) were analysed and H:L ratio was calculated as a stress indicator. At 35 d of age, broilers fed with dose 2 was found to be growed much better at the rate of 6.6%, 4.5% and 7.1% whenever compared control, dose 1 and dose 3 groups, respectively (P<0.01). The right ventricle weight was similar among the dietary groups whereas the total ventricle weight was found to be the lowest in the control group (8.1 g, P<0.05). A similar RV:TV ratio ranged between 0.18 – 0.24%, was observed for the dietary groups (P>0.05). The H:L ratio was found to be lower in lignocellulose supplementation groups than the control. It was found as 1.49 in the control, and 1.02, 1.18 and 1.14 in dose 1, dose 2 and dose 3 groups, respectively. As a conclusion, current findings showed that dietary suppmenetation of lignocellulose have a stimulating effect for growth rate and a protective effects against stressfulness in broilers..

Key words: *broiler, lignocellulose, growth rate, ascites, heterophil/lymphocyte ratio.*

INTRODUCTION

Currently, there is a growing interest for new feeding strategies for stimulation of growth performance with an efficient feed utilization in broiler production. Recently, many studies have focused on the possible effects of fiber supplementation for various parameters in broilers (Hussein et al., 2017; Kheravii et al., 2017; Zhou et al., 2018; Makivic et al., 2019; Zeitz et al., 2019). Preliminary studies have verified that lignocellulose supplementation in various amounts can be very beneficial for both performance, the digestive function and health of both broiler and layer chickens (Mateos et al., 2012; Farran et al., 2013; He et al., 2015; Jimenez-Moreno et al., 2016).

This study focused on the effects of lignocellulose rich fiber source that contained 90% lignin, on on growth rate, ascites incidence and heterophil/lymphocyte ratio as a stress indicator in broilers.

MATERIALS AND METHODS

A total of 720 one-day old Ross 308 broiler chickens were were weighed using a balance (±0.1 g precision), and then randomly assigned to four treatment groups: control (no supplementation), dose 1 (0,5 kg/tonnes), dose 2 (1 kg/tonnes) and dose 3 (2 kg/tonnes) dietary supplementation of lignocellulose. The lignocellulose is a product containing 90% lignin processed at low temperature. Six replicates containing 30 chicks (15 female, 15 male) were used for each dietary group.

The chicks received a standard commercial crumbled broiler starter diet between days 1-14, and then a grower diet between days 15-35. Water and feed were offered ad libitum throughout the experiment. Room temperature was 33°C at 1 d of age and was decreased by 3°C every week until maintained at 20°C and 50-60% relative humidity till the end of the experiment. Wood shavings were used as litter material, which was laid at a thickness of 7-8 cm on the floors of the pens. Lighting schedule was applied according to the company's management guides and recommendations (Ross 308 Management Guide, 2013).

During experimental period, growth rate of broilers was determined for 1-14 days and 15-35 days. To assess ascites susceptibility, the hearts were removed to obtain heart weight, and right ventricle (RV) and total ventricle (TV) weights to calculate RV:TV ratio as an indicator of ascites (n = 12 broilers from each treatment group). At 35 d of age, serum biochemical parameters (heterophil, lymphocytes, monocytes, eosinophils, basophils) were analysed and H:L ratio was calculated as a stress indicator.

In this study, data of growth rate, ascites incidence and heterophil/lymphocyte ratio were subjected to variance analysis (SAS, 2003), utilizing ANOVA procedures for balanced data. Significant differences among the treatment means were determined by Duncan's multiple range test. All values are presented as mean ± standard error. In all cases, a difference was considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

The effects of dietary supplementation of lignocellulose on growth performance are presented in Table 1. Body weight of chicks on day 1 were similar and changed between 45.1 g and 46.2 g among the treatment groups ($P>0.05$). Ay day 14 and 35 days of age, broilers in dose 2 group had the heaviest body weight with values of 489.6 g and 2305.0 g respectively compated to the other groups ($P<0.01$). On the other hand, growth rate of birds found to be similar during growing period ($P>0.05$). Feed conversion rate was found to be more efficient in dose 2 group with a value of 1.06 on day 14 and 1.50 on day 35 of experimental period ($P<0.05$). This findings are supported by Sarikhan et al. (2010) who reported that broilers fed with diets supplemented with insoluble fiber between weeks 3 and 6 had heavier body weight and more efficient FCR. Observed iincrement in growth performance could be related with a stimulating effect of lignocellulose supplementation for digestive tract functionality and gidestibility of protein and fats (Bogulawska-Tryk et al., 2016; Makivic et al., 2019).

Table 1. The effect of lignocellulose supplementation on growth performance parameters in broilers

Parameters	Treatment groups				P Value
	Control	Dose 1	Dose 2	Dose 3	
Body weight (g)					
Day 1	45.1	45.9	45.1	46.2	NS
Day 14	460.0^{c}	474.5^{b}	489.6^{a}	471.4^{bc}	<0.01
Day 35	2154.0^{bc}	2201.0^{b}	2305.0^{a}	2141.7^{c}	<0.01
Growth rate (%)					
Days 1-14	1020.2	1009.2	1053.6	1008.2	NS
Days 14-35	340.4	352.1	358.0	359.3	NS
CFC (g)	3325.3^{ab}	3250.7^{b}	3391.7^{a}	3259.0^{b}	<0.01
FCR					
Day 14	1.13^{a}	1.08^{ab}	1.06^{b}	1.09^{ab}	<0.05
Day 35	1.58^{a}	1.51^{b}	1.50^{b}	1.55^{ab}	<0.05

CFC: Cumulative feed consumption, FCR: Feed conversion rate

^{a,b,c} Means bearing different superscripts within the same line are significantly different. NS: Not significant

n: 6 replicate pens per treatment group (30 broilers/pen). Control: no supplementation of lignocellulose, dose 1: 0.5 kg supplementation/tone feed, dose 2: 1 kg supplementation/tone feed, dose 3: 2 kg supplementation/tone feed.

The effect of lignocellulose supplementation on heart development and ascites incidence over 35 days of age in broilers are presented in Table 2. Any significant effect was observed for right ventricle, ratio between right ventricle and total ventricle, and relative weight of heart. Total ventricle weight showed a variation between treatment groups ($P<0.05$), this could be related with final body weight of broilers in treatment groups. It could be emphasized that the supplementation of lignocellulose had any effects for heart development and ascites incidence.

Table 2. The effect of lignocellulose supplementation on heart development and ascites incidence over 35 days of age in broilers

Parameters	Treatment groups				P Value
	Control	Dose 1	Dose 2	Dose 3	
RV (g)	1.9	2.1	2.9	2.7	NS
TV (g)	8.1 ^{b}	11.9 ^{a}	13.3 ^{a}	12.8 ^{a}	<0.05
RV/TV	0.24	0.18	0.22	0.21	NS
Heart (%)	0.36	0.49	0.53	0.52	NS

RV: Right ventricle, TV: Total ventricle

^{a,b,c} Means bearing different superscripts within the same line are significantly different. NS: Not significant

n: 12 broilers per treatment group (30 broilers/pen). Control: no supplementation of lignocellulose, dose 1: 0.5 kg supplementation/tone feed, dose 2: 1 kg supplementation/tone feed, dose 3: 2 kg supplementation/tone feed.

The effect of lignocellulose supplementation on some serum biochemical parameters over 35 days of age in broilers are presented in Table 3. Results showed that the supplementation of lignocellulose cause a decline in H/L value compared to the groups. This could be an indicator for protective effect of fiber supplementation for broilers.

Table 3. The effect of lignocellulose supplementation on some serum biochemical parameters over 35 days of age in broilers

Parameters	Treatment groups				P Value
	Control	Dose 1	Dose 2	Dose 3	
Basophils (%)	7.5	7.8	9.0	8.7	NS
Eosinophils (%)	6.7	6.8	6.3	7.4	NS
Monocytes (%)	16.5	16.2	16.2	15.9	NS
Heterophils (%)	41.5	35.0	37.1	36.2	NS
Lymphocytes (%)	27.8	34.2	31.4	31.8	NS
H/L	1.49^{a}	1.02^{c}	1.18^{b}	1.14^{b}	<0.05

^{a,b,c} Means bearing different superscripts within the same line are significantly different. NS: Not significant

n: 12 broilers per treatment group (30 broilers/pen).

Control: no supplementation of lignocellulose, dose 1: 0.5 kg supplementation/tone feed, dose 2: 1 kg supplementation/tone feed, dose 3: 2 kg supplementation/tone feed.

CONCLUSIONS

Unfortunately, any scientific papers focused on the possible effects of lignocellulose on ascites incidence and H/L as a stress indicator in broilers. Therefore, more detailed research should be beneficial to understand the possible affecting mechanism of lignocellulose supplementation. Based on the current findings, it should be recommended 1 kg supplementation of lignocellulose per tone feed as optimum lignocellulose level for growing performance of broilers.

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DETERMINATION OF POTENTIAL NUTRITIVE VALUE OF SOME LEGUME SEED

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Abstract

The aim of the current experiment was to determine the potential nutritive value of some legume seed using chemical composition and in vitro gas production. Species had a significant effect on chemical composition, gas production, methane production, metabolisable energy and organic matter digestibility of legume seeds. Crude ash, ether extract and crude protein contents of legume seed 2.90 to 4.63% and 1.47 to 17.26 and 21.75 to 35.19 % respectively. Crude ash, ether extract and crude protein contents of Glycine max were significantly higher than the other legume seeds. Neutral detergent fiber and acid detergent fiber contents of legume seeds ranged from 22.44 to 37.04 and 8.85 to 23.58 % respectively. Neutral detergent fiber and acid detergent fiber contents of Lathyrus sativus and Vicia narbonensis were significantly higher than the other legume seeds. Gas production ranged from 42.47 to 58.58 ml. The gas production from Vicia pregrina was significantly higher than the other legume seed. Methane production ranged from 6.96 to 9.90 ml. Metabolisable energy and organic matter digestibility ranged from 9.36 to 11.76 MJ/ kg DM, 64.40 to 79.66 % respectively. Metabolisable energy and organic matter digestibility from Lathyrus sativus was significantly higher than the other legume seeds. As a conclusion there are considerable variation among legume seed in terms of chemical composition, gas production, methane production, metabolisable energy and organic matter digestibility.

Key words: *legume seeds, gas production, methane production, metabolisable energy, organic digestibility*

INTRODUCTION

Ruminant nutrition system in the Mediterranean area largely depends on low quality forages and crop residues which are usually deficient in crude protein and have a low digestibility. Therefore the productivity of ruminant is generally low. Soybean meal has been widely used to improve the efficiency of ruminant nutrition in Turkey as in many parts of world.. Recently interest for cheap and alternative locally available protein sources has increased due to increase in price and fluctuation of soybean production. Recently some researchers clearly showed that under utilised legume seeds instead of soybean can be used in fish diet to some extent without compromising of grow performance, feed intake and feed conversation ratio (Buyukcapar and Kamalak 2010; Buyukcapar 2012). Therefore underutilised legume seeds seem to be a potential source of supplement in ruminant diet to improve the efficiency of ruminant nutrition. Therefore available information in the literature on the nutritive value of some legume seeds should be increased to make accurate decision during the ration preparation for ruminant diets. The aim of the current experiment was to determine the potential nutritive value of some legume seed using chemical composition and *in vitro* gas production.

MATERIALS AND METHODS

Seed of *Lathyrus sativus*, *Vicia narbonensis*, *Vicia peregrina*, *Vicia sativa* and *Pisum elatius* were obtained from plant in natural pasture at seeding maturity in 2018. The seed from *Glycine max* was obtained commercial feed company. Legume seeds were milled through a 1 mm sieve after drying. Chemical compositions of seeds were determined using the methods described by AOAC (1990). Neutral detergent fiber and acid detergent fiber content of seed were determined with the method described by Van Soest (1991). *In vitro gas* production of seed was determined for 24 h (Menke et al. 1979). Rumen fluid was obtained from three fistulated sheep fed twice daily with a diet containing alfalfa hay (60%) and concentrate (40%). after incubation.

Methane content of gas produced was determined using the infrared methane analyzer (Goel et al., 2008). The metabolisable energy (MJ/kg DM) and organic matter digestibility (OMD) of seeds were calculated using equations of Menke and Steingass (1988) as follows:

ME (MJ/kg DM) = 2.20 + 0.1357 GP + 0.057 CP + $0.002859EE^2$
OMD (%) = 14.88 + 0.8893 GP + 0.448 CP + 0.651CA.

One-way analysis of variance (ANOVA) was used to determine the effect of species on chemical composition, gas, methane production, metabolisable energy and organic matter digestibility of legume seeds. Significance between individual means was identified using the Tukey's multiple range tests. Mean differences were considered significant at $p<0.05$

RESULTS AND DISCUSSION

The effects of species on the chemical composition of some legume seeds:

The effects of species on the chemical composition of some legume seeds were given in Table 1. Species had a significant effect on chemical composition of some legume seeds. Crude ash, ether extract and crude protein contents of legume seed 2.90 to 4.63% and 1.47 to 17.26, 21.75 to 35.19 % respectively. Crude ash, ether extract and crude protein contents of *Glycine max* were significantly higher than the other legume seeds. Neutral detergent fiber and acid detergent fiber contents of legume seeds ranged from 22.44 to 37.04 and 8.85 to 23.58 % respectively. Neutral detergent fiber and acid detergent fiber contents of *Lathyrus sativus* and *Vicia narbonensis* were significantly higher than the other legume seeds.

The chemical composition of *Vicia peregrine* is consistent with that reported by Buyukcapar (2012) who reported that dry matter, crude protein, crude fat and crude ash of *Vicia peregrine* were 93.02, 25.31, 1.53 and 3.89% respectively. The chemical composition of *Pisum elatius* is consistent with that reported by Buyukcapar and Kamalak (2010) who reported that dry matter, crude protein, crude fat and crude ash of *Pisum elatius* were 88.99, 23.34, 2.53 and 2.81 respectively. The chemical composition of *Vicia sativa* is consistent with that reported by Huang et al. (2017) who reported that dry matter, crude protein, crude fat, NDF, ADF and crude ash of *Pisum elatius* were 90.6, 28.3, 1.58, 24.19, 9.3 and 3.43% respectively.

The CP content of seed of *Vicia sativa* was consistent with that reported by Canbolat and Bayram (2007) who reported that CP content of seed of *Vicia sativa* was 28.3% of DM whereas NDF content of *Vicia sativa* was considerably higher than that reported by by Canbolat and Bayram (2007) who reported that NDF and ADF contents of *Vicia sativa* was 17.6 and 9.9 % of DM. The CP content of seed of *Lathyrus sativus* was considerably lower than that by Canbolat and Bayram (2007) who reported that CP content of seed of *Lathyrus sativus* was 28.3 of DM whereas NDF and ADF contents of seed of *Lathyrus sativus* was significantly lower than those reported by Canbolat and Bayram (2007). The differences among studies are possibly associated with growing conditions and climatic conditions where the experiments were carried out.

The effects of species on gas production, methane production, metabolisable energy and organic matter digestibility of legume seeds

The effects of species on gas production, methane production, metabolisable energy and organic matter digestibility of legume seeds were given in Table 2. Species had a significant effect on chemical composition of some legume seeds. Gas production ranged from 42.47 to 58.58 ml. The gas production from *Vicia pregrina* was significantly higher than the other legume seed. Methane production ranged from 6.96 to 9.90 ml. The gas production from *Glycine max* and *Lathyrus sativus* were significantly lower than the other legume seeds. Metabolisable energy and organic matter digestibility ranged from 9.36 to 11.76 MJ/ kg DM, 64.40 to 79.66 % respectively. Metabolisable energy and organic matter digestibility from *Lathyrus sativus* was significantly higher than the other legume seeds. The differences in gas, methane, metabolisable energy and OMD of legume grains reflect differences in their chemical composition.

The ME content of *Vicia sativa* is consistent with those reported by Valentine and Bartch (1996.) and Budag and Bolat (2010) who showed that the ME content of Vicia sativa ranged from 8.67 to 12.50 MJ/kg DM. The

OMD content of *Vicia sativa* is consistent with that reported by Hadjipanayiotou et al (1985) who indicated that OMD ranged from 81 to 91%.

Table 1. The effects of species on the chemical composition of some legume seeds

Species	DM	CA	EE	CP	NDF	ADF
Glycine max	89.42^{d}	4.63^{a}	17.26^{a}	35.19^{a}	22.44^{b}	12.12^{c}
Lathyrus sativus	94.12^{a}	3.08^{b}	1.47^{b}	21.75^{d}	37.04^{a}	23.58^{a}
Vicia narbonensis	94.35^{a}	3.08^{b}	2.30^{b}	24.61^{c}	34.70^{a}	19.27^{b}
Vicia peregrina	92.10^{c}	3.07^{b}	1.73^{b}	23.87^{cd}	27.25^{b}	9.80^{cd}
Vicia sativa	93.5^{b}	2.90^{b}	1.82^{b}	29.62^{b}	26.51^{b}	8.85^{d}
Pisum elatius	94.42^{a}	2.90^{b}	2.30^{b}	22.11^{d}	22.44^{b}	12.12^{c}
sem	0.170	0.127	0.665	0.646	1.672	0.788

^{ab}Column means with common superscripts do not differ (p>0.05), sem: Standard error mean, DM: Dry matter(%), CA: Crude ash (%), EE; Ether extract (%), CP: Crude protein (%), ADF: acid detergent fiber(%),NDF: Neutral detergent fiber(%).

Table 2. The effects of species on gas production, methane production, metabolisable energy and organic matter digestibility of legume seeds

Species	Gas	CH$_4$(ml)	CH$_4$ (%)	ME	OMD
Glycine max	45.66^{d}	7.26^{c}	15.90^{d}	11.43^{a}	74.30^{b}
Lathyrus sativus	42.47^{d}	6.96^{c}	16.41^{cd}	9.36^{c}	64.40^{c}
Vicia narbonensis	49.75^{c}	9.07^{ab}	18.23^{a}	10.60^{b}	72.13^{b}
Vicia pregrina	58.58^{a}	9.90^{a}	16.89^{bc}	11.76^{a}	79.66^{a}
Vicia sativa	53.75^{b}	9.41^{a}	17.51^{ab}	11.43^{a}	77.83^{a}
Pisum elatius	50.94^{bc}	8.34^{b}	16.37^{cd}	10.63^{b}	71.96^{b}
sem	1.142	0.289	0.269	0.165	1.036

^{ab}Column means with common superscripts do not differ (p>0.05), sem: Standard error mean, ME: Metabolisable energy(MJ/kg DM), OMD: Organic matter digestibility (%).

CONCLUSIONS

As a conclusion there are considerable variation among legume seed in terms of chemical composition, gas production, methane production, metabolisable energy and organic matter digestibility of legume seeds. Although crude protein content of the legume seeds studied in the current experiment was considerably lower than that *Glycine max*, the ME and OMD of legume seeds was comparable with *Glycine max.* Therefore the legume seeds studied in the current experiment seem to have a potential to use in the ruminants diets. However further investigation is required to determine the effect of legume seeds on ruminant performance.

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EFFECT OF *MELIA AZEDARACH* SEED EXTRACT ON GAS AND METHANE PRODUCTION OF ALFALFA HAY

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Abstract

The aim of the current experiment was to determine the effect of Melia azedarach seed extract on the gas and methane production of alfalfa hay. Melia azedarach seed were chopped and extracted at 10 g seed / 80 ml of solvent mixture (10 methanol, 10 ml ethanol and 80 ml distilled water) for 72 h soaking time. Melia azedarach seed extract was tested at four doses (0.0, 0,6, 1.2 and 1.8 ml/g DM) in four replicates for each treatment using in vitro gas production technique. Melia azedarach seed extract had a significant effect on the gas and methane production. Gas and methane production ranged from 38.3 to 45.44 ml and 7.25 to 10.87 ml respectively. Melia azedarach seed extract decreased gas production while increased methane production. On the other hand Melia azedarach seed extract significantly decreased metabolisable energy and organic matter digestibility. Metabolisable energy of alfalfa hay ranged from 7.40 to 8.37 MJ /kg DM and 62.50 to 68.90% respectively. Therefore supplementation of Melia azedarach seed extract is not recommended to mitigate enteric methane production since Melia azedarach seed extract decreased metabolisable energy and organic matter digestibility and increased methane production.

Key words: *Melia azedarach, gas production, methane production, alfalfa*

INTRODUCTION

Recently considerable attention has been given to manipulate the efficiency of ruminal fermentation using the leaf and seed of tree leaves to mitigate enteric fermentation. The methane production from ruminant animal has a considerable contribution to the global worming during the fermentation. It was also reported that during the ruminal fermentation 2- 12 % of dietary energy intake is lost as methane (Jonhson and Johnson 1995). Recently *in vitro* gas production technique has been used to test the anti-methanogenic activity of some additives (Pellikaan et al. 2011, Hassanat and Benchar 2013). *Eucalyptus globulus* is one of the shrubs grown in Turkey and producing considerable amount of leaves and seed. However there is no information on the effect of *Eucalyptus globulus* leaf and seed extract on ruminal fermentation *in vitro*. The aim of the current experiment was to determine the effect of *Melia azedarach* seed extract on gas and methane production of alfalfa hay in vitro.

MATERIALS AND METHODS

Commercially available alfalfa hay sample was harvested at flowering stage. Alfalfa hay sample was milled through a 1 mm sieve after drying. Chemical composition of hays was determined using the methods described by AOAC (1990). Neutral detergent fiber and acid detergent fiber content of alfalfa hay were determined with the method described by Van Soest (1991). The composition of alfalfa hay was (g/kg DM), 905 organic matter, 166 g crude protein, 12 g ether extract, 407 g neutral detergent fiber and 326 g acid detergent fiber. *Melia azedarach* seed were chopped and extracted at 10 g seed / 80 ml of solvent mixture (10 methanol, 10 ml ethanol and 80 ml distilled water) for 72 h soaking time. *Melia azedarach* seed extract was tested at four doses (0.0, 0,6, 1.2 and 1.8 ml/g DM) in four replicates for each treatment using *in vitro* gas production technique. (Menke *et al.*, 1979). Gas production was determined for 24 h. Rumen fluid was obtained from three fistulated sheep fed twice daily with a diet containing alfalfa hay (60%) and concentrate (40%). after incubation. Methane content of gas produced was determined using the infrared methane analyzer (Goel et al., 2008). Gas production and methane production were determined using additive specific blank, which is a syringe without substrate containing only inoculums, incubation medium and *Melia azedarach* seed extract. The metabolisable energy (MJ/kg DM) and organic matter digestibility (OMD) of alfalfa hay were calculated using equations of Menke and Steingass (1988) as follows:

$$ME\ (MJ/kg\ DM) = 2.20 + 0.1357\ GP + 0.057\ CP + 0.002859EE^2$$

$$OMD\ (\%) = 14.88 + 0.8893\ GP + 0.448\ CP + 0.651CA.$$

One-way analysis of variance (ANOVA) was used to determine the effect of *Melia azedarach* seed extract seed extract on gas, methane production, metabolisable energy and organic matter digestibility of alfalfa hay. Significance between individual means was identified using the Tukey's multiple range tests. Mean differences were considered significant at $p<0.05$

RESULTS AND DISCUSSION

The effects of *Melia azedarach* seed extract on gas production, methane production, metabolisable energy and organic matter digestibility were given in Table 1. *Melia azedarach* seed extract had a significant effect on the gas and methane production. Gas and methane production ranged from 38.3 to 45.44 ml and 7.25 to 10.87 ml respectively. *Melia azedarach* seed extract decreased gas production while increased methane production. The percentage of methane ranged from 16.0 to 27.92 % and increased with supplementation of *Melia azedarach* seed extract in a dose dependent manner. On the other hand *Melia azedarach* seed extract significantly decreased metabolisable energy and organic matter digestibility. Metabolisable energy of alfalfa hay ranged from 7.40 to 8.37 MJ /kg DM and 62.50 to 68.90% respectively.

Table 1. The effects of *Melia azedarach* seed extract on gas production, methane production, metabolisable energy and organic matter digestibility of alfalfa hay

	Doses of *Melia azedarach* seed extract				
Parameters	0.0	0.6	1.2	1.8	sem
Gas(ml)	45.40^a	41.55^b	38.3^c	39.30^c	0.489
CH_4(ml)	7.25^d	8.40^c	9.65^b	10.87^a	0.316
CH_4(%)	16.0^d	20.27^c	25.27^b	27.92^a	0.751
ME (MJ)	8.37^a	7.82^b	7.40^c	7.50^c	0.069
OMD(%)	68.9^a	65.42^b	62.5^c	63.15^c	0.440

^{abcd}Row means with common superscripts do not differ ($p>0.05$), sem: Standard error mean, ME: metabolisable energy(MJ/ kg DM), OMD: organic matter digestibility(%)

These results obtained in the current experiment are consistent with finding of Hassanat and Benchaar (2013) and Pellikaan et al. (2011) who showed that several tannins obtained from different sources have significant effect on the gas and methane production. They also indicated that the decrease in gas and methane production is possible associated with decrease in acetate concentration

CONCLUSIONS

Supplementation of *Melia azedarach* seed extract is not recommended to mitigate enteric methane production since *Melia azedarach* seed extract decreased metabolisable energy and organic matter digestibility and increased methane production.

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EFFECTS OF *EUCALYPTUS GLOBULUS* LEAF AND SEED EXTRACTS ON GAS AND METHANE PRODUCTION OF ALFALFA HAY

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Abstract

The aim of the current experiment was to determine the effects of extracts of leaf and seed of Eucalyptus globulus on the gas and methane production of alfalfa hay. Leaves and seeds of Eucalyptus globulus were chopped and extracted at 10 g seed and leaf / 80 ml of solvent mixture (10 ml methanol, 10 ml ethanol and 80 ml distilled water) for 72h soaking time. Leaf and seed extracts of Eucalyptus globulus were tested at 1.2 ml/g DM of alfalfa hay in four replicates for each treatment using in vitro gas production technique. The seed extract of Eucalyptus globulus had a significant effect on the gas production whereas the leaf extract had no significant effect on gas production of alfalfa hay. Gas production ranged from 43.55 to 46.25 ml. On the other hand leaf and seed extracts of Eucalyptus globulus had a significant effect on methane production of alfalfa hay. The methane production ranged from 7.25 to 11.67 ml. Supplementation of the leaf and seed extracts of Eucalyptus globulus increased methane production of alfalfa hay by 60% whereas supplementation of the leaf and seed extracts on the gas, ME OMD are very small. Therefore supplementation of the leaf and seed extracts is not recommended to mitigate enteric methane production.

Key words: *eucalyptus globulus, gas production, methane production, alfalfa*

INTRODUCTION

Recently considerable attention has been given to manipulate the efficiency of ruminal fermentation using the leaf and seed of trees to mitigate enteric fermentation. The methane production from ruminant animal has a considerable contribution to the global warming during the fermentation. It was also reported that during the ruminal fermentation 2-12 % of dietary energy intake is lost as methane (Jonhson and Johnson 1995). Recently *in vitro* gas production technique has been used to test the anti-methanogenic activity of some additives (Pellikaan et al. 2011, Hassanat and Benchar 2013). *Eucalyptus globulus* is one of the shrubs grown in Turkey and producing considerable amount of leaf and seed. However there is no information on the effects of *Eucalyptus globulus* leaf and seed extracts on ruminal fermentation *in vitro*. The aim of the current experiment was to determine the effects of *Eucalyptus globulus* leaf and seed extracts on gas and methane production of alfalfa hay *in vitro*.

MATERIALS AND METHODS

Commercially available alfalfa hay sample was harvested at flowering stage. Alfalfa hay sample was milled through a 1 mm sieve after drying. Chemical composition of hay was determined using the methods described by AOAC (1990). Neutral detergent fiber and acid detergent fiber contents of alfalfa hay were determined with the method described by Van Soest (1991). The composition of alfalfa hay was (g/kg DM), 905 g organic matter, 166 g crude protein, 12 g ether extract, 407 g neutral detergent fiber and 326 g acid detergent fiber. Leaf and seed of *Eucalyptus globulus* were chopped and extracted at 10 g seed and leaf / 80 ml of solvent mixture (10 ml methanol, 10 ml ethanol and 80 ml distilled water) for 72h soaking time. Leaf and seed extracts of *Eucalyptus globulus* were tested at 1.2 ml/g DM of alfalfa hay in four replicates for each treatment using *in vitro* gas production technique. (Menke et al., 1979). Gas production was determined for 24 h. Rumen fluid was obtained from three fistulated sheeps fed twice daily with a diet containing alfalfa hay (60%) and concentrate (40%). After incubation methane content of gas produced was determined using the infrared methane analyzer (Goel et al., 2008). Gas production and methane production were determined using additive specific blank, which is a syringe without substrate containing only inoculums, incubation medium and *Eucalyptus globulus* leaf and seed extracts.

The metabolisable energy (MJ/kg DM) and organic matter digestibility (OMD) of alfalfa hay were calculated using equations of Menke and Steingass (1988) as follows:

ME (MJ/kg DM) = 2.20 + 0.1357xGP + 0.057xCP + 0.002859xEE2
OMD (%) = 14.88 + 0.8893xGP + 0.448xCP + 0.651xCA.

One-way analysis of variance (ANOVA) was used to determine the effects of *Eucalyptus globulus* leaf and seed extracts on gas, methane production, metabolisable energy and organic matter digestibility of alfalfa hay. Significance between individual means was identified using the Tukey's multiple range tests. Mean differences were considered significant at $p<0.05$

RESULTS AND DISCUSSION

The effects of leaf and seed extracts of *Eucalyptus globulus* on gas production, methane production, metabolisable energy and organic matter digestibility were given in Table 1. The seed extract of *Eucalyptus globulus* had a significant effect on the gas production whereas the leaf extract had no significant effect on gas production of alfalfa hay. Gas productions ranged from 43.55 to 46.25 ml. On the other hand leaf and seed extracts of *Eucalyptus globulus* had a significant effect on methane production of alfalfa hay. The methane productions ranged from 7.25 to 11.67 ml.

These results obtained in the current experiment are consistent with finding of Hassanat and Benchaar (2013) and Pellikaan et al. (2011) who showed that several tannins obtained from different sources have significant effect on the gas and methane productions. They also indicated that the decrease in gas and methane production is possible associated with decrease in acetate concentration.

Table 1. The effects of leaf and seed extracts of *Eucalyptus globulus* on gas production, methane production, metabolisable energy and organic matter digestibility of alfalfa hay

Parameters	AH	AH+LE	AH+SE	SEM
Gas (ml)	45.40^a	46.25^a	43.55^b	0.645
CH_4 (ml)	7.25^b	11.67^a	11.60^a	0.741
CH_4 (%)	16.01^b	25.18^a	26.65^a	1.509
ME (MJ)	8.37^{ab}	8.47^a	8.10^b	0.098
OMD (%)	68.90^a	69.62^a	67.17^b	0.580

^{a,b,} Row means with common superscripts do not differ ($p>0.05$), SEM: Standard error mean, AH: alfalfa hay, AH+LE: alfalfa hay +leave extract, AH+SE: alfalfa hay +seed extract, ME: metabolisable energy(MJ/ kg DM), OMD: organic matter digestibility(%).

CONCLUSIONS

Supplementation of the leaf and seed extracts of *Eucalyptus globulus* increased methane production of alfalfa hay by 60% whereas supplementation of the leaf and seed extract on the gas, ME OMD are very small. Therefore supplementation of the leaf and seed extracts is not recommended to mitigate enteric methane production.

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DETERMINATION OF POTENTIAL NUTRITIVE VALUE OF SOME FORAGES USED RUMINANT NUTRITION IN TURKEY

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Abstract

The aim of the current experiment was to determine the potential nutritive value of some forages using chemical composition and in vitro gas production. Species had a significant effect on chemical composition, gas production, methane production, metabolisable energy and organic matter digestibility of forages. Crude ash, and crude protein contents of some forages ranged from 7.76 to 10.76 % and 2.13 to 13.50 % respectively. Crude ash of vetch-rye mixture was significantly higher than the other forages while CP content of alfalfa hay was significantly higher than the other forages. Neutral detergent fiber and acid detergent fiber contents of some forages ranged from 50.53 to 75.83 and 39.86 to 51.30 % respectively. Neutral detergent fiber and acid detergent fiber contents of barley straw and wheat straw were significantly higher than the other forages. Gas production, metabolisable energy and organic matter digestibility of alfalfa hay was significantly higher than the other forages. As a conclusion there are considerable variation in the chemical composition, gas production, metabolisable energy and organic matter digestibility of forages. The differences in chemical composition were reflected in gas production, metabolisable energy and organic matter digestibility of forages.

Key words: forage, gas production, methane production, metabolisable energy, organic digestibility.

INTRODUCTION

Forages are very important component of ruminant diets, which provide protein, energy and minerals for ruminant animals. The ruminal animal required forages for proper rumen function. Preliminary experiment showed that there is considerable variation among forages in terms of chemical composition. Although forages is very important for ruminants the information about the chemical composition and energy value of forages is limited due to lack of facilities. Recently *in vitro* gas production technique with chemical composition have been widely used to evaluate the potential nutritive value of previously uninvestigated forages since in vitro gas production technique is quick, cheap, less time consuming (Kamalak et al. 2005, Kamalak and Canbolat 2010). In addition, in vitro gas production technique was used to screen the feedstuffs in terms of their methane reduction potential [Jayanegara et al 2009, Lin et al 2013). Methane production during rumen fermentation is one of important contributors to global warming (Lassey 2007). The aim of the current experiment was to study the effect of species on the chemical composition, in vitro gas production, methane production, metabolisable energy and organic matter digestibility.

MATERIALS AND METHODS

Commercially available forage samples were purchased from local market and milled through a 1 mm sieve. Chemical compositions of forage samples were determined using the methods described by AOAC (1990). Neutral detergent fiber and acid detergent fiber contents of forage samples were determined with the method described by Van Soest (1991).

Forage samples were incubated in four replicates for 24 h to determine gas production (Menke *et al.,* 1979). Rumen fluid was obtained from three fistulated sheep fed twice daily with a diet containing alfalfa hay (60%) and concentrate (40%). after incubation. Methane content of gas produced was determined using the infrared methane analyzer (Goel et al., 2008). The metabolisable energy (MJ/kg DM) and organic matter digestibility (OMD) of forage samples were calculated using equations of Menke and Steingass (1988) as follows:

$$\text{ME (MJ/kg DM)} = 2.20 + 0.1357\ GP + 0.057\ CP + 0.002859EE^2$$
$$\text{OMD (\%)} = 14.88 + 0.8893\ GP + 0.448\ CP + 0.651CA.$$

One-way analysis of variance (ANOVA) was used to determine the effect of species on chemical composition, gas, methane production, metabolisable energy and organic matter digestibility of forage samples. Significance between individual means was identified using the Tukey's multiple range tests. Mean differences were considered significant at $p<0.05$

RESULTS AND DISCUSSION

The effects of type forages on the chemical composition of some forages were given in Table 1. Type had a significant effect on chemical composition of some forages. Crude ash, ether extract and crude protein contents of forage samples 7.76 to 10.76%, 0.80 to 1.23 and 2.13 to 13.50% respectively. Crude ash of Vetch-Rye mixture was significantly higher than the others. Crude protein contents of alfalfa hay were significantly higher than the other forages.. Neutral detergent fiber and acid detergent fiber contents of forages ranged from 50.53 to 75.83 and 39.86 to 49.56 % respectively. Neutral a detergent fiber and acid detergent fiber contents of Barley straw and *wheat straw* were significantly higher than the other forages.

Table 1. The effects of species on the chemical composition of some forages used in ruminant diets in Turkey

Forages	DM	CA	EE	CP	NDF	ADF
Alflfa hay	93.03ab	9.93b	1.23	13.50a	50.53c	39.86c
Vetch-Rye mixture	92.73bc	10.76a	1.10	8.53b	61.60b	41.50bc
Lentil straw	93.00bc	7.80d	0.96	6.66c	53.43b	44.26b
Barley straw	93.96a	8.23c	0.80	2.13f	75.83a	49.20a
Wheat straw	93.76ab	10.06b	1.00	4.16d	73.73a	49.56a
Chickpea straw	91.80c	7.76d	1.26	3.60e	65.73c	51.30a
sem	0.357	0.127	0.145	0.112	1.446	1.140

^{ab}*Column means with common superscripts do not differ (p>0.05), sem: Standard error mean, DM: Dry matter(%), CA:Crude ash (%), EE; Ether extract (%), CP: Crude protein (%), ADF: acid detergent fiber(%),NDF: Neutral detergent fiber(%)*

The effects of species on gas production, methane production, metabolisable energy and organic matter digestibility of forages were given in Table 2. Species had a significant effect on chemical composition of forages.

Gas production ranged from 16.68 to 43.71 ml. The gas production from alfalfa was significantly higher than the other forage samples. Methane production ranged from 3.08 to 7.63 ml. The methane productions from alfalfa and lentil straw were significantly higher than the other forage samples. There is no significant difference among forages in terms of percentage of methane in gas produced for 24 h incubation.

Metabolisable energy and organic matter digestibility of forages ranged from 4.58 to 8.90 MJ/ kg DM, 36.02 to 66.27 % respectively. Metabolisable energy and organic matter digestibility of alfalfa was significantly higher than the other legume seeds.

Table 2. The effects of species on gas production, methane production, metabolisable energy and organic matter digestibility of legume seeds

Forage	Gas	CH_4(ml)	CH_4 (%)	ME	OMD
Alflfa hay	43.71a	7.63a	17.45	8.90a	66.27a
Vetch-Rye mixture	35.95bc	5.81b	19.80	7.57b	57.68b
Lentil straw	37.96b	7.31ab	19.29	7.73b	56.70b
Barley straw	16.68d	3.08c	18.49	4.58d	36.02e
Wheat straw	21.28d	3.89c	18.34	5.33d	42.21d
Chickpea straw	32.79c	6.04ab	18.48	6.86c	50.70c
sem	1.519	0.535	1.533	0.206	1.351

^{ab}*Column means with common superscripts do not differ (p>0.05), sem: Standard error mean, ME: Metabolisable energy(MJ/kg DM), OMD: Organic matter digestibility (%).*

CONCLUSIONS

There are considerable variation among forages in terms of gas production, methane production, metabolisable energy and organic matter digestibility of forages. Although nutritive value of alfalfa hay is significantly higher than the other forages studied in the current experiment, Lentil straw is better nutritive value when compared with other straws.

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PREDICTION OF RELATIVE FEED VALUE OF OAK LEAVES USED RUMINANT NUTRITION IN TURKEY

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Abstract

The aim of the current experiment was to predict the relative feed value of some oak leaves Species had a significant effect on chemical composition, dry matter digestibility (DDM), dry matter intake (DMI) and relative feed value (RFV). Crude ash, and crude protein contents of some forages ranged from 1.6 to 7.33 % and 8.20 to 9.76 % respectively. Crude ash of Quercus infectoria was significantly higher than the other oak leaves. Neutral detergent fiber and acid detergent fiber contents of oak leaves ranged from 50.60 to 55.73 and 40.06 to 51.70 % respectively. Neutral detergent fiber of Quercus coccifera were significantly higher than the other oak leaves. Dry matter digestibility (DMD) and dry matter intake (DMI) of oak leaves ranged from 48.63 to 57.70%. Dry matter digestibility of leaves of Quercus infectoria and Quercus coccifera were significantly higher than that of Quercus cerris while relative feed value of Quercus infectoria was significantly higher than those of Quercus coccifera and Quercus cerris. As a conclusion, there are considerable variation among oak leaves in terms of chemical composition, dry matter digestibility, dry matter intake and relative feed value.

Key words: *oak leaves, chemical composition, digestibility, dry matter intake, relative feed value*

INTRODUCTION

During the critical period of year, small ruminant animal meet their requirements by grazing the leaves of tree and shrub in Mediterranean woodlands and shrublands (Kababya et al., 1998; Perevolotsky et al., 1998). Oak tree is the one of the important trees in Mediterranean woodlands and shrublands. Oak tree provides considerable amount of leaves and nut which are often grazed by animals or loped to use as livestock fodder during lean periods (Singh et al., 1996). Approximately 7 million ha of forest in Turkey were covered by oak trees (Kayacik, 1996). Although oak leaves are an important source of forage for ruminants in most part of Turkey during critical periods of the year when quality and quantity of pasture herbages is limited, there is little information on the relative value of oak leaves (Kamalak et al., 2004). The aim of the current experiment was to predict the relative feed value of some oak leaves Species had a significant effect on chemical composition, dry matter digestibility (DDM), dry matter intake (DMI) and relative feed value (RFV).

MATERIALS AND METHODS

Leaves from *Quercus infectoria, Quercus coccifera and Quercus.cerris were collected in July in 2018.* Leave samples were dried and milled were milled through a 1 mm sieve after drying. Chemical composition of hays was determined using the methods described by AOAC (1990). Neutral detergent fiber and acid detergent fiber content of alfalfa hay were determined with the method described by Van Soest (1991).
Dry matter digestibility (DDM), dry matter intake (DMI) and relative feed value (RFV) were estimated as follows (Rohweder et al 1978).

$$\% DDM = 88.9 - (0.779 * \%ADF)$$
$$DMI \% \text{ of } BW = 120 / \%NDF$$
$$RFV = (\%DDM * \%DMI) /1.29$$

One-way analysis of variance (ANOVA) was used to determine the effect of species on chemical composition, digestibility, feed intake and relative feed value of oak leaves. Significance between individual means was identified using the Tukey's multiple range tests. Mean differences were considered significant at $p<0.05$

RESULTS AND DISCUSSION

The effects of species on the chemical composition, dry matter digestibility (DDM), dry matter intake (DMI) and relative feed value (RFV) were given in Table 1. Species had a significant effect on chemical composition, dry matter digestibility (DDM), dry matter intake (DMI) and relative feed value (RFV). Crude ash, and crude protein contents of some forages ranged from 1.6 to 7.33 % and 8.20 to 9.76 % respectively. Crude ash of *Quercus infectoria* was significantly higher than the other oak leaves. Neutral detergent fiber and acid detergent fiber contents of oak leaves ranged from 50.60 to 55.73 and 40.06 to 51.70 % respectively. Neutral detergent fiber of *Quercus coccifera* was significantly higher than the other oak leaves. Dry matter digestibility (DMD) and dry matter intake (DMI) of oak leaves ranged from 48.63 to 57.70%.

Dry matter digestibility of leaves of *Quercus infectoria* and *Quercus coccifera* were significantly higher than that of *Quercus cerris* while relative feed value of *Quercus infectoria* was significantly higher than those of *Quercus coccifera* and *Quercus cerris*. As a conclusion, there are considerable variation among oak leaves in terms of chemical composition, dry matter digestibility, dry matter intake and relative feed value.

The crude contents of *Q. infectoria* and *Q.cerris* were in agreement with the findings of Kamalak et al. (2004) although The crude contents of *Q.coccifera* was considerable higher than that reported by Kamalak et al. (2004). The crude ash of *Q. infectoria* was consistent with that reported by Kamalak et al. (2004) although the crude ash contents of *Q.coccifera* and Q.cerris was considerable higher than that reported by Kamalak et al. (2004). The differences in chemical composition of oak leaves between two studies are possibly associated with differences in growing conditions and harvesting stages.

Table 1. The effects of species on the chemical composition, digestibility, dry matter intake and relative feed value of some oak leaves used in ruminant diets in Turkey

	Species			
	Q. infectoria	*Q.coccifera*	*Q.cerris*	sem
DM	48.96b	50.40a	46.16c	4.537
CA	7.33a	4.30b	1.6c	0.158
HP	9.76a	8.66b	8.20b	0.229
HY	5.06a	5.50a	3.40b	0.475
NDF	50.60b	55.73a	51.73b	0.979
ADF	40.06b	42.96b	51.70a	1.006
DDM	57.70a	55.44a	48.63b	0.789
DMI	2.37a	2.32a	2.15b	0.043
RFV	106.10a	92.60b	87.53b	1.811

^{ab}Row means with common superscripts do not differ (p>0.05), sem: Standard error mean, DM: Dry matter(%), CA:Crude ash (%), EE; Ether extract (%), CP: Crude protein (%), ADF: acid detergent fiber(%),NDF: Neutral detergent fiber(%)

CONCLUSIONS

Species has a significant on the chemical composition, dry matter digestibility (DDM), dry matter intake (DMI) and relative feed value (RFV). As a conclusion, there are considerable variation among oak leaves in terms of chemical composition, dry matter digestibility, dry matter intake and relative feed value.

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MOLECULAR IDENTIFICATION OF LIGULA INTESTINALIS PARASITE DIAGNOSED IN ALBURNUS SP.

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Abstract

This study comprises the molecular identification of Ligula intestinalis parasite diagnosed in Alburnus sp caught in Menzelet Dam Lake in Kahramanmaras province. For that purpose, parasite samples have been obtained from the intestines of fish species caught in 2013. The collected parasites have been stored in sample vials containing 70% alcohol. 12 Ligula intestinalis have been diagnosed using morphological and staining methods. DNA isolation of Ligula intestinalis has been done using special tissue sets for parasites. Specific primers have been utilized in molecular identification of Ligula intestinalis using Polymerase Chain Reaction (PCR) technique. Thus, it has been possible to verify that all of the parasites had Ligula intestinalis molecules. In conclusion, it has been identified that Ligula intestinalis parasite is observed in the fish species caught in Kahramanmaras province. Identification of Ligula intestinalis parasite using morphological and staining methods takes a long time, but with PCR technique it was successfully performed in a short time. The success of this study will shed light on the diagnosis of fish parasitic diseases, treatment follow-up and epidemiological studies.

Key words: *Ligula intestinalis, PCR, parasite, fish*

INVESTIGATION OF BACTERIAL DISEASES CAGE BUSINESS RAINBOW TROUTS (ONCORHYNCHUS MYKISS) OF DOWN FIRAT BASIN

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Abstract

In this study, It aimed to determine the effect of antibiotics and the screened for bacterial diseases the frequent especially in fish in business, determination of fishing facilities made aquaculture and production in cages operating in Down Fırat Basin. For this purpose, farms different trout between january 2018 and june 2019 were taken in samples the gut and kidney, spleen, liver, eye rainbow trout weighing 250-300 g. For isolation bacterial disease agents; Tryptic Soy Agar (TSA), Nutrient Broth (NB), Brain Heart (Infusion) Agars (BHIA) KF Streptococcus Agar Base, Yersinia Selective Agar Base and Tryptone Yeast Extract Salt Agar (TYESA) were used. This medium was incubated at an incubator for 24-72 hours in 15-24oC. The pure strains obtained from these samples from trout farms were evaluated phenotypic characteristics applied with Biolog System Connection (The Genie biolog micro plate) and Biochemical tests. Consequently In this study, according to the phenotypic and biochemical characteristics of bacteria were identified and isolated strain Vibrio anguillarum, Flavobacterium psychrophilum, Yersinia ruckeri, Lactococcus garvieae designated.

Key words: *Rainbow trout, Oncorhynchus mykiss, Phenotypic, Lactococcus garvieae, Yersinia ruckeri, Flavobacterium psychrophilum, Vibrio anguillarum, BIOLOG GEN III.*

THE STUDIES ABOUT TENDERIZATION OF SQUID

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Abstract

Tenderization has been used for improving the taste, aroma and textural qualities of meat and fishery products. Many processing technologies have been used for tenderization of these products such as cooking, freezing, marination, radiation, using enzymes, blade tenderization, muscle stretching techniques, shockwave, ultrasound, pulsed electric field, low-frequency ultrasonication, high hydrostatic pressure etc. The texture of squid is very tough to be eaten. For this reason, tenderization process is essential for squid before they are consumed. In Turkey, squid is generally sold in fish markets and bazaars as fresh or as frozen in the form of whole or annular. After taken, tenderization of squid with marination has been made by consumers. Before frying process, squid have been marinated by using carbonate, sugar, lemon juice, and mineral water according to the traditional method to improve the taste and textural qualities of squid. Many marinades have been sold in Turkish markets. These products are produced from fish and fishery products such as sardines, anchovies, mussels, octopus, shrimps etc. in different formulations. Mix seafood marinated salads containing mussel, octopus, squid are also produced and sold in markets. They are consumed as appetizer with meals in Turkey. There have been made many studies about tenderization of meat products by using different technologies. However, limited studies have been done about tenderization of squid. As a result; It is advised that the number of studies about the tenderizing of squid by using different technologies should be increased. Because of very tough textural characteristic, squid should be tenderized before eaten. This process, which has been made by consumers gives rise to the limit of consumption, waste of time and the difficulties in the preparation etc. In addition to this; tenderizing squids, which are in the form of ready to eat, have not been found and sold in Turkish markets. Therefore; it is essential that new developments would be obtained about tenderization of squid and these products would be placed in markets in the future. In this review; tenderization processes and the studies about tenderization of squid have been handled.

Key words: *tenderization, squid, processing*

INTRODUCTION

Cephalopods are a nutritious, healty and sustainable source of seafood (Faxholm et al., 2018). Due to the texture of squid rather tough, many new consumers possess a negative opinion of eating quality of squid (Melendo et al., 1997).Tenderness is one of the most important aspects of meat quality particularly for beef, lamp, pork etc. (Morton et al., 2019). Each piece of meat has physical properties, with varying importance in their contribution to texture which depends on the inherent hierarchical structure of the muscle, including the contribution of connective tissue proteins, structural proteins within the muscle cell, and degradation or disruption of these proteins (Warner et al., 2017). Innovative and new processing and cooking technologies, such as high pressure processing, shockwave, ultrasound, pulsed electric field, Smartstretch, Pi-Vac Elasto-Pack, and sous vide cooking can be used to tenderize meat postmortem (Warner et al., 2017; Hopkins, 2018). There are many studies about tenderization of meat products such as marination, cooking, freezing, radiation, using enzymes, shockwave, high intensity ultrasound, high hydrostatic pressure, pulsed electric fields, low-frequency ultrasonication technologies etc. (Stanley and Smith, 1984; Melendo et al., 1997; Collignan and Montet, 1998; Okitani et al., 2009; Bolumar et al., 2013; Kanatt et al., 2015; Sikes and Warner, 2016; Bolumar and Toepfl, 2016; Zou et al., 2018). The texture of squid are very tough to be eaten. For this reason, processing technologies are essential for squid before they are consumed. Therefore, the aim of this study was to review the tenderization processes and the studies about tenderization of squid and also food products by using different technologies.

MATERIALS AND METHODS

The methods used for tenderization of meats

The two protein classes that are among the most important direct responsibles for tenderization: sarcomeric proteins and proteolytic enzymes (Lana and Zolla, 2016). Meat tissue consists of numerous molecules which denaturate, interact, and solubilize at different temperatures and time scales (Warner et al., 2017). Blade tenderization (BT) were reported to be used in the beef industry to enhance tenderness by (Thomas et al., 2019). The tenderization of goosemeat was made by using $CaCl_2$ treatments (Li et al., 2017). Ginger and cumins extracts could be used as effective alternative to papain for tenderization of buffalo meat with marination described by (Naveena et al., 2004).The tenderization effect of a new elastase from *Bacillus sp.* EL31410 was investigated on beef meat (Qihe, et al., 2006). The suitability of using salt-fermented shrimp sauce prepared from processing by-product (head, shell,and tail) of southern rough shrimp (*Trachypena curvirostris*) as a meat tendizer was investigated by (Kim et al., 2005). The effect of low-frequency ultrasonication on tenderness of goose breast muscle were studied by(Zou et al.,2018).Ultrasound was succesfully used for improving processes such as mass transfer and marination, tenderization of meat, explained by (Alarcon-Rojo et al., 2019). The effect of shockwave on other aspects of meat quality such as functionality of meat proteins were described by (Bolumar and Toepfl, 2016). The effect of radiation processing (0, 2.5, 5 and 10 kGy) on the tenderness of three types of popularly consumed meat in India namely chicken, lamb and buffalo was investigated by (Kanatt et al., 2015). High Intensity Ultrasound (HIU) application after storage has potential as a safe method for tenderizing bovine meat when handled under the conditions reported by (Pena- Gonzalez et al., 2019). Pulsed Electric Fields (PEF) treatment had the ability to improve several processes such as preservation, tenderization and aging was done by (Gomez et al., 2019).

The methods used for tenderization of squid

With the aim of reducing squid toughness, exogenous proteases such as commercial bromelain and a crude lysosomal extract from bovine spleen were used by (Melendo et al., 1997). In another report the authors said that the very tough muscle tissues of squid (*Illex coindetii*) caused considerable marketing limitations. In this study the most probable of tenderization process of squid was determined by (Collignan and Montet, 1998). Freezing produced a tendency for fibers separated by homogenization to lose their outer membranes and for the underlying myofibrils to become shredded explained by (Stanley and Smith, 1984). Tenderization effect of tumbling in cephalopod muscles was studied by (Gokoglu et al., 2017a). The effects of using bromelain and papain enzymes on the textural properties of squid examined by (Gokoglu et al., 2017b).

RESULTS AND DISCUSSIONS

Cooking is the most obvious processing changes (Stanley and Smith, 1984). It was observed in one study that the time required for the steak to reach 71.1˚C significantly influenced by weight, thickness and muscle type on the cooking time required for mechanically tenderized beef steaks (Saha et al., 2019). The effects of HIU, applied after storage, on the physical, microstructural and sensory characteristics of beef were evaluated. Samples of four beef m. *Longissimud dorsi* muscle were stored in vacuum at 4˚C for 0,7 or 14 days and then ultrasonicated (40 KHz, $11W/cm^2$) for 60 min. Application of HIU of meat after meat was stored affected microstructural and textural properties and accelerated aging (Pena-Gonzalez et al., 2019).Radiation processing of meat at a dose of 2.5 kGy improved its texture and had acceptable odour (Kanatt et al., 2015). The authors investigated whether actin liberation from myofibrils occurs during the heating of various muscles, as well as squid mantle muscle at temperatures, such as 60°C, employed for vacuum cooking of meats.The liberation of actin from myofibrils may contribute to the greater tenderness of vacuum-cooked meat (meat heated at a low temperature for long time),as compared with meat prepared by cooking at a higher temperature (Okitani et al., 2009). Gerelt et al.(2002) reported that the dipping in 150 mM calcium chloride solution after dehydration could be applied to improve meat tenderness. In one study; the squid mantles were transversally cut as rings, and marinaded with the enzyme at pH 7.0 and 37˚C for 30 min. prior to cooking. Both Atlantic squids *Loligo vulgaris* and *Illex coindetii* were significantly tenderized by effect of bromelain and bovine spleen lysosome-enriched extract (Melendo et al., 1997). In another study; the maximum level of tenderization was determined by incubating squid mantle rings at 45˚C and pH 2. Products processed at 45˚C were found to be significantly more tender and less crispy than unprocessed squids according to (Collignan and Montet, 1998). Gokoglu et al. (2017a) exhibited that tumbling was a convenient method to tenderize squid muscle. In another study; use of bromelain and papain enzymes was found as effective method to tenderize of squid muscle. When compared with bromelain, better results were obtained with papain described by (Gökoglu et al., 2017b)

CONCLUSION

In conclusion many studies have been done about tenderizing of meat products by using different technologies. However, limited studies have been done about tenderizing of squid. It is advised that the number of studies about the tenderizing of squid should be increased. Because of very tough textural characteristic, squid should be tenderized before eaten. This process gives rise to the limit of consumption, waste of time and the difficulties in the preparation etc. In addition to this tenderizing squid, which is ready for consuming, have not been found and sold in Turkish market. Therefore; it is essential that new developments would be obtained about tenderization of squid and these products would be placed in markets in the future.

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THE COMPARISON OF USING REAL-TIME POLYMERASE CHAIN REACTION (PCR) AND TRADITIONAL METHODS FOR IDENTIFICATION OF PATHOJEN *VIBRIO SPP.* IN FISHERY PRODUCTS

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Abstract

Polymerase Chain Reaction (PCR) method is very useful for identifition of bacteria in fishery products. When compared with traditional method, real-time PCR method is very rapid, sensitive, reliable and easy. The species of Vibrio especially Vibrio chlorea, Vibrio vulnificus and Vibrio parahaemolyticus are mostly found in fishery products that can be caused infections, diseases, sometimes death. In this approach the identification of pathojen bacteria in fishery products by using rapid methods such as real-time PCR method is very important for human health. So, in this review; the meaning of real-time PCR, the principle of this method, the advantage and disadvantages of real-time PCR, the comparison of real-time PCR with traditional method, the analyses stages of real-time PCR and traditional methods, the identification of Vibrio spp. in fishery products by using these methods, the microbiological criteria of Vibrio spp. in fishery products according to the Turkish Food Codex have been examined. In conclusion; Real-Time PCR method should be used for all types of fish and fishery products that is rapid, sensitive, it gives rise to examine more samples in a short time, practical and easy to use for persons, studies in laboratories, research institutes, universities, fish processing factories etc. PCR devise is also very suitable for the control of the pathogen bacteria in exported fish and fishery products due to its features. Day by day new methods have been developed by researches. When compared these methods with traditional methods. These new methods have more advantages than traditional methods. PCR method is the one of these methods. The price of PCR device is more higher than traditional method. However, more samples can be examined in a short time by using PCR. In near future, new methods will be developed by researches that may be decreased the time of the identification of pathogen bacteria in a more shorter time than nowadays.

Key words: *Real-Time PCR, traditional method, identification, Vibrio spp., in fishery products*

INTRODUCTION

Food safety is very common subject for human health. With the increasing focus on the food safety, rapid methods for detection of pathogen microorganisms are crucial for both industry and regulatory agencies. Recently, many molecular methodologies with diverse technologies have been introduced (Hu et al., 2018). The proposed method provides a rapid and simple means of detecting and identifying bacteria in foods with complex microbial communities (Diaz et al., 2016). PCR represents a significant advance for aquaculture by providing easy, fast and low-cost method for identification of several closely related pathogens that are difficult to discriminate by traditional approaches (Cano-Gomez, 2015). This method proved to be rapid, sensitive, and specific and successfully eliminate false-negative results for the detection of *V. alginolyticus*, *V. parahaemolyticus*, *V. vulnificus* and *V. cholera* (Wei, et al., 2014). The real-time PCR assay developed in this study was found to be rapid, sensitive and reliable diagnosis tool that could allow the presence of pathogenic microorganisms and the monitorization of the quality of the water (Fernandez- Alvarez, et al., 2019). Rapid identification of the genus and species of bacteria in foods and clinical specimens is important (Chiang et al., 2006). *Vibrio* spp., are pathogenic, diverse and abundant group of aquatic organism. Aquatic animals like fish, mollusks, shrimp harbors pathogenic and non-pathogenic form of *Vibrio* spp. (Kumar et al., 2019). Vibrios are widely distributed in aquaculture environment and further risk assessment is needed to conduct (Yan et al., 2019). *V. parahaemolyticus*, *V. chlorea* and *V. vulnificus* are the most notorious fish-borne pathogens responsible for several deadly diseases in humans (Bonny et al., 2018). According to The Turkish Food Codex Regulation on Microbiological Criteria

No. 28157 dated December 29, 2011, *V.cholerae* and *V.parahaemolyticus* should not be present in fishery products that are grown/caught from the waters. Therefore, rapid and accurate diagnostic methods are needed for identification of the pathogen *Vibrio spp*. (Chiang et al., 2012).

The aim of this review study is to the comparison of using real-time polymerase chain reaction (PCR) and traditional methods for identification of pathogenic *Vibrio* spp. in fishery products.

MATERIALS AND METHODS

The analyses stages of PCR

The analyses of PCR consists of three stages. These are Preenrichment, extraction and loading device. Preenrichment; 25 g samples are added into 225 ml alkaline peptone water and incubated 22-24 h at 37 °C for BAX® System Lysis Sample Preparation and Real-Time PCR Assay. Extraction; After that the BAX® heating blocks are turned on. A rack file in the BAX® system software are created. Lysis reagent by adding 150 µl of protease to one 12 ml bottle of lysis buffer are prepared and then transferred 200 µl of lysis reagent to each cluster tube. A 5 µl samples from enriched alkaline peptone water to the corresponding cluster tube are added. The rack of cluster tubes on a pre-warmed heating block are placed at 37°C for 20 min and transferred rack to a pre-warmed heating block at 95°C for 10 min. The cluster tubes to a cooling block are transferred and allowed to cool for 5 min. Loading device:The BAX® System Instrument are initialized. *Vibrio* Real-Time PCR Assay tubes in a chilled PCR cooling block are placed. The caps from the strip of tubes with the decapping tool are removed and transferred 30 µl of lysate from the cluster tubes to corresponding PCR tubes.New optical caps on the strip of tubes and secure tightly are placed and repeated this for all samples. Tubes into the BAX® System Q7 instrument are loaded and runned the program. After approximately 1 h, the results are readed for each species *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus* (Anonymous, 2008-2009).

The analyses of conventional method

25 g of samples are placed into 225 ml of alkaline saline peptone water (ASPW) and incubated at 41,5±1ºC for 6 ±1 h. Enrichment are performed with employing 1 ml of the first enrichment and 9 ml of ASPW. This broth culture are incubated at 41,5±1°C for 18 h. After enrichment procedure, samples are plated onto thiosulphate-citrate-bile salt sucrose (TCBS) agar and incubated at 37 ±1 °C for 24 ±3 h. At least five colonies (green, blue–green or yellow–green colonies, 2–3 mm in diameter on TCBS agar plates) presumptively selected as *V. parahaemolyticus* colonies are transferred onto Saline Nutrient Agar plates (NaCl 1%) and incubated at 37 ±1 °C for 24 ±3 h. After the incubation period, the isolates are subjected to the Gram stain, the oxidase test using Oxidase Sticks, Triple-Sugar-Iron (TSI) for further biochemical identification (ISO/TS 21872-1:2007)

RESULTS AND DISCUSSION

There have been many studies about the isolation of *Vibrio* spp. in fish and fishery products by using traditional method. In one report; the samples were tested for the presence of *Vibrio* spp. The most frequently found was *V. parahaemolyticus* (9.40%), *V. vulnificus* (6.84%) and *V. alginolyticus* (3.42%) (Jaksic et al., 2002). Although culture-based methods are accessible and inexpensive, they lack the specificity needed to definitively identify *Vibrio* spp. The goal of this study was to develop a more accurate, culture-based method for the initial detection of *Vibrio* species, thereby decreasing or eliminating the requirement for confirmatory molecular tests (Williams et al., 2013). Another study is about the comparison of cultured-based method with the PCR method. In this study; cultured -based method ISO 21872-1:2007 only the detection of total *V. parahaemolyticus* is possible and this takes at least 3 days followed by complementary techniques to identify pathogenic serotypes. (Garrido et al., 2012). The authors said in one report that the examined shellfish may carry the potential human health risk associated with the presence of pathogenic *V. parahaemolyticus* that the reliable molecular detection methods should be included in the routine seafood examination in addition to the conventional bacteriological methods (Abd-Elghany and Sallam., 2013). In another study the authors reported that PCR method could be reliable to detect toxigenic-pathogenic strains of *V. cholera* (Imani et al., 2013). Zarei et al. (2012) reported that all the TCBS isolates were firstly identified to the genus level with PCR and then identified to the species level using battery of biochemical reactions and tests. Robert-Pillot et al. (2010) reported that PCR can be used to quantity *V.*

parahaemolyticus in natural shrimp samples. DNA-based survey for the identification of fish and fish roes was also used in sushi products commonly available (But et al., 2019). DNA fingerprinting of *V. parahaemolyticus* in another study indicated that a high genetic diversity among the isolates and PCR was able to distinguish the isolates (Paydar et al., 2013).

CONCLUSION

Day by day new methods have been developed by researches. When compared these methods with traditional methods. These new methods are rapid, sensitive, more reliable than traditional method. PCR method is the one of these methods. The price of PCR device is more higher than traditional method. However, more samples can be examined in a short time by using PCR in a short time. In near future, new methods will be developed by researches that may be decreased the time of the identification of pathogen bacteria in a more shorter time than nowadays.

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NATURAL ANTIMICROBIAL AGENTS USED FOR SEAFOOD PRESERVATION

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Abstract

Fish and other seafoods are highly susceptible to spoilage due frequently to microbiological growth and lipid oxidation. Seafood can also undergo rapid microbial contamination and growth as a consequence of inadequate handling and storage. Fresh or processed seafood can contain pathogens (Vibrio parahaemolyticus, Clostridium botulinum, Staphylococcus aureus, Listeria monocytogenes and Escherichia coli O157:H7, etc) that cause infections or intoxications. To prevent microbial growth in seafood, various synthetic antimicrobials have been used. However, in recent years, with growing concerns regarding the safety of synthetic antimicrobials, foods preserved with natural additives have become very popular. This increasing demand for natural preservatives is encouraging the use of antimicrobial agents derived from plants, animals, or microflora, mainly lactic acid bacteria. These agents include propolis (bee glue), lactoperoxidase (milk), lysozyme (egg white, figs), saponins and flavonoids (herbs and spices), organic acids and bacteriocins (lactic acid bacteria) and chitosan (shrimp shells). The antimicrobials can be directly added into the product formulation, coated on its surface or incorporated into the packaging material.

Key words: *seafood, natural antimicrobials, lactic acid bacteria, flavonoids, propolis*

MICROBIOLOGICAL SPOILAGE AND RISKS IN SEAFOOD

Fresh and lightly preserved fish products are more likely spoiled by psychrotrophic Gram negative bacteria such as *Shewanella* sp. (*S. putrefaciens, S. baltica*), *Aeromonas* sp., *Pseudomonas* sp. (*P. fragi, P. fluorescens, P. putida, P. lundensis*, etc.), *Photobacterium* sp. (*P. phosphoreum, P. illiopiscarium*), *Enterobacteriaceae* (*Serratia proteomaculans, Hafnia alvei*, etc.), or *Brochothrix thermosphacta* (Gram and Huss, 1996; Gram and Dalgaard, 2002; Leroi, 2014; Løvdal, 2015). *Lactic acid bacteria* (LAB), mainly *Carnobacterium* sp. (*C. maltaromaticum, C. divergens*), *Lactobacillus* sp. (*L. curvatus, L. sakei, L. farciminis, L. plantarum*) can also be found in high proportion and may contribute to seafood spoilage (Cortesi et al., 2009; Pilet and Leroi, 2011;Wiernasz et al. 2017).

Microbial growth is a major concern because some microorganisms can potentially cause food-borne illness (Lucera et al., 2012). Seafood could involve bacteria, viruses and parasites, which can cause illnesses ranging from mild gastroenteritis to life-threatening diseases. Some of these pathogens are naturally present in the aquatic environment, while others can be introduced through animal or human fecal shedding and sewage pollution (Amagliani et al., 2012). Contamination of water and sediments is due to the presence of naturally occurring pathogens (e.g. *Vibrio* spp., *Aeromonas* spp., spores of *Clostridium botulinum* type F) or enteric bacteria (e.g. non-typhi *Salmonella*, and *Campylobacter*). Microbial agents of major concern in seafood are repoted as norovirus, *Vibrio* and *Salmonella* spp., whilst minor agents of concern include hepatitis A virus, *Shigella, Listeria monocytogenes, C. botulinum*, and microbial toxins such as staphylococcal enterotoxin C and staphylococcal enterotoxin A including those produced enterically such as cholera toxin produced by *Vibrio cholera* (Elbashir et al., 2018). *V. parahaemolyticus, V. vunificus*, and *V. cholerae* constitute a significant human health hazard causing outbreaks and sporadic foodborne illnesses associated with the consumption of raw or undercooked contaminated seafood (Scallan et al., 2011; Nishibuchi and DePaola, 2005). Food-poisoning with *V. parahaemolyticus* is most commonly reported from countries with both a high ambient temperature and where seafood is consumed raw (Borresen, 2008).

Salmonella infections from consumption of seafood products are most commonly associated with raw, undercooked, and/or poorly cooked finfish and crustaceans (NACMCF, 2008; Elbashir et al., 2018). Globally, *A. hydrophila* has been isolated from aquaculture products, including crabs and fishes (Nielson et al., 2001). Smoked mussels, salmon, and other fish have been associated with outbreaks of *Listeria* (Thigeel et al., 2014), and *Salmonella* outbreaks have been linked to the consumption of Sushi (Canaven, 2013; Mizan et al., 2015).

Wound infections, caused particularly by mycobacteria, *Streptococcus iniae, Erysipelothrix rhusiopathiae* or *Photobacterium damselae* and *V. alginolyticus* are seen after injury during handling fish or after exposure of open wounds to water environment. In the case of poor hygiene, the contamination of fish and fish products may increase due to unsanitary procedures, the rotation of the assigned duties of workers, and airborne microorganisms during packing of the product (Novotny et al., 2004).

CONTROL OF MICROBIAL GROWTH OF SEAFOOD

Control of microbial food spoilage and pathogens growth are currently representing a crucial challenge, as they have been evaluated to be responsible for the loss of 25% of all post-harvesting food production (Gram and Dalgaard, 2002). In addition to industrial traditional technologies and to face the consumers' demand for minimally processed food, new trends such as biopreservation, high hydrostatic pressure, pulsed electric fields, superchilling, chitosan coating, and active packagings are promising complementary ways to extend food shelf-life and reduce microbial risks (Devlieghere et al., 2004; Cortesi et al., 2009; Wiernasz et al., 2017).

Prevention of nutritional and sensory losses caused by microbiological, enzymatic, or chemical changes, and shelf-life extensionof food are usually achieved by chemical preservatives, such as sodium benzoates, sodium nitrite, and sulfur dioxide. Nonetheless, accumulation of these synthetic preservatives in tissues can be detrimental to health (Ozdemir et al., 2012; Olatunde and Benjakul, 2018). Natural antimicrobial systems found in plants, animals, and microorganisms can be used with developing the technology (Ogunkalu et al. 2018).

NATURAL ANTIMICROBIALS

PLANT ESSENTIAL OILS AND EXTRACTS

Numerous essential oils, and extracts from plants have been tested for their antimicrobial properties against various food-borne microorganisms (Hsieh et al., 2001; Burt, 2004). Antimicrobials from plants can be used as an alternative to chemical preservatives in order to satisfy consumers' demand for safe, convenient and wholesome food (Boziaris et al., 2011). Compounds derived from the secondary metabolism of plant materials, such as leaves, shrubs, fruits, or peel, having one or morephenolic rings, can be regarded as plant polyphenols (Parr and Bolwell, 2000). Phenolic compounds in plant extracts or essential oils can disrupt the cell membrane integrity by interacting with membrane proteins of bacteria or fungi. By increasing the permeability of the cell membrane, these compounds cause the leaching of potassium ons and other cytoplasmic structures and ultimately, cell death (Simoes et al., 2009). However, the antimicrobial efficiency of essential oils depends upon the pH, storage temperature, and concentration of oxygen (Jiang and Li, 2001; Arshad and Batool, 2017).

Essential oils from oregano, rosemary, thyme, laurel, sage, cinnamon, clove, and basil have been the most used antimicrobial and antioxidant agents in fish and seafood products. The use of thyme (Kostaki et al., 2009; Mastromatteo et al., 2010), clove (*Eugenia caryophyllata*) (Emir Çoban and Patir, 2013), oregano (Lee et al., 2016), sage (*Salvia officinalis L.*) (Emir Çoban et al., 2016), turmeric and lemongrass (Masniyom et al., 2012), and lemon (Alfonzo et al., 2017) essential oils applied to the fish or other seafoods alone or in combination with other preservation methods, was effective in improving the quality and extending the shelf life of the treated products (Hassoun and Çoban, 2017).

The carvacrol and thymol are reported to be the main compounds responsible for the antimicrobial and antioxidant activity of oregano essential oil (Rodriguez-Garcia et al., 2016). Rosemary (*Rosmarinus officinalis*) essential oils has been reported to exhibit an effective antioxidant and antimicrobial activity, which is mainly related to phenolic diterpenes compounds such as carnosol and carnosic acid (Bozin et al., 2007; Kenar et al., 2010; Makri, 2013; Hassoun and Çoban, 2017). Rosemary, sage, basil, oregano, thyme, cardamom, and clove essential oils are very effective against many pathogenic and spoilage microorganisms like *Salmonella, Escherichia coli, L. monocytogenes, Campylobacter* spp., and *Staph. aureus* and help to increase their quality and shelf stability (Arshad and Batool, 2017). Lee et al. (2016) reported a decreased growth of inoculated *L. monocytogenes* and *Salmonella typhimurium* in tuna meat by wrapping the product in a composite film (1% gelatin+4% red pepperseed meal protein+0.5% oregano essential oil) was noticeable when oregano essential oil was used (Alparslan et al., 2016).

Bensid et al. (2014) found that anchovy in ice containing thyme (0.04% w/v), oregano (0.03% w/v), and clove (0.02% w/v) extracts had longer (12 days) shelf-life than that kept in traditional ice (5 days). The shelf-life of frozen chub mackerel (–20°C) treated with bay leaf, grape seed, and flaxseed EOs (1%) was extended to 7 months while the remaining treated and untreated samples had a shelf-life of 6 months (Erkan and Bilen, 2010). The reduction in fecal coliform and lactic acid bacterial count in vacuum-packed European anchovy by 1% lemon balm (LB) or lavender (LD) ethanol extracts was below 1 log cfu/g (Ozogul et al., 2017). The extracts of myrtle and laurel inhibit oxidation of lipids and growth of bacteria in vacuum-packaged European eel (*Anguilla anguilla*) at 4 °C (Ozogul et al., 2014).

LACTIC ACID BACTERIA AND THEIR ANTIMICROBIAL COMPOUNDS

Lactic acid bacteria (LAB) possess a major potential for use in biopreservation because most LAB are generally recognized as safe, and they naturally dominate the microflora of many foods (Ghanbari et al., 2013). LAB is generally includes *Lactococcus, Streptococcus, Lactobacillus, Pediococcus, Leuconostoc, Enterococcus, Carnobacterium, Aerococcus, Oenococcus, Tetragenococcus, Vagococcus*, and *Weisella* genera of micro-organismsm. LAB had abilities to produce different inhibitory compounds such as bacteriocins, organic acids as lactic acid, hydrogen peroxide, diacetyl, and carbon dioxide (Vieco-Saiz et al., 2019).

The commonly used bacteriocins in foods are lantibiotics nisin and lacticin, pediocin-like bacteriocins and, enterocin AS-48. Bacteriocins can be applied in three forms i.e. *in situ* production by starter or protective cultures, as an ingredient of fermentation of a bacteriocinogenic strain and as an additive in a semi- or purified preparation (Singh, 2018). Bacteriocins have distinct mechanisms of action and can be divided into those that promote a bactericidal effect, with or without cell lysis, or bacteriostatic, inhibiting cell growth (Da Silva Sabo et al., 2014; Silva et al., 2018). Although many LAB-produced bacteriocins such as nisin and pediocin have been approved by the competent authorities and widely used in food products (Turovskiy et al., 2007), the inability to inhibit Gram-negative pathogens, main causes of foodborne diseases, limits their applications (Gillor et al., 2008; da Costa et al., 2019).

CHITOSAN AND CHITIN

Although chitin is synthetized by many categories of organisms, the main sources for chitin extraction are crab and shrimp shells (Matica et al., 2017). In general, chitosan can be produced by deacetylation of chitin, which is a structural element in crustacean exoskeleton. Chitosan is one of the most abundant natural polymers and owing to its unique biological activities has important commercial applications in biomedical, food and chemical industries (Harish Prashanth and Tharanathan, 2007; Rinaudo, 2006; Beck et al. 2019).

Some authors have stated that chitosan generally showed stronger effects for gram-positive bacteria than for gram-negative bacteria (Coma et al., 2003; No et al., 2002). Conversely, it has been demonstrated that hydrophilicity in gram-negative bacteria is significantly higher than in gram-positive bacteria, making them most sensitive to chitosan (Chung et al., 2004; Goy et al., 2009). Studies on *Bacillus cereus, E. coli, Staph. aureus, Pseudomonas aeruginosa, Salmonella enterica, Bacillus subtilis, L. monocytogenes and Klebsiella pneumoniae* have shown that the chitosans with smaller moleculer weight have a higher antibacterial activity, as small polymers have higher mobility and stronger interactions with the bacterial walls, than the chitosans with high molecular weights (Goy et al., 2009; Matica et al., 2017).

Chitosans chemically extracted from *Metapenaeus stebbingi* shells showed antimicrobial effects on nearly all types of the bacteria (*Pseudomonas putida*, *Pseudomonas fluorescens, V. parahaemolyticus, Staphylococcus epidermidis, Staph. aureus, E. coli, L. monocytogenes, Ent. faecalis, Aeromonas caviae*) (Kucukgulmez et al., 2011). Chitosan exhibited antibacterial activity against Streptococcus iniae at concentrations of 0.1% and above and was lethal at a concentration of 0.4% and higher (Beck et al. 2019)

Chitosan has been widely used for developing nano materials due to excellent properties such as biodegradability and biocompatibility. Due to its film-forming ability, a chitosan solution is widely used as an outer coating to protect seafood from environmental factors and microbial invasion (Olatunda and Benjakul, 2018). Pretreatment of fish fillets (*Oncorhynchus nereka*) with 1% chitosan solution (high deacetylation degree) for 3h retarded the counts for mesophiles, psychrotrophs, coliforms, *Aeromonas* spp., and *Vibrio* spp. (Tsai et al., 2002). Growth of aerobic bacteria in Atlantic bonito (*Sarda sarda*) fillets packaged with chitosan film was slower than in the fillets of control and vacuum groups during storage (Alak et al., 2010). Divergicin-loaded chitosan (100 kDa, 94.7% de-acetylated) film reduced *L. monocytogenes* to below the detection limit (<50 cfu/g) and kept total counts below 104 cfu per g of cold-smoked wild salmon compared to 109 cfu per g in control samples (Benabbou et al., 2018). Fish gelatin-chitosan edible films with D-limonene exhibited strong antibacterial activity against *E. coli* (Yao et al. 2019).

PROPOLIS

Propolis (bee glue) is a sticky resinous material applied by honey bees *Apis mellifera* L. as a building material in their hives (Bankova et al., 2016). Propolis contain natural mixtures of different secondary metabolites that are responsible for various bioactivity such as antibacterial, anti-angiogenic, antiulcer, anti-inflammatory, antioxidant, and anti-viral activities (De Figueiredo et al., 2015). Flavonoids, prenylated flavanones, benzopyranes, benzophenones, caffeic acid esters, triterpenoids, benzoic acid and cinnamic acid derivatives, and naphthoquinone epoxides were commonly found antimicrobial and antioxidant compounds in propolis (Campos et al., 2011).

Casquete et al. (2015) indicated that incorporation of ethanolic extract of propolis in a food susceptible to *Listeria* contamination may be an interesting alternative to existing chemical preservatives. The strongest antibacterial activity of propolis was reported against *Staph. aureus*, with a MIC value of 0.5 mg/mL, and *L. monocytogenes*, with a MIC as low as 0.1 mg/mL (Ristivojević et al. 2016). The ethanol extraction of propolis at doses of 0.8% showed antimicrobial activity against some pathogenic microorganisms (*Staph. aureus*, *Salmonella*, *E. coli* and *Clostridium* spp.) (Gutiérrez-Cortés et al., 2014). Propolis extracts (0.8 and 1.2%) could be effective for the control of Gram positive bacteria and some Gram negative bacteria that are present in cachama fillets (Saurez et al. 2014). Fish paste prepared by adding the propolis had high quality and functionality (Kim et al. 2008).

Propolis have also a food applications as a constituent of the socalled active packaging materials, based on the incorporation of active substances, mainly natural antimicrobial and antioxidant agents, in food biopackaging materials (Mascheroni et al., 2010). Rollini et al. (2017) indicated that active packaging materials prepared in the combination of chitosan, propolis and micro fibrillated cellulose resulted in 1 log cycle reduction on growth of *Listeria innocua.* Chitosan films containing 0, 2.5, 5, 10 and 20% w/w propolis extract were developed by Siripatrawan and Vitchayakitti (2016). The characterization of mechanical properties and antimicrobial activities against *Staph. aureus, Salmonella Enteritidis*, *E. coli* and *P. aeruginosa* suggested the benefits of adding propolis into chitosan films and the potential of using the developed film as active food packaging.

LYSOZYME AND LACTOPEROXIDASE

Lysozyme is a hydrolytic enzyme which has been purified from cells, secretions and tissues of virtually all living organisms and viruses. Lysozyme has been shown to have antimicrobial activities and is currently used as a preservative in many foods, such as cheese, fish and meat (D'Amato et al., 2010). The presence of lysozyme in food, on food surface or on packaging surface contributes to control of *L. monocytogenes* (Bower et al., 1998; Gill et al., 2000). Pańczyk and Kołodziejska (2009) examined the inactivation of some Gram-positive and Gram-negative bacteria exposed to the pressure of 193 MPa at –20 C in the presence of lysozyme or nisin at concentration of 400 mg/ml. The highest effect of pressure at subzero temperature and lysozyme was found with pressure sensitive *Pseudomonas fluorescens* (Pańczyk and Kołodziejska, 2009). Lactoperoxidase derived from bovine milk has been shown to generate beneficial effects as a bactericidal and bacteriostatic agent (Abbes et al., 2013; Abbes et al., 2018). The lactoperoxidase system has attracted the attention of scientists as a natural biopreservative with generally recognized as safe (GRAS) status (Alegbeleye et al., 2018). This system generates hypothiocyanite, an active compound against Gram-positive and Gram-negative bacteria, including *Escherichia coli* (Kennedy et al., 2000; Garcia-Graells et al., 2000; Al-baari 2019). Lactoperoxidase purified from bovine rennet whey incorporated alginate films showed the good potential in forming a natural antimicrobial mechanism in different foods (Mecitoğlu and Yemencioğlu, 2007). Yener et al. (2009) investigated the antimicrobial effect of lactoperoxidase incorporated alginate films on *E. coli*, *Listeria innocua* and *Pseudomonas fluorescens* in presence of different concentrations of H_2O_2 (0.2, 0.4, and 0.8 mM) and KSCN (1, 2, and 4 mM). The decreasing order of the resistance of studied bacteria to lactoperoxidase system was reported as *E. coli*, *L. innocua*, and *P. fluorescens*, respectively. Lactoperoxidase incorporated into edible whey protein films exerted the good potential of this enzyme for use in antimicrobial packaging for different microorganisms and smoked salmon (Min and Krochta, 2005a; Min et al., 2005b; Min et al., 2005c). Elotmani and Assobhei (2003) reported that combination of nisin (100 IU mlG) and lactoperoxidase was significantly more effective than lactoperoxidase or nisin alone against all strains, excepting *Aeromonas salmonicida* subsp. *salmonicida* and *Vibrio alginolyticus*. Combination of nisin and lactoperoxidase could be of great interest as biopreservatives for fish and fish products (Jooyandeh et al., 2011).

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INVESTIGATION OF ANTIBIOTIC SUSCEPTIBILITY OF SOME IMPORTANT LACTIC ACID BACTERIA

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Abstract

The interest in lactic acid bacteria has increased recently since they have an important role on food fermentation. They have also probiotic properties due to their health promoting capability for human and inhibiting undesirable bacteria in food. On the other hand, the intensive use of the antibiotics in human and animal health has enabled bacteria to resist towards antibiotics. Thus, the aim of present study was to investigate of antibiotic susceptibility against 17 different antibiotics of some important lactic acid bacteria such as of Lactobacillus pentosus, Lactobacillus plantarum, Pediococcus acidilactici, Lactobacillus paraplantarum, Lactobacillus fermentum, Lactococcus rafinolactis, Pediococcus pentosaceus and Leuconostoc mesenteroides by disc diffusion method. The results of the study showed that Leu. mesenteroides were more resistant to all of the antibiotics except for ampicillin. Although most of bacteria exhibited high resistance to tested antibiotics, some of strains were found to have intermediate resistant and sensitivity. The project was financially supported by the Scientific Research Projects Unit of Cukurova University (TSA-2018-11188).

Keywords*: lactic acid bacteria, antibiotic resistance, antibiotics, sensitivity*

INTRODUCTION

Wide range of fermented foods have been protected with natural processes of fermentation and thus providing longer shelf life of these products (Ananou et al., 2007; Temmerman et al., 2003). *Lactic acid bacteria* (LAB) play a key role in food fermentations. One of the most important properties of LAB is their ability to produce antimicrobial substances such as bacteriocin and organic acids. LAB also produce aroma compounds, exopolysaccharides and several enzymes (Boulares et al., 2012; Jose et al., 2015; Servin, 2004). However, the intensive use of the antibiotics in human and animal health has caused bacteria to become resistant. Probiotic organisms are known to accumulate the resistance genes and transfer these to pathogenic bacteria (Ammor et al., 2007; Devirgiliis et al., 2014; Flórez et al., 2014). Although LAB are considered as GRAS (Generally Recognized as Safe), their potential to transfer antibiotic resistance to pathogenic bacteria must not be ignored since some LAB strains were demonstrated to have acquired transferable antibiotic resistance properties (Pan et al., 2011). Thus, they may have represented a potential source for the spread of resistance genes when they were added to different kinds of fermented foods as starter culture (D'Aimmo et al., 2007). LAB are largely susceptible to antibiotics inhibiting the synthesis of proteins, such as chloramphenicol, erythromycin, clindamycin and tetracycline, and more resistant to aminoglycosides (neomycin, kanamycin, streptomycin and gentamicin) (Charteris et al., 1998b; Zhou et al., 2005). Various LAB species isolated from different fermented food products have been studied to determine their resistance against different antibiotics including vancomycin, kanamycin, erythromycin (Coton et al., 2018; Danielsen and Wind, 2003; Fons et al., 1997; Gfeller et al., 2003; Lin and Chung, 1999; Sudhamani et al., 2008; Tannock et al., 1994) and chloramphenicol (Park et al., 2004). D'Aimmo et al. (2007) also reported that different LAB species are sensitive against ampicillin, bacitracin, clindamycin, dicloxacillin, erythromycin, novobiocin, penicillin G, rifampicin and resistant against aztreonam, cycloserin, kanamycin, nalidixic acid, polymyxin B and spectinomycin.

The aim of present study was to investigate of antibiotic susceptibility against of some important fermentative lactic acid bacteria towards 17 different antibiotics.

MATERIAL AND METHOD

The important fermentative lactic acid bacteria including *Lb. pentosus*, *Lb. plantarum*, *P. acidilactici*, *Lb. paraplantarum*, *Lb. fermentum*, *Lc. rafinolactis*, *P. pentosaceus* and *Leu. mesenteroides* were used in the present investigation. Susceptibility of LAB strains against clinically important antibiotics including ceftazidime (CAZ) (30µg) ampicillin (AM) (10µg), amoxicillin (AX) (25 µg), ceftriaxone (CRO) (30µg), cefotaxime (CTX) (30µg), imipenem (IPM) (10 units), vancomycin (VA) (30µg), meropenem (MEM) (10µg), aztreonam (ATM) (30µg), tetracycline (TE) (30µg), ciprofloxacin (CIP) (5µg), rifampin (RA) (30µg), gentamicin (GM) (30 µg), kanamycin (K) (30µg), erythromycin (E) (15µg), streptomycin (S) (10µg) and amikacin (AK) (30µg) were determined by using disc diffusion method (Bauer et al., 1966) . LAB strains were inoculated into MRS broth and incubated at 37 °C for 24 h. A volume of 100 µl of overnight grown culture suspension of isolates was adjusted 0.5 McFarland standard, which equivalent to cell density of 10^8 cfu/ml. After that, a sterile cotton wool swap dipped into the bacterial suspension and swab with test bacterial suspension was used to spread bacteria on the surface of the MRS agar plates. The inoculated MRS agar plate was allowed to dry before placing the antibiotics disc and then antibiotic discs were placed on the surface of agar plates. Each disc was firmly pressed to ensure complete contact with agar surface. The plates were incubated at 37 °C for 24 h. The inhibition zone diameter obtained around the antibiotic disc were measured at end of the 24 h incubation and the results were expressed as resistant ($R \leq 14$ mm), intermediate resistant (IR; 15-19 mm) or susceptible ($S \geq 20$ mm) as the recommended standards (Bauer et al.1996). The results were interpreted according to the Clinical antimicrobial susceptibility testing for antimicrobial susceptibility testing (Clinical and Laboratory Standards Institute, 2012) as explained by Charteris et al. (2000).

RESULT AND DISCUSSION

All isolates including *Lb. fermentum*, *Lb. paraplantarum*, *Lb. pentosus*, *Lb. plantarum*, *L. rafinolactis*, *Leu. mesenteroides*, *P. acidilactici* and *P. pentosaceus* were evaluated for antibiotic susceptibility against 17 different antibiotics (Table 1). All isolates of *Lb. fermentum* were found to be resistant to ceftazidime, ceftriaxone, meropenem, aztreonam, rifampin, kanamycin and streptomycin antibiotics, while they were found to be intermediate resistance to cefotaxime, ciprofloxacin, gentamicin, erythromycin. All *Lb. fermentum* isolates exhibited sensitivity to ampicillin, amoxicillin, imipenem, vancomycin and tetracycline. Furthermore, *Lb. fermentum* isolates of 21 and 23 showed intermediate resistance to amikacin, whereas isolate of 19 showed high resistance. *Lb. paraplantarum* isolates showed high level of resistance against ceftazidime, meropenem, aztreonam, rifampin and kanamycin. Besides all the tested *Lb. paraplantarum* isolates were highly sensitive to ampicillin, amoxicillin, imipenem, vancomycin, tetracycline. However, *Lb. paraplantarum* isolate 12 showed sensitivity to ceftriaxone and ceftazidime, while other isolates of 9, 21 and 30 showed high and intermediate resistance to the same antibiotics. Likewise, these isolates exhibited a multi sensitivity to erythromycin, streptomycin, amikacin, gentamicin, ciprofloxacin. Sharma et al. (2016) tested the antibiotic susceptible of various probiotic *Lactobacillus* isolates including *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus reuteri*, *Lb. plantarum* and *Lb. fermentum* against 45 antibiotics. Their results indicated that the isolates showed high level of resistance toward nalidixic acid, vancomycin, kanamycin, teicoplanin, co-trimoxazole, amikacin, streptomycin, norfloxacin, cefepime and nitrofurantoin, whereas the isolates exhibited a low level of resistance toward tobramycin, gentamicin, ampicillin, cefaclor, methicillin, penicillin, tetracycline, levofloxacin, azithromycin, chloramphenicol, amoxyclav, sulbactam, oxacillin, ofloxacin, ciprofloxacin, cloxacillin and novobiocin. On the other hand, all the isolates were found to be susceptible against ceftazidime, ceftriaxone, cefadroxil, cefotaxime, cephalothin, cefoperazone and netillin, although none of the strains showed resistant to erythromycin, linezolid, quinupristin/dalfopristin and doxycycline.

In the current study, the strains of *Lb. pentosus* were resistant to ceftazidime, ceftriaxone, cefotaxime, meropenem, aztreonam, kanamycin and streptomycin. On the other hand, the majority of these isolates displayed resistance and intermediate resistance against imipenem, ciprofloxacin, rifampin, gentamicin, erythromycin and amikacin, but some of strains showed sensitivity. These isolates were also remarkable sensitive against ampicillin, amoxicillin, vancomycin and tetracycline (Table 1). All of the *Lb. plantarum* strains were highly resistant to ceftazidime, ceftriaxone, cefotaxime, meropenem, aztreonam, kanamycin and streptomycin while it was intermediate resistant to gentamicin and amikacin. They were intermediate and high resistant against rifampin and erythromycin. All of the strains were observed to have sensibility against ampicillin, amoxicillin, imipenem, vancomycin and tetracycline. Similar results were reported by Han et al. (2015) who found seven *Lb. plantarum* strains resistant to vancomycin, streptomycin, kanamycin and gentamicin. However, one strain was resistant to ampicillin, while others were intermediate resistant to ampicillin.

Table 1. Assessment of LAB strains for each species against 17 different antibiotics

Isolates source	Bacteri strains	Ceftazidime CAZ	Ampicillin AM	Amoxicillin AX	Ceftriaxone CRO	Cefotaxime CTX	Imipenem IPM	Vancomycin VA	Meropenem MEM	Aztreonam ATM	Tetracycline TE	Ciprofloxacin CIP	Rifampin RA	Gentamicin CN	Kanamycin K	Erythromycin E	Streptomycin S	Amikacin AK
Smoked salmon	*Lb. fermentum*	R	S	S	R	IR	S	S	R	R	S	IR	R	IR	R	IR	R	IR
Smoked Salmon	*Lb. fermentum*	R	S	S	R	IR	S	S	R	R	S	IR	R	IR	R	IR	R	IR
Marinated Smoked Mackerel	*Lb. fermentum*	R	S	S	R	IR	S	S	R	R	S	IR	R	IR	R	IR	R	R
'Lor' whey cheese	*Lb. paraplantarum*	R	S	S	S	S	S	S	R	R	S	R	R	S	R	IR	R	IR
Butter	*Lb. paraplantarum*	R	S	S	IR	R	S	S	R	R	S	IR	R	IR	R	IR	IR	R
Butter	*Lb. paraplantarum*	R	S	S	IR	IR	S	S	R	R	S	IR	R	S	R	R	R	R
Butter	*Lb. paraplantarum*	R	S	S	IR	IR	S	S	R	R	S	S	R	IR	R	IR	R	R
Marinated Smoked Mackerel	*Lb. pentosus*	R	S	S	R	IR	S	S	R	R	S	IR	R	IR	R	IR	R	R
Marinated Smoked Mackerel	*Lb. pentosus*	R	S	S	R	IR	S	S	R	R	S	IR	R	IR	R	IR	R	R
Marinated Mackerel	*Lb. pentosus*	R	S	S	R	R	IR	S	R	R	S	S	R	IR	R	R	R	R
Marinated Mackerel	*Lb. pentosus*	R	S	S	R	R	IR	S	R	R	S	S	R	IR	R	R	R	R
Smoked Mackerel	*Lb. pentosus*	R	S	S	R	R	IR	S	R	R	S	IR	R	IR	R	IR	R	IR
Smoked Mackerel	*Lb. pentosus*	R	S	S	R	R	S	S	R	R	S	IR	R	IR	R	R	R	IR
Smoked Mackerel	*Lb. pentosus*	R	S	S	R	R	IR	S	R	R	S	S	IR	IR	R	IR	R	IR
Smoked Mackerel	*Lb. pentosus*	R	S	S	R	R	IR	S	R	R	S	S	IR	IR	R	IR	R	IR
Marinated Smoked Mackerel	*Lb. plantarum*	R	S	S	R	R	S	S	R	R	S	S	IR	IR	R	IR	R	IR
Marinated Smoked Mackerel	*Lb. plantarum*	R	S	S	R	R	S	S	R	R	S	S	IR	IR	R	IR	R	IR
Smoked Salmon	*Lb. plantarum*	R	S	S	R	R	S	S	R	R	S	IR	R	IR	R	R	R	IR
Smoked Salmon	*Lb. plantarum*	R	S	S	R	IR	S	S	R	R	S	IR	R	IR	R	R	R	IR
Keffir	*Lc. rafinolactis*	R	S	S	R	IR	S	S	R	R	S	IR	R	IR	R	IR	R	R
Keffir	*Lc. rafinolactis*	R	S	S	R	IR	S	S	R	R	S	IR	R	IR	R	IR	R	R
'Lor' whey cheese	*Leu. mesenteroides*	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Somoked Salmon	*P. acidilactici*	R	S	S	S	S	S	S	R	R	S	IR	R	S	R	S	R	R
'Lor' whey cheese	*P. acidilactici*	R	S	S	S	S	S	S	R	R	S	IR	IR	S	R	S	R	R
Marinated Mackerel	*P. acidilactici*	R	S	S	S	S	S	S	R	R	S	IR	IR	S	R	S	R	R
'Lor'whey cheese	*P. acidilactici*	R	S	S	S	S	S	S	R	R	S	IR	R	S	R	S	R	R
'Lor' whey cheese	*P. acidilactici*	R	S	S	S	S	S	S	R	R	S	IR	R	S	R	S	R	IR
'Lor'whey cheese	*P. acidilactici*	R	S	S	S	S	S	S	R	R	S	IR	R	S	R	S	R	IR
'Lor' whey cheese	*P. acidilactici*	R	S	S	S	S	S	S	R	R	S	IR	R	S	R	S	R	IR
Keffir	*P. pentosaceus*	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Keffir	*P. pentosaceus*	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R

Three of these seven strains were resistant to tetracycline, two were sensitive and other two had intermediate resistance. Six *Lb. pentosus* strains showed resistance to vancomycin, streptomycin, kanamycin and gentamicin. However, four of the six strains showed intermediate resistance to AM and other two strains showed resistance. Liasi et al. (2009) reported that the lactic acid bacteria such as *Lb. casei, Lb. plantarum* and *Lb. paracasei* isolated from fermented fish products (known as Budu) were resistant to colestin sulhate, streptomycin, amikacin, norfloxacin, nalidixic acid, mecilinam, trimethoprim/sulphanethoxazole, kanamycin, neomycin, bacitracin and gentamicin while they were susceptible to erythromycin, penicilin, chloramphenicol, tetracycline, ampicillin and nitrofurantion. Two isolates of *Lb. rafinolactis* in this study were observed to have high resistant to ceftazidime, ceftriaxone, meropenem, aztreonam, rifampin, kanamycin, streptomycin and amikacin. Besides, they were low resistant against cefotaxime, ciprofloxacin, gentamicin and erythromycin. These isolates were susceptible to ampicillin, amoxicillin, imipenem, vancomycin and tetracycline. *Leu. mesenteroides* were observed to be high resistant to all of the antibiotics except for ampicillin. *Leuconostoc* species were reported to be resistant to nalidixic acid, gentamicin, kanamycin, streptomycin, nitrofurantoin, sulphadiazine and trimethoprim (Flórez et al., 2005; Katla et al., 2001). *Leuconostoc lactis* strains causing a human ventriculitis infection were found to be resistant to carbapenem antibiotics (Deye et al., 2003). All of the isolates of *P. acidilactici* except for 2 isolates were resistant to ceftazidime, meropenem, aztreonam, kanamycin, streptomycin and amikacin. However, they showed sensitivity against other all of the antibiotics. On the other hand, the other 2 isolates exhibited resistant to all of the antibiotics (Table 3). This result in the current study are in accordance with the results of study by Zarazaga et al. (1999). They reported that *Pediococcus species* were resistant to gentamicin, erythromycin, rifampin, clindamycin. The susceptibility of LAB to antibiotics is thought to be related to LAB species (Danielsen et al., 2007).

CONCLUSION

Although most of bacteria exhibited high resistance to antibiotics, some of strains were found to have intermediate resistant and sensitivity. The knowledge on the antibiotic resistance of LAB is still restricted because of the differences in resistance spectra and the large number of species. Therefore, susceptibility testing should be performed against different antibiotics.

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SUSTAINABILITY OF AFRICAN CATFISH IN SOUTH-WEST NIGERIA

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Abstract

Eriwe fish farming village has been described as one of the highest fish producing village that reduces poverty through youth empowerment and cooperative scheme since its establishment in 2002. This study investigated and compared the economic, social and environmental sustainability of extensive and intensive systems at the fish farm village, using primary and secondary data and proposed indicators in existing aquaculture literatures. The study found out that fish farming is relatively economically sustainable with annual gross margin of ₦16,148,938 and ₦9,382,705 and net income of ₦16,089,057 and ₦9,325,799 for intensive and extensive systems respectively. The huge profit observed could be attributed to the subsidized cost of inputs, such as land, security and credit facilities. The study found that NPVs of both the intensive and extensive systems are positive, and with BCR of 2.35 and 2.16 respectively, both systems are economically sustainable. Intensive and extensive systems have a remuneration of 0.43 and 0.46 respectively, which is below the global standard of 1, indicating average labour in the village receives less than the country's minimum wage. Intensive systems generated a total of 31 employment annually, while extensive systems generated 51 employments. Environmental sustainability indicators revealed that all the farmers released waste water into the River untreated, and a total of 61,050 fish were reported to have escaped into the wild in the last one year. 100% of the farmers in the village contributed to annual road upgrade and security assurance of the village. The study showed that little or no attention was given to social and environmental sustainability of fish farming practice in this village. Thus, this study recommends an urgent intervention to include a comprehensive social and environmental guidelines in line with best management practices to protect the interest of labour, community and ecological status of Eriwe river.

Key words: *Fish, NPV, Clarias, catfish, economic, social, environment.*

mtDNA-A POWERFUL TOOL FOR PHYLOGENETIC AND BIODIVERSITY SEARCH IN CHICKENS

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Abstract

The origin and the population structure of the chickens, the most breeding species in the farm animal genetic resources, are still cliff-hanging subjects in animal genetics. In the past, morphological features were used to identify the genetic variation in chicken breeds. However, with the development of molecular genetic techniques, genetic mapping and genetic characterization studies on animal breeds have become more effective and understandable in the identification of breeds. Molecular markers such as RAPD (Random Amplified Polymorphic DNA: Randomly Amplified Polymorphic DNA), AFLP (Amplified Fragment Length Polymorphism), microsatellite markers and SNP (Single Nucleotide Polymorphism: Single nucleotide polymorphism) and mtDNA are used for genetic characterization of chicken breeds. In contrast to mammals, due to the heterogametic structure of hens, mtDNA is preferred as a highly effective phylogenetic marker in determining the maternal inheritance. Because of its evolutionary conservative structure, mtDNA, which is preferred as a powerful marker in phylogenetic identification of chicken breeds, has great advantages in determining indigenous chicken breeds in unrestrainedly crossed and no pedigreed populations. Mitochondrial DNA is preferred to determine the evolution process and the origin of poultry because mtDNA D-loop region, which does not have the enzyme or protein equivalent of the transcription promoters in mtDNA, shows a high degree of variation. In this review, phylogenetic studies and mtDNA D - loop region will be discussed as molecular tools in investigations of chickens' origin.

Key words: *Phylogenetic Study, Domestication, Mitochondrial DNA, Evolution Process, D-Loop Region.*

PILOT STUDY ON CIRCULATING MICRORNAS PROFILE IN PIEDMONTESE CATTLE DURING DIFFERENT GROWTH PERIODS

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Abstract

MicroRNA (miRNA) are small (21-23 nt) non-coding, highly conservative and tissue specific RNA that regulate gene expression after transcription and their circulating levels can be measured and thus could be potentially used as biomarker for various physiological changes. However, the role of circulating miRNAs in livestock species has not been fully studied. Therefore, our pilot study aimed to explore and describe expression profile of miRNAs in plasma samples during different growth periods. Out of 24 RNA samples processed, we have used one RNA sample from each age group (newborn calf, 5 month/weaning, 11 month and 16 month of age) for sequencing. Using small RNA sequencing (NGS), we have identified total 1031 miRNAs of which 369 miRNAs were expressed in all samples (n = 4 bovine). Among miRNAs identified in plasma, we have found a lot of miRNAs closely related to myoblast proliferation or myocyte differentiation such as miR-1, miR-206, miR-26a and miR-27b. These results provide novel information on bovine circulating miRNAs in Piedmontese breed but this is pilot study and further analysis is needed.

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Key words: *cattle, circulating miRNA, skeletal muscle*

INTRODUCTION

Piedmontese cattle is a native-breed of North-West Italy and is characterized by a peculiar muscular hypertrophic development called double-muscle. Genesis of skeletal muscle is a complex process comprising of proliferation, differentiation, and formation of myotubes and myofibers. A set of postnatal muscle stem cells (satellite cells) produce myoblast cells that can fuse with the existing myofibers, thus increasing their size (Robelin et al, 1993). These molecular events are regulated by myogenic regulatory factors (e.g. MyoD) and microRNAs (miRNAs) (Bukingham et al, 2014). MiRNAs are small non-coding RNAs, 21-23 nt in length, that regulate gene expression and play key roles in various physiological and developmental processes (Bartel et al, 2004). miRNAs highly enriched in muscle are called myomiRNAs. The main known myomiRNAs are miR-1, miR-133a/b and miR-206. These miRNAs have important roles in regulating myogenesis and differentiation of skeletal muscle (Kim et al., 2006). Other miRNAs such as miR-24, miR-26a, miR27b, miR125b, miR148a, miR-181, and miR-214 have been reported to regulate myoblast physiology but are not particularly abundant in the muscle tissue (Liu et al., 2010). MiRNAs can be also released from the cell and circulate in the blood or other body fluids in a stable cell-free form (ci-miRNAs). Recent studies show that ci-miRNAs participate in cell-to-cell communication to regulate normal and pathological processes indicating that ci-miRNAs can be used as biomarkers of physiological or pathological processes (Stenfeldt et al, 2017; Turchinovich et al, 2012). In livestock, ci-miRNAs have not been totally explored. So, studying the role of ci-miRNAs associated with the skeletal muscle growth could be useful for developing strategies to improve production performance in livestock.

Previous studies indicated that circulating levels of tissue specific miRNAs could be measured and used as biomarker for tissue functions and production traits and also for the early diagnosis of animal diseases (Ioannidis, 2018; Farrell, 2015). Therefore, we hypothesize that plasma miRNA expression profiles are affected by age and associated with production traits in beef cattle and that ci-miRNAs could be promising biomarkers for the genetic selection of meat-purpose animals for future breeding.

The aim of this pilot study was to explore and define a panel of expression of ci-miRNAs in the plasma samples collected from the Piedmontese breed cattle just after the birth and during the course of skeletal muscle growth until slaughter. This study is part of a larger project that aims to identify if molecular factors such as specific ci-miRNAs could be effective biomarkers associated with skeletal muscle growth and production traits.

MATERIALS AND METHODS

Animals and collection of samples

For our project, blood samples are to be collected from 72 animals falling into four age groups (new born calf, 5 month/weaning, 11 month and 16 month of age) with 18 animals in each group and so far samples have been collected from 24 animals (6 samples for each age group) in Plasma EDTA vacutainers. Sample size was calculated using the online software- Power and Sample Size Calculation-PS, Vanderbilt University.

Animals were housed in the animal facility of the Dept of Veterinary Science, University of Turin, under the same management conditions. All the experiments involving Piedmontese cattle were approved by the Ethical Animal Welfare Committee of the Dept. of Veterinary Science, UniTO (Prot. No. 663).

MiRNA and total RNA extraction

Blood samples were processed within 2 hr of collection according to a standardized operating procedure (centrifugation at 3500 rpm for 10 min at 22°C) to collect plasma. MiRNA and total RNA were extracted with miRNeasy Plasma/serum kit (Qiagen, Germany) according to the manufacturer's protocol where Ce_miR39 was added as spike-in control. Quantification of miRNA was done using QuBit 3.0 fluorometer (Invitrogen, Life technologies, USA) and the samples with sufficient quantity of miRNA were immediately reverse transcribed into cDNA (Qiagen/Exiqon). The quality of RNA extraction and reverse transcription was checked with Real-time qPCR using Ce_miR39 primer assay.

Library preparation and sequencing

TruSeqSmallRNA Sample Prep kit (Illumina, San Diego, CA) was used for library preparation following the manufacturer's instructions. Just before the library preparation, RNA samples were again quantified and quality tested by Agilent 2100 Bioanalyzer RNA (Agilent technologies, Santa Clara, CA) or by Caliper RNA LabChip GX (Caliper Life Sciences, Hopkinton, MA).

Libraries were then prepared for sequencing and sequenced on single-end 75 bp mode on NextSeq500 (Illumina, San Diego, CA). Final libraries were quantified using the Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, CA) and quality tested by Agilent 2100 Bioanalyzer (Agilent technologies, Santa Clara, CA) or by Caliper RNA LabChip GX (Caliper Life Sciences, Hopkinton, MA).

Sequencing data analysis

The Bcl2Fastq 2.0.2 version of the Illumina pipeline was used to process raw data for both format conversion and de-multiplexing. Whole data was normalized using the standard method by DESeq2.

RESULTS AND DISCUSSION

Out of 24 RNA samples processed, we used one RNA sample from each age group for next generation sequencing (NGS).

The quality of sequencing reads was assessed by the read length (Figure 1)

Table 1. Expression (reads in million) of three myo-miRNAs and other miRNAs

Age in months	0 month or newborn	05 month	11 month	16 month
miR-1 (Koutsoulidou et al, 2011)	32.14	10.02	35.3 ↑	14.5
miR-206 (Kim et al, 2006)	9.84	0.52	76.49 ↑	1.61
miR-133a (Koutsoulidou et al, 2011)	30.18	3.16	76.48 ↑	5.23
miR-27b (Christ et al, 2009)	85319.92	121582.8	72960.99	122924.14 ↑
miR-221	7699.87	7435.64	6724.81	29248.14 ↑
miR-181 b	9322.38 ↑	7613.92	6654.21	5923.51
miR-26a	263241.72	333975.31	46997.20	582001.53 ↑
miR-214	33.46 ↑	20.04	5.88	27.80
miR-24	349.70	504.78 ↑	141.20	408.89

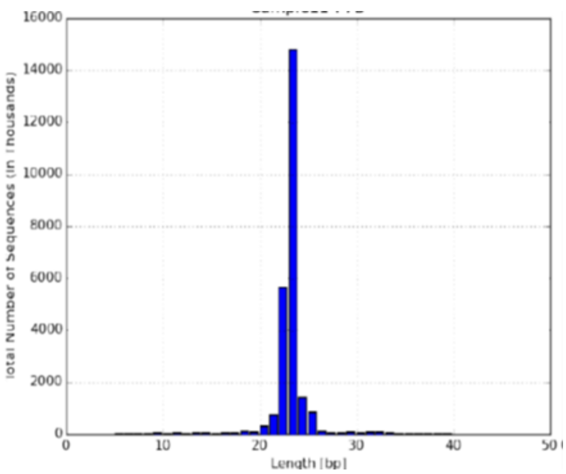

Figure 1. The length distribution of all sequence reads (example of one sample)

Identified miRNAs matching sequence reads as determined with up to two mismatches allowed are as follows

Age group	Known	Known matched	miRNAs Matched %
Newborn	3424	1932	56.43
05 month	3424	2327	67.97
11 month	3424	895	26.14
16 month	3424	1171	34.20

Total 1031 miRNAs were identified and 369 miRNAs were expressed in all samples.

The top 20 most abundant miRNAs of total miRNA expression in bovine blood plasma are listed here

- bta-miR-286
- bta-miR423-5p
- bta-miR-191
- bta-miR-25
- bta-miR-92a
- bta-miR-30d
- bta-miR-148a
- bta-miR-186
- bta-miR-27b
- bta-miR-30e-5p
- bta-miR-151-3p
- bta-miR-146b
- bta-miR-26a
- bta-miR-660
- bta-miR-6529a
- bta-miR-150
- bta-miR-30a-5p
- bta-miR-451
- bta-let-7i
- bta-let-7c

Among miRNAs identified in plasma in all samples sequenced, we have found miRNAs involved in either myoblast proliferation or myocyte differentiation. (Table 1)

Table 1 Expression (reads in million) of three myo-miRNAs and other miRNAs based on NGS results during four periods of muscle growth. Arrow shows the higher expression of a miRNA at a particular period of growth.

Based on the expression profile of different miRNAs, validation will be done Real time qPCR.

All these miRNAs are known to be involved in skeletal muscle growth and differentiation processes. MyomiRNA such as miR-1 and miR-206 have specific roles in modulating bovine skeletal muscle satellite cell proliferation and differentiation through the positive regulation of Pax7 and the downregulation of HDAC4 (Dai et al, 2016) High expression levels of miR-26a are required to promote differentiation of satellite cells (Wang et al., 2018). The miR-27 family influences many cellular processes and plays an important role in down regulation of Myostatin, a negative regulator of muscle growth, thus regulation of post-natal myogenesis and promotes skeletal muscle satellite cell proliferation in animal production species (Huang et al, 2012; Zhang et al, 2018).

CONCLUSIONS

MiRNAs are necessary for skeletal muscle development and have regulatory roles throughout growth and differentiation. Little is known about role of ci-miRNA in cell-to-cell communication to regulate normal skeletal muscle processes such as the regulation of muscle differentiation. Nevertheless, to our knowledge, no studies have examined ci-miRNA expression profiles during different growth periods in bovine. Thus, the primary objective of this study was to identify if a panel of miRNA-centered on regulation in the context of bovine skeletal muscle cell growth and differentiation was represented.

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CARCASS TRAITS OF SOME TURKISH NATIVE GOAT BREEDS

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Abstract

Carcass characteristics of Angora, Hair, Honamli and Kilis goat kids were investigated in this study. Male kids of Angora (n=6), Hair (n=6), Honamli (n=6) and Kilis (n=6) pure breeds were used as experimental animals. All kids were slaughtered at 3 months of weaning age. There were no significant differences between kids in terms of birth weight, but slaughter weight of Hair kids were higher ($p<0.05$) than Angora, Honamli and Kilis kids. Hot and cold carcass weight of Hair and Honamli kids were higher ($p<0.05$) than Angora and Kilis kids. Angora kids had lower ($p<0.05$) hot and cold carcass yield than those of other breeds. However, chilling loss and chilling loss percentage of Kilis kids were lower ($p<0.05$) than those of other breeds. Angora kids had higher ($p<0.05$) relative internal fat, total fat and pelt weight than those of other breeds. Additionally, testis weight of Hair kids was relatively higher ($p<0.05$) than those of other breeds. There were no significant differences between kids in terms of other non-carcass components. It was concluded that carcass traits and non-carcass components of male kids born to Angora, Hair, Honamli and Kilis Turkish native goat breeds exhibit differences.

Key words: *kid, goat, native breeds, carcass traits, non-carcass components*

INTRODUCTION

Turkey has different animal domestic genetic resources and has a considerable potential in livestock production of Europe. Turkey is also one of the most important countries in the world in terms of animal genetic resources. Turkey has 10.9 million goats in 2018 and goat population of Turkey represents 46 % of the European goat population (TUIK, 2018; FAO, 2018). Although Turkey is an important goat breeding country of Europe, it has had a dramatically reduce in the number of goats, especially in the last 20 years (TUIK, 2018). Generally, reduce in number of goats has been caused by reasons such as social and economic problems in rural areas, migrations to urban areas, a traditional farming system, inadequate government support, lack of cooperation among breeders, reluctance of young people to sustain traditional goat farming, lack of demand for goat products and difficulties in finding shepherds (Ertuğrul et al., 2010).

The importance of goats as meat-producing animals is increasing as their meat has been accepted in many new markets (Mahgoub et al., 2011). The breeding of goat, which have a large share in the production of red meat around the world, is carried out under extensive conditions and there is almost no such additional or supplementary feeding practices (Zervas and Tsiplakou, 2011; Sen et al., 2016). Pasture grazing is the most common practice for managing goat flocks worldwide (Zervas and Tsiplakou, 2011). Therefore, determination of quality and nutrient content of the meat in native goat breeds used in meat production may help offer alternative meat varieties to the consumers. For this reason, studies are needed to determine the growth and carcass performance of local breeds.

Turkey has about 10 million goats and 9 different breeds (TUIK, 2018). Therefore, the goats are an important source for meat production. Turkey has local goat breeds, which have a variety of geographic and climatic conditions. The most commonly raised native goat breeds in Turkey are Hair, Kilis, Angora and Honamli. These breeds constitute approximately 92% of the goat population in Turkey (TUIK, 2018). Numerous studies have examined carcass characteristics of sheep, cattle and pig, but there is little data from comparative studies for carcass traits of kids born to Hair, Angora, Kilis and Honamli Turkish indigenous goat breed.

The present study was, therefore, conducted to comparatively determine carcass traits of kids born to Hair, Angora, Kilis and Honamli Turkish indigenous goat breed. The results of present study will contribute to the improvement of the carcass characteristics Turkish indigenous goat breed, which is chosen fast growth, high meat production, and adaptability for slaughter.

MATERIALS AND METHODS

A total of 24 kids of Angora (n=6), Hair (n=6), Honamli (n=6) and Kilis (n=6) breeds were used as experimental. All animals were obtained from the national sheep and goat breeding project in Ankara (Angora), Tokat (Hair), Antalya (Homanlı) and Kilis (Kilis) provinces of Turkey. Birth weight of kids was recorded within 12 h after parturition. Feed and water were withdrawn overnight for determination of fasting live weight in the next morning at 90 days of weaning age. All kids were slaughtered following a standard commercial slaughter procedure.

Hot carcass weights were measured after removing all internal organs. The pelt, head, feet, internal organs (spleen, lungs, liver, kidney and heart), empty reticulo rumen, empty intestine and testis were weighed. In addition to weight of internal, kidney and total fats were recorded. Carcasses were chilled for 24 h at 4°C and reweighed to determine cold carcass weight.

The statistical analysis was conducted on completely randomized design for traits. The statistical analyses were performed using SPSS statistical software. Significant differences between means were tested by Duncan's multiple comparison tests. Results were computed as mean ± SE and statistical significance was determined at the level of $p<0.05$.

RESULTS AND DISCUSSION

Slaughter and carcass characteristics of male kids born to Turkish native goat breeds are present in Table 1. There were no significant differences between kids in terms of birth weight, but slaughter weight of Hair kids were higher ($p<0.05$) than Angora, Honamli and Kilis kids. Hot and cold carcass weight of Hair and Honamli kids were higher ($p<0.05$) than Angora and Kilis kids. Angora kids had lower ($p<0.05$) hot and cold dressing percentage than those of other breeds. However, chilling loss and chilling loss percentage of Kilis kids were lower ($p<0.05$) than those of other breeds. Various factors affect the growth performance of kids. Birth weight is one of these factors, especially in terms of increasing the survival rate of kids. In the present study, birth weight of similar and this results in agreement with the previous studies (Atay et al., 2010; Alaşahan and Öztürk, 2010; Saatçi and Akbaş, 2016).

Table 1. Slaughter and carcass characteristics of male kids born to Turkish native goat breeds.

	Angora	Hair	Honamli	Kilis
Birth weight (kg)	2.95 ± 0.11	2.90 ± 0.29	3.16 ± 0.30	2.70 ± 0.16
Slaughter weight (kg)	16.17 ± 0.36^{bc}	20.90 ± 2.4^{a}	18.19 ± 2.2^{ab}	14.68 ± 0.38^{c}
Hot carcass weight (kg)	6.58 ± 0.18^{b}	9.08 ± 1.14^{a}	8.74 ± 1.34^{a}	6.29 ± 0.16^{b}
Hot carcass yield (%)	40.73 ± 1.23^{b}	43.37 ± 0.72^{ab}	47.52 ± 2.37^{a}	45.46 ± 0.61^{ab}
Cold carcass weight (kg)	6.32 ± 0.19^{b}	8.81 ± 1.15^{a}	8.46 ± 1.35^{a}	6.15 ± 0.16^{b}
Cold carcass yield (%)	39.17 ± 1.20^{b}	41.98 ± 0.70^{ab}	45.91 ± 2.59^{a}	44.04 ± 0.56^{ab}
Chilling loss (kg)	0.25 ± 0.02^{a}	0.27 ± 0.02^{a}	0.28 ± 0.03^{a}	0.14 ± 0.04^{b}
Chilling loss percentage (%)	3.85 ± 0.29^{a}	3.22 ± 0.49^{a}	3.49 ± 0.65^{a}	1.42 ± 0.12^{b}

a,b,c The differences indicated by different letters on the same line are significant

Additionally, there were positive correlation It was found that hot and cold carcass yield were approximately 40.73%-47.52% and 39.17%-45.91% based on slaughter weight, respectively. Koyuncu et al. (2007) reported higher dressing percentages based on slaughter weight than this study. On the other hand, Özcan et al. (2010) reported lower dressing percentages. However, Dhanda et al. (2003) and Daskiran et al. (2010) reported similar results to this study. In agreement with this study, some authors reported a significant effect of genotype on dressing percentage (Koşum et al., 2003; Dhanda et al., 2003; Özcan et al., 2010). However, Cameron et al. (2001) did not find significant effects of different genotypes. Because this study was conducted in extensive conditions, the fattening performances of the kids were not determined. Dressing percentages were affected by various factors such as genotype, sex, age, preslaughter weight, management, and feeding and varied between 35% and 53%. Therefore, it was thought that dressing percentages would be higher with better management systems.

Non-carcass components (as % of slaughter weight) of male kids born to Turkish native goat breeds are present in Table 1. Relative internal fat, total fat and pelt weight of Angora kids were higher ($p<0.05$) compare to kids born to Hair, Honamli and Kilis breeds in the present study. Morever, Hair kids had relatively higher ($p<0.05$) testis weight compare to kids born to Hair, Honamli and Kilis breeds in the present study. There were no significant differences between kids in terms of other non-carcass components.

Table 2. Non-carcass components (as % of slaughter weight) of male kids born to Turkish native goat breeds.

	Angora	Hair	Honamli	Kilis
Internal fat	0.86±0.08^{a}	0.38^{b}±0.07	0.50±0.12^{b}	0.83±0.06^{a}
Kidney fat	0.25±0.03	0.21±0.04	0.21±0.05	0.26±0.03
Total fat	1.11±0.09^{a}	0.60±0.09^{b}	0.71±0.17^{b}	1.08±0.07^{a}
Head	6.87±0.05	6.53±0.36	7.09±0.16	7.33±0.35
Pelt	13.14±0.53^{a}	8.10±0.28^{b}	8.25±0.55^{b}	7.81±0.20^{b}
Feet	2.89±0.04	3.37±0.15	3.93±0.10	3.78±0.13
Small intestine	2.38±0.10	2.87±0.30	2.03±0.30	2.42±0.08
Reticulo rumen	3.04±0.14	3.44±0.15	2.15±0.22	3.20±0.08
Lung	1.71±0.09	1.62±0.13	2.10±0.33	1.8±0.07
Liver	2.21±0.06	2.10±0.15	2.21±0.23	2.06±0.09
Kidney	0.43±0.02	0.40±0.04	0.48±0.04	0.49±0.02
Heart	0.66±0.03	0.57±0.03	0.55±0.04	0.45±0.01
Spleen	0.40±0.05	0.27±0.03	0.25±0.02	0.22±0.01
Testis	0.23±0.02^{ab}	0.28±0.05^{a}	0.18±0.02^{b}	0.21±0.01^{ab}

a,b,c The differences indicated by different letters on the same line are significant

In the present study, the significant differences between breeds in terms of pelt were similar to the study conducted by Özcan et al. (2010), and Akbaş and Saatci (2016). The differences between skin percentages might be specifically associated with the denser and longer hair production of Angora kids compared to kids born to Hair, Honamli and Kilis breeds. Oman et al. (2000) reported that Angora goats had lighter live and hot carcass weights than other studied goat breeds and crossbreeds (Spanish, Boer × Spanish and Spanish×Angora) when slaughtered at a given age. Moreover, in the study of Oman et al. (2000) primal carcasses cuts of Angora breeds were always the fattest or among the fattest compare to other studied goat breeds and crossbreeds. In the current study internal fat and total fat weight of Angora kids were higher than kids born to Hair, Honamli and Kilis breeds. These observations are in agreement with the argument of Oman et al. (2000). Wood (1990) reported that 2 to 3% of body fat is needed to ensure the organoleptic qualities of meat; therefore, Angora kids potentially presented a better fat distribution in carcass.

CONCLUSIONS

In conclusion, the results obtained from present study indicate that there is an influence of breed on carcass traits and non-carcass components in weaning kids born to Turkish indigenous goat breeds. Differences among Turkish indigenous goat breeds in terms of meat carcass traits and non-carcass components may be help production of alternative kid meat or meat product for the consumer. Additionally, carcass traits and non-carcass components of all breeds were commercially acceptable for consumer and requirements of the market.

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IDENTIFICATION OF CANDIDATE SIGNATURE GENES AND KEY REGULATORS ASSOCIATED WITH TRYPANOTOLERANCE IN THE ETHIOPIAN SHEKO CATTLE BREED

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Abstract

African Animal trypanosomiasis (AAT) is caused by a protozoan parasite that affects the health of livestock. Livestock production in Ethiopia is severely hampered by AAT; various controlling measures were not successful to eradicate the disease. AAT affects the indigenous breeds in varying degrees. The Ethiopian Sheko breed, which is considered to be of taurine origin, is known for its high trypanotolerance level. The reasons for the tolerance of Sheko are not well understood. Since taurine breeds are known to be rather trypanotolerant in general, we first determine the degree of taurine admixture to the Sheko genome by comparing it with eleven African breeds. Using genotype data from a total of n = 265 animals obtained with the Ilumina BovineHD genotyping Bead chip, we found that the Sheko genome is largely of indicine orign with substantial taurine admixture. Further, applying three different statistical methods (iHS, CLR and Rsb) to discover selective sweeps in the Sheko genome, we identified 99 genomic regions harboring altogether 364 signature genes. Based on a literature review, we identified 15 genes which appear to be good candidate genes for trypanotolerance. We also revealed 13 overrepresented pathways and ten master regulators in Sheko using the TRANSPATH database in the geneXplain platform. Most of these pathways are related with oxidative stress responses indicating a possible selection response against the induction of oxidative stress following trypanosomiasis infection. Furthermore, we present for the first time master regulators involved in trypanotolerance of not only the Sheko breed but of cattle breeds in general. Our findings show that the master regulator Caspase is a key protease which plays a major role for the emergence of adaptive immunity in harmony with other master regulators. The design and the implementation of genetic intervention strategies is necessary to improve the performance of trypanosusceptible breeds. We believe that the master regulators that we identified in our study represent interesting potential therapeutic targets for the development of new drugs for trypanosomiasis treatment.

Key words: *trypanosomiasis, trypanotolerant, selection signature, candidate signature genes, master regulators, overrepresented pathways.*

EFFECT OF TRANSHUMANCE ON ENVIRONMENTAL RESILIENCE

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Abstract

Archeological evidence of animal domestication and the subsequent development of mobile pastoralism has been discovered dating back 7,500 years in ancient Mesopotamia. Since that time ecological impacts of livestock production on the natural environment can easily be documented in numerous scientific studies. Most recent records indicate that approximately 30%, namely 3.5 billion hectares of ice-free land on earth has been used for pastoral grazing. Transhumance dating back to the ancient times has since expanded to virtually every terrestrial continent on the globe. The production system has proven to be an important management tool for the conservation of fragile dry-land ecosystems. Yet the loss of pastureland to cropping and sedentism has reduced mobile pastoralism numbers to marginal levels over recent centuries. Our study shows that transhumance and nomadic pastoralism has a lot to offer in sustainable resource management to these marginal regions. The traditional values and the flexible adaptation to physical constraints of the mobile pastoralist system provides a major conservation advantage among arid and semi-arid rangeland resources.

Key words: *transhumance, sustainability, Turkey, resource management.*

INTRODUCTION

Garret Hardin’s “Tragedy of Commons” brought a serious message on conservation of the world’s finite resources and it ended in the concept of sustainable development. Yet, there is an enormous absence of the understanding of the value of our habitats and of nature as an environment of culture and human presence. It is predominantly guided by the philosophy of consumable development. This structure of living is not only observed in the developed world, but also in less developed world. Almost every corner in the world formulation of the policies in order to minimize the environmental degradation has failed to address the livelihood issues of marginal communities; as such transhumance production systems.

Transition from hunter-gathering to farming and herding in the society have been the most important milestone in the one-million year history of humanity. “Settled” life began around 10000 BC, initially with farming and later by domesticating animals. Climate change, lack of resources and dependency of production on natural resources has forced migration of human being between continents, thus a nomadic lifestyle emerged. Transhumance, also referred to as mobile pastoralism has been practiced since the Neolithic, the late stone age period (Nandris, 1985; Arnold and Greenfield, 2006). With the onset of agricultural evolution among prehistoric humans, people have started to move for better and more feed for their domesticated animals, so called nomadic/transhumant practices. The term ‘‘Transhumance’’ can be described as “a way of living which practice seasonal and cyclical movement of people with their animals between different ecological zones/pasture lands. Transhumant systems have been common for centuries in many regions of the Old World, and it is a traditional farming practice that shapes the landscape and benefits ecosystem conservation (Ruiz and Ruiz, 1986; MacDonald et al., 2000; Fortina et al., 2001; Garzon, 2001). As periodic movement allows the adaptation of grazing pressure to the carrying capacity of pasturelands, mobile pastoralism has historically been the dominant type of livestock management strategy in semi-arid tropics, deserts, and highlands. Particularly in semi-arid countries, animal migratory systems are critical for making efficient use of the primary productivity of ecosystems across seasons (Alerstam et al. 2003, Manzano-Baena and Casas 2010). Many mountain areas in Europe where transhumant livestock spend most of the year have developed highly diverse ecosystems that play a significant role in conserving biodiversity (MacDonald et al., 2000). Transhumance flocks usually moves from areas that are vulnerable, changeable and mariginal. It is a way of adapting to these unbalanced conditions and making use of ecological complementarities between regions. Today they are still the main livelihood, alleviating rural poverty, in certain regions of the world (Moktan et al. 2008). Transhumance in Turkey has been declining dramatically in the last decades. This decline are caused by social, economic and political factors. As such, the ecological and climate crisis that severely affects water and feed availability on the migration routes; tension between transhumant and other farmers-massive pressure on land and natural resources and decentralization and taxes for the native pastures. Nevertheless, transhumance in Turkey remains still dynamic in the less favored and mountainous areas. The loss of this environment use model means decrease in economic and natural resources, as well as a unique folklore which developed over centuries.

In this study we aim to highlight environmental dynamics that transhumance provide and introduce their holistic contribution on biological resilience which is an additional approach for understanding the benefits of those communities for natural resource management.

MATERIALS AND METHODS

This research was carried out on the central part of the Taurus mountains located in the Mediterranean region of Turkey (36°08′44.9″ N, 33°14′50.4″E). The prevailing climate of the study area is a typical Mediterranean climate with an altitude of about 670 m above the sea level. The mean annual temperature and precipitation in 2017 were 19.1°C and 592.3 mm, respectively. About 70% of precipitation falls during the winter and spring (November–May). Dominant tree species of these sparse forests are *Pinus nigra, Pinus brutia, and Cedrus libani*, which are replaced by *Juniperus excelsa* in the process of regressive succession due to human disturbances (Celik, 2005).

The name of the tribe is Sarıkeçililer and have been breeding Black goat (hair goats) since centuries. They begin migration in early April covering 400-500 km every year to the Central Anatolian lowlands (Fig 1) and return in October back to the Mediterranean highlands. The main factor determining the migration route these days is solely water availability. Main data sources came from semi-structured walk and talk interviews (Moktan et al.,2008) and participant observations while living and migrating with transhumants.

Figure 1. One of the migration route of goat transhumance in Turkey

RESULTS AND DISCUSSION

Comparative advantages of Transhumance on the environmental stability

Transhumance has been a traditional practice in Turkey, dating back to the antient times. Today they account for around 3,5% of the total sheep and goat population while in the 1950's it was more than 32%. Pastures in Turkey can be classified into 6 main ecological regions or biomes; steppes, high steppes or high pastures, anthropic steppe, alpine and sub-alpine pastures, forest and maquis scrubland or garrigue (Atalay, 2000). Aiming to maximize the utilization of resources within these eco-regions through grazing, the transhumant moves livestock between winter and summer pastures showing that the transhumant has practiced agro ecological principles as part of their grazing process for centuries in the region. There is strong proof that, soil enrichment and native pasture improvement has definitely resulted through natural urine and manure deposits constantly left behind by the migrating herds. Resultantly, good vegetation cover and no overgrazing has ensured soil conservation due to lack of erosion helping to improve water quality in the respective catchment areas.

Periodic livestock movements and the successive occupation of different territories have helped develop various interesting fauna-flora symbiosis in the Anatolian steppes. For instance one of the main shrub species *Quercus coccifera*; the kermes oak, has developed spiny-serrated coriaceous leaves limiting the forage browsing by goats and sheep and yet for a short period early in its development can provide valuable nutrient intake. The same applies for the various perennial *Euphorbia spp*.; the spurges, which again are toxic to most herbivores but in small quantities at certain times of the year, has anti-inflammatory properties for various small ruminant ailments, the goats seem to know exactly how many bites to take without reaching the toxic threshold.

The Anatolian alpine pasture system is a product of the ancient livestock vegetation interaction that has evolved with the transhumance lifestyle through many millennia. The ingested pasture seeds that are later deposited within the fertilizer rich manure of the travelling sheep or goat have meant higher germination ensuring a better spread of the established species. If we are to assume that we can identify potential areas of intervention for sustainable natural resource management for the livestock sector in Turkey, we must first accept that there is much to be learned from existing knowledge on transhumance in present day Anatolia. *Pinus brutia*, commonly referred to as 'Turkish pine' a very common forest tree in the East Mediterranean steppes and very prone to debilitating forest fires has performed far better in the historical transhumant routes than in other regions where livestock interaction was abated. By browsing the undergrowth and low scrub, the herd maintains the brush and heath to manageable levels where fires are far less intense if they occur due to natural causes. As well by constant stomping whilst browsing the understory, the herd buries the volatile pine needles into the soft forest soil, further deterring the likelihood of forest fires.

Consequently, thousands of years of history, the dynamics of vegetation and pastoral land use management have been highly inter-reliant. In this social-ecological system the landscape both shapes and is shaped by the land-use, livelihood and culture of pastoralism. Transhumance requires deep knowledge of the location and availability of natural resources, including spatial and temporal patterns, ecosystem types, responses to disturbances such as diseases, and formal and informal institutions that regulate transhumance (Oteros-Rozas et al, 2014).

CONCLUSIONS

Transhumance has made use of this rich resource over the years and has paid particular attention to preserving the ecology. The study showed that the wandering grazing patterns of the mobile transhumant herd have helped spread the seeds of the local endemic varieties and shape the unique ecosystems of the Mediterranean region and maintain the interesting floristic composition of the Anatolian steppes with a high environmental resilience. Transhumance enable preservation of animals as a capital, thus economical sustainability with low production costs while building cultural resilience with their unique production prototypes.

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A PRELIMINARY STUDY ON GRAZING PATTERN OF SHEEP TRANSHUMANCE IN TURKEY

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Abstract

Grazing activities of indigenous sheep breed of the Turkish breed Karakaş and Koçeri which were transhumance flocks were investigated in a typical unimproved mountainous pasture in the East of Turkey. Data were collected from two different flocks on main daily activities (grazing, resting, moving or standing) every fifteen minutes. The characteristics of the pastures around the routes were assessed according to CORINE Land Cover classification system. The animals concentrated their activity mostly on grazing while resting and moving occurred in specific times during the whole grazing period. Standing was a residual activity in both study paths. Once the animals were heading back to their main settlement areas to be milked, grazing activity have been increased. Further studies on grazing behavior and changes in land cover will help to adjust the management strategies to the available natural resources.

Key words: *natural resources, grazing behavior, sheep, transhumance, Turkey*

INTRODUCTION

Grazing is the most important daily activity that ruminant livestock undertake. The ability to detect and understand the grazing patterns of free-ranging livestock is critical for monitoring the weight gain of individual animals, managing available biomass within the landscape (Delagarde and Lamberton, 2015) (Ueda et al., 2011). Rangelands (dominated by dry grasslands and grass/shrublands) cover 22.9 million ha in Turkey (Koc et al., 2014) and seasonal grazing have been historically the way of livestock production. These pasturelands are still critically important to livestock production, an activity which contributes to 12 % of the agricultural output in Turkey, accomplished in small-scale farms in which domestic breeds with low productivity but high adaptation to the harsh environmental conditions are managed (FAO, 2016). Most of the land is under the ownership and authority of the state. Anatolian highlands have been exposed to systematic grazing over the last ten thousand years by a high degree of variability in degree of mobility, land use and animal preferences and herd management strategies (Hammer and Arbuckle, 2017). Sheep transhumance in Turkey, which has a thousand years of history experiencing tragedy of commons and is not perceived as a self-sufficient and environmental friendly production at national scale. The transhumant farmers are seasonally moving from south east Anatolia to the Eastern mountains and vice versa for better feed availability.

Mountainous pastures, which located up to 600 m, are characterized by a rough topography and great variability of plant communities and herbage cover (Aldezabal et al. 1999). Their flora composed mainly by perennial grasses and glass-like plants as well as of some forbs and shrubs (Figure 1). They are grazed seasonally, from mid- spring to autumn, while they are covered by snow during winter (Sarlis 1998).

Figure 1. Koçeri breed in unimproved mountain pasture of Ağrı province.

Grazed pastures represent a specific, but synthetic ecological system with crucial dependent variables such as nutrition and environmental controls influencing the dominant plant and animal species (Squires 1975). In recent years, it has been increasingly realized that grazing of sheep considerably contributes to a sustainable management of agricultural areas and to landscape preservation (Brüne & Stumpf 2004,Fischer et al. 2007).Turkey has around 31 million sheep (FAO, 2017) and majority of them are still linked to seasonal transhumance production systems. Those sheep flocks have been adopted since millennia to the unique pastoral areas and landscapes with conservation value which prevail in the Eastern (sheep transhumance) and Mediterranean region (goat transhumance) of Turkey (Figure 2).

Figure 2. Karakaş breed in Erzurum mountain pastures.

There are scarce studies which have tackled transhumant sheep grazing patterns in relation with landscape characteristics in a given moment in these rangelands. There are different systems adapted to several regions, yet no studies have been conducted on their grazing patterns. This paper aimed to describe the nocturnal grazing behavior of sheep flocks' during summer period in the Eastern of Turkey. Assessing their grazing behavior within a specific landscape context is a key aspect to develop appropriate management systems which maximize animal performances while controlling environmental impact. The paper presents a preliminary case study on sheep grazing patterns in transhumance production system linked to specific land covers in Eastern Anatolia, Turkey.

MATERIALS AND METHODS

Study site

The study was conducted in Erzurum and Ağrı provinces in East Anatolia, Turkey (Fig. 3) during the year 2017.

The pastures in the mountain elevating from 1975-2062 m in Ağrı and 2503-2810 m in Erzurum. The climate of the whole area is continental with long winters and cold limiting dormant-season grazing to south-facing slopes. According to the meteorological station of Erzurum, the mean annual temperature was 16.6□C and the total regional annual precipitation ranged between 400 mm and 650 mm, and it intensifies from autumn through spring. Vegetation cover is dominated by cool-season grasses that are dormant during the summer, especially in August and early September. The mountain steppe has a dense coverage of short grasses, mainly sheep fescue and some legumes. The pasture in Agri Tutak Döşkaya pasture was composed of; Medicago spp., Onobrychis sativa, Dactylis glomerata, Koeleria cristata, Agropyron spp., Festuca ovina, Astragalus spp., Poa bulbosa, Bromus tectorum, Astragalus spp., Achillaa millefolium, Eryngium campestre, Centaurea kilaea, Saponaria officinalis and Anthemis. In Erzurum the botanical composition was; Ori ganum onites,Melitotus Officinalis, Onorbrychis sp., Poa Pratensis, Lotus corniculatus, Pona pila, Dactylis glomerata L. Astragalus membran aceus, Verbascum thapsus.

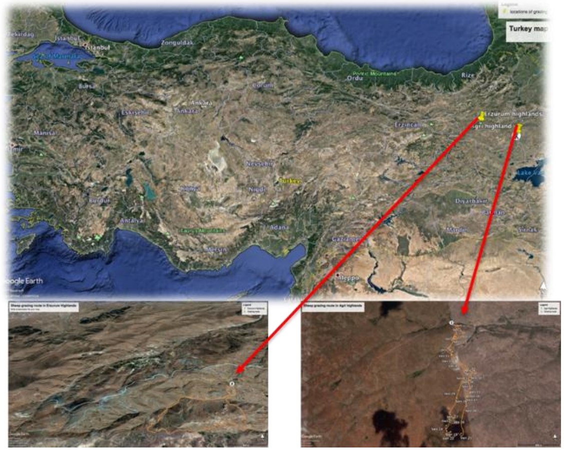

Figure 3. Location of study sites in Turkey.

GIS Route

The routes of the flock were recorded via Wikiloc®2017 and transferred to Google Earth. The land cover surrounding each pastureland (25 km radius) was analysed according to CORINE Land Cover classification (Büttner et al., 2012), and a vector based geographic information system, ArcGis 10.3 (ESRI, 1996).

Sampling and experimental analysis

While farmers were in their summer settlement areas, two transhumant flocks were followed on foot in order to record daily grazing routes and grazing patterns in August 2017. Karakaş (Fig. 4a) and Koçeri (Fig.4b) were the sheep breeds in Erzurum and Ağrı flocks respectively. Karakaş and Koçeri are indigenous multipurpose Turkish breeds with fat tail. Average flock size for both flocks were approximately 400 ewes. Grazing activities of min 20 sheep of each flock were randomly observed every 15 minutes by 2 trained observers equipped with hand chromometers and cameras and the number of animals which were grazing, walking, standing and resting was recorded in each time.

Figure 4. a) Karakaş sheep in Erzurum highlands. b) Koçeri sheep in Ağrı highlands.

Grazing in the eastern pastures are performed mainly at night due to belief that cooler temperatures are more suitable for grazing according to the local shepherds. Therefore observations were made from 19.00'to 8.15' due to the normal grazing practice. Grazing activities were recorded as grazing time (grazing or browsing while walking or standing), resting (simply sitting for rest), moving (simply walking, running) and standing (simply standing doing nothing). The flocks were followed during night until morning and shepherds were interviewed before and after the routes in order to gather information about their management strategies. Chi square test were used in order to test the dependency between the vaiables.

RESULTS AND DISCUSSION

The average distances covered by the herds during their daily routes were 6.3 and 8.4 km in Ağrı and Erzurum, respectively. Maximum and minimum altitudes were reached at 2062 m and 1975 m in Ağrı and, at 2801 m and 2503 m in Erzurum, respectively.

The flocks moved across a landscape dominated by natural grasslands (especially in Erzurum, Table 1) followed by sparsely vegetated areas in both areas. Nevertheless, the relevance of other land covers was variable in each case (Table 1). Regarding Ağrı path, the main land covers (agglutinating around 75% of all surfaces) natural grasslands, sparsely vegetated areas, arable land and salines. As for Erzurum path, landscape is dominated by natural grassland, sparsely vegetated areas and transitional woodland-scrubland. Forests were more abundant in the Erzurum surroundings (6%).

Land use devoted to agriculture and arable land cover a wider surface in Agri than in Erzurum mountains. These marginal territories offer limited options but enough for small ruminants which had lower nutritional requirements. An alternative production systems such as livestock grazing of bigger species like cattle or horses could be unfeasible (Rosa García et al., 2013).

Table 1. Percentage of main land-cover categories for both studied paths according to the CORINE land cover information.

CORINE Land Cover (25km radius)	**Agri**	**Erzurum**
Discontinuous urban fabric	0,15	0,16
Construction sites	0,00	0,01
Non-irrigated arable land	18,96	1,59
Permanently irrigated land	0,85	0,86
Complex cultivation patterns	2,36	3,98
Agriculture land with significant natural vegetation	9,69	7,77
Broad-leaved forest	0,00	1,76
Coniferous forest	0,00	3,52
Mixed forest	0,00	0,86
Sparsely vegetated areas	19,64	22,04
Transitional woodland-scrub	0,32	11,89
Pastures	1,12	0,00
Natural grassland	21,06	41,71
Beaches, dunes, sands	0,38	0,00
Bare rocks	3,80	3,51
Inland marshes	0,10	0,34
Salines	17,18	0,00
Water courses and bodies	4,39	0,00

Grazing was the main activity (55.4%) followed by moving (18.5%) during each observation in both study sites, while resting and standing were recorded only in 10-13.8% of the sheep in each occasion. Shepherds stopped twice to rest and most of the animals lie down during those two periods in both areas. The grazing activity intensified in three periods along the night in Erzurum although 5:45 a.m. onwards most of the animals focused on this activity. The moving activity increased in several periods during night and some of them with a decrease in grazing. The resting frequency fluctuated and increased in two occasions whereas it was a minority activity by the end of the routes (Table 2,3). The standing activity inconsistent with a little peak between 21:15 p.m. and 21:30 p.m. (Figure 5).

Grazing intensified during early day time in Ağrı (Table 3). Similarly, Loridas et al. 2011 found higher grazing time of sheep in the highlands. With regards to the night grazing or grazing in the cool hours, there is an agreement with Shinde et al. 1997 that ruminants tend to avoid grazing during the hot hours of the day and thus reducing their daily grazing time. Resting had two peaks between 20:45 p.m.-21:30 p.m. and 2:30 a.m. and 2:45 a.m. The standing activity was scarce with no remarkable peaks (Figure 6).

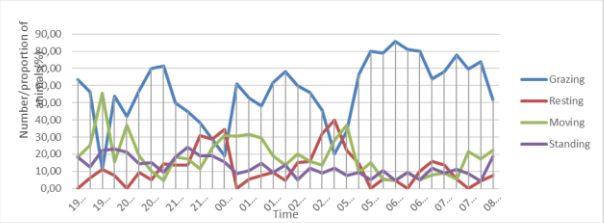

Figure 5. Daily variation of grazing patterns in Erzurum.

Table 2. Variation of grazing patterns in Erzurum highlands.

Pattern		Time		Total
	19:45-21:30	00:00-02:45	04:30-07:15	
Grazing (number of animals/%)	10 (47,6)	12 (52,2)	14 (66,7)	36 (55,4)
Resting (number of animals/%)	3 (14,3)	3 (13)	2 (9,5)	8 (12,3)
Moving (number of animals/%)	4 (19)	5 (21,7)	3 (14,3)	12 (18,5)
Standing (number of animals/%)	4 (19)	3 (13)	2 (9,5)	9 (13,8)
Total	21 (100)	23 (100)	21 (100)	65 (100)
χ^2: 2,004 P=0,919				

Table 3. Variation of grazing patterns in Ağrı highlands.

Pattern		Time		Total
	19:45 – 21:30	00:00 – 02:45	04:30 – 07:15	
Grazing (number of animals/%)	9 (52,9)	10 (52,6)	14 (60,9)	33 (55,9)
Resting (number of animals/%)	2 (11,8)	3 (15,8)	2 (8,7)	7 (11,9)
Moving (number of animals/%)	4 (23,5)	4 (21,1)	5 (21,7)	13 (22)
Standing (number of animals/%)	2 (11,8)	2 (10,5)	2 (8,7)	6 (10,2)
Total	17 (100)	19 (100)	23 (100)	59 (100)
χ^2: 0,726 P=0,994				

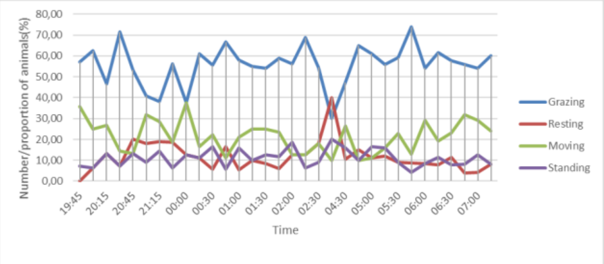

Figure 6. Daily variation of grazing patterns in Agri.

CONCLUSIONS

It is concluded that sheep spent more grazing time and less resting and standing during the whole grazing period. Standing was a residual activity in both study paths. Once animals were heading back to tents to be milked, their grazing activity increased. Further observations together with studies focused on diet selection and animal performances in other periods are needed to detect any seasonal/daily changes in grazing patterns. Those aspects will be useful to adjust the management techniques to a more-efficient use of the natural resources. A deeper analysis on the changes of the land cover along time will help to evaluate the potential of these transhumant activities in the future.

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ESTIMATION OF EGG YIELD BY NARUSHIN-TAKMA MODELS

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Abstract

The aim of this study is to estimate egg yield of laying hens with Narushin-Takma nonlinear model. Total of 1980 laying hens were used and 9900 egg numbers were collected through five weeks to predict egg yield of a commercial sire line. The hens were housed in individual cages and selected based on records made between 22 and 40 week of age as known as early part of record. Eggs were collected daily and individually.
Narushin-Takma nonlinear model equation is taken with five parameters, which of both have biological meanings. Levenberg-Marquardt estimation method was used with 1000 iterations. The coefficient of determination (R2) was detected as 0.786. In conclusion, Narushin-Takma nonlinear model can be applied to breeding programs for determining the egg yield from early part of record.

Keywords: *Narushin-Takma nonlinear model, laying hen, egg yield*

INTRODUCTION

The main objective of poultry breeding is to raise individuals with higher egg laying ability, lower body weight and sexual maturity at an early age. When choosing highly efficient individuals at the beginning of the breeding period, they elected indirectly reaching sexual maturity early (Tijan, 1982).
Partial recording is using as a selection criterion at the earlier period of life's of individuals beside reducing the generation interval, may help increase the annual progress (Mostageer et al., 1978). Foster (1981), implied that there are many advantages of selection based on partial records due to shortening of the generation interval and the appropriate time of caging, mating and hatching, selection, always occurs at the same time of year and less affected by environmental variation (Boukila et al., 1987).
Partial egg production can be considered as longitudinal data records and can be analyzed using linear throughout an animal's life by using linear (Al-Samarai et al., 2008) or nonlinear function during the life by using linear (Al-Samarai et al., 2008) or nonlinear functions (Fialho and Ledur, 1997; Grossman et al., 2000, Wolc et al., 2007) or multi-trait (Pires et al., 2002; Wolc et al., 2008) models (Venturini et al., 2012).

In this study, it is aimed to estimate the egg yield of laying hens using Narushin-Takma nonlinear model.

MATERIALS AND METHODS

The material of this study consisted of egg yield records from six generations of artificially inseminated commercial sire line (L1) were used as a data set. Total of 1955 layers from 43 sires and per sire 8 dams at four hatching periods were utilized in the analysis. The period of data collection was extended from the 22nd–40th week of age that known as early part of record. Eggs were collected daily and individually.
Narushin and Takma (2003) introduced 3 models for evaluating production and growth in laying hens. These models are named with Narushin-Takma NT1, NT2, and NT3, are rational polynomials consisting of 5, 6, and 7 parameters, respectively. In the study, NT1 NT2, and NT3 models were used for estimating of the egg yield. The used models are given below (Narushin and Takma, 2003):

$$y = \frac{at^3 + bt^2 + ct + d}{t + e}$$

$$y = \frac{at^3 + bt^2 + ct + d}{t^2 + et + f}$$

$$y = \frac{at^3 + bt^2 + ct + d}{t^3 + et^2 + ft + g}$$

where,

y is the output variable; t is age of the laying hen (week); and a, b, c, d, f, g, and h are coefficients of proportionality.

The NLIN procedure (Marquart algorithm) of BM SPSS v25 statistical programme.

RESULTS AND DISCUSSION

The parameter estimates with standard errors and their confidence interval values are given in Table 1.

Table 1. Model results

Parameter Estimates

Param.	Estimate	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
a	-161835714.19	3174976753917.41	-6223800320061.43	6223476648633.06
b	1534009280.02	30094987257804.12	-58991124961375.78	58994192979935.83
c	-4165683003.92	81724524860773.20	-160201841905653.72	160193510539645.90
d	3835083967.06	75238662647062.44	-147480153825029.44	147487823992963.56
f	19561766.58	383772877121.68	-752257961007.78	752297084540.94

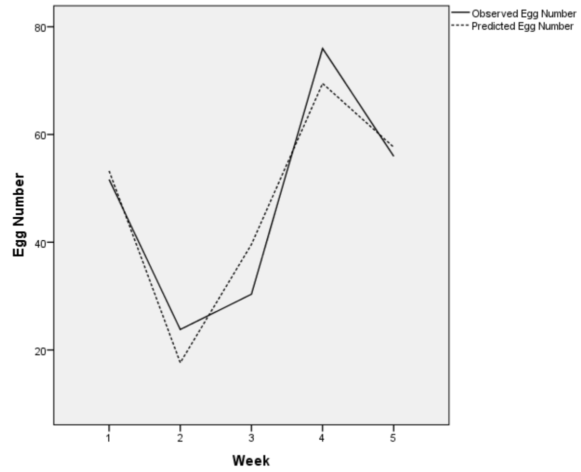

Figure 1. The predicted and observed egg curve

Generally, the predicted and observed egg number curve shows similar tendency. However, Narushin-Takma model underestimate egg number at second week and fourth weeks.

Determination coefficients (R^2) of the model was found as 0.79. The model can be used for the accurate the estimation of egg yield with a minimum mathematical calculation.

Several researchers were used Narushin-Takma model for estimating egg yield (Minvielle et al., 2000; Faridi et al., 2011; Narinç et al., 2014). They all found that Narushin-Takma models are mostly fit the curves, needs a minimum mathematical complexity.

CONCLUSIONS

Researchs with egg production modelling have been carried out for a long time. Nonlinear models are frequently used in egg yield estimation due to its biological structure. The model to be used should contain more biologically important parameters. For this reason, Narushin-Takma1 model, which is the simplest, is used. Because of the similar tendency with observed and predicted values this model can be applied to breeding programs for determining the egg yield from early part of record.

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AN REVIEW ON NATIONAL SHEEP AND GOAT BREEDING PROJECT OF TURKEY

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Abstract

Turkey has different small ruminant genetic resources and has a considerable potential in livestock production of Europe. Turkey has 35.2 million sheep and 10.9 million goats in 2018. Sheep and goat population of Turkey represents 28 % and 46 % of the European sheep and goat population, respectively. Sheep and goat breeding is very popular and different sheep and goat breeds are raised with different breeding system at different geographical conditions in Turkey. Some of the Turkish sheep breeds are Akkaraman, Morkaraman, Awassi, Karayaka, Kıvırcık and Chios; they constitute approximately 80% percentage of the sheep population in Turkey. Additionally, the most commonly raised local goat breeds are Hair, Kilis, Angora and Honamli; they constitute approximately 92% of the goat population in the Turkey. Sheep and goat productions are insufficient genetic improvement programs, traditional breeding methods, inadequate organizational structure and poor technical capacity of breeders. Therefore, Sheep and Goat Breeders Association in Turkey has been supported to develop better organizational structure for breeders. The purpose of this review is to analysis National Sheep and Goat Breeding Project of Turkey.

Key words: *sheep, goat, production, association, Turkey*

INTRODUCTION

Turkey located in the eastern Mediterranean has been bridge between European and Asian continents. Total land area of Turkey is over 785000 km^2 and its population is about seventy six million (TUIK, 2018). Turkey is mainly divided into seven regions named as Black sea, Marmara, Aegean, Central Anatolia, Mediterranean, Eastern Anatolia and Southeast Anatolia. Turkey has different animal domestic genetic resources and has a considerable potential in livestock production of Europe. Turkey is also one of the most important countries in the world in terms of animal genetic resources. Turkey has 17.1 million cattle, 178 thousand Anatolian buffalo, 35.2 million sheep and 10.9 million goats in 2018. Sheep population of Turkey represents 28 % of the European sheep population and goat population 46 % (TUIK, 2018; FAO, 2018).

Turkey has 11 sheep breeds named as Akkaraman, Morkaraman, Awassi, Hemşin, Cihos, Kivircık, Karayaka, Karagül, Herik, Dağlıç and Norduz. Akkaraman, Morkaraman, Awassi, which are classified as fat-tail breeds, and Karayaka, Kıvırcık, Chios, which are classified as thin-tail breeds, are commonly raised indigenous sheep breeds. Thus, they constitute approximately 80% percentage of the sheep population in Turkey. Additionally, Turkey has twelve native goat breeds named as Angora, Norduz, Honamlı, Hair, Kilis, Damascus, Gürcü, Ispir, Kaçkar, Abaza, Malta and Ak keçi. The most commonly raised local goat breeds in Turkey are Hair, Kilis, Angora and Honamli. Thus, they constitute approximately 92% of the goat population in the Turkey (Sen et al., 2011; Sirin et al., 2017; Aksoy et al., 2018; Sirin, 2018; Aksoy et al., 2019;

Although sheep and goat productions have many advantages in Turkey, the level of milk production for per head, growth and carcass yield of animals are very low and not enough for intensive production level (Sen et al., 2011; Yilmaz et al., 2012; Daskiran and Koluman, 2014; Sirin, 2018). Main reasons for low yield in sheep and goat productions are insufficient genetic improvement programs, traditional breeding methods, inadequate organizational structure and poor technical capacity of breeders (Daskiran et al., 2009; Daskiran et al., 2012; Daskiran and Ayhan, 2014). Sheep and Goat Breeders Association in Turkey has been supported to develop better organizational structure for breeders. Therefore, the purpose of this review is to analysis National Sheep and Goat Breeding Project of Turkey.

NATIONAL SHEEP AND GOAT BREEDING PROJECT (NSGBP)

Currently Sheep and Goat Breeders Association have approximately 250 thousand members and 35 million registered sheep and goats in eighty different provinces of Turkey (Anonymous, 2018). It is the biggest non-governmental association of Turkey. Because of low educational level, inconvenient socio-economic capacities and lack of organizational infrastructure of small ruminant breeders, these conditions do not allow them to be open to innovations in animal production. Above mentioned facts and issues seems a big barrier for Sheep and Goat Breeders Association and are waiting to solve.

To improve organizational infrastructure of Sheep and Goat Breeders Association, the government has taken some steps through increasing technical background of breeders and set up small ruminant registration system. Sheep and Goat Breeders Association had to needed to be supported by Ministry of Agriculture and Forestry. For this aim, Ministry of Agriculture and Forestry have started National Sheep and Goat Breeding Programme with collaboration to Sheep and Goat Breeders Association.

Ministry of Agriculture and Forestry has initiated National Sheep and Goat Breeding Programme in the field conditions to solve some of the main problems in the sheep and goat production sector. In the beginning of the project there were only two local breeds (Akkaraman sheep and Angora goat), which were selected as base animal material, within two provinces of Turkey. After National Sheep and Goat Breeding Programme is started, it is improved by being supported from different partners and other breeders organizations. The development of Sheep and Goat Breeders Association has been stimulated by the start of this project in two thousand six the involvement of researchers at the Universities and Ministry of Agriculture and Forestry Research Institutes (Daskiran et al., 2009; Daskiran et al., 2012; Daskiran and Ayhan, 2014).

PROJECT AIM OF NSGBP

The aim of national sheep and goat breeding project of Turkey is improvement of different product yields characteristics such as milk, meat, hair and prolificacy of national or local sheep and goats breed in the breeder conditions. While improving yield characteristics of small ruminants, infrastructure system of breeders organization, setting up animal registration systems and thereby increase the awareness of livestock breeders will be provided and local genetic resources will be protected (Daskiran et al., 2009; Daskiran et al., 2012; Daskiran and Ayhan, 2014).

The justification of national sheep and goat breeding project of Turkey is lack of small ruminant registration system, lack of sheep and goat breeders' organization system, low sheep and goat performance, requirement of high capacity ram and bucks and improvement socio-economic level of sheep and goat breeders (Daskiran et al., 2009; Daskiran et al., 2012; Daskiran and Ayhan, 2014).

PROJECT METHOD OF NSGBP

Principe of the programme is pure breeding and basic selection method. This method is summarized in Figure 1. Animal material of the project were divided into 3 main groups such as elite, semi elite and base flocks. Elite flock provides high quality rams and bucks for the semi elite flock. Animals in the elite flock are under full control. All the activities about mating are recorded by the breeders and technical staff of the project. The collected data are sent to the project leader for genetic evaluation to select the parents of next generation. Sheep and goat breeders as project partner have to allow ram and bucks to be transferred among flocks based on the protocol with the association (Daskiran et al., 2009; Daskiran et al., 2012; Daskiran and Ayhan, 2014).

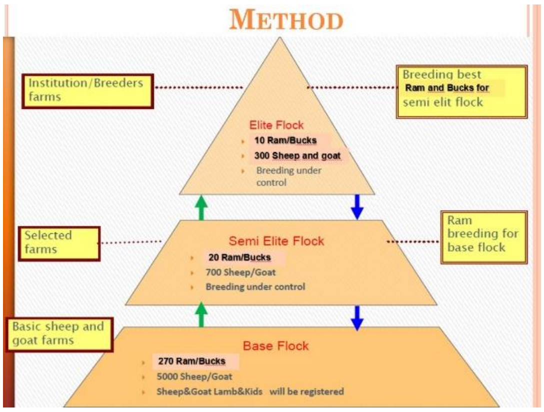

Figure 1. Pure breeding and basic selection method

Main activities of the project are based on the data collection from animals. Thus, all teams in the project focus on correct and regular data collection and analysis. A technical staff such as zootechnician, veterinary, animal or veterinary operator is hired for each project and his salary is paid from budget of the project. Ministry of Agriculture and Forestry provides financial support to breeder for each animal. Project leader works with project technical staff, breeder association and General Directorate of Agriculture Research and Policies, Livestock and Fisheries Department. Breeder associations have to provide logistic support to project leader, project technical staff and share all responsibilities. Project leader collects needed data for improving yield characteristics of animals and carries out the data analysis. General Directorate of Agriculture Research and Policies, Livestock and Fisheries Department holds on annual meeting for coordinating project. All project leaders attend to this meeting and they present own data, results, problems and solutions. Annual evaluation meeting provides good opportunities all researchers and project leaders for sharing their experiences. The plan of the next year project is also determined in this meeting. The final report of annual meeting is prepared by General Directorate of Agriculture Research and Policies, Livestock and Fisheries Department experts and sent to all project partners (Daskiran et al., 2009; Daskiran et al., 2012; Daskiran and Ayhan, 2014).

PROJECT PARTNERS OF NSGBP

For efficiency of the project and productivity of the project partners, network is very important. The all sector partners collaborated with in this project. Researchers from Universities and Institutes of Ministry of Agriculture and Forestry, Breeder Associations and sheep and goat breeders are gathering for the first time in this project scope. All of them try to develop breeding strategies, and improve small ruminant production. Actually, National Sheep and Goat Breeding Project collaborate with twenty-five universities all over Turkey and fourth project leaders working on the projects. Benefits of project leaders from the projects are to provide real situation for researches and to use the project budget for different scientific researches. The results from these researches are being used to improve animal materials of the projects. Except for using scientific material, they do not take any extra consultancy fee. Project leaders from Ministry of Agriculture and Forestry Research Institutes are also taking active role in the project. Coordination of the project is provided by General Directorate of Agriculture Research and Policies, Livestock and Fisheries Department of Ministry of Agriculture and Forestry. Most important partners of the project are sixty-four breeder associations. They have been responsible for all of implementation process of the project, collection of data, works of technical staff in the project and breeder training activities (Daskiran et al., 2009; Daskiran et al., 2012; Daskiran and Ayhan, 2014).

PERSPECTIVES OF NSGBP

The perspectives of national sheep and goat breeding project of Turkey are tracking and control system will be improved, livestock policies will be developed for increasing animal production, small ruminants registration systems and databases will be enhancement, animal health services will be furthered, livestock input costs will be decreased, marketing systems will be improved, new financing cost will be developed, sheep and goat organization infrastructure will be strength and effective training systems will be set up for sheep and goat breeders. General Directorate of Agriculture Research and Policies, Livestock and Fisheries Department is planning to continue the project between two thousand eighteen and two thousand eighteen twenty one and some of them will be transferred to sheep and goat associations. In this term Ministry of Agriculture and Forestry is developing new project vision and supporting systems based on regional or country prioritization. On the other hand within targeted time, animal registration system must be set up and started to apply selection (Daskiran et al., 2009; Daskiran et al., 2012; Daskiran and Ayhan, 2014).

CONCLUSIONS

Main goal of this project is to establish high quality breeding sheep and goat farms and develop high genetic capacity ram and bucks in Turkey. As seen from this goal, the first and most important rule is to set up and sustainable animal registering system. The second important aim is to support the animal breeder organizations and set up infrastructure organization. Project supports for breeders to take active role in breeding and improve awareness. During the implementation period of the project, the project technical staff gathers data and training breeders on animal feeding, housing, health and breeding methods. Project capacity items such as animal, project area, human resources is large and coordination is the most important instrument for reach the targeted aim.

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PATH ANALYSIS FOR PLACENTAL TRAITS ON LAMB BIRTH WEIGHT IN AKKARAMAN SHEEP

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Abstract

Path analysis is used to describe the directed dependencies among a set of variables and application of path analysis in animal breeding improve breeding practices. The aim of this study was to examine the direct and indirect effect of some placental traits on lamb birth weight born to Akkaraman sheep breed using path analysis. For this aim, relationship between lamb birth weight and seven traits (placental weight; PW, total cotyledon numbers; TCN, total cotyledon surface area; TCSA, cotyledon efficiency; CE, total cotyledon volume; TCV, volumetric cotyledon efficiency; VCE, plancenta efficiency; PE) were studied in 42 singleton - bearing sheep. Birth weight, lamb sex and placental measurements were recorded within 12 h after parturition. The direct effects of PW, PE on birth weight were found statistically significant (p<0.05). Additionally, the indirect effects of PW on birth weight were found statistically significant (p<0.05). While PE was found with highest direct effect (0.660) on BW, the highest total indirect effect (-0,493) on BW was found in PW variable. The direct effect (-0.079) of VCE was lowest on BW. The results showed that PW was the most explanatory variable for birth weight of Akkaraman lambs.

Key words: *path analysis, lamb birth weight, placental traits, direct effect, indirect effect, Akkaraman*

INTRODUCTION

Placental characteristics are important indicators of the postnatal mortality of offspring in small ruminants (Dwyer et al., 2005). Mellor and Stafford (2004) reported that the postnatal viability of newborns is associated with placental growth and development during gestation. The exchange capacity of ovine placenta between maternal and fetal systems depends on placental size and number of placentomes (Ocak et al., 2013; Sen and Onder, 2016). Therefore, placental size, which is related to the nutrient transfer capacity of the placenta, plays a pivotal role in determining the prenatal growth trajectory of the fetus and hence birth weight and postnatal viability (Sen et al., 2013). Placental growth and development support fetal development during mid- to late gestation (Redmer et al., 2004; Sen et al., 2013). Previous studies indicated that there are significant relationships between placental weight and birth weight of the newborn (Osgerby et al., 2003; Dwyer et al., 2005; Sen et al., 2013; Sen and Onder, 2016).

In general, the aim of animal breeding is to genetically improve populations of livestock so that they produce more efficiently under the expected future production circumstances (Önder and Abacı, 2015). Genetic improvement for economic traits is achieved by selecting the best individuals of the current generation and by using them as parents of the next generation (Dekkers et al., 2004). In many cases, the offspring with higher birth weight are selected as future breeding material or these criteria are used to valorize the animals. Placental traits have important effect on birth weight of the newborn (Osgerby et al., 2003; Dwyer et al., 2005; Sen et al., 2013; Sen and Onder, 2016). Generally, to evaluate relationship between placental characteristics and birth-related traits data relational statistics such as regression and correlation are used (Ocak et al., 2013; Sen et al., 2013; Sen and Onder, 2016). Birth weight of the newborn is selected as response variable and placental characteristics are selected as explanatory variables. Therefore, it is aimed to explain the response variable from explanatory variables. However, indirect effects of explanatory variables on response variable should be considered beside the direct effects (Arı and Önder, 2013). Path analysis is used to describe the directed dependencies among a set of variables (Önder and Abacı, 2015). Therefore, application of path analysis in animal breeding practices began to increase (Önder and Abacı, 2015). In addition, there are lack of studies that examined the direct and indirect effects of placental characteristics on birth weight of the newborn. The aim of the current study was therefore to investigate direct, indirect and total effects of placental characteristics on lamb birth weight in Akkaraman ewes.

MATERIALS AND METHODS

The study was conducted on 42 singleton-bearing Akkaraman ewe in a private dairy farm in Kirsehir, Turkey in the normal breeding season. All Akkaraman ewes were housed and cared for under the same conditions in the stockyard and were allowed to graze for 5 h daily during gestation. Birth weight (BW) and the sex of lambs were recorded within 12 h after parturition. Each Akkaraman ewe was left to deliver the placenta naturally and placentas were collected immediately after delivery. Placental weight (PW) was measured and recorded after removing placental fluid. The total cotyledon numbers (TCN) and total cotyledon weights (TCW) of placental cotyledons dissected from the chorioallantois were also counted and determined. Cotyledon length (CL), depth (CDe), and width (CWi) were measured with a digital compass and 30 cotyledons of the same size were selected (small, <10 mm diameter; medium, 10–30 mm diameter; large, >30 mm diameter). Placental efficiency (PE) was calculated for each Akkaraman ewe, as the ratio of lamb birth weight to placental weight (PW). Cotyledon density (CD) was calculated as the number of cotyledons per gram PW. Cotyledon efficiency (CE) was defined as the ratio of kid BW in grams to the total cotyledon surface area (TCSA). TCSA was calculated after the measurements of all the cotyledons in individual placenta as cm^2 with the following formula: radius squared of cotyledon $[((CWi + CL) / 4)^2] \times 3.14\ (\pi) \times TCN$. PE was calculated as the ratio of kid BW to PW for each Akkaraman ewe.

SPSS (2004) statistical software was used to analyze the data with the license of Ondokuz Mayıs University. Every linear model has a direct effect and amount of indirect effect which is number of explanatory variables minus one. The general expression of multiple regression model formed for the measurements (one response and p explanatory variables) is given in Equation 1.

$$y_k = \beta_0 x_{k_1}^{\beta_1} x_{k_2}^{\beta_2} x_{k_3}^{\beta_3} \ldots x_{k_p}^{\beta_p} e_i; \quad i = 1,2,\ldots,n \qquad (1)$$

The multiple linear regression model adopted was

$$\hat{y}_k = b_0 + b_1 x_{k1} + b_2 x_{k2} + b_3 x_{k3} \qquad (2)$$

where:

$\hat{y}_k$ = response variable (BW),

b_0 = intercept,

b_i = standardized regression coefficients,

x_{kp} = explanatory variables (CG, BL, HS)

A path coefficient (P) is a standardized regression coefficient (b) showing the direct effect of an independent variable on a dependent variable in the path model (Garson, 2008; Önder and Abacı, 2015). Path coefficient, which indicates the effect of one standard deviation change of any explanatory variable X versus on response variable Y, can be calculated as (Mendes et al., 2005).

$$Pyx_k = b\frac{Sx_k}{S_y} \qquad (3)$$

Here; Pyx is the path coefficient which indicates the direct effect of X explanatory variable on response variable Y,Sx indicates the standard deviation of X, Sy indicates the standard deviation of Y and b indicates the partial regression coefficient. Path coefficients can be shown with path diagrams. One way and two way arrows are used in path diagrams. One way arrows which named as direct effects are drawn from explanatory variable to response variable and two way arrows which showed correlations are drawn between explanatory variables (Tahtali et al., 2011).

To obtain the path coefficients should be replaced in linear equation system as given in Equation 4.

$$\begin{bmatrix} P_{YX_1} \\ P_{YX_2} \\ P_{YX_3} \end{bmatrix} = \begin{bmatrix} 1 & r_{X_1X_2} & r_{X_1X_3} \\ r_{X_2X_1} & 1 & r_{X_2X_3} \\ r_{X_3X_1} & r_{X_3X_2} & 1 \end{bmatrix}^{-1} * \begin{bmatrix} r_{YX_1} \\ r_{YX_2} \\ r_{YX_3} \end{bmatrix} \qquad (4)$$

In the Equation 4, coefficients given by P_{YXi} were path coefficients (direct effects) between explanatory variable and response variable and $r_{xixj}P_{YXi}$ represented indirect effects of explanatory variable ith on response variable via explanatory variable jth, r_{xixj} represented pearson correlation coefficients between ith an jth traits (Topal et al., 2008)..

RESULTS AND DISCUSSION

Direct and indirect effects of explanatory variables on birth weight in Akkaraman ewes are present Table 1. The direct effects of PW, PE on birth weight were found statistically significant ($p<0.05$). Additionally, direct effects of TCN, TCSA, CE, TCV, VCE and PE on birth weight were not found statistically significant. The indirect effects of PW on birth weight were found statistically significant ($p<0.05$). Moreover, direct effects of TCN, TCSA, CE, TCV, VCE and PE on birth weight were not found statistically significant in the present study. While PE was found with highest direct effect (0.660) on BW, the highest total indirect effect (-0,493) on BW was found PW variable. The direct effect (-0.079) of VCE was lowest on BW.

Table 1. Direct and indirect effects of explanatory variables on birth weight in Akkaraman ewes.

Trait	Direct effects	Indirect effect
PW	0.532*	-0.493*
TCN	-0.116	-0.088
TCSA	0.046	0.018
CE	0.027	0.229
TCV	-0.040	0.134
VCE	-0.079	0.266
PE	0.660*	-0.184

PW= placental weight, TCN= total cotyledon numbers, TCSA= total cotyledon surface area, CE= cotyledon efficiency, TCV= total cotyledon volume, VCE= volumetric cotyledon efficiency, PE= plancenta efficiency. * $p<0.05$

Placental traits, such as placental weight, cotyledon numbers, cotyledon efficiency, plancenta efficiency etc., are important indicators of the postnatal survive of lambs in sheep (Dwyer et al., 2005; Sen et al., 2013). Therefore, some factors affecting lambs birth weight should be determined. Owing to this, the path analysis is very important for determining factors affecting lamb birth weight (Önder and Abacı, 2015). In this study, which aimed to investigate the direct, indirect and total effects of placental characteristics on birth weight in Akkaraman lambs. Mostly, evaluation of relationship between placental characteristics and birth-related traits data relational statistics such as regression and correlation are used (Ocak et al., 2013; Sen et al., 2013; Sen and Onder, 2016). Birth weight of the lambs were selected as response variable and placental characteristics were selected as explanatory variables in the present study. Therefore, path analysis was used to describe the directed dependencies among a set of variables in the present study. Onder et al. (2017) direct and total effects on lamb birth weight were supported by studies in Akkaraman lambs. Some direct and total effects of placental traits on lamb birth weight were similar with results of Onder et al. (2017).

CONCLUSIONS

As a result, it was concluded that PW could be used for management decisions and as indirect selection criteria for selection on lamb birth weight due to PW had the highest direct and the lowest indirect effect lamb birth weight.

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ESTABLISHMENT OF EFFECTIVE PLACENTAL CHARACTERISTICS ON BIRTH WEIGHT IN KARAYAKA SHEEP BREED WITH PATH ANALYSIS

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Abstract

Path analysis is used to describe the directed dependencies among a set of variables and application of path analysis in animal breeding improve breeding practices. The aim of this study was to examine the direct and indirect effect of some placental traits on lamb birth weight born to Karayaka sheep breed using path analysis. For this aim, relationship between lamb birth weight and seven traits (placental weight; PW, total cotyledon numbers; TCN, total cotyledon surface area; TCSA, cotyledon efficiency; CE, total cotyledon volume; TCV, volumetric cotyledon efficiency; VCE, plancenta efficiency; PE) were studied in 26 singleton - bearing sheep. Birth weight, lamb sex and placental measurements were recorded within 12 h after parturition. The direct effects of CE on birth weight were found statistically significant (p<0.05). Additionally, the indirect effects of VCE on birth weight were found statistically significant (p<0.05). While CE was found with highest direct effect (0.489) on BW, the highest total indirect effect (0,437) on BW was found in VCE variable. The direct effect (-0.026) of TCV was lowest on BW. Similarly, the indirect effect (-0.150) of TCV was lowest on BW. The results showed that CE and VCE were the most explanatory variable for birth weight of Bafra lambs.

Key words: *path analysis, lamb birth weight, placental traits, direct effect, indirect effect, Karayaka*

INTRODUCTION

Placental characteristics are important indicators of the postnatal mortality of offspring in small ruminants (Dwyer et al., 2005). Mellor and Stafford (2004) reported that the postnatal viability of newborns is associated with placental growth and development during gestation. The exchange capacity of ovine placenta between maternal and fetal systems depends on placental size and number of placentomes (Ocak et al., 2013; Sen and Onder, 2016). Therefore, placental size, which is related to the nutrient transfer capacity of the placenta, plays a pivotal role in determining the prenatal growth trajectory of the fetus and hence birth weight and postnatal viability (Sen et al., 2013). Placental growth and development support fetal development during mid- to late gestation (Redmer et al., 2004; Sen et al., 2013). Previous studies indicated that there are significant relationships between placental weight and birth weight of the newborn (Osgerby et al., 2003; Dwyer et al., 2005; Sen et al., 2013; Sen and Onder, 2016).

In general, the aim of animal breeding is to genetically improve populations of livestock so that they produce more efficiently under the expected future production circumstances (Önder and Abacı, 2015). Genetic improvement for economic traits is achieved by selecting the best individuals of the current generation and by using them as parents of the next generation (Dekkers et al., 2004). In many cases, the offspring with higher birth weight are selected as future breeding material or these criteria are used to valorize the animals. Placental traits have important effect on birth weight of the newborn (Osgerby et al., 2003; Dwyer et al., 2005; Sen et al., 2013; Sen and Onder, 2016). Generally, to evaluate relationship between placental characteristics and birth-related traits data relational statistics such as regression and correlation are used (Ocak et al., 2013; Sen et al., 2013; Sen and Onder, 2016). Birth weight of the newborn is selected as response variable and placental characteristics are selected as explanatory variables. Therefore, it is aimed to explain the response variable from explanatory variables. However, indirect effects of explanatory variables on response variable should be considered beside the direct effects (Arı and Önder, 2013). Path analysis is used to describe the directed dependencies among a set of variables (Önder and Abacı, 2015). Therefore, application of path analysis in animal breeding practices began to increase (Önder and Abacı, 2015). In addition, there is lack of studies that examined the direct and indirect effects of placental characteristics on birth weight of the newborn. The aim of the current study was therefore to investigate direct, indirect and total effects of placental characteristics on lamb birth weight in Karayaka ewes.

MATERIALS AND METHODS

The study was conducted on 26 singleton-bearing Karayaka ewes in a private dairy farm in Tokat, Turkey in the normal breeding season. All Karayaka ewes were housed and cared for under the same conditions in the stockyard and were allowed to graze for 5 h daily during gestation. Birth weight (BW) and the sex of lambs were recorded within 12 h after parturition. Each Karayaka ewe was left to deliver the placenta naturally and placentas were collected immediately after delivery. Placental weight (PW) was measured and recorded after removing placental fluid. The total cotyledon numbers (TCN) and total cotyledon weights (TCW) of placental cotyledons dissected from the chorioallantois were also counted and determined. Cotyledon length (CL), depth (CDe), and width (CWi) were measured with a digital compass and 30 cotyledons of the same size were selected (small, <10 mm diameter; medium, 10–30 mm diameter; large, >30 mm diameter). Placental efficiency (PE) was calculated for each Karayaka ewe, as the ratio of lamb birth weight to placental weight (PW). Cotyledon density (CD) was calculated as the number of cotyledons per gram PW. Cotyledon efficiency (CE) was defined as the ratio of kid BW in grams to the total cotyledon surface area (TCSA). TCSA was calculated after the measurements of all the cotyledons in individual placenta as cm^2 with the following formula: radius squared of cotyledon $[((CWi + CL) / 4)^2] \times 3.14\ (\pi) \times TCN$. PE was calculated as the ratio of kid BW to PW for each Karayaka ewe.

SPSS (2004) statistical software was used to analyze the data with the license of Ondokuz Mayıs University. Every linear model has a direct effect and amount of indirect effect which is number of explanatory variables minus one. The general expression of multiple regression model formed for the measurements (one response and p explanatory variables) is given in Equation 1.

$$y_k = \beta_0 x_{k_1}^{\beta_1} x_{k_2}^{\beta_2} x_{k_3}^{\beta_3} \dots x_{k_p}^{\beta_p} e_i ; \quad i = 1,2,\dots,n \qquad (1)$$

The multiple linear regression model adopted was

$$\hat{y}_k = b_0 + b_1 x_{k1} + b_2 x_{k2} + b_3 x_{k3} \qquad (2)$$

where:

$\hat{y}_k$ = response variable (BW),

b_0 = intercept,

b_i = standardized regression coefficients,

x_{kp} = explanatory variables (CG, BL, HS)

A path coefficient (P) is a standardized regression coefficient (b) showing the direct effect of an independent variable on a dependent variable in the path model (Garson, 2008; Önder and Abacı, 2015). Path coefficient, which indicates the effect of one standard deviation change of any explanatory variable X versus on response variable Y, can be calculated as (Mendes et al., 2005).

$$Pyx_k = b \frac{Sx_k}{S_y} \qquad (3)$$

Here; Pyx is the path coefficient which indicates the direct effect of X explanatory variable on response variable Y,Sx indicates the standard deviation of X, Sy indicates the standard deviation of Y and b indicates the partial regression coefficient. Path coefficients can be shown with path diagrams. One way and two way arrows are used in path diagrams. One way arrows which named as direct effects are drawn from explanatory variable to response variable and two way arrows which showed correlations are drawn between explanatory variables (Tahtali et al., 2011).

To obtain the path coefficients should be replaced in linear equation system as given in Equation 4.

$$\begin{bmatrix} P_{YX_1} \\ P_{YX_2} \\ P_{YX_3} \end{bmatrix} = \begin{bmatrix} 1 & r_{X_1X_2} & r_{X_1X_3} \\ r_{X_2X_1} & 1 & r_{X_2X_3} \\ r_{X_3X_1} & r_{X_3X_2} & 1 \end{bmatrix}^{-1} * \begin{bmatrix} r_{YX_1} \\ r_{YX_2} \\ r_{YX_3} \end{bmatrix} \qquad (4)$$

In the Equation 4, coefficients given by P_{YXi} were path coefficients (direct effects) between explanatory variable and response variable and $r_{xixj}P_{YXi}$ represented indirect effects of explanatory variable ith on response variable via explanatory variable jth, r_{xixj} represented pearson correlation coefficients between ith an jth traits (Topal et al., 2008)..

RESULTS AND DISCUSSION

Direct and indirect effects of explanatory variables on birth weight in Karayaka ewes are present Table 1. The direct effects of CE on birth weight were found statistically significant (p<0.05). Additionally, direct effects of PW, TCN, TCSA, TCV, VCE and PE on birth weight were not found statistically significant. The indirect effects of VCE on birth weight were found statistically significant (p<0.05). Moreover, direct effects of PW, TCN, TCSA, CE, TCV and PE on birth weight were not found statistically significant in the present study. While CE was found with highest direct effect (0.489) on BW, the highest total indirect effect (0,437) on BW was found PW variable. The direct effect (-0.026) of TCV was lowest on BW. Similarly, the indirect effect (-0.150) of TCV was lowest on BW.

Table 1. Direct and indirect effects of explanatory variables on birth weight in Karayaka ewes.

Trait	Direct effects	Indirect effect
PW	0.159	0.372
TCN	-0.079	0.189
TCSA	0.390	-0.810
CE	0.489*	0.290
TCV	-0.026	-0.150
VCE	0.147	0.437*
PE	-0.043	0.104

PW= placental weight, TCN= total cotyledon numbers, TCSA= total cotyledon surface area, CE= cotyledon efficiency, TCV= total cotyledon volume, VCE= volumetric cotyledon efficiency, PE= plancenta efficiency. * p<0.05

Placental traits, such as placental weight, cotyledon numbers, cotyledon efficiency, plancenta efficiency etc., are important indicators of the postnatal survive of lambs in sheep (Dwyer et al., 2005; Sen et al., 2013). Therefore, some factors affecting lambs birth weight should be determined. Owing to this, the path analysis is very important for determining factors affecting lamb birth weight (Önder and Abacı, 2015). In this study, which aimed to investigate the direct, indirect and total effects of placental characteristics on birth weight in Karayaka lambs. Mostly, evaluation of relationship between placental characteristics and birth-related traits data relational statistics such as regression and correlation are used (Ocak et al., 2013; Sen et al., 2013; Sen and Onder, 2016). Birth weight of the lambs were selected as response variable and placental characteristics were selected as explanatory variables in the present study. Therefore, path analysis was used to describe the directed dependencies among a set of variables in the present study. Onder et al. (2017) direct and total effects on lamb birth weight were supported by studies in Akkaraman lambs. Some direct and total effects of placental traits on lamb birth weight were similar with results of Onder et al. (2017).

CONCLUSIONS

As a result, it was concluded that CE and VCE could be used for management decisions and as indirect selection criteria for selection on lamb birth weight due to CE and VCE had the highest direct and TCV had the lowest direct and indirect effect lamb birth weight in Karayaka sheep breed.

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COMPARISON OF DIFFERENT LACTATION MODELS FOR TEST DAY MILK YIELDS OF HOLSTEIN FRIESIAN COWS

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Abstract

In this study, total 161 test day milk yields of Holstein Friesian cows were used. For this purpose, three different lactation curve models (Wood, Wilmink and Cobby and Le Du) were fitted to test day milk yields. For the comparison of the models, the corrected coefficient of determination ($\bar{R}^2$), root mean square error (RMSE), mean absolute deviation (MAD) and mean absolute percentage (MAPE) were used. The corrected determinations of coefficients for the three models were 0.828, 0.77 and 0.829, respectively. The MAD values for found Wood, Wilmink and Cobby lactation curve models were 129.7911, 154.34 and 128.933, respectively; MAPE values were obtained as 9.85, 11.54 and 9.64; The RMSE values were found 1.69, 1.94 and 1.70, respectively. When the models were compared in terms of these criterion considered, it was observed that the Cobby Le Du model was more compatible with the other models.

Keywords: *Holstein Friesian, Lactation curve, Wood, Wilmink, Cobby and Le Du*

INTRODUCTION

According to International Farm Comparison Network data, world milk production is estimated to be 867 million tons in 2017. Cows and buffalo milk account for 96% (833 million tons) of this amount. As can be seen, the share of cattle in milk production is large.

The changes in milk yield, which are shaped by the effects of genetic and environmental factors, are called lactation curves in cows. By mathematical modeling of milk yield over time, lactation curves are estimated and thus information about the milk yield of the cow is obtained according to the changes in milk yield during lactation. In fact, the low slope of the lactation curve shows that the cow's milk yield continuity is good. Accordingly, it is preferred that milk yield does not change much during lactation (Wood, 1967; Batra, 1986).

On the other hand, knowing the structure of the lactation curve of cows in a herd is beneficial in many ways. Curves of cows of different breeds and different age groups also differ. It would be appropriate to use the group with more variation as breeding material. In addition, it can be used in the early stages of lactation and the planning of optimum feeding programs for low milk yield cows.

The lactation curve can also be used to estimate the total lactation yield from lactation records (Soysal et al., 2005). Several models are used to estimate the lactation curve (Goel and Tomar, 1985).

In this study, it is aimed to determine the best mathematical model that can explain the lactation curve by comparing the models that define the lactation curves in Holstein Friesians.

MATERIALS AND METHODS

The material of this study consisted of milk yields of 161 Holstein Friesian cows, which were reared in Tahirova farm, which is connected to The General Directorate of Agricultural Enterprises in Turkey, on the first lactation test day records.

Levenberg-Marquardt iterative procedure was used for the parameter estimations for the milk yields of Holstein Friesian cows. Starting values of parameters were taken from previous analyses. 1.0E-8 was used as convergence criterion.

The coefficient of determination, Root Mean Square Error (RMSE), Mean Absolute Deviation (MAD) and Mean Absolute Percentage Error (MAPE) were used to determine the best fit model.

The following three lactation curve models with their functions were shown in Table 1.

Table 1. The lactation curve models and functions

Model	**Functions**
Wood	$at^{b}e^{-ct}$
Wilmink	$a + be^{-kt} + ct$
Cobby and Le Du	$a-bt-a e^{-ct}$

Where, Y is test day milk yield (kg), t is the days in milk and a, b, c and d are the coefficients that define the shape of the lactation curve. Depending on the characteristics of the model, a parameter is the initial milk yield, b parameter is the pre-peak increase rate, c parameter is the post-peak decrease rate and k parameter is the coefficient associated with the peak time (Macciotta et al., 2005).

According to these criteria high $\bar{R}^2$ and low RMSE, MAD and MAPE values determine the model that fits well.

These equations and the terms in the equations are given below.

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}(\hat{y}_i - y_i)^2}{n}}$$

$$MAD = \frac{\sum_{i=1}^{n}|y_i - \hat{y}_i|}{n}$$

$$MAPE = \frac{\sum_{i=1}^{n}\left|\frac{y_i - \hat{y}_i}{y_i}\right|}{n} x100, (y_i \neq 0)$$

$$\bar{R}^2 = 1 - (1 - R^2)\frac{(n-1)}{(n-p-1)}$$

Here, n: Number of records, y_i: Observed value, $\hat{y}$: Estimated value, p: Numbers of independent variables.

All statistical analyses were done with IBM SPSS v25 statistical programme.

RESULTS AND DISCUSSION

In this study, three different lactation curve models were used. The parameter estimates (a, b and c) and their standard errors of model were given in Table 2. The parameters estimated as 21.72, 0.38 and 0.18 for Wood, 21.72, 0.38 and 0.18 for Wilmink, 23.92, 1.59, and 1.74 for Cooby Le Du, respectively.

Table 2. Parameter estimates (a, b and c) and standard errors for different lactation curves

Param.	Estimate	Error	95% Conf. Interval	
			Lower	Upper
		Wood		
a	21.72	0.14	21.47	21.96
b	0.38	0.013	0.36	0.41
c	0.18	0.004	0.18	0.19
		Wilmink		
a	565.6	3032.6	-5382.9	6514.2
b	-546.5	3032.8	-6495.5	5402.5
c	11.7	34.08	-55.2	78.5
k	-0.02	0.06	-0.13	0.09
		Cooby Le Du		
a	23.92	0.14	23.64	24.20
b	1.59	0.02	1.54	1.63
c	1.74	0.04	1.65	1.82

Mean Absolute Percentage Error (MAPE), Mean Absolute Deviation (MAD), Root Mean Square Error (RMSE), and the corrected determination of coefficient were calculated and given in Table 3. The MAPE values were for found Wood, Wilmink and Cobby lactation curve models were 9.85, 35.94 and 9.64, respectively; MAD values were obtained as 129.79, 458.52 and 128.33; The RMSE values were found 1.69, 32.65 and 1.68. The corrected coefficients of determination were estimated 0.828, 0.828 and 0.829, respectively.

Table 3. MAPE, MAD, RMSE and $\bar{R}^2$ values for the models.

	Wood	Wilmink	Cobby Le Du
MAPE	9,85	35,94	9,64
MAD	129,79	458,52	128,33
RMSE	1,69	32,65	1,68
$\bar{R}^2$	0,828	0,828	0,829

Whereas Soysal et al. (2016) were found higher corrected determination of coefficients, on the other hand Olori et al. (1999) and Osman and Ertuğrul (1999) were reported lower values. Moreover, some researchers found similar results with our study (Sosyal et al., 2005; Koncagül and Yazgan, 2011; Soysal et al., 2016). They found the Wood model as the best-fitted model.

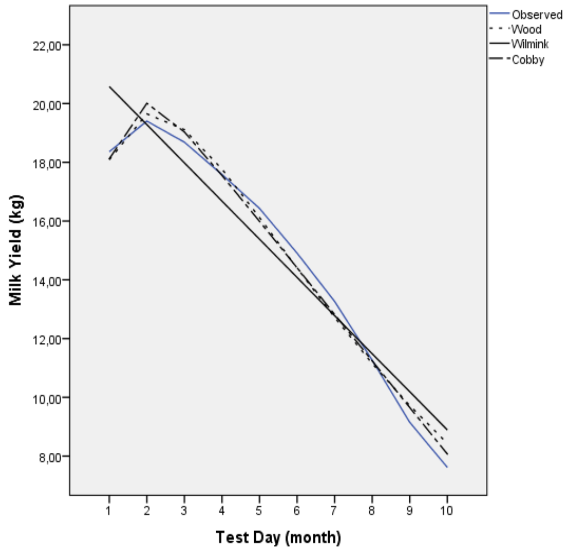

Figure 1. Observed and estimated milk yields of Holstein Cows

Observed and predicted lactation curves obtained from Wood and Cobby Le Du models were generally similar (Figure 1). However, the Wood model overestimate milk yield at the end of lactation. Wilmink model gave overestimates at the beginning and end of lactation.

CONCLUSIONS

Successful herd management and accurate in dairy cattle in order to make selection decisions, the shape of the lactation curve, the selection of the appropriate model and the correct estimation of the model parameters are important. It is found that the Cobby Le Du model was the best fitted model among the models.

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COMPARISON OF SOME STATISTICAL PACKAGE PROGRAMS

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Abstract

In scientific research studies, the analysis of the data collected during the research takes an important place in order to reach a hypothesis or solution. Data sets of data obtained from research are often too large to be manually analyzed or long enough to be manually analyzed. Nowadays, there are many statistical analysis package programs used in data analysis that will eliminate this problem of researchers. Researchers carry out the analysis of their data by using statistical analysis package programs that are appropriate to the data set to be analyzed or that contain the analysis methods they want to do. The vast majority of statistical analysis package programs do not include all analysis methods or to a limited extent. In addition, the algorithms used by packet programs in calculations may vary. These differences in algorithms may affect the result of the analysis to a certain extent. In this study, in order to give researchers a different perspective about statistical analysis programs, the most used paid and unpaid statistical analysis programs were selected and the same analyzes were performed on the same data sets and the results were compared.

Key words: *statistic, program.*

TRANSFORMATION TO SOME GROWTH MODELS WIDELY USED

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Abstract

A mathematical model is a tool used to obtain information about the behavior of a system. The mathematical models can be used to have a preliminary knowledge about the functioning of a system, reducing the product costs and improving the performance. The aim of this study is related to how some of the sigmoidal models widely used in agriculture are converted to mechanistic models which have biological meaning. For this purpose, the commonly used ten different models with sigmoidal structure were used.

Key words: *Transformation, Model.*

DETERMINATION OF SOME EFFECTIVE FACTORS ON EGG BREAKING RESISTANCE BY REGRESSION TREE ANALYSIS

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Abstract

The egg is a nutrient with high biological value, and almost all of it is used by the body as a protein source. Consuming a high-quality egg is important for consumers. But the eggshell is susceptible to abrupt fractures due to its precision. Egg quality is reduced due to the broken eggshell and cannot be consumed. Therefore, it is important to determine the factors affecting the fracture resistance of the egg. This study investigated some traits on fracture resistance of the egg using CRT algorithm of regression tree analysis. For this aim, used data were egg fracture resistance, egg weight, hen age (25-72 weeks), egg width, egg length, egg whites height, egg whites length, egg flux width, egg yolk height, egg yolk diameter measurements are taken from 613 pieces of organically grown Lohmann Brown hen eggs. As a result of regression tree analysis, it was determined that egg shell thickness was effective in egg breaking. Chicken age was found to be effective in second place. Crust resistance was found to be 2.602 kg / cm² for the eggs with a thickness of less than 0.355 mm or equal, and as 3.475 kg / cm² for those larger than 0.355 mm. In the eggs with a shell thickness greater than 0.355 mm, the fracture resistance was found to be 3.105 kg / cm² after 47.5 weeks, while it was determined to be 3.797 kg / cm² at 47.5 weeks and below. At the same time, the eggs obtained from chickens under the age of 47.5 weeks have a break resistance of 4.107 kg / cm² in the yellow diameter of 40.850 mm and below.

Key words: *Protein, Egg, Layer Hen, CRT, Breaking resistance.*

USE OF MARS DATA MINING ALGORITHM FOR EGG WEIGHT ESTIMATION

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Abstract

*In this study, goodness of fit criteria were determined by using multivariate adaptive regression spline (MARS) data mining algorithm in determining egg weight. For this purpose, the eggs of the Lohmann LSL Classic white hybrid drive (n = 31831) were used. In the study, weekly egg yields were evaluated from 22 weeks to 62 weeks. In order to estimate the egg weight, which is determined as dependent and continuous variable, independent variables such as shape index, refractive index, container weight, container thickness, diameter-height-weight of egg yolk, width-height-height of egg whites, and egg yolk color were used. Pearson correlation coefficient values , Akaike information criterion, corrected Akaike information criterion, square root of error squares mean, Mean error, Mean absolute deviation, standard deviation rate, Global relative approximate error, coefficient of variation (VK%) and Mean absolute percentage error, statistical values of goodness of fit were estimated. As a result, in order to prevent excessive goodness of fit, in the "earth" package of the R program, the definitions of penalty = -1, degree = 2, nfold = 10 and nk = 60 were made and one of the calculated values, pearson correlation coefficient (r) 0.82 was found. As a result of the research, "Egg weight = 61.4-1.17 * h (75-Shape index) + 1.05 * (group a2 * h (75- Shape index)) -0.45 * h (13-color) 0.45 + 13.45 * h (White high) -7.4) - 0.18 * (Shape index * h (White high-7.4)) - 5.27 * (group a2 * h (7.4- White high)) - 0.26 * (group a3 * h (Shape index-75)) " obtained equality was determined as a mars estimation model.*

Key words: *MARS, data mining, egg weight*

MULTIVARIATE FACTOR ANALYSIS OF ANATOLIAN BUFFALOES MILK COMPOSITION

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Abstract

The aim of this study was to explain the structure of relationships between milk components and Somatic cell counts of Anatolian Buffaloes. The explication of the several traits that contribute to defining milk quality is difficult due to the high degree of correlation among them. In this case, one of the best methods of statistical processing is factor analysis, which belongs to the multivariate groups; for present study this particular statistical approach was employed. The analysis grouped the milk components into three latent or common factors. In the present research some milk components and somatic cell count of 288 Anatolian buffaloes were used. Results of the present study showed that the averages of solid non fat, fat, protein, lactose, casein, acidity, freezing point, free fat acids and somatic cell count in Anatolian Buffaloes raw milk samples were 10.92±0.003%, 5.97±0.010%, 4.89±0.004%, 5.18±0,002%, 3.60±0.003%, 8.13±0.014(oSH), 0.54±0.001(0C), 0.53±0.0.23(mmol/10 L) and 139 774±1.41 cells/ml respectively. Analysis shoved that the total variation were explained in three groups. The first, second and third foctors were 51.2%, 36.2% and 12.6% respectively.

Key words: *Factor analysis, Buffaloes, milk composition, somatic cell score.*

DETERMINATION THE EFFECT OF BREEDING ON HYGIENIC BEHAVIOR IN HONEY BEES BY PROBIT REGRESSION

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Abstract

Honey bees are exposed to many damaging pathogens and parasites. Hygiene is a behavioral trait of honey bees (Apis mellifera) conferring economically useful and heritable resistance to damaging pathogens and parasites. For selection on hygienic behavior ≥95% cleaning success is expected to get the colony as breeding material. In this study, it was aimed to determine the effect of selection on hygienic behavior in honey bees by using Probit regression analysis. Time (hour) was used as explanatory variable and cleaning success was used as response variable. Two generations were evaluated for this purpose. Results showed that the optimal solutions have been found for both generations for Probit regression with less than 10 iterations and regression models were statistically significant (P<0.001). At first generation 50%, 95% and 99% cleaning success has been reached at 5.36, 45.59 and 62.25 hours, respectively. At the second generation 50%, 95% and 99% cleaning success has been reached at 10.29, 43.51 and 57.28 hours, respectively. Results indicated that selection has positively affected the cleaning success about 5% and 8% for 95% and 99% cleaning success. This results also showed the Probit regression can be used for studies on efficiency of hygienic behavior.

Key words: *hygienic behavior, honey bee, probit, cleaning success*

INTRODUCTION

Honey bees are exposed to many damaging pathogens and parasites. Hygiene is a behavioral trait of honey bees (*Apis mellifera*) conferring economically useful and heritable resistance to damaging pathogens and parasites. Hygienic bees are able to detect, uncap and remove diseased brood. The removal of freeze-killed brood by colonies is the general test of hygiene, related relatively well with removal of Varroa-infested brood (Rinderer et al., 2010). Hygienic behavior is probably the most successful achievement in breeding bees because of controlled by two recessive genes. It's been very well studied and proven to be effective against chalkbrood, American foulbrood, and Varroa. Hygienic behavior is described as the detection and removal of unhealthy or dead brood by workers. Early research on this behavior was limited to its impact on the transmission of the bacterial brood disease American foulbrood. However, subsequent studies demonstrated that this behavior also limits the growth of fungal brood diseases, and even, to some extent, invertebrate parasite populations (Leclercq et al., 2017). For a recessive trait to be expressed, a worker needs to be homozygous for the gene. Homozygous means that it gets the same allele from the mother and father. Heterozygous means that the bee has one of the alleles and so is a carrier, but the trait is not expressed (Boylu and Onder, 2019).
The heritability of hygienic behavior was estimated as ~0.2 by Boecking et al. (2000) and as ~0.6 by Harbo and Harris (1999) and Lapidge et al. (2002). According to Spötter at al. (2016) 6 SNP markers had highly significant associations with the Varroa specific defense behavior (Boylu and Onder, 2019). For selection on hygienic behavior ≥95% cleaning success is expected to get the colony as breeding material.
The effect of animal breeding on interested trait can be measured by many ways. In this study we use Probit Regression to measure the effect of breeding on hygienic behavior in honey bees.

MATERIALS AND METHODS

The animal studies were conducted in the research farm of Ondokuz Mayıs University in the years of 2012 and 2013. Hygienic behavior has been investigated by use of approximately 300-350 ml of liquid nitrogen was poured into the cylindrical metal template covering 165 pupae cells (Figure 1). Photographed by a digital camera, these pictures were loaded onto the computer, later the removed cells counted and recorded on the colony cards (Figure 2). Honey bee colonies that remove dead pupae greater than or equal to 95% in at least two applications are considered as hygienic (Bıyık and Guler, 2016).

Figure 1. Application of liquid nitrogen.

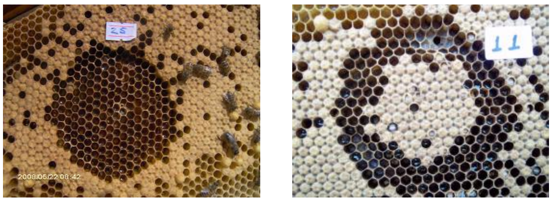

Figure 2. Determining the hygienic behavior (25: hygienic, 11: non-hygienic).

Probit analysis is a model used as an alternative to logistic regression to find the effect of one or more explanatory variables on a categorical response variable (right, dead; working, not working, sold or not sold, etc.). The cumulative normal distribution is used in the Probit, which is basically the inverse of the additive standard normal distribution. Probit coefficient β is the standard deviation increase of β in the Probit score by a one-unit increase in the estimate. The Probit coefficient measures the effect of the independent variable at the standard z-value of the dependent variable. The numerical magnitudes of these coefficients have no significance and no special interpretation, they only determine the direction and degree of the relationship (Arı and Onder, 2013).

In this study, time (hour) was used as explanatory variable and cleaning success (ratio of removed cells to 165 pupae cells) was used as response variable. Statistical analysis was performed by using SPSS with the license of Ondokuz Mayıs University.

RESULTS AND DISCUSSION

Results showed that the optimal solutions have been found for both generations for Probit regression with less than 10 iterations and regression models were statistically significant ($P<0.001$). At first generation 50%, 95% and 99% cleaning success has been reached at 5.36, 45.59 and 62.25 hours, respectively. At the second generation 50%, 95% and 99% cleaning success has been reached at 10.29, 43.51 and 57.28 hours, respectively. Results indicated that selection has positively affected the cleaning success about 5% and 8% for 95% and 99% cleaning success. This results also showed the Probit regression can be used for studies on efficiency of hygienic behavior.

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BIOLOGICAL CONTROL OF GASTROINTESTINAL HELMINTHES OF LIVESTOCK

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Abstract

Gastrointestinal helminths cause considerable production loss in ruminants globally and constraint to optimum livestock production. Extensively managed animals often host gastrointestinal parasites. Guidelines for sustainable control of parasites in sheep (SCOPS) have been produced in the UK which include recommendations for treatments to ewes. Traditionally control has been achieved through the application of broad spectrum anthelmintic; however, resistance is the major issue. In terms of parasitic nematodes in sheep, widespread resistance has been identified to the three available broad-spectrum anthelmintic classes with reports of increasing multi-class resistance was reported. Anthelmintic treatments can reach up to eight for lamb finishing. Resistance to anthelmintic can be reached to 88%. The trend towards nonchemical parasite control methods (ecological, organic, green) farming of livestock. Biological control of nematode parasites of livestock is the rising issue of animal science. In this study, biological control of nematode parasites of livestock has been reviewed.

Key words: *Gastrointestinal helminthes, livestock, Biological control*

INTRODUCTION

Global food security and an increasing demand to produce more animal protein, due to growing human populations, will require the production of more food with more efficient land use, and reduced waste (Learmount et al., 2015). Gastrointestinal helminthes cause considerable production loss in livestock all over the world (Easton et al., 2018). Healthy adult ewes are mostly immune to infection with gastrointestinal parasites and as a result there is rarely a requirement for treatment with anthelmintic drugs. Around lambing, however, this immunity is lost, worm burdens increase, faecal egg counts (FECs) rise and farmers are more likely to treat the ewes to improve condition and also to reduce the potential contamination of pastures that will be grazed by naive lambs with little resilience. Anthelmintic treatments around lambing time have become common practice on UK farms as well as in other countries with a large sheep producing industry such as New Zealand. These treatments have been shown to be high risk for selection for anthelmintic resistance (Learmount et al., 2018). With increasing reports of anthelmintic resistance worldwide, control strategies for gastrointestinal nematodes that minimize the use of anthelmintic are of increasing importance. Research, particularly in the UK, Australia, South Africa and New Zealand, has resulted in a growing awareness of the problems of anthelmintic resistance and recognition of potential resistance-delaying strategies, which can be used on farms (Learmount et al., 2015). Control has traditionally been achieved through the frequent application of broad spectrum anthelmintics; however, this has led to the emergence and spread of resistance. Maintaining production and economic viability in the face of resistance is an increasing challenge (Easton et al., 2018).

The trend towards nonchemical parasite control methods (ecological, organic, green) farming of livestock. Biological control of nematode parasites of livestock is the rising issue of animal science. In this study, biological control of nematode parasites of livestock has been reviewed.

GASTROINTESTINAL HELMINTHS

There are many kinds of gastrointestinal helminths such as *Haemonchus contortus, Cysticercus tenuicollis, Teladorsagia circumcincta Chabertia, Cooperia, Haemonchus, Nematodirus, Oesophagostomum, Teladorsagia, Trichostrongylus* and *Trichuris spp.* cause considerable production loss in ruminants. The most common ones are;

Haemonchus contortus

Haemonchus contortus, also known as the barber's pole worm, is a very common parasite and one of the most pathogenic nematodes of ruminants. Adult worms attach to abomasal mucosa and feed on the blood. This parasite is responsible for anemia, oedema, and death of infected sheep and goats, mainly during summer in warm, humid climates. Females may lay over 10,000 eggs a day, which pass from the host animal in the faeces. After hatching from their eggs, H. contortus larvae molt several times, resulting in an L3 form that is infectious for the animals. The host ingests these larvae when grazing. The L4 larvae, formed after another molt, and adult worms suck blood in the abomasum of the animal, potentially giving rise to anaemia and oedema, which eventually can lead to death.

The ova is yellowish in color. The egg (Figure 1) is about 70–85 μm long by 44 μm wide, and the early stages of cleavage contain between 16 and 32 cells. The adult female (Figure 2) is 18–30 mm long and is easily recognized by its trademark "barber pole" coloration. The red and white appearance is because H. contortus is a blood feeder, and the white ovaries can be seen coiled around the blood-filled intestine. The male adult worm is much smaller at 10–20 mm long, and displays the distinct feature of a well-developed copulatory bursa, containing an asymmetrical dorsal lobe and a Y-shaped dorsal ray.

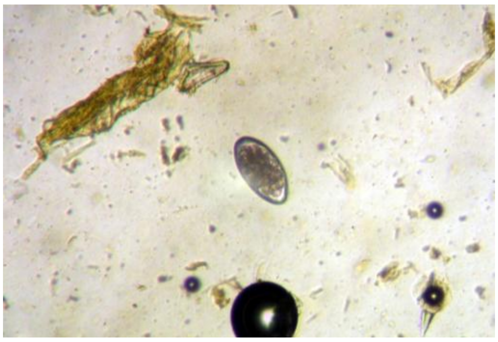

Figure 1. Haemonchus contortus egg.

Figure 2. Haemonchus contortus adult females were taken from one sheep infected with a single strain of this worm species.

The infection, called haemonchosis, causes large economic losses for farmers around the world, especially for those living in warmer climates. Anthelminthics are used to prevent and treat these, and other, worm infections, but resistance of the parasites against these chemicals is growing. Some breeds, such as the West African Dwarf goat and N'Dama cattle, are more resistant than other breeds to H. contortus (haemonchotolerance).

Clinical signs are largely due to blood loss. Sudden death may be the only observation in acute infection, while other common clinical signs include pallor, anemia, oedema, ill thrift, lethargy, and depression. The accumulation of fluid in the submandibular tissue, a phenomenon commonly called "bottle jaw", may be seen. Growth and production are significantly reduced.

Prophylactic anthelmintic treatment necessary to prevent infection in endemic regions, but wherever possible, a reduction on reliance on chemical treatment is warranted given the rapid rise of anthelmintic resistance (URL1).

Cysticercus tenuicollis

Cysticercus tenuicollis is the larval stage of the canine tapeworm Taenia hydatigena (Figure 3, Figure 4), the presence of which has been reported in wild and domestic ruminants all over the world. It is a common parasite of small ruminants in the north of Portugal. C. tenuicollis is generally seen attached to the omenta, the mesenteries or also found in the liver. In the ewe, tissue lesions have been associated with degenerative cysts or with oncosphere migrations. Infected carnivores eliminate T. hydatigena eggs with their faeces. Herbivores become infected with the eggs on account of having fed on contaminated pastures. Possible intermediate hosts for C. tenuicollis are squirrels, cattle, sheep, goats and other wild ruminants and also swines. After ingestion, the egg's shell is digested and the oncospheres become free to migrate through the intestinal walls, reaching the liver through the hepatic portal system. The oncospheres may remain in the liver or migrate to the omenta, the mesenteries and the serosal surface of the peritoneal cavity, where they attach and initiate post- oncospheral development (Payan- Carreira et al., 2008).

Like any other disease, the symptoms vary from its bodily location and when pertaining to the density of larvae. Many of the major symptoms are the result of inflammation during larval degeneration or a mass effect from the parasite. Neurocysticercosis is a serious form of cysticercosis. Common symptoms include chronic headaches and seizures. Other symptoms include: nausea, vomiting, vertigo, ataxia, confusion or other changes in mental health, behavioral abnormalities, progressive dementia, and focal neurologic signs (URL2).

Figure 3. Taenia hydatigena in dog.

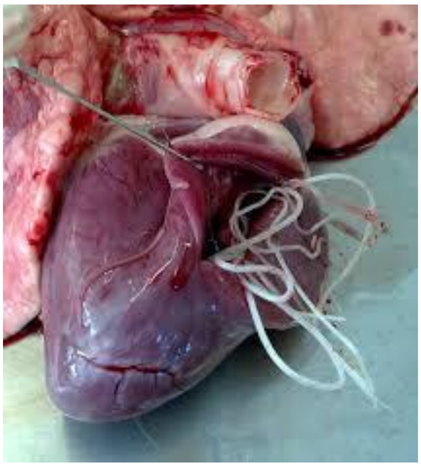

Figure 4. Taenia hydatigena in Sheep.

In the normal lifecycle of Taenia, cysticerci develop in the muscles of the intermediate hosts such as pigs, cattle, and sheep. In these animals, they do not cause severe symptoms. They are transmitted to humans when their infected meats are eaten.[8][9] However, T. solium is unusual because its cysticerci can develop in humans. Due to accidental consumption of the eggs from contaminated foodstuffs, cycticerci in humans produce clinical symptoms. Thus, humans are accidental intermediate hosts (URL2).

Best prevention consists in preventing livestock feed (fresh pasture as well as hay, silage, and other stored feed) or water from being contaminated with dog feces that may contain tapeworm eggs. It must be considered that the eggs may remain infective in the feed after silage or other processing of hey or fodder (pelletizing, fermentation, etc.). The regular use of anthelmintics is not indicated for preventing horse or livestock infections with Cysticercus tenuicollis. There are reports that albendazole and praziquantel are effective, but only at dosis higher than the usual therapeutic ones, and results can be unreliable. There are so far no vaccines that would protect sheep or goats, other livestock or horses against Cysticercus tenuicollis. To learn more about vaccines against parasites of livestock. Biological control of Taenia tapeworms respectively Cysticercus tenuicollis (i.e. using its natural enemies) is so far not feasible (URL3).

Teladorsagia circumcincta

Teladorsagia circumcincta (Figure 5) is a nematode that is one of the most important parasites of sheep and goats. It was previously known as Ostertagia circumcincta and is colloquially known as the brown stomach worm. It is common in cool, temperate areas, such as south-eastern and south-western Australia and the United Kingdom. There is considerable variation among lambs and kids in susceptibility to infection. Much of the variation is genetic and influences the immune response. The parasite induces a type I hypersensitivy response which is responsible for the relative protein deficiency which is characteristic of severely infected animals. There are mechanistic mathematical models which can predict the course of infection. There are a variety of ways to control the infection and a combination of control measures is likely to provide the most effective and sustainable control.

Adults are slender with a short buccal cavity and are ruddy brown in colour. The average worm size varies considerably among sheep. Females range in size from 0.6 to 1.2 cm with males typically about 20% smaller.

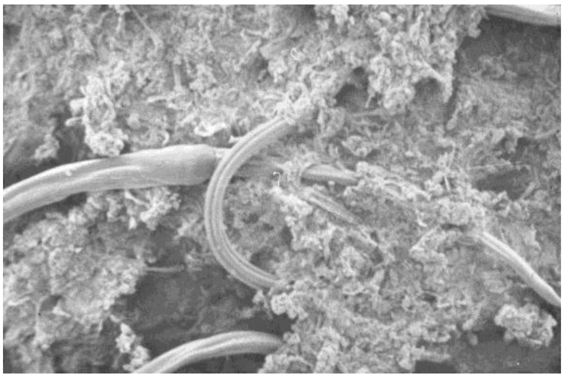

Figure 5. A scanning electron micrograph of Teladorsagia circumcincta.

The life cycle is relatively simple. Male and female adults breed on the abomasal surface. Eggs are passed in the faeces and develop through first (L1), second (L2) and third stage larvae (L3). Third stage larvae are unable to feed and are the infective stage. Following ingestion, third-stage larvae rapidly moult within 48 hours into fourth stage larvae (L4) which develop in the gastric glands. About 8 days after infection, the young adults emerge from the gastric glands, mature and breed. The prepatent period can be as short as 12 days. The number of eggs in the uterus of mature females can vary from less than 10 to more than 60 and is heavily dependent on the host immune response. The number of eggs produced per day by an adult female worm has been estimated as ranging from 0 to approximately 350, with longer females laying more eggs.

It is responsible for considerable economic losses in sheep. It is believed to also cause severe losses in goats although there is a relative dearth of research in this species. In most countries, infection with third-stage larvae resumes in the spring and is triggered by the production of large numbers of eggs by periparturient females. The key trigger appears to be a relative protein deficiency in the ewe and the periparturient rise may be prevented by feeding supplementary protein. In the stereotypical pattern, egg production (assessed by faecal egg counts in the lambs) rises till midsummer then declines. The development of free-living nematodes is influenced by temperature and moisture and there is considerable variation within and among among years in the number of infective larvae available for ingestion. Consequently, the pattern of egg production during the year also varies among years. The number of infective larvae gradually dies down at the end of the grazing season when grass growth also declines. The end of the season varies among countries depending upon their climate and is also likely to vary from year to year within countries depending upon local weather but is poorly documented. For example, in Scotland, the season usually ends about late October.

There is considerable variation among animals in faecal egg counts and much of this variation is genetic in origin. The sources of variation are dynamic and their relative importance changes over the course of the year. Other sources of variation, apart from inherited effects include maternal and common environmental effects and effects specific to each individual, including variation in intake and non-additive genetic effects. The gender of the lamb, type of birth (single or twin), date of birth and intensity of early exposure to nematode infection have relatively minor but still important effects. The inevitable variation between the observed count and the true faecal egg count also contributes to the observed variation. Both the natural history of infection and the sources of variation among individuals are relatively well understood for T. circumcincta compared to most parasites.

Essentially all grazing animals are exposed to infection and most animals will carry some nematodes either as adults or arrested early fourth-stage larvae or both. For disease control, the aim is not to diagnose infection but to identify animals or flocks that are sufficiently heavily infected to show reduced production, decreased animal welfare or parasitic gastroenteritis. Heavily infected animals are relatively protein deficient. Clinical signs include reduced appetite, poor growth performance, weight loss and intermittent diarrhoea. In addition to clinical signs, faecal egg counts and the timing of infection are used to identify severely affected animals. However, lambs with very high numbers of worms produce very few eggs; there are strong density-dependent effects of worm number on egg production.

The methods to control nematode infection in livestock can be grouped into 6 categories:

anthelmintic treatment, grazing management, biological control, nutritional supplementation, vaccination and genetic resistance. Each method has its advantages and disadvantages. Anthelmintic treatment usually involves treatment with one or more of five classes of broad spectrum drugs. Drugs: benzimidazoles, levamisole, macrocyclic lactones, amino acetonitrile derivatives and spiroindoles. Narrow spectrum drugs are also available. Drug formulations vary among countries and only some countries allow combinations of drugs to be used. Anthelmintic treatment is cheap, simple and effective but is threatened by the development of drug resistance in parasite populations. There are a number of strategies to mitigate the development of drug resistance including the use of combinations to delay the development of drug resistance in parasite populations. Drugs have been so cheap and effective that it was usually easier and cheaper to treat all animals in a population. However, not all individuals require treatment and best practice is now to target treatment on selected animals to delay the development of drug resistance. The criteria to decide which animals are to be treated and the size of the untreated population are matters of active research (URL4).

Biological control with spores of the fungus Duddingtonia flagrans has recently been licensed in Australia and can be very effective but requires regular, possibly daily, feeding (Waller and Thamsborg, 2004).

CONTROL OF GASTROINTESTINAL HELMINTHS

There are many kind of control approaches for gastrointestinal helminths such as chemical control, grazing management, feed – nutrients, feed – antinutrients, ethnoveterinary remedies, genetics and breeding, vaccination and so on (Gray et al., 2012; Hoste and Torres-Acosta, 2011).

BIOLOGICAL CONTROL OF GASTROINTESTINAL HELMINTHS

Nematophagous fungi present themselves as natural enemies of gastrointestinal helminth parasites. They can be found in diverse environments and have been shown to be effective as biocontrol agents. In addition, an abundance of natural antagonists of helminths, including protozoa, bacteria, viruses, mites, beetles and fungi, have already been described as biological controllers. These organisms (nematophagous fungi) comprise different types of fungi and are cosmopolitan, occurring in natural soil, agricultural soils and in all types of decaying organic matter. In the environment these fungi are biologically very important, since they play a role in the recycling of carbon, nitrogen and other elements that originate from the degradation of the nematode (Braga and de Araujo, 2014). Nematophagous fungi are divided into three groups;

(1) Endoparasites are seen mainly as spores, but sometimes as chlamydospores (resistant spores) that are released at the time of the disintegration of the nematode. Their activity is infective, but they do not produce extensive fungal mycelium. Furthermore, they are not capable of growing in the soil, making them impossible to be proposed as inocula for the environmental control of a target nematode. Another point of great importance is their dependence on free water, which tends to be the main limiting factor for routine use under laboratory conditions as biological controllers of organisms (Stirling and West, 1991; Braga and de Araujo, 2014)

(2) Predators. This group includes the main species of nematophagous fungi — the genera Arthrobotrys, Duddingtonia and Monacrosporium. Their main feature is the production of traps and they have the advantage of greater potential for industrialization.

(3) Ovicidal or opportunistic fungi are those that parasitize eggs, cysts and nematode females. This group of fungi has been studied for a long time, and the first studies showed that these fungi would be

promising in reducing populations of nematodes and helminths.

Duddingtonia flagrans

Currently, the work on biological control of nematode parasites of livestock is almost exclusively associated with the nematode-destroying microfungus Duddingtonia flagrans (Waller and Thamsborg, 2004). The microfungus has three very important attributes: (a) the ability to survive gut passage of livestock; (b) the propensity to grow rapidly in freshly deposited dung; and (iii) the possession of a voracious nematophagous capacity (Larsen, 1999). This fungus thus breaks the life cycle by capturing infective larval stages before they migrate from dung to pasture, where they would otherwise be acquired by grazing animals (Waller and Thamsborg, 2004). D. flagrans spore material; second, long-term field trials using D. flagrans have shown no adverse effects on the environment; and third, it has been established that D. flagrans is ubiquitous and that very close genetic similarity exists between isolates from all regions of the world (Waller, 2003; Waller and Thamsborg, 2004). Buzatti et al (2015) showed that D. flagrans reduced the number of infective larvae on fecal egg counts and the biological control with the predacious fungi D. flagrans is a promising free-living parasite regulator alternative to be use in livestock.

Duddingtonia flagrans (Figure 6) can be used against Barber’s Pole Worm or Wire Worm (Haemonchus spp.), Black Scour Worm (Trichostrongylus spp.), Small Brown Stomach Worm (Teladosgia (Ostertagia) spp.), Medium Brown Stomach Worm (Ostertagia ostertagi), Nodule Worm (Oesophagostomum spp.), Hair Worm (Trichostrongylus spp.), Intestinal Worm (Cooperia spp.), Thin Necked Intestinal Worm (Nematodirus spp.) and Hook Worm (Bunostomum spp.) with great achievements (URL5).

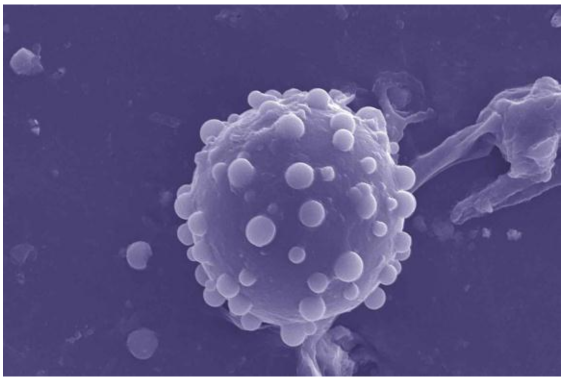

Figure 6. Duddingtonia flagrans.

Arthrobotrys

This genus is said to be ‘classic’ and was first described in the 19th century. It should also be mentioned that predatory fungi belonging to this group produce an extensive hyphal system along which are produced organelles capable of capturing nematodes. It is still valid to remember that among the predatory fungi that capture and kill nematodes, Arthrobotrys is probably one of the most important genera and certainly the most promising.

Monacrosporium

Fungi of the Monacrosporium genus are able to survive passage through the gastrointestinal tract of ruminants. This important premise regarding its activity, that it does not lose its predatory action, is of prime importance in the biological control of helminths. Monacrosporium also has proven action on gastrointestinal nematodes, which makes it a promising candidate to be used in the biological control of these organisms. The ability of M. thaumasium and M. sinense to survive passage through the gastrointestinal tract of domestic animals without losing predatory activity has been an important prerequisite for their use in biological control (Araújo et al. 1999; Braga and de Araujo, 2014).

Ovicidal fungi

These organisms, in addition to feeding on parasite eggs, cysts and females of nematodes and helminths, are saprophytic and, therefore, do not depend on the presence of the parasite in the soil for their survival and are thus easily cultured in the laboratory. This is a promising group of fungi to be employed in the biological control of helminths, mainly because they reduce levels of viable eggs in the soil (Braga and de Araujo, 2014).

Pochonia

This genus of ovicidal fungi is one of the most studied in the control of helminths that are potentially harmful to agriculture, and, currently, has also been standing out in combating their eggs. Again, it is noteworthy that experiments with this fungal genus include ‘scientific enthusiasm’ in their results, especially with regard to the effective control of nematode populations. P. chlamydosporia has been implicated as a suppressor of several genera of helminths, parasitizing their eggs by structures known as ‘appressoria’, formed from undifferentiated hyphae when in contact with these eggs (Braga and de Araujo, 2014).

Paecilomyces

Paecilomyces is a cosmopolitan filamentous fungus that exists worldwide and which inhabits soil, decaying plants, and food. In relation to the biological control of eggs from gastrointestinal parasites, research is advancing, but the main obstacle is the potential harmful ‘element’ that this genus and some strains can cause in man and animals (Braga and de Araujo, 2014).

Predatory fungi

Factors such as soil type, pasture, temperature, humidity, oxygen and predators determine a microclimate around the eggs and larvae on pasture, directly influencing the availability of infective larvae (L3). This microclimate is formed initially in the faecal matter, which is the source of larvae for the contamination of pastures. After transforming themselves into L3, larvae migrate to the adjacent pastures (within 90 cm of faecal matter and 15 cm at the base of the plants), where they can be ingested by the host. This migration usually occurs in the morning and late afternoon, when the light intensity is lower. The larvae often use water drops (such as dew) to migrate to pasture. Thus, the passage of nematophagous fungi through the gastrointestinal tract of domestic animals acts as an ‘inundative biological control type’, which consists of increasing concentrations of antagonist organisms already present in the soil (Braga and de Araujo, 2014).

RESULTS AND DISCUSSION

Biological control with fungi is a viable and safe alternative for animals, humans and the environment. Gastrointestinal helminthes which caused considerable production loss in livestock can be controlled by biological methods instead of chemical control. More studies should be conducted to understand the effect and mechanisms of biological agents on helminths and effect of then on the environment.

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COMPARISON OF LEAST SQUARE METHOD, RIDGE AND LIU ESTIMATORS IN SAANEN GOATS

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Abstract

In this study, it is aimed to compare the reliability of the Least Squares Method (LS), Ridge and Liu Estimator for the data has non-normal distribution, multicollinearity. For this aim, we taken into consideration a data set which consist of body weight and body measurements taken from Saanen kids for 6 months, where body weights are estimated from various body measurements with different level of multicollinearity. The data were obtained from a private farm in Bafra province. As a result of the analysis, Liu estimator is more applicable than LS and Ridge estimator when the data has multicollinearity, non-normal distribution.

Key words: *Least Square Method, Ridge Estimator, Liu Estimator, Saanen goat, multicollinearity.*

PROTECTIVE ACTIVITY OF NIGELLA SATIVA, THYMOQUINONE AND NA BENTONITE ON AFLATOXICOSIS IN BROILERS

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Abstract

It is aimed to determine the preventive efficacy of black seed (Nigella sative L.), thymoquinone, and Na bentonite single and in combination on tissue degeneration in liver and other organs induced by aflatoxicosis in broilers. One hundred 1-d-old broiler chicks were used and divided into 10 equal groups and fed for 21 days. Animals were received feed with 2 mg/kg total AF (83.34% AFB1, 9.28% AFB2, 5.37% AFG1 and 2.01% AFG2), Black seed (BLS; 5%), Thymoquinone (TMK; 300 mg/kg), Na bentonite (BNT; 1%) single and in combination. At the end of the experiment, blood samples were taken; and then the animals were euthanized and liver tissue samples were collected. Transforming Growth Factor β (TGF-β), Hepatocyte Growth Factor (HGF), 8-hydroxy-2′-deoxyguanosine (8-OHdG) and Total Antioxidant Capacity (TAC) were analyzed by ELISA method. Compared to control, AF administration significantly increased serum and liver 8-OHDG, serum TGF-β and liver TAC levels whereas it significantly decreased liver TGF-β and serum TAC levels. However, no change was observed in the serum and liver HGF levels by AF. Single and combined addition of BLS, BNT and TMK to AF-contaminated feed significantly improved the AF-related changes in liver 8-OHDG. Serum 8-OHDG levels showed improvement only in AF+BNT+TMK group. Addition of BLS, BNT and TMK alone to AF-contaminated feed significantly ameliorated the changes in serum TGF-β due to AF. Also, AF+BLS, AF+TMK, AF+BNT+BLS and AF+BNT+TMK groups showed significant improvement in AF-related changes in liver TGF-β. On the other hand, there was a significant decrease in serum TAC levels in AF+TMK and AF+BNT+TMK groups compared to all groups including AF group. Single and combined addition of BLS, BNT and TMK to AF-contaminated feed significantly improved the AF-depending change in liver TAC levels. Single addition of BLS, BNT and TMK to AF-free diet did not cause any significant change compared to the control in terms of the parameters that were examined, with little exception. As a result, BLS and TMK supplementation to the AF-contaminated feed may be beneficial against aflatoxicosis in broilers. However, these data should be evaluated together with other parameters that reflect the effects of AF on target organs and tissues.

Key words: *transforming growth factor β, hepatocyte growth factor, 8-hydroxy-2′-deoxyguanosine, total antioxidant capacity, aflatoxicosis*

SEROPREVALENCE OF SOME ARBOVIRAL DISEASES IN CATTLE IN THE AFYONKARAHISAR PROVINCE OF TURKEY

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Abstract

This study is supported by the Scientific Research Project Directorship of Hatay Mustafa Kemal University, Hatay, Turkey (Project No: 18.M.058). Aim of the study: Arboviral diseases are infections caused by viruses spread to animals by the bite of infected arthropods such as mosquitoes, sand flies, midges or ticks. Animals infected by arboviruses, may suffer diseases ranging from sub-clinical to mild, characterized by loss in milk production, lameness, abortion, congenital malformations, astasia and ataxia. The aim of the study was to investigate the seroprevalence of akabane virus (AKAV), bovine ephemeral fever virus (BEFV) and schmallenberg virus in cattle in the Afyonkarahisar Province. Material and Methods: Blood samples were collected from randomly selected cattle (n= 270) from epidemiologically independent herds (n=80) in the Afyonkarahisar Province in the Aegean region of Turkey. Commercial competitive ELISA kits were used for the detection of AKAV anti-G1, SBV nucleoprotein antibodies and a sandwich ELISA kit was used for the detection of BEFV specific antibodies. Results and Conclusion: Antibodies against AKAV and SBV were detected in 26 (9.6%) and 24 (8.9%) cattle, respectively. Antibodies against BEFV were not detected in investigated samples. Among the 80 flocks, 10 (12.5%) had one or more AKAV or SBV seropositive animals. Seropositivity rate of SBV was higher in female animals than males ($P<0.05$). The higher AKAV and SBV seroprevalence rates were found among cattle older than 36 months. The results of this study indicate that AKAV and SBV infections are prevalent in the Afyonkarahisar Province. Therefore, a control program is needed against to AKAV and SBV infections.

Key words: *Seroprevalence, Cattle, Akabane virus, Bovine ephemeral fever virus, Schmallenberg virus*

ANALYSES OF POSTERIOR SEGMENT OF THE EYE BY SPECTRAL DOMAIN OPTICAL COHERENCE TOMOGRAPHY IN TAWNY OWLS (STRIX ALUCO)

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Abstract

Spectral Domain Optical Coherence Tomography (SD-OCT) is an imaging system to directly scan cross section of the posterior segment of the eye. Present study shows a qualitative and quantitative evaluation of the structures in posterior segment of tawny owl's (Strix aluco) eyes with SD-OCT technique. In the present study, six eye globes taken from three healthy and adult owls were used. All the owls were clear in respect of any vision and eye problems. Eyes were scanned with SD-OCT on retina mode (high-resolution spectral surface OCT). It was found that retina has ten different layers and area centralis rotunda is the only centre throughout the retina. Right and left macula thickness were averagely measured as 224 µm. Subfovea choroid thickness of eyes was found as 284 µm and 306 µm on right and left eyes, respectively. Sclerotic ring and/or ossicles layer were detected on between sclerae and choroid layer which were not be found on the eye of mammalians. Pecten oculi was observed on the region where the optic nerves entered on retina. Consequently, morphology of posterior segment of eyes of tawny owls were investigated with OCT technique in the present study. It was presented that these results might be used for avian ophthalmology clinical applications and further scientific research in the field as a basic information source.

Key words: *Tawny owl (Strix aluco), optical coherence tomography, pecten oculi, posterior segment, retinal thickness.*

CHANGES OF OXIDATIVE STATUS AND BIOCHEMICAL PARAMETERS ON CALVES PERSISTENTLY INFECTED WITH BOVINE VIRAL DIARRHEA VIRUS

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Abstract

The aim of this study was to investigate changes of antioxidant capacity and biochemical parameters [Albumin, ALP (Alkaline phosphatase), ALT (Alanine transaminase), AST (Aspartate aminotransferase), cholesterol, CK-MB (Creatine kinase), GGT (Gama-glutamyl transferase), T-Protein, Triglyceride, BUN (Blood Urea Nitrogen)] on calves persistently infected (PI) with Bovine Viral Diarrhea Virus (BVDV) in Konya. Blood samples were collected from 600 animals in 25 farms in Konya. These samples were evaluated for BVDV twice at regular intervals and 6 animals were determined persistently infected with BVDV. Blood samples (both sera and leukocyte) were collected from the PI calves. The samples were analyzed for BVDV antigen and antibodies and then determined levels of antioxidant capacity and biochemical parameters by Enzyme Linked Immunosorbent Assay (ELISA) kits. In the present study, ALB, ALP, Cholesterol, CK-MB, TP levels were statistically significant differences while MDA and TAC values no statistically significant difference between control and study groups. As a result it may be mentioned that persistently BVDV infection can be damage in the heart, duodenum and liver. The changes in biochemical parameters and antioxidant capacities in PI animals can give some information about the pathogenesis of the infection. In conclusion, PI animals should be removed from the farms because they can cause economic loss and also as a source of infection for other animals in the farms. This study was produced from project which supported by University of Selcuk Scientific Research Projects Coordinator (Project number: 17401075)

Key words: *Persistently infected, BVDV, MDA, TAC, Biochemical Parameters.*

SEROLOGICAL INVESTIGATION OF AKABANE VIRUS INFECTION IN KONYA REGION, TURKEY

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Abstract

Akabane disease is a viral disease of ruminants that is mainly characterized by fetal damage. This disease causes epidemics of abortions and congenital malformations manifested as arthrogryposis and hydranencephaly or microanencephaly in cattle. Outbreaks of the Akabane virus have been reported from many countries in Southeast Asia, Middle East, Africa and Australia. A cross-sectional study was carried out in in the surrounding region of the southeast of Konya province in Turkey to determine seroprevalence and to assess risk factors associated with Akabane virus (AKAV) infection in dairy herds. In this study, a total of 282 blood samples were collected from clinically healthy dairy cattle in the surrounding region of the southeast of Konya province and tested for antibodies against AKAV using ELISA. The ELISA test was performed according to the producer instructions. The serological results and other information gathered during this investigation such as sex (female/male), rearing system (semi-intensive/ intensive) and age (0-1 / 1-3 / >3 years) of the sampled animals were edited and analyzed statistically using statistical package SPSS and R Program. The association between age, sex and rearing system were analyzed by Chi-square test and logistic regression. Differences were considered statistically significant when $p < 0.05$. A total of 4 cattle were found seropositive with overall prevalence of 1.41% (4/282). The sero-prevalences were 1.1 and 8.3% in female and male, respectively. All the age groups of cattle tested had seropositive animals, 0-1 year (1/41, 2.43%), 1-3 years (2/188, 1.06%) and >3 years (1/53, 1.88%), respectively. Statistical analysis showed sex is significantly associated with infection ($p<0.05$) but age and rearing system are not significantly associated with infection ($p>0.05$). Finding obtained shall provide information for other studies that may be conducted on the Epidemiology of Akabane Disease. It also provides an overview about the risk factors associated with the disease at the examined localities. This will facilitate early detection and aid the development of appropriate control measures against the disease in Turkey.

Key words: *Akabane virus, Epidemiology, Prevalence, Cattle, Turkey.*

THE RELATIONSHIP BETWEEN PLACENTAL CHARACTERISTICS AND BIRTH-RELATED FACTORS IN WATER RESTRICTED AWASSI EWES

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Abstract

The aim of present study was to determine relationship between placental characteristics and birth-related factors in water restricted Awassi ewes during pregnancy. Pregnant Awassi ewes were randomly assigned to free water (C; n=20) group received ad libitum water and water restricted (WR; n=20) group received 50% less of C group, after the first month of pregnancy. Birth weight, lamb's sex and placental measurements were recorded within 12 h after parturition. There were positive correlation between cotyledons number and medium cotyledons weight (0.877; P<0.01), and placental weight and large cotyledons weight (0.514; P<0.05), and placental weight and cotyledon efficiency (0.563; P<0.05), and lamb birth weight and large cotyledons weight (0.774; P<0.01), and placental efficiency and large cotyledons weight (0.744; P<0.01) in WR group, whilst not correlation in C group. Moreover, there were negative correlation between cotyledon efficiency and cotyledons number (-0.737; p<0.05), and average cotyledon surface area and cotyledons number (-0.779; p<0.05) in WR group, whilst not correlation in C group. Contras to, there were negative correlation between placental efficiency and placental weight (-0.816; p<0.05) in C group, whilst not correlation in WR group. The results suggest that water restriction during pregnancy influence relationships between placental characteristics and birth-related factors in Awassi ewes.

Key words: *water restriction, placental characteristics, lamb birth weight, correlation, Awassi*

INTRODUCTION

The water shortage will affect livestock production specifically grazing animals, which are depending on sparsely spaced watering points in future (Alamer, 2006). The ability of animals to endure dehydration, preventing pasture loss in the vicinity of the dispersed water sites and extension of the radius of grazing area around the water spots is essential for animal survival under such conditions (Silanikove, 1994; 2000; Megersa et al., 2014). The intrauterine existence of fetus is dependent on the placenta and placental components, which are support normal fetal development (Biswas and Ghosh, 2008; Sen and Onder 2016) through respiratory gases, nutrients and wastes exchange between maternal and fetal systems, and thereby determining the weight of the fetus (Meschia, 1983; Wulff et al., 2003; Reynolds et al., 2005; Sen et al., 2013). Thus, it is crucial to have balanced placental elements both in the maternal and fetal side, adequate vascularization and ultimately blood flow to the placenta for normal fetal growth (Igwebuike, 2010). There are many factors that affect fetal and placental development such as genotype, parity, litter size, environmental stress and nutrition deprivation (Ocak et al., 2014; Sen et al., 2013; Ocak et al., 2015; Sen and Onder 2016; Sen et al., 2016). Hence, it is particularly important to understand the impacts of the maternal environment on placental growth and development which directly affects fetal growth. Previous studies showed that maternal nutrition level during pregnancy affects the size and development of both placenta and fetus (Redmer et al., 2004; Sen et al., 2013). Additionally water restriction, like water deprivation, causes a reduction in feed intake and utilization in various ruminant species (Bohra and Ghosh, 1977; More and Sahni, 1981) and may resulting in adversely effect on fetal and placental development.

The Awassi breed, indigenous to the Middle East Region, is known for its good adaptability to the semi-arid environments, namely long periods of drought and high temperatures (Jaber et al., 2004). Moreover, there have been numerous studies investigating the effects of water restriction on physiological and chemical responses in this breed (Alamer and Al-Hozab, 2004; Jaber et al., 2004; Hamadeh et al., 2006; Ghanem et al., 2008). However, most of the studies on adaptation to drought were carried out on non-productive or non-pregnant animals (Silanikove, 1994). Therefore, the aim of present study was to determine relationship between placental characteristics and birth-related factors in water restricted Awassi ewes during pregnancy.

MATERIALS AND METHODS

The study was conducted on pregnant Awassi ewes, which were randomly assigned to free water (C n=20) group received ad libitum water and water restricted (WR n=20) group received 50% less of C group. Average daily water consumption (ADWC) of all ewes was measured on first month of pregnancy (4.4 l/ewe/day). At the end of the first month, the ewes were randomly allocated according to body weight into two group. From the second month of pregnancy and onward, ewes in the WR group received 2.2 l/ewe/day until lambing. C and WR ewes were held as two separate groups in a semi-open barn; allowed to graze between 7.00-11.00 am and 14.00-18.00 pm on the same paddock without access to water in the field during the full term of pregnancy. After returning from the afternoon grazing, they had access to the same amount of feed supplementation, which was composed from 300 g/day of concentrates and 0.5 kg/day of alfalfa hay.

Lambs birth weight (LBW) and sex were recorded within 12 h after parturition. Each ewes was left to deliver the placenta naturally and placentas were collected immediately after delivery; care was taken to ensure that any placental weight (PW) taken were of the total placenta with any fluid being removed before weighting. The total numbers (TCN) of placental cotyledons dissected from the chorioallantois were also counted and determined. Diameter of cotyledon were measured with a digital compass and divided into two categories as medium (10–30 mm) and large (>30 mm). Placental efficiency (PE) was calculated for each ewe, as the ratio of lamb birth weight to PW. Cotyledon efficiency (CE) was defined as the ratio of lamb birth weight to cm^2 total cotyledon surface area (TCSA), according to Ocak et al. (2015).

Relationships between variable traits for data were determined with Pearson correlation analysis at the 95% confidence interval in the SPSS package program.

RESULTS AND DISCUSSION

Pearson correlation coefficients of placental characteristics and birth-related factors in C and WR groups are presented Table 1 and 2, respectively. There were positive correlation between cotyledons number and medium cotyledons weight (0.877; P<0.01), and placental weight and large cotyledons weight (0.514; P<0.05), and placental weight and cotyledon efficiency (0.563; P<0.05), and lamb birth weight and large cotyledons weight (0.774; P<0.01), and placental efficiency and large cotyledons weight (0.744; P<0.01) in WR group, whilst not correlation in C group.

Table 1. Pearson correlation coefficients of placental characteristics and birth-related factors in C group.

	PW	CN	MCW	LCW	LBW	CE	PE	ACSA
CN	0,231							
MCW	-0,233	0,175						
LCW	0,412	0,311	-0,791**					
LBW	-0,311	0,135	-0,358	0,183				
CE	-0,122	0,082	-0,687*	0,596*	0,824**			
PE	-0,819**	0,008	-0,032	-0,140	0,781**	0,548*		
ACSA	0,244	0,062	-0,826**	0,868**	0,217	0,718**	-0,041	
TCSA	0,327	0,416	-0,669*	0,920**	0,181	0,638*	-0,080	0,927**

PW= placenta weight (g); CN= cotyledon number; MCW= medium cotyledon weight; LCW= large cotyledon weight; LBW= lamb birth weight; CE= cotyledon efficiency; PE= placental efficiency; ACSA= average cotyledon surface area; TCSA= total cotyledon surface area
*p<0.05, **p<0.01

Additionally, there were positive correlation between average and total surface cotyledon area and placental weight (0.532; 0.505; P<0.05), lamb birth weight(0.616; P<0.05, 0.822; P<0.01), placental efficiency (0.506; P<0.05, 0.791; P<0.01), respectively. Moreover, there were negative correlation between cotyledon efficiency and cotyledons number (-0.737; p<0.05), and average cotyledon surface area and cotyledons number (-0.779; p<0.05) in WR group, whilst not correlation in C group. Contras to, there were negative correlation between placental efficiency and placental weight (-0.816; p<0.05) in C group, whilst not correlation in WR group. The relationships between other remaining traits showed a similar trend.

Table 2. Pearson correlation coefficients of placental characteristics and birth-related factors in WR group.

	PW	CN	MCW	LCW	LBW	CE	PE	ACSA
CN	-0,138							
MCW	-0,045	0,877**						
LCW	0,514*	-0,385	-0,622*					
LBW	0,703	-0,306	-0,373	0,774**				
CE	0,563*	-0,737**	-0,764**	0,779**	0,859**			
PE	0,289	-0,314	-0,475	0,744**	0,881**	0,780**		
ACSA	0,532*	-0,779**	-0,824**	0,836**	0,616*	0,864**	0,506*	
TCSA	0,505*	-0,221	-0,536*	0,918**	0,822**	0,748**	0,791**	0,662*

PW= placenta weight (g); CN= cotyledon number; MCW= medium cotyledon weight; LCW= large cotyledon weight; LBW= lamb birth weight; CE= cotyledon efficiency; PE= placental efficiency; ACSA= average cotyledon surface area; TCSA= total cotyledon surface area
*p<0.05, **p<0.01

The significance of PE depends on the inter-relations between fetal weight and placental weight whereas the total cotyledon surface area and vascularity has another important effect on growth of the fetus (Igwebuike, 2010; Sen et al., 2016). Although WR group had poor cotyledon morphology and angiogenesis formation in the placenta, the ewes physiologically become more resilient to keep the fetus alive, suggesting possibly yet another breed characteristic. Less water intake and circulation in the present study may have caused a decrease in the amount of blood flow between mother and fetus and that might adversely affected the vascular angiogenesis and cotyledon formation. Adequate blood flow to the placenta is critical for placental mass and formation of the components, which are associated with cotyledons. Different colorations in the WR placentas therefore might be explained due to the less umbilical blood flow. Until now, we have known that development of placental vascular beds is highly dependent on umbilical blood flow, and that this dependence is a result of high metabolic demands associated with tissue growth. Thus, tissue growth of the utero placenta and the importance of vascular development to placental function have long been recognized (Meschia, 1983; Reynolds et al., 2005; Igwebuike, 2010). Placenta seem to support pregnancy in WR group with poor morphology, less cotyledon number and less total cotyledon surface area due to the greater efficiency of placenta. The constrained fetal development resulting in low lamb birth weight in the WR group was mainly due to the limited fetal glucose uptake and tissue formation was resulting from restricted water intake of the ewe, which ultimately reduced blood flow and vascular bed development in the placenta. However although the WR ewes had lower parturition, placenta and birth weight as mentioned earlier, the PE and CE for the WR group were markedly higher. Authors are confident that the WR ewes mobilized most of the energy obtained by feed and deposited in the tail to the placenta in order to cope with the stress conditions to maintain pregnancy.

CONCLUSIONS

In conclusion, the results of the present study imply that water restriction during pregnacy influence placental development and exchange capacity of placenta to fetus and change relationships between placental characteristics and birth-related factors, which reflect variations in birth weight of lambs, in Awassi ewes.

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PATH ANALYSIS FOR PLACENTAL TRAITS ON LAMB BIRTH WEIGHT IN AWASSI SHEEP

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Abstract

Path analysis is used to describe the directed dependencies among a set of variables and application of path analysis in animal breeding improve breeding practices. The aim of this study was to examine the direct and indirect effect of some placental traits on lamb birth weight born to Awassi sheep breed using path analysis. For this aim, relationship between lamb birth weight and seven traits (placental weight; PW, total cotyledon numbers; TCN, total cotyledon surface area; TCSA, cotyledon efficiency; CE, total cotyledon volume; TCV, volumetric cotyledon efficiency; VCE, plancenta efficiency; PE) were studied in 40 singleton - bearing sheep. Birth weight, lamb sex and placental measurements were recorded within 12 h after parturition. The direct effects of PE on birth weight were found statistically significant (p<0.05). Additionally, the indirect effects of VCE on birth weight were found statistically significant (p<0.05). While PE was found with highest direct effect (0.532) on BW, PE had lowest (-0.067) indirect effect on BW. The highest total indirect effect (0,431) on BW was found in VCE variable. Additionally, the direct effect (-0.188) of TCV was lowest on BW. The results showed that PE and VCE were the most explanatory variable for birth weight of Awassi lambs.

Key words: *path analysis, lamb birth weight, placental traits, direct effect, indirect effect, Awassi*

INTRODUCTION

Placental characteristics are important indicators of the postnatal mortality of offspring in small ruminants (Dwyer et al., 2005). Mellor and Stafford (2004) reported that the postnatal viability of newborns is associated with placental growth and development during gestation. The exchange capacity of ovine placenta between maternal and fetal systems depends on placental size and number of placentomes (Ocak et al., 2013; Sen and Onder, 2016). Therefore, placental size, which is related to the nutrient transfer capacity of the placenta, plays a pivotal role in determining the prenatal growth trajectory of the fetus and hence birth weight and postnatal viability (Sen et al., 2013). Placental growth and development support fetal development during mid- to late gestation (Redmer et al., 2004; Sen et al., 2013). Previous studies indicated that there are significant relationships between placental weight and birth weight of the newborn (Osgerby et al., 2003; Dwyer et al., 2005; Sen et al., 2013; Sen and Onder, 2016).

In general, the aim of animal breeding is to genetically improve populations of livestock so that they produce more efficiently under the expected future production circumstances (Önder and Abacı, 2015). Genetic improvement for economic traits is achieved by selecting the best individuals of the current generation and by using them as parents of the next generation (Dekkers et al., 2004). In many cases, the offspring with higher birth weight are selected as future breeding material or these criteria are used to valorize the animals. Placental traits have important effect on birth weight of the newborn (Osgerby et al., 2003; Dwyer et al., 2005; Sen et al., 2013; Sen and Onder, 2016). Generally, to evaluate relationship between placental characteristics and birth-related traits data relational statistics such as regression and correlation are used (Ocak et al., 2013; Sen et al., 2013; Sen and Onder, 2016). Birth weight of the newborn is selected as response variable and placental characteristics are selected as explanatory variables. Therefore, it is aimed to explain the response variable from explanatory variables. However, indirect effects of explanatory variables on response variable should be considered beside the direct effects (Arı and Önder, 2013). Path analysis is used to describe the directed dependencies among a set of variables (Önder and Abacı, 2015). Therefore, application of path analysis in animal breeding practices began to increase (Önder and Abacı, 2015). In addition, there are lack of studies that examined the direct and indirect effects of placental characteristics on birth weight of the newborn. The aim of the current study was therefore to investigate direct, indirect and total effects of placental characteristics on lamb birth weight in Awassi ewes.

MATERIALS AND METHODS

The study was conducted on 40 singleton-bearing Awassi ewe in a private dairy farm in Yozgat, Turkey in the normal breeding season. All Awassi ewes were housed and cared for under the same conditions in the stockyard and were allowed to graze for 5 h daily during gestation. Birth weight (BW) and the sex of lambs were recorded within 12 h after parturition. Each Awassi ewe was left to deliver the placenta naturally and placentas were collected immediately after delivery. Placental weight (PW) was measured and recorded after removing placental fluid. The total cotyledon numbers (TCN) and total cotyledon weights (TCW) of placental cotyledons dissected from the chorioallantois were also counted and determined. Cotyledon length (CL), depth (CDe), and width (CWi) were measured with a digital compass and 30 cotyledons of the same size were selected (small, <10 mm diameter; medium, 10–30 mm diameter; large, >30 mm diameter). Placental efficiency (PE) was calculated for each Awassi ewe, as the ratio of lamb birth weight to placental weight (PW). Cotyledon density (CD) was calculated as the number of cotyledons per gram PW. Cotyledon efficiency (CE) was defined as the ratio of kid BW in grams to the total cotyledon surface area (TCSA). TCSA was calculated after the measurements of all the cotyledons in individual placenta as cm^2 with the following formula: radius squared of cotyledon [((CWi + CL) / 4)^2] × 3.14 (π) × TCN. PE was calculated as the ratio of kid BW to PW for each Awassi ewe.

SPSS (2004) statistical software was used to analyze the data with the license of Ondokuz Mayıs University. Every linear model has a direct effect and amount of indirect effect which is number of explanatory variables minus one. The general expression of multiple regression model formed for the measurements (one response and p explanatory variables) is given in Equation 1.

$$y_k = \beta_0 x_{k_1}^{\beta_1} x_{k_2}^{\beta_2} x_{k_3}^{\beta_3} \ldots x_{k_p}^{\beta_p} e_i ; \quad i = 1,2,\ldots,n \qquad (1)$$

The multiple linear regression model adopted was

$$\hat{y}_k = b_0 + b_1 x_{k1} + b_2 x_{k2} + b_3 x_{k3} \qquad (2)$$

where:

$\hat{y}_k$ = response variable (BW),

b_0 = intercept,

b_i = standardized regression coefficients,

x_{kp} = explanatory variables (CG, BL, HS)

A path coefficient (P) is a standardized regression coefficient (b) showing the direct effect of an independent variable on a dependent variable in the path model (Garson, 2008; Önder and Abacı, 2015). Path coefficient, which indicates the effect of one standard deviation change of any explanatory variable X versus on response variable Y, can be calculated as (Mendes et al., 2005).

$$Pyx_k = b \frac{Sx_k}{S_y} \qquad (3)$$

Here; Pyx is the path coefficient which indicates the direct effect of X explanatory variable on response variable Y,Sx indicates the standard deviation of X, Sy indicates the standard deviation of Y and b indicates the partial regression coefficient. Path coefficients can be shown with path diagrams. One way and two way arrows are used in path diagrams. One way arrows which named as direct effects are drawn from explanatory variable to response variable and two way arrows which showed correlations are drawn between explanatory variables (Tahtali et al., 2011).

To obtain the path coefficients should be replaced in linear equation system as given in Equation 4.

$$\begin{bmatrix} P_{YX_1} \\ P_{YX_2} \\ P_{YX_3} \end{bmatrix} = \begin{bmatrix} 1 & r_{X_1X_2} & r_{X_1X_3} \\ r_{X_2X_1} & 1 & r_{X_2X_3} \\ r_{X_3X_1} & r_{X_3X_2} & 1 \end{bmatrix}^{-1} * \begin{bmatrix} r_{YX_1} \\ r_{YX_2} \\ r_{YX_3} \end{bmatrix} \qquad (4)$$

In the Equation 4, coefficients given by P_{YXi} were path coefficients (direct effects) between explanatory variable and response variable and $r_{xixj}P_{YXi}$ represented indirect effects of explanatory variable i^{th} on response variable via explanatory variable j^{th}, r_{xixj} represented pearson correlation coefficients between i^{th} an j^{th} traits (Topal et al., 2008)..

RESULTS AND DISCUSSION

Direct and indirect effects of explanatory variables on birth weight in Awassi ewes are present Table 1. The direct effects of PE on birth weight were found statistically significant (p<0.05). Additionally, direct effects of PW, TCN, TCSA, CE, TCV, VCE and PE on birth weight were not found statistically significant. However, PW had tend to be the direct effect on birth weight (0.340). The indirect effects of VCE on birth weight were found statistically significant (p<0.05). Moreover, direct effects of PW, TCN, TCSA, CE, TCV and PE on birth weight were not found statistically significant in the present study. While PE was found with highest direct effect (0.532) on BW, PE had lowest (-0.067) indirect effect on BW. The highest total indirect effect (0,431) on BW was found in VCE variable. Additionally, the direct effect (-0.188) of TCV was lowest on BW.

Table 1. Direct and indirect effects of explanatory variables on birth weight in Awassi ewes.

Trait	Direct effects	Indirect effect
PW	0.340	-0.379
TCN	0.049	0.059
TCSA	0.296	-0.22
CE	0.160	0.081
TCV	-0.188	0.106
VCE	-0.151	0.431*
PE	0.532*	-0.067

PW= placental weight, TCN= total cotyledon numbers, TCSA= total cotyledon surface area, CE= cotyledon efficiency, TCV= total cotyledon volume, VCE= volumetric cotyledon efficiency, PE= plancenta efficiency. * p<0.05

Placental traits, such as placental weight, cotyledon numbers, cotyledon efficiency, plancenta efficiency etc., are important indicators of the postnatal survive of lambs in sheep (Dwyer et al., 2005; Sen et al., 2013). Therefore, some factors affecting lambs birth weight should be determined. Owing to this, the path analysis is very important for determining factors affecting lamb birth weight (Önder and Abacı, 2015). In this study, which aimed to investigate the direct, indirect and total effects of placental characteristics on birth weight in Awassi lambs. Mostly, evaluation of relationship between placental characteristics and birth-related traits data relational statistics such as regression and correlation are used (Ocak et al., 2013; Sen et al., 2013; Sen and Onder, 2016). Birth weight of the lambs were selected as response variable and placental characteristics were selected as explanatory variables in the present study. Therefore, path analysis was used to describe the directed dependencies among a set of variables in the present study. Onder et al. (2017) direct and total effects on lamb birth weight were supported by studies in Akkaraman lambs. Some direct and total effects of placental traits on lamb birth weight were similar with results of Onder et al. (2017).

CONCLUSIONS

As a result, it was concluded that PE and VCE could be used for management decisions and as indirect selection criteria for selection on lamb birth weight due to PE had the highest direct and the lowest indirect effect lamb birth weight in Awassi sheep breed.

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LIFE ANALYSIS AND APPLICATION IN LIVESTOCK

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Abstract

Survival analysis is a collection of analysis methods used to examine the time elapsed until the emergence of any phenomenon identified by the researcher. In medical studies, such as clinical trials comparing two or more trials; time is taken as the beginning of the individual's participation in the experimental study. Life analysis is researched not only in the field of medicine but also in many branches such as economy, social life, zoology and botany. Explain the methods that use the formation processes of some situations in the population.
The purpose of survival analysis is to obtain survival probability estimates at different times, to estimate survival time distribution, and to compare life time distributions of different patient groups. In this study, the results obtained by applying life analysis to the data set obtained from animal husbandry will be explained.

Key words: *Survival analysis, Kaplan-meier, time series, livestock*

INTRODUCTION

Survival analysis is the analysis of the time period until the occurrence of so-called failure, such as death and deterioration. For living things, the concept of death and for the inanimate are often mentioned (Miller, 2011). Survival analysis is mostly used in medicine, engineering and social sciences. This study investigates the application of survival analysis in the field of Animal Science. Thus, it is shown that life analysis can be used in zootechnics as well as in medicine (Cox, 2018). In this study, the survival analysis of small ruminants selected from the field of Animal Science was introduced and a practical application of the Kaplan-Meier test was performed (Efron, 1988).
Survival analysis is the realization of an event depending on time and the importance of the factors affecting this event during the realization process. Life analysis can be applied to any time-dependent event rather than just death and illness (Sutton et al., 2000). The analysis made for this purpose is also called monitoring or observation analysis.
Any material other than living material, such as human and animal, can also be used as the material in survival analysis (Zhang,1997). Furthermore, the most important feature that distinguishes life analysis from other analyzes is that the event is not necessarily defined for all subjects or samples. Especially in animal studies and animal breeding, genetic parameter estimation allows survival analysis to be a usable analysis (Vukasinovic, 1988). In studies on animals, quantitative yield characteristics such as meat, milk yield, egg yield and economic yield level durations depending on time and survival analysis and methods can be examined in determining the characteristics that affect this period (Dusrocq, 1997).
The aim of this study is to define the survival analysis and to examine an application in the field of Animal Science. In addition to the widespread use of survival analysis in the medical field, it also shows good results when used in other fields. In this study, Kaplan-Meier test of survival analysis was applied to the data obtained from livestock and interpreted in tables (Cox, 1972).

MATERIALS AND METHODS

In this study, milk data of Damaskus crossbreed and Pure Damaskus goat herd consisting of two different races were used to make an application of survival analysis in the field of animal husbandry (Seker et al., 2004; Van et al., 2014). In this flock, the identification code for the last lactation of goats of different ages was analyzed for survival analysis (Harman etal., 1996). In milk goat, the life expectancy of milk yield of

Damaskus hybrid and Pure Damaskus races was analyzed by Kaplan-Meier test (Rich et al., 2010). IBM SPSS Statistic 23 package program was used for data analysis. Survival analysis methods investigate the events occurring at predetermined time intervals (Li et al., 1994). This method creates completed and incomplete events. Events that cannot be obtained in cases where the event of interest does not occur within the specified time interval or if the event is excluded from the investigation is called an incomplete event. The expression of survival is the state in which the event in the monitoring process after a certain starting point is investigated or attained the desired state. One feature that distinguishes survival analysis from other analyzes is the absence of incomplete data. The data generated in the survival analysis may not occur within the defined periods of the identified event. The data obtained in these cases is called incomplete data (Guyot et al., 2012).

Kaplan-Meier method was used for life analysis. Kaplan-Meier method was used to compare the races Log-Rank test. Time is very important in Kaplan-Meier method (Jager et al., 2008; Goel et al., 2010). The Kaplan-Meier method is the general form of survival curves. Log-Rank test is used to compare these curves. In the Kaplan-Meier method, a probability is estimated for each event. The distinguishing feature the Kaplan-Meier method from life analysis is that it evaluates each time of death separately (Ture et al., 2009; Smith et al., 2001). Thus, survival occurs in the form of probabilities and step function.

The assumptions of Kaplan-Meier method; when the subjects began to work, the date of occurrence of the events and the occurrence of losses should be known. In addition, the event and losses should not occur at the same time (Vukasinovic et al., 2001).

RESULTS AND DISCUSSION

Widely used in the determination of productive life length in animal husbandry, the lifestyle of various breeds of goats helps to manage the milk yield age (Zırhlıoğlu and Kara, 2004). In this study, the survival of two different breeds in milk yield was analyzed by Kaplan-Meier test. Table 1 shows the completed and incomplete data of two different breeds. According to Table 1, 10 out of 18 goats from Damaskus race and 7 out of 27 goats from pure damaskus race were evaluated as incomplete observation.

Table 1. Kaplan Meier analysis according to the complete and incomplete observations and percentages

Group	Total N	N of Events	Censored	
			N	Percent
Damascus	18	10	8	44.4%
Pure Damascus	27	7	20	74.1%
Overall	45	17	28	62.2%

Table 2. As a result of Kaplan-Meier analysis of the average of two different races and median life expectancy. In Table 2, the average milk yield age was 5 in the Damaskus breed, while the average milk yield age was 7 in the Pure Damaskus breed. In addition, while the standard error of the Damaskus race was 0.497, the standard error of the pure Damaskus race was 0.612. Thus, it is seen that the length of life of the pure Damaskus race on milk yield is longer than the Damaskus hybrid race (Vukasinovic et al., 1997).

Table 2. Kaplan-Meier Analysis and Average Life Expectancy.

Group	Mean			
			95% Confidence Interval	
	Estimate	Standart Error	Lower Bound	Upper Bound
Damascus	5.00	0.497	4.348	6.295
Pure Damascus	7.00	0.612	5.869	8.268

In Table 3, it is stated that two different races are equal in terms of survival. In addition, as a result of the Log rank test, the chi square test statistic was calculated as 1.838 and p> 0.05.

Table 3. Survival distributions for the different levels of Grup

	Chi-quare	df	Sig.
Log Rank (Mantel-Cox)	1.838	1	0.175

Finally, Graph 1 shows the milk yield ages of two different breeds. According to Table 1, it is seen that the milk dam age is higher than that of the pure damaskus race, which is the second group.

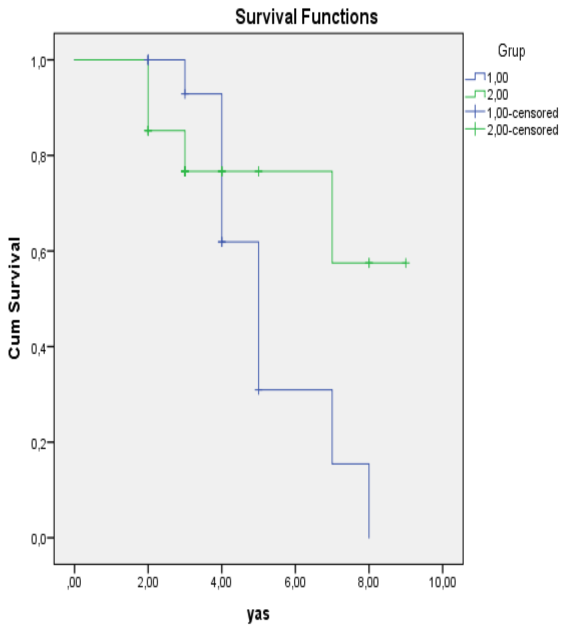

Figure 1. Survival times of two different races. 1. Group: Damascus, 2. Group Pure Damascus

As a result, it was seen that the length of life of the pure damask race on milk yield was longer than the Damaskus hybrid race. In other words, the results of the Log-rank test statistics for the data obtained as a result of the study were significant according to the insignificant level of 0.05. In addition, according to Kaplan-Meier life analysis hypothesis, the mean life milk yield age was different between the groups.

CONCLUSIONS

In this study, Kaplan-Meier test was used to determine the survival rate of milk yields of different breeds. As a result of the analysis, it is shown that the length of life of the pure damask race on milk yield is longer than that of the Damaskus hybrid race. In addition, as a result of the Log rank test, the chi square test statistic was found to be 1.838 and $p > 0.05$ was statistically insignificant.

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MODELING OF LACTATION MILK YIELD WITH SPLINE REGRESSION METHOD

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Abstract

In this thesis, modeling of lactation milk yields by means of the Spline Regression method was examined comparatively with a classical model (Wood). The thesis data were obtained from the Izmir Cattle Breeders' Association. 24960 milk yield record of 832 Holstein-Friesian dairy cows was used. From the available data, both general averages and individual modeling were performed separately for the animals in lactates 1, 2 and 3. 7 different Cubic Spline Regression models were compared with Wood model. According to the results obtained, the Spline Regression method showed successful results in individual curve modeling. In addition, it is concluded that lactation milk yields do not form a standard cubic curve and can be a successful modeling for curves with undulating yields.

Key words: *spline regressions, wood model, holstein-friesian, lactation curves*

INTRODUCTION

Modeling of lactation curves has become a necessary issue for researchers who will make dairy farming and breeding studies. Many classic models have been developed and developed for years. Modeling can be used to estimate lactation curves, the amount and shape of the milk yield curves. Nowadays, individual modeling of lactation curves is a more preferable method. Spline regression method, which is seen as rising value in breeding studies in recent years, has a superior estimation success because it has high coefficient of determination and low error squares mean values in modeling both individually and in general averages.

In this study, 24960 milk yield records of 832 Holstein-Friesian cattle in 1, 2 and 3 lactation were used. Separate modeling of each animal's milk yield was done by using both regressions and Wood model which is a classic model for comparison.

Numerous studies have been done on modeling and application of piecewise regressions. In these studies, it is possible to express spline regressions under more than one heading. It can be named by different names such as linear spline, polynomial spline, cubic spline and restricted cubic spline. In parametric or non-parametric regression analyzes, analyzes can be performed using either appropriate or appropriate methods. In the next section, comparative and / or explanatory studies using spline regressions are summarized.

MATERIALS AND METHODS

The study was conducted with data from 832 cattle of Holstein-Friesian breed from Izmir Cattle Breeders Association. The cattle which are the study material consist of females who gave birth between 2005-2013. The lactation curves obtained by using the values of 305 milk yield day 305 of 305-day milk yields obtained from lactations 1, 2 and 3 of cattle were analyzed on an individual basis and the results were obtained.

For each cattle included in the study, the yields obtained on the milk yield day were recorded. Only 305-day milk yields were taken into consideration and animals yielding more than or less than 305 days were not included in the study. In the analysis of the data, it was commented on the results obtained from the data processed by SAS 9.0 using cubic spline regression method.

In this study, individual lactation curves obtained from milk yields of 1, 2 and 3 lactations of calf cows between 10th month of 2005 and first month of 2013 were modeled by individual cubic spline regression method and the results were compared with Wood model. 1, 2 and 3 lactations were also compared with the results of the average yield.

In the study, Wood model is used with its own name, while the model used for the spline regression method has 7 different functions since it is a cubic spline regression. Due to the formation of different nodes on the curve and therefore different intervals are used as Cubic1, Cubic2, Cubic3, Cubic4, Cubic5, Cubic6 and Cubic7. For the Cubic1 function, the 2nd milk yield day (60th day) is determined as the node, 90th day for Cubic2, 120th day for Cubic3, 150th day for Cubic4, 180th day for Cubic5, 210th day for Cubic Day 240 is determined as the joint. In the comparison of the results, the values to be taken into consideration are the values of Wood and Cubic7.

In this study, the common error squares mean, determination coefficient, Akaike Information Criteria and Durbin Watson (autocorrelation) test statistic were taken into consideration in comparing the models and evaluating the model fit.

RESULTS AND DISCUSSION

In the light of this study, while Wood model is successful in individual modeling of lactation curves, the real success is that piecewise regression yields very good results in yields different from the expected and known standard lactation curve.

The results showed that the classical Wood model gave very good results in individual modeling of lactation curves. Compared to the Wood model, the results of the spline regression model on the basis of general averages are also satisfactory.

When the lactation sequences are compared, the coefficients of determination resulting from the modeling of the 3rd lactation data are high and the mean error squares are low. This result showed that modeling with spline regressions was more consistent and consistent with the structure of 3rd lactation data consisting of non-scattered data.

When the results were interpreted in terms of Durbin-Watson values, it was found that autocorrelation was mostly absent for the wood model while it was mostly in the region of instability.

When the Akaike Information Criteria is considered, it is concluded that the spline model is more appropriate than Wood. Due to the fluctuating data structure of the overall means of the 2nd lactation, the Wood model appeared to be more appropriate than the spline model, albeit with a slight difference, but the superiority of the spline model in terms of AIC values is indisputable.

CONCLUSIONS

Lactation curves while to find its place in world literature in recent years has not been demonstrated to be modeled as an individual of this modeling study in Turkey. Therefore, the study will serve as an example for further research.

In conclusion, it is shown that estimation of individual lactation curves by continuous cubic segment regression method gives very successful results.

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This study is summarized from the master thesis.

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SHEWHART CUSUM AND EWMA CONTROL GRAPHICS AND AN APPLICATION

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Abstract

In this study, SHEWHART (control charts for variable quantities), CUSUM (cumulative sum control charts), EWMA (exponential weighted moving average control charts) control graphics have been examined. These statistical Qualıty charts (SQC) techniques have been applied to the data set received from the company and prospective predictions have been made for production process.

Key words: *SQC charts, Shewhart, Cusum, Ewma.*

ESTABLISHMENT OF EFFECTIVE PLACENTAL CHARACTERISTICS ON BIRTH WEIGHT IN BAFRA SHEEP BREED WITH PATH ANALYSIS

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Abstract

Path analysis is used to describe the directed dependencies among a set of variables and application of path analysis in animal breeding improve breeding practices. The aim of this study was to examine the direct and indirect effect of some placental traits on lamb birth weight born to Bafra sheep breed using path analysis. For this aim, relationship between lamb birth weight and seven traits (placental weight; PW, total cotyledon numbers; TCN, total cotyledon surface area; TCSA, cotyledon efficiency; CE, total cotyledon volume; TCV, volumetric cotyledon efficiency; VCE, plancenta efficiency; PE) were studied in 40 singleton - bearing sheep. Birth weight, lamb sex and placental measurements were recorded within 12 h after parturition. The direct effects of PE on birth weight were found statistically significant (p<0.05). Additionally, the indirect effects of PW on birth weight were found statistically significant (p<0.05). While PE was found with highest direct effect (0.602) on BW, the highest total indirect effect (-0,468) on BW was found in PW variable. The direct effect (-0.046) of TCN was lowest on BW. Also, the indirect effect (0.093) of PE was lowest on BW. The results showed that PW and PE were the most explanatory variable for birth weight of Bafra lambs.

Key words: *path analysis, lamb birth weight, placental traits, direct effect, indirect effect, Bafra*

INTRODUCTION

Placental characteristics are important indicators of the postnatal mortality of offspring in small ruminants (Dwyer et al., 2005). Mellor and Stafford (2004) reported that the postnatal viability of newborns is associated with placental growth and development during gestation. The exchange capacity of ovine placenta between maternal and fetal systems depends on placental size and number of placentomes (Ocak et al., 2013; Sen and Onder, 2016). Therefore, placental size, which is related to the nutrient transfer capacity of the placenta, plays a pivotal role in determining the prenatal growth trajectory of the fetus and hence birth weight and postnatal viability (Sen et al., 2013). Placental growth and development support fetal development during mid- to late gestation (Redmer et al., 2004; Sen et al., 2013). Previous studies indicated that there are significant relationships between placental weight and birth weight of the newborn (Osgerby et al., 2003; Dwyer et al., 2005; Sen et al., 2013; Sen and Onder, 2016).

In general, the aim of animal breeding is to genetically improve populations of livestock so that they produce more efficiently under the expected future production circumstances (Önder and Abacı, 2015). Genetic improvement for economic traits is achieved by selecting the best individuals of the current generation and by using them as parents of the next generation (Dekkers et al., 2004). In many cases, the offspring with higher birth weight are selected as future breeding material or these criteria are used to valorize the animals. Placental traits have important effect on birth weight of the newborn (Osgerby et al., 2003; Dwyer et al., 2005; Sen et al., 2013; Sen and Onder, 2016). Generally, to evaluate relationship between placental characteristics and birth-related traits data relational statistics such as regression and correlation are used (Ocak et al., 2013; Sen et al., 2013; Sen and Onder, 2016). Birth weight of the newborn is selected as response variable and placental characteristics are selected as explanatory variables. Therefore, it is aimed to explain the response variable from explanatory variables. However, indirect effects of explanatory variables on response variable should be considered beside the direct effects (Arı and Önder, 2013). Path analysis is used to describe the directed dependencies among a set of variables (Önder and Abacı, 2015). Therefore, application of path analysis in animal breeding practices began to increase (Önder and Abacı, 2015). In addition, there is lack of studies that examined the direct and indirect effects of placental characteristics on birth weight of the newborn. The aim of the current study was therefore to investigate direct, indirect and total effects of placental characteristics on lamb birth weight in Bafra ewes.

MATERIALS AND METHODS

Experimental animals were of the Bafra breed, 3–5 years of age, and maintained at the Sheep Farm of Ondokuz Mayis University, Samsun, Turkey. The study was conducted on 42 singleton-bearing Bafra ewes in the normal breeding season. All Bafra ewes were housed and cared for under the same conditions in the stockyard and were allowed to graze for 5 h daily during gestation. Birth weight (BW) and the sex of lambs were recorded within 12 h after parturition. Each Bafra ewe was left to deliver the placenta naturally and placentas were collected immediately after delivery. Placental weight (PW) was measured and recorded after removing placental fluid. The total cotyledon numbers (TCN) and total cotyledon weights (TCW) of placental cotyledons dissected from the chorioallantois were also counted and determined. Cotyledon length (CL), depth (CDe), and width (CWi) were measured with a digital compass and 30 cotyledons of the same size were selected (small, <10 mm diameter; medium, 10–30 mm diameter; large, >30 mm diameter). Placental efficiency (PE) was calculated for each Bafra ewe, as the ratio of lamb birth weight to placental weight (PW). Cotyledon density (CD) was calculated as the number of cotyledons per gram PW. Cotyledon efficiency (CE) was defined as the ratio of kid BW in grams to the total cotyledon surface area (TCSA). TCSA was calculated after the measurements of all the cotyledons in individual placenta as cm^2 with the following formula: radius squared of cotyledon $[((\mathrm{CWi} + \mathrm{CL}) / 4)^2] \times 3.14\ (\pi) \times \mathrm{TCN}$. PE was calculated as the ratio of kid BW to PW for each Bafra ewe.

SPSS (2004) statistical software was used to analyze the data with the license of Ondokuz Mayıs University. Every linear model has a direct effect and amount of indirect effect which is number of explanatory variables minus one. The general expression of multiple regression model formed for the measurements (one response and p explanatory variables) is given in Equation 1.

$$y_k = \beta_0 x_{k_1}^{\beta_1} x_{k_2}^{\beta_2} x_{k_3}^{\beta_3} \ldots x_{k_p}^{\beta_p} e_i; \quad i = 1,2,\ldots,n \qquad (1)$$

The multiple linear regression model adopted was

$$\hat{y}_k = b_0 + b_1 x_{k1} + b_2 x_{k2} + b_3 x_{k3} \qquad (2)$$

where:

$\hat{y}_k$ = response variable (BW),

b_0 = intercept,

b_i = standardized regression coefficients,

x_{kp} = explanatory variables (CG, BL, HS)

A path coefficient (P) is a standardized regression coefficient (b) showing the direct effect of an independent variable on a dependent variable in the path model (Garson, 2008; Önder and Abacı, 2015). Path coefficient, which indicates the effect of one standard deviation change of any explanatory variable X versus on response variable Y, can be calculated as (Mendes et al., 2005).

$$Pyx_k = b\frac{Sx_k}{S_y} \qquad (3)$$

Here; Pyx is the path coefficient which indicates the direct effect of X explanatory variable on response variable Y,Sx indicates the standard deviation of X, Sy indicates the standard deviation of Y and b indicates the partial regression coefficient. Path coefficients can be shown with path diagrams. One way and two way arrows are used in path diagrams. One way arrows which named as direct effects are drawn from explanatory variable to response variable and two way arrows which showed correlations are drawn between explanatory variables (Tahtali et al., 2011).

To obtain the path coefficients should be replaced in linear equation system as given in Equation 4.

$$\begin{bmatrix} P_{YX_1} \\ P_{YX_2} \\ P_{YX_3} \end{bmatrix} = \begin{bmatrix} 1 & r_{X_1X_2} & r_{X_1X_3} \\ r_{X_2X_1} & 1 & r_{X_2X_3} \\ r_{X_3X_1} & r_{X_3X_2} & 1 \end{bmatrix}^{-1} * \begin{bmatrix} r_{YX_1} \\ r_{YX_2} \\ r_{YX_3} \end{bmatrix} \qquad (4)$$

In the Equation 4, coefficients given by P_{YXi} were path coefficients (direct effects) between explanatory variable and response variable and $r_{xixj}P_{YXi}$ represented indirect effects of explanatory variable ith on response variable via explanatory variable jth, r_{xixj} represented pearson correlation coefficients between ith an jth traits (Topal et al., 2008).

RESULTS AND DISCUSSION

Direct and indirect effects of explanatory variables on birth weight in Bafra ewes are present Table 1. The direct effects of PE on birth weight were found statistically significant (p<0.05). Additionally, direct effects of PW, TCN, TCSA, CE, TCV and VCE on birth weight were not found statistically significant. The indirect effects of PW on birth weight were found statistically significant (p<0.05). Moreover, direct effects of TCN, TCSA, CE, TCV, VCE and PE on birth weight were not found statistically significant in the present study. While PE was found with highest direct effect (0.602) on BW, the highest total indirect effect (-0,468) on BW was found PW variable. The direct effect (-0.046) of TCN was lowest on BW. Also, the indirect effect (0.093) of PE was lowest on BW.

Table 1. Direct and indirect effects of explanatory variables on birth weight in Bafra ewes.

Trait	Direct effects	Indirect effect
PW	0.371	-0.468*
TCN	0.046	0.303
TCSA	0.115	0.178
CE	0.068	0.197
TCV	-0.088	0.188
VCE	-0.089	0.428
PE	0.602*	0.093

PW= placental weight, TCN= total cotyledon numbers, TCSA= total cotyledon surface area, CE= cotyledon efficiency, TCV= total cotyledon volume, VCE= volumetric cotyledon efficiency, PE= plancenta efficiency. * p<0.05

Placental traits, such as placental weight, cotyledon numbers, cotyledon efficiency, plancenta efficiency etc., are important indicators of the postnatal survive of lambs in sheep (Dwyer et al., 2005; Sen et al., 2013). Therefore, some factors affecting lambs birth weight should be determined. Owing to this, the path analysis is very important for determining factors affecting lamb birth weight (Önder and Abacı, 2015). In this study, which aimed to investigate the direct, indirect and total effects of placental characteristics on birth weight in Bafra lambs. Mostly, evaluation of relationship between placental characteristics and birth-related traits data relational statistics such as regression and correlation are used (Ocak et al., 2013; Sen et al., 2013; Sen and Onder, 2016). Birth weight of the lambs were selected as response variable and placental characteristics were selected as explanatory variables in the present study. Therefore, path analysis was used to describe the directed dependencies among a set of variables in the present study. Onder et al. (2017) direct and total effects on lamb birth weight were supported by studies in Akkaraman lambs. Some direct and total effects of placental traits on lamb birth weight were similar with results of Onder et al. (2017).

CONCLUSIONS

As a result, it was concluded that PW and PW could be used for management decisions and as indirect selection criteria for selection on lamb birth weight due to PW had the highest direct and PE the lowest indirect effect lamb birth weight in Bafra sheep breed.

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DIFFERENCES BETWEEN SHEWHART CUSUM AND EWMA CONTROL GRAPHICS

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Abstract

The aim of this study is to explain the use of quality control charts to provide process control. In the study control charts for variables (Shewart), cumulative sum quality control charts (CUSUM) and exponentially weighted moving average control charts (EWMA) are used. The differences between them are explained with the sample data.

Key words: *SQC charts, Shewhart, Cusum, Ewma.*

A MONTE CARLO SIMULATION STUDY ROBUSTNESS OF MANOVA TEST STATISTICS IN NORMAL AND STUDENT-T DISTRIBUTION

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Abstract

The aim of this study is to compare the robustness of Manova test statistics against the Type I error rate using the Monte Carlo simulation in Normal and Student-t distribution. In the method, numbers have been generated according to constant and increasing variance for g=3,4,5 group p=3,5,7 dependent variables n=10, 20, 50 sample size using the RStudio. The specified combinations have been repeated 10,000 times. As the result Pillai Trace test statistic has been the least deviating from the nominal α =0.05 value. Wilks' Lambda and Hotelling-Lawley Trace test statistics results have been close to each other. While researchers conclude their analysis, they can decide on the results of the comparison proposed in this study.

Key words: *Monte-Carlo, simulation, normal distribution, Student-t, distribution*

INTRODUCTION

The one-way multivariate analysis of variance (one-way MANOVA) is used to determine whether there are any differences between independent groups on more than one dependent variable. The most important assumptions are multivariate normality and homogeneity of variance-covariance matrices. The most well known and widely used MANOVA test statistics are Wilk’s Λ, Pillai, Lawley-Hotelling, and Roy’s test.

Wilk’s Λ: Wilks' lambda (Wilks,1932) is a test statistic used in multivariate analysis of variance (MANOVA) to test whether there are differences between the means of identified groups of subjects on a combination of dependent variables. Wilks' lambda is the oldest multivariate test statistic, and is the most widelyused (Johnson and Wichern, 1982)

Let,

T: Total sums of squares and cross-products matrix
B: Between-group sums of squares and cross-products matrix
W: Within-group sums of squares and cross-products matrix
p: Number of dependent variables in each group
g: The number of groups $g \geq 2$.
$\bar{x}$: Overall sample mean vectors
n_i : sample size for the i-th group
S_i : sample covariance matrix for the $i-$th sample

Thus B and W matrix can be expressed by

$$B = \sum_{i=1}^{g} n_i(\bar{x}_i - \bar{x})(\bar{x}_i - \bar{x})' \quad W = \sum_{i=1}^{g}(n_i - 1)\, S_i \qquad (1)$$

The Wilks' Lambda statistic is the ratio of the within generalized dispersion to the total generalized dispersion

$$\Lambda = \frac{|W|}{|B+W|} = \frac{|W|}{|T|} \qquad (2)$$

takes values between zero and one. The Wilks’ Lambda can be obtained as a product of eigenvalues which can be obtained by the eigenvalues of the matrix of BW^{-1} by following method

$$\Lambda = \prod_{i=1}^{s} \frac{1}{1+\lambda_i} \qquad (3)$$

where s = min(p, g−1) and the rank of the B matrix and the expression λ_i are eigenvalues of the BW^{-1} matrix. According to Johnson and Wichern the Wilks’ Lambda performs, in a multivariate setting, with a combination of dependent variables - the same role as the F-test performs in a one-way analysis of variance. Bartlett (1954) using a chi-square test instead of an F-distribution test. Bartlett's test is a modification of the corresponding likelihood ratio test designed to make the approximation of the chi-square distribution better at all stages as formuled

$$V = -[N - 1 - (p + g)/2]ln\Lambda \qquad (4)$$

denotes the χ^2 distribution of p (g-1) degrees of freedom if $V > \chi^2_{Tablo[p(g-1)];\alpha}$ there is a difference between the mean vectors. The Wilks Lambda statistic can salso be calculated with the help of the F distribution. In different groups, variables and observation numbers, approach to F distribution and degrees of freedom are available.

Hotelling-Lawley Trace (T): The Hotelling ve Lawley Trace (1938) statistic which defined as follows (Seber 1984).

$T = trace(BW^{-1}) = \sum_{i=1}^{s} \lambda_i$

The F distribution can be used to test the T statistic (Stevens 1986). T is the trace of the BW $^{-1}$ matrix (Hotelling 1931; Lawley 1939).

Pillai''s Trace (V): Pillai (1955) trace statistic can be interpreted as the proportion of variance in the dependent variables which is accounted for by variation in the independent variables. The V statistics where s, m, n parameters are as follows;

s=$min\ (g\text{-}1,p)$, $m = \frac{|p-(g-1)|-1}{2}$,$n = \frac{N-p-g-1}{2}$, $\frac{2n+s+1}{2m+s+1} \times \frac{V}{s-V}$

closed F distribution with s(2m+s+1) and (2n +s+1) degrees of freedom (Morrison 1976).

Roy's Largest Root (R): If the big eigenvalue of the matrix of BW $^{-1}$ is denoted by λ_{max} Roy's R statistic is given by $R = \sum_{i=1}^{s} \frac{\lambda_{max}}{1+\lambda_{max}}$.

This value is compared to the Heck graph value with parameter s, m, n. If the R statistic is greater than the Heck graph value, it is said to be the difference between the mean vectors (Alpar 2013). When s = 1, R shows exact F distribution (Kanık 1999).

MATERIALS AND METHODS

This investigation deals mainly to assess the robustness of MANOVA. To do is the Multivariate Normality assumption is violated to see if that will affect Type I error rate. In order to evaluate the robustness of MANOVA the virtual experiment was designed in the following way. For the significance test of difference between the groups, the number of groups was determined as g=3, g=4, g=5. Dependent variable numbers were set at p= 3, p=5, p=7 for each group. Sample size determined as n=10, 20, 50. That simulation was based on 10,000 replications. The Monte Carlo study manipulated in equal variance (σ12 = σ22=…= σg2) and unequal variance (σ12 < σ22…< σg2). When establishing the unequal variance, the variance of a dependent variable was first set, then the other dependent variables were multiplied by 3, that mean variance ratio is (1:3).

All of the statistical methods were conducted using R (MVNormTest written by Slawomir on 04/12/2012: Normality test for multivariate variables package). In order to test the hypothesis used to compare the mean of more than two groups the Wilks' Lambda(W), Pillai's Trace(V), Hotelling-Lawley Trace(T), Roy's Largest Root test(R) statistics values and their Type I error rate were calculated. If p-value was less than 0.05, the nominal alpha level, the null hypothesis was rejected. The data are produced in the Normal and Student-t distribution. Scenarios were prepared in 54 different combinations for each test statistic. These operations were repeated 10,000 times and the number of null hypothesis rejections was determined for each test statistic. Experimental Type I error rates were calculated for each test statistic with dividing the rejection number by the repeat number.

RESULTS AND DISCUSSION

Monte Carlo test result for R,V,T,W test statistics is given respectively Table 1, Table 2, Table 3 and the comments are below.

Table 1. For g=3, p=3, 5, 7; sample size n=10,20,50 experimental Type I error rate with 10000 raplicate.

g	p	variance	n	Roy (R)		Pillai Tracks (V)		Hotelling-Lawley (T)		Wilks Lambda (W)	
				N	t	N	t	N	t	N	t
3	3	constant	10	0,0453	0,0847	0,0523	0,0814	0,0495	0,0759	0,0468	0,077
			20	0,0463	0,0724	0,0493	0,0763	0,0469	0,0753	0,0486	0,0724
			50	0,0499	0,0697	0,0478	0,0692	0,0517	0,0699	0,0486	0,0716
		Increase	10	0,0509	0,0794	0,0514	0,0792	0,0528	0,0771	0,0506	0,0822
			20	0,0505	0,0733	0,0524	0,0749	0,0507	0,0716	0,0525	0,0756
			50	0,0455	0,071	0,0493	0,0712	0,048	0,0745	0,0476	0,0729
	5	constant	10	0,0563*	0,1002	0,0512	0,0983	0,048	0,0967	0,0528	0,1065
			20	0,0495	0,0875	0,0532	0,0908	0,0491	0,0915	0,048	0,091
			50	0,049	0,0838	0,0495	0,088	0,0487	0,0777	0,0492	0,0879
		Increase	10	0,0496	0,0988	0,0485	0,1028	0,0483	0,0981	0,0527	0,1031
			20	0,052	0,0904	0,052	0,0911	0,0466	0,0895	0,0468	0,0939
			50	0,05	0,0854	0,0486	0,0842	0,051	0,082	0,0504	0,0839
	7	constant	10	0,0464	0,1235	0,0502	0,1249*	0,0432*	0,1251*	0,0484	0,1192
			20	0,051	0,1024	0,0452*	0,1089	0,0521	0,098	0,05	0,1075
			50	0,0495	0,0932	0,0533	0,1004	0,0477	0,0976	0,0516	0,0947
		Increase	10	0,0472	0,1262*	0,0496	0,1228	0,0482	0,1236	0,0533	0,1247*
			20	0,0531	0,1041	0,0494	0,1085	0,05	0,1074	0,0482	0,1103
			50	0,0497	0,1011	0,0463	0,0921	0,054	0,1007	0,0508	0,101

When group number is g=3, for all values of p, observations are interpreted in Normal and Student-t distribution according to sample size for Roy Largest Root test statistics with Figure 1.

For the Roy test statistic in Normal Distrubition, constant and increasing variance; when the sample size and the number of variables increased, it was seen that deviations from Type I error decreased. For Roy test statistic in g=3, the highest deviation was seen in all scenarios when p=5 n=10,constant variance with 0.0563. In Student-t distribution ıt was observed that deviations from 0.05 are generally high and between 0,068-0,126. The highest deviation in Student-t distribution was seen in all scenarios when p=7 n=10,increasing variance with 0.1262.

When group number is g=3, for all values of p, observations are interpreted in Normal and Student-t distribution according to sample size of Pillai's Trace test statistics with Figure 2.

For Pillai in Normal distribution when p=3, both constant and increasing variance, deviations from nominal α =0.05, increase as the sample size (n value) increase. For Pillai test statistic in g=3, the highest deviation was seen in all scenarios when p=7, n=20, in constant variance with 0.0452 value. In Student-t distribution for Pillai test statistic in g=3, the increase of p negatively affected the deviations.

The highest deviation was seen in all scenarios when p=7, n=10, in increasing variance with 0.1249.

When group number is g=3, for all values of p, observations are interpreted in Normal and Student-t distribution according to sample size of Hotelling-Lawley test statistics with Figure 3.

For Hotelling-Lawley in Normal distrubition when p=3 as sample size grows, deviations decrease and p=5 per group, both constant and increasing variance, deviations for all sample sizes are minimum. The highest deviation is seen when p= 7 n=10, constant variance with 0,0432. In Student-t distribution, it was observed that the deviation increases when the number of variables (p) increases. For Hotelling-Lawley test statistic deviation sfrom 0,05 are between 0,069-0,13. The highest deviation was seen in all scenarios when p=7, n=10, in constant variance with 0.1251. It was observed that as the sample size increased, the deviations decreased for all variable values.

When group number is g=3, for all values of p, observations was interpreted in Normal and Student-t distribution according to sample size of Wilks’ Lambda test statistics with Figure 4.

Wilks’ Lambda in Normal Distrubition when p=3, both constant and increasing variance, deviations from nominal α =0.05, increase as the sample size (n value) increase. As p=5 the highest deviation is seen; when n =20 for increasing variance. The highest deviation was seen in all scenarios when p=7, n=10, in constant variance with 0,0533. In Student-t distrubition, as p=3, p=5, p=7, both constant and increasing variance it was seen that deviations are decrease when sample size increase. The highest deviation was seen in all scenarios when p=7, n=10, in increasing variance with 0.1247 value.

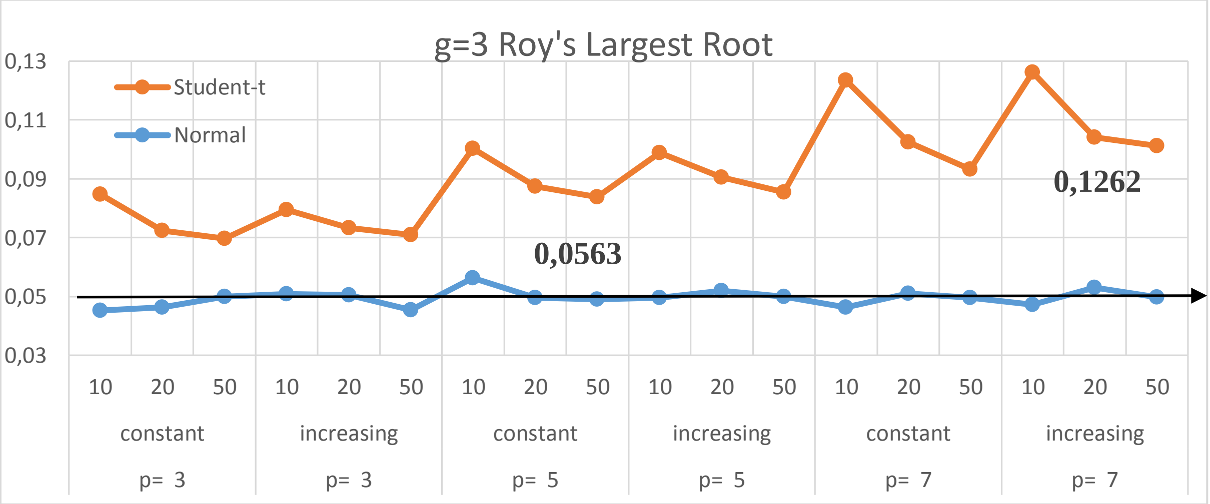

Figure 1. Type I error rates for Roy Largest Root test statistic in g=3

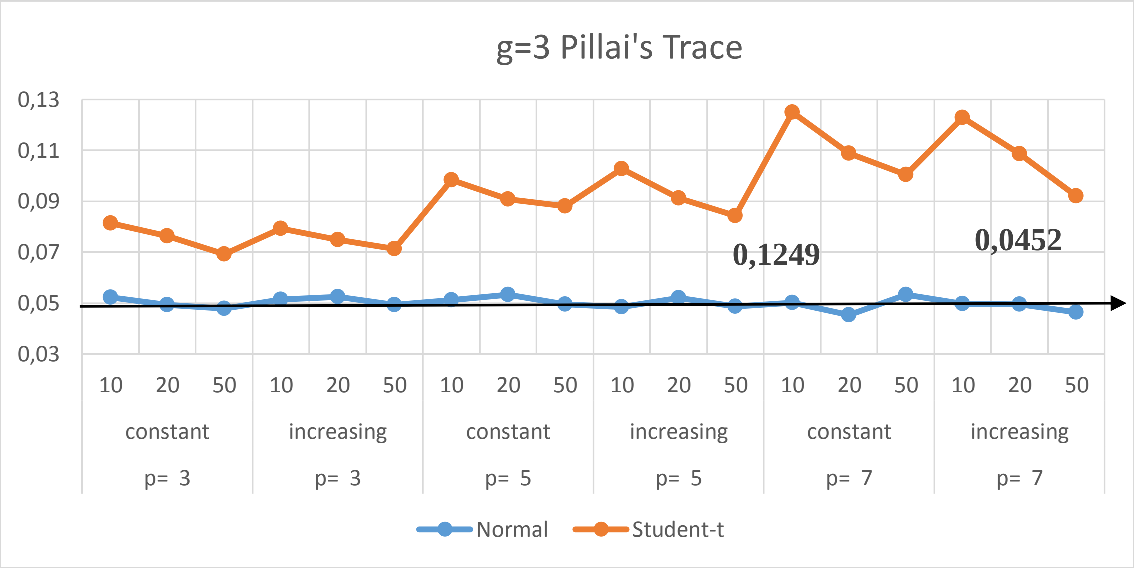

Figure 2. Type I error rates for Pillai’s Trace test statistic in g=3

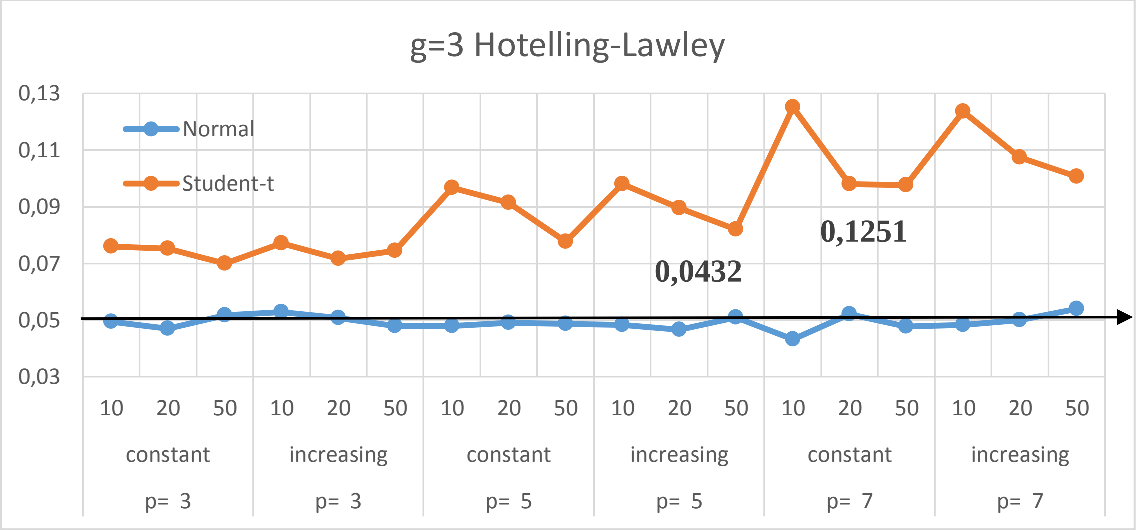

Figure 3. Type I error rates for Hotelling-Lawley test statistic in g=3

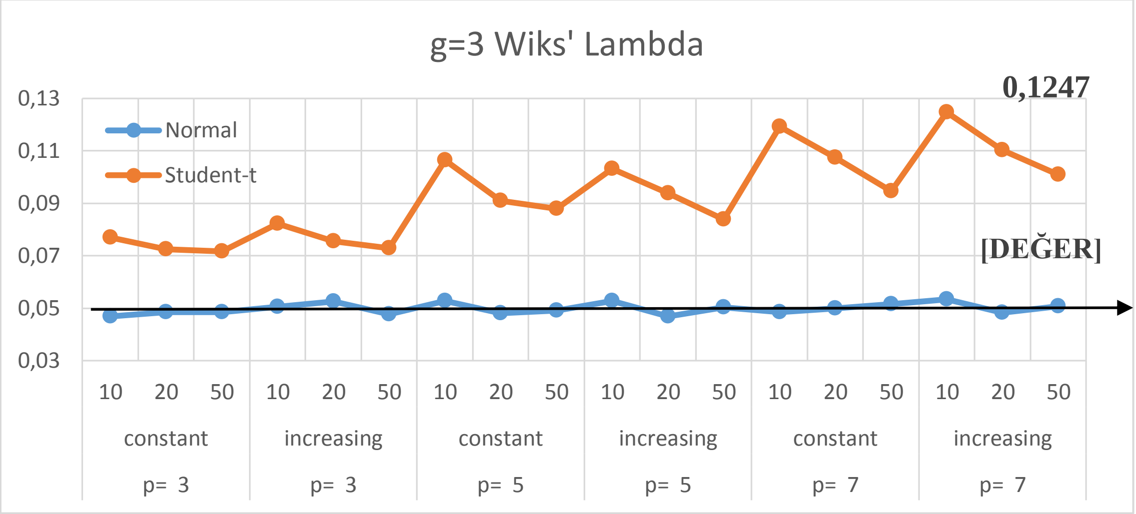

Figure 4. Type I error rates for Wilks’Lambda test statistic in g=3

Table 2. For g=4, p=3, 5, 7; sample size n=10,20,50 experimental Type I error rate with 10000 raplicate

g	p	variance	n	Roy (R)		Pillai Tracks (V)		Hotelling-Lawley (T)		Wilks Lambda (W)	
				N	t	N	t	N	t	N	t
4	3	constant	10	0,0526	0,0783	0,0509	0,0778	0,0465	0,0817	0,0516	0,081
			20	0,0495	0,0764	0,0486	0,0744	0,0492	0,0773	0,0476	0,0741
			50	0,0496	0,0736	0,0498	0,0745	0,0487	0,0744	0,0472	0,0713
		Increase	10	0,048	0,0787	0,0499	0,0786	0,0458	0,0779	0,0538	0,0759
			20	0,0528	0,0783	0,0454	0,0753	0,0495	0,0747	0,0526	0,072
			50	0,0491	0,0745	0,0502	0,0726	0,0503	0,0751	0,0482	0,0735
	5	constant	10	0,0483	0,0974	0,0518	0,0937	0,0493	0,0978	0,0478	0,1007
			20	0,0456	0,0917	0,0506	0,0911	0,0482	0,0923	0,047	0,0928
			50	0,0465	0,0842	0,0479	0,0837	0,052	0,0877	0,0499	0,0855
		Increase	10	0,0517	0,098	0,0491	0,0976	0,0532	0,0949	0,0493	0,098
			20	0,0553*	0,0916	0,0485	0,0916	0,0463	0,0873	0,0503	0,0931
			50	0,0493	0,0885	0,0492	0,0851	0,0462	0,0839	0,0444*	0,087
	7	constant	10	0,0498	0,1136*	0,0568*	0,1142*	0,0514	0,1137*	0,0511	0,1136
			20	0,0506	0,1029	0,0521	0,1092	0,0498	0,1054	0,0518	0,1051
			50	0,0484	0,0999	0,0512	0,0952	0,0504	0,1052	0,0501	0,1013
		Increase	10	0,0515	0,1114	0,0508	0,1124	0,0562	0,1119	0,0491	0,1145*
			20	0,0521	0,1076	0,0506	0,1039	0,05	0,099	0,0517	0,1038
			50	0,0498	0,0943	0,0508	0,0974	0,0477	0,0972	0,0508	0,0969

When group number is g=4, for all values of p, observations are interpreted according to sample size of Roy Largest Root test statistics with Figure 5.

For the Roy test statistic, it was observed that the deviations for all variable values and sample size were quite low. As the p = 5 the highest deviation is seen when n=20 for increasing variance in all scenarios with 0,0553. In Student-t distribution the results closest to 0.05. Deviations are between 0,073-0,1136. The highest deviation in the was observed when p = 7 n = 10, and this deviation was the highest one in all scenarios with 0,1136.

When group number is g=4, for all values of p, observations are interpreted according to sample size of Pillai's Trace test statistics with Figure 6.

For Pillai'sTrace when p=3, big deviations is seen when the sample size n=20 for the increasing variance. For Pillai test statistic in g=4, the highest deviation was seen in all scenarios when p = 7 n = 10 with 0,0568. In Student-t distribution, deviations are increased as variable values increased. The deviations for each variable value are inversely proportional to the sample size. As sample size grows, deviations decrease. The highest value 0,1142, while p = 7, n = 10 while constant variance was observed.

When group number is g=4, for all values of p, observations are interpreted according to sample size of Hotelling-Lawley test statistics with Figure 7.

For Hotelling-Lawley in Normal distrubition when p=3 both constant and increasing variance as sample size grows, deviations decrease and as p=5 deviations are big for increasing variance. The highest deviation is seen when p=7 n=10. In Student-t distrubition the closest results to the nominal α =0.05 value were seen when p = 3 both at constant and increasing variance. Also 0,1137 is which is the highest value in Student-t distrubition all scenarios. For p=5 deviation ranged from 0,8-0,9. For p=7 deviation ranged from 0,9-0,11.

When group number is g=4, for all values of p, observations are interpreted according to sample size of Wilks'Lambda test statistics with Figure 8.

For Wilks' Lambda in Normal distribution when p=3, both constatnt and increasing variance, deviations decreased while sample size increased. For Wilks Lambda test statistic, the highest deviation was seen in all scenarios when p=5, n=10, in increasing variance with 0,0567 value. In the Student-t distribution, In the case of increasing and constant variance, deviations with sample size for all variable values were inversely proportional. the closest results to the nominal α =0.05 value were seen when p = 3 both at constant and increasing variance. For p=5 deviation ranged from 0,8-0,10. For p=7 deviation ranged from 0,9-0,11.

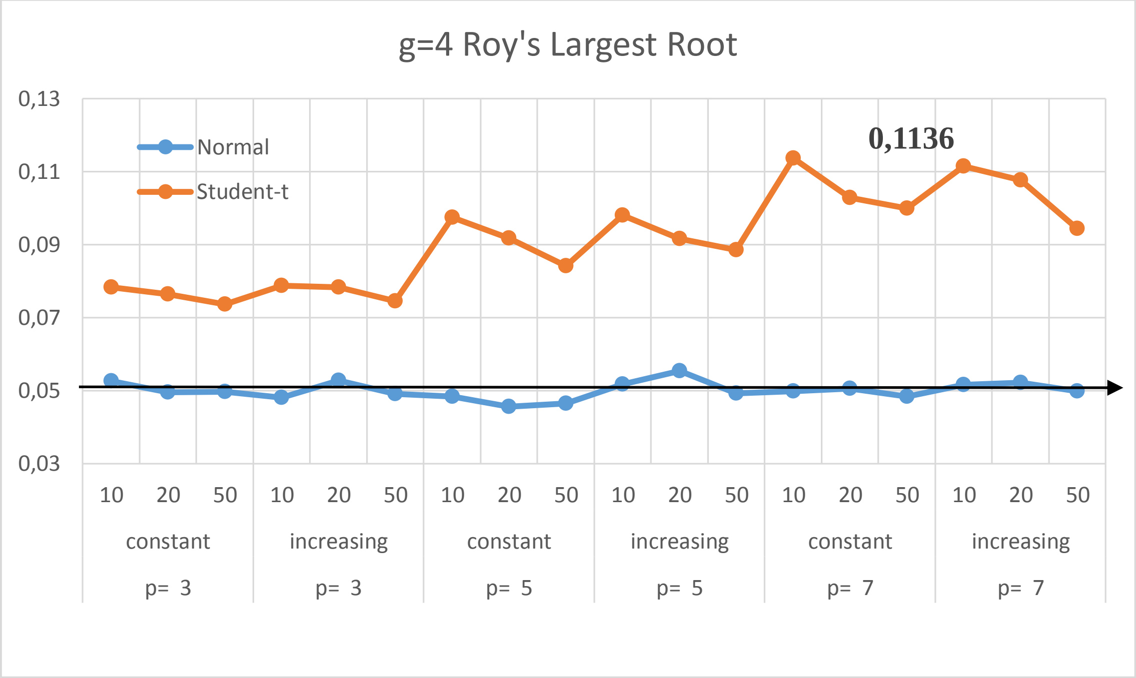

Figure 5. Type I error rates for Rooy Largest Root test statistic in g=3

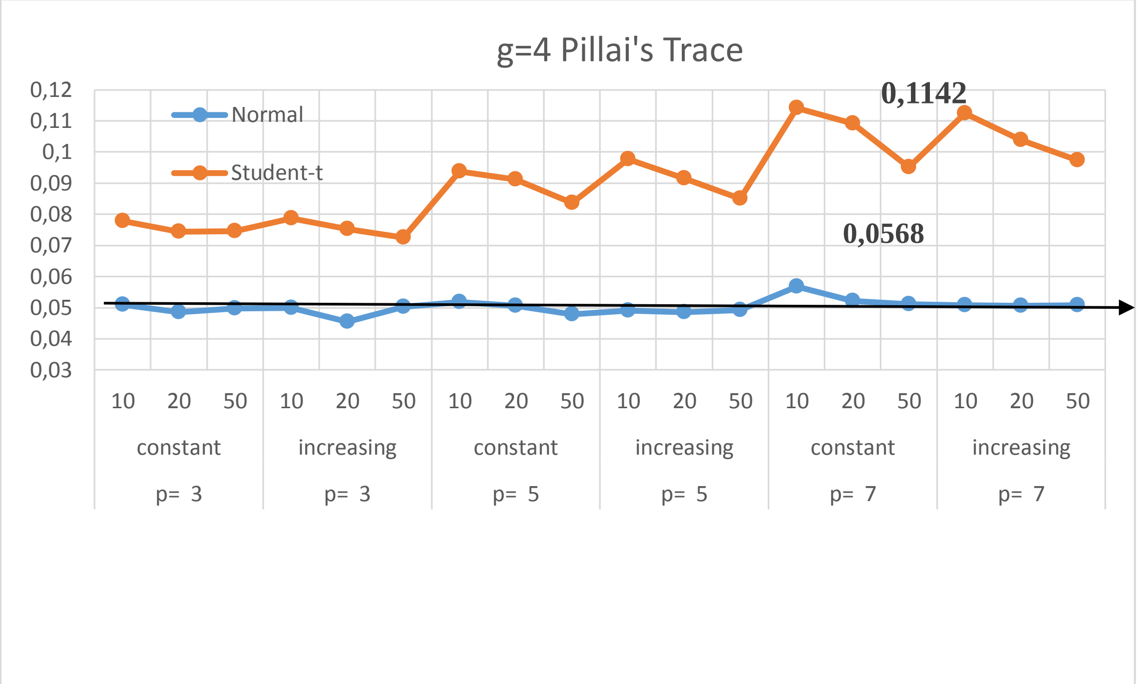

Figure 6. Type I error rates for Pillai's Trace test statistic in g=4.

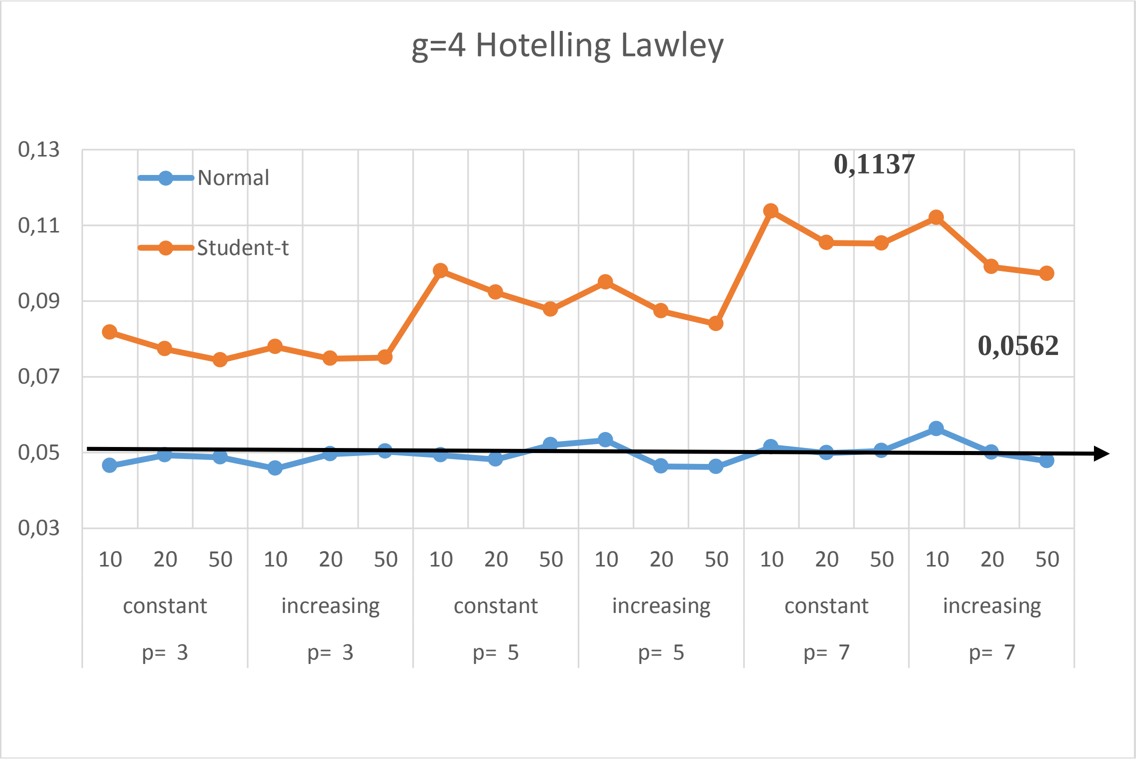

Figure 7. Type I error rates for Hotelling-Lawley test statistic in g=4

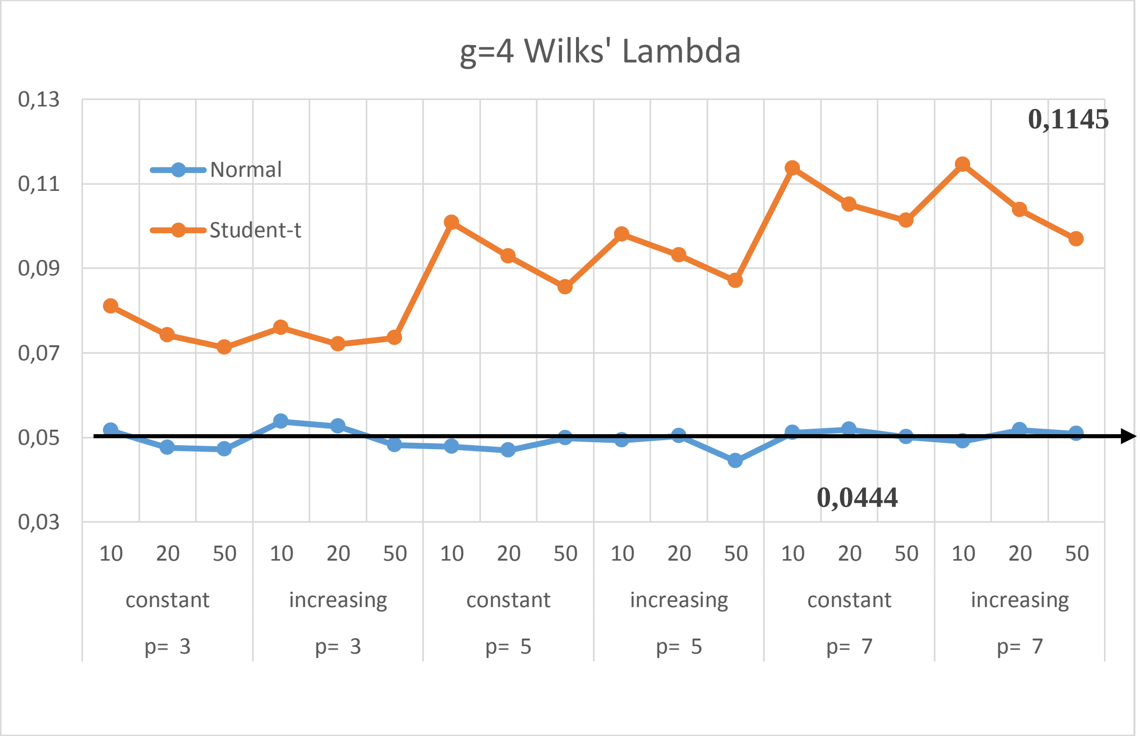

Figure 8. Type I error rates for Wilks' Lambda test statistic in g=4

Table 3. For g=5, p=3, 5, 7; sample size n=10,20,50 experimental Type I error rate with 10000 raplicate

g	p	variance	n	Roy (R)		Pillai Tracks (V)		Hotelling-Lawley (T)		Wilks Lambda (W)	
				N	t	N	t	N	t	N	t
5	3	constant	10	0,0505	0,075	0,0515	0,0706	0,0482	0,0759	0,049	0,0719
			20	0,0491	0,0699	0,0491	0,0741	0,0576	0,074	0,0502	0,0734
			50	0,0516	0,0752	0,0529	0,0713	0,0498	0,0683	0,0474	0,071
		Increase	10	0,049	0,0773	0,0503	0,0765	0,0528	0,0721	0,0491	0,0723
			20	0,048	0,0764	0,0537	0,0752	0,0498	0,0758	0,0501	0,0758
			50	0,0479	0,0709	0,0458	0,0742	0,0492	0,0707	0,0514	0,0752
	5	constant	10	0,0499	0,0912	0,0487	0,0889	0,0486	0,0875	0,053	0,0907
			20	0,0489	0,0863	0,0492	0,0888	0,0502	0,0896	0,0485	0,086
			50	0,0494	0,0892	0,0438*	0,0871	0,0528	0,0801	0,0493	0,0843
		Increase	10	0,0549	0,0894	0,0499	0,0912	0,0466	0,0905	0,0517	0,0884
			20	0,0508	0,085	0,0518	0,085	0,0499	0,0916	0,0513	0,0866
			50	0,0514	0,0847	0,052	0,087	0,0487	0,0827	0,0507	0,0842
	7	constant	10	0,0508	0,1049*	0,0465	0,1053*	0,051	0,0989	0,0492	0,1067
			20	0,0487	0,0973	0,0461	0,1014	0,0469	0,1022	0,0472	0,1069
			50	0,047	0,0943	0,0494	0,0967	0,0551	0,0982	0,0482	0,0971
		Increase	10	0,0469	0,101	0,0494	0,1027	0,0507	0,1041*	0,0521	0,107*
			20	0,0515	0,0964	0,0506	0,1019	0,0495	0,1033	0,0496	0,1054
			50	0,0482	0,0992	0,051	0,0927	0,0466	0,0952	0,048	0,0957

When group number is g=5, for all values of p, observations are interpreted according to sample size of Roy Largest Root test statistics with Figure 9.

For Roy in Normal distrubition it is observed that the nominal α=0,05 value is maintained relatively. For Roy test statistic in Student-t distrubition the highest deviation was seen in all scenarios when p=7, n=10, in constant variance 0.1049. In Student-t distribution, the deviation increases as the number of variables increases.

When group number is g=5, for all values of p, observations are interpreted according to sample size of Pillai’s Trace test statistics with Figure 10.

For Pillai test statistic deviations are usually below 0.05 for all variable values. In g=5, the highest deviation was seen in all scenarios when p=5, n=50, in constant variance with 0,0438. In the Student-t distribution deviations ranged between, 0,07-0,10.

When group number is g=5, for all values of p, observations are interpreted according to sample size of Hotelling-Lawley test statistics with Figure 11.

For Hotelling-Lawley in Normal distribution when p=3 per group, both constant and increasing variance, the highest deviation is seen when n=20. As p=5 the highest deviation is seen; when n = 10 for the constant variance and when n=10 for the increasing variance. As the number of variables p = 7 the highest deviation is seen; when n=50 for both constant and increasing variance. For Hotelling-Lawley test statistic in g=5, the highest deviation was seen in all scenarios when p=3, n=10, in constant variance with 0,0576 value. In Student-t distribution, the deviation increases as the number of variables increases and also 0,1041 is the highest deviation as p=7,n=10 in increasing variance.

When group number is g=5, for all values of p, observations are interpreted according to sample size of Wilks’Lambda test statistics with Figure 12.

For Wilks’Lambda in Normal distribution when p=3 the highest deviation was seen; when n = 50 for both constant and increasing variance. When p = 5, the greatest deviation was seen when n=10 for the constant variance.

When p=7 the highest deviation was seen; increasing variance. For Wilks Lambda test statistic in g=5, it is observed that the nominal α=0,05 value is maintained relatively. In Student-t distrubition, deviations from the nominal value were increased as the variable values increased. In large variable values when n = 20 for constant variance, n=10 deviations are big. When p=3 both increasing and constant variance the deviation is around 0.7. When p=5 the deviations are ranged 0,08-0,09. When p=5 the deviations are ranged 0,09-0,10.

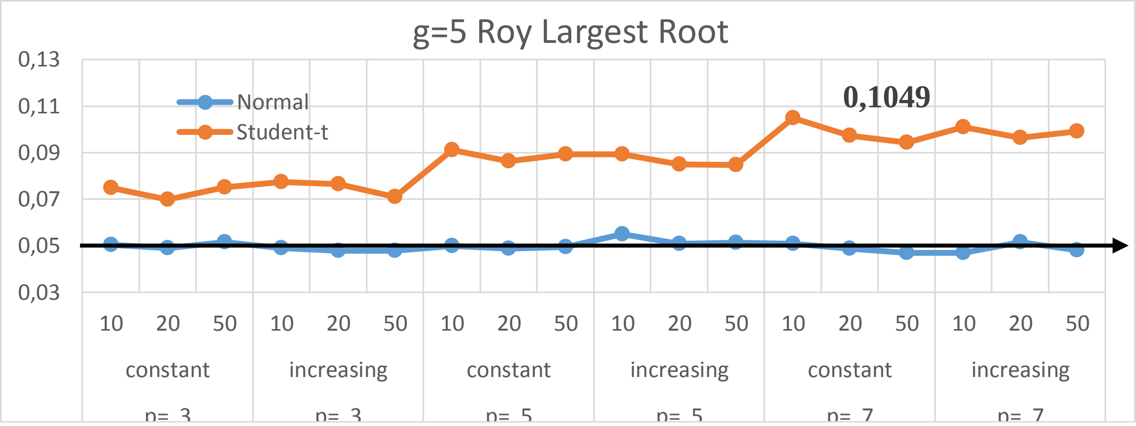

Figure 9. Type I error rates for Roy Largest Root test statistic in g=5

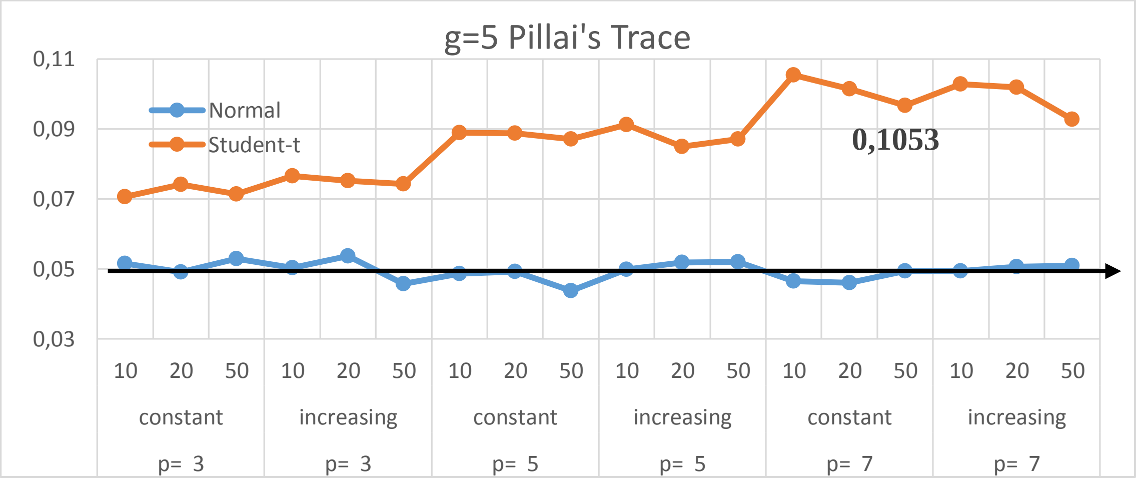

Figure 10. Type I error rates for Pillai’s Trace test statistic in g=5

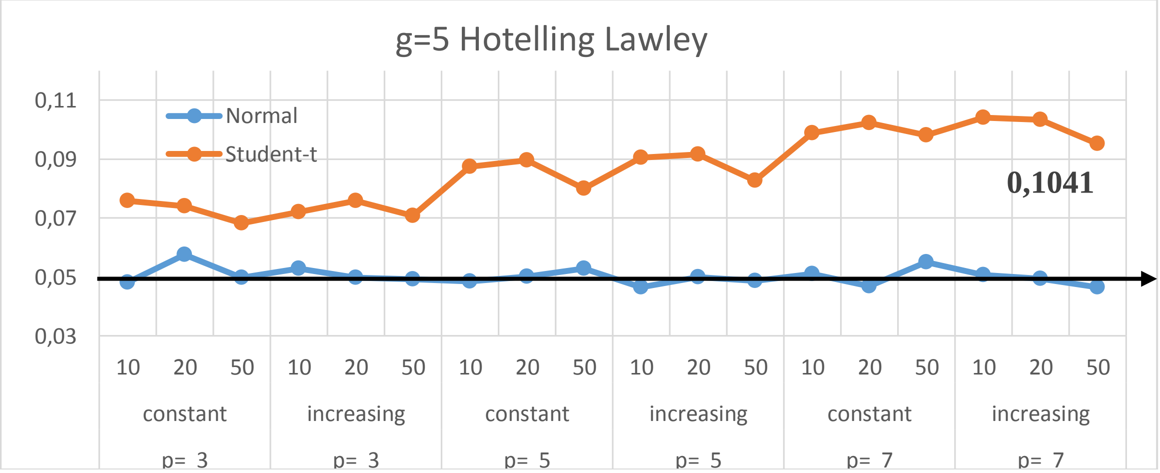

Figure 11. Type I error rates for Hotelling-Lawley test statistic in g=5.

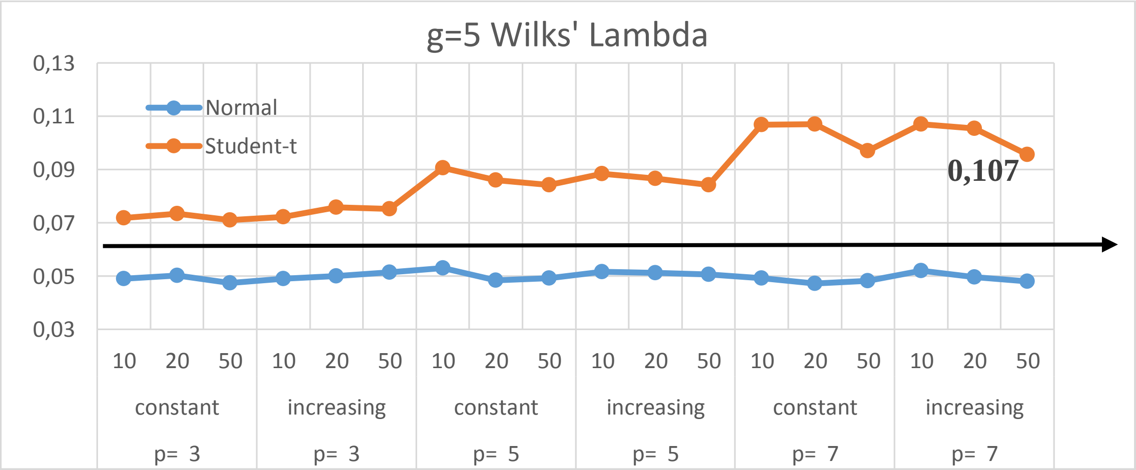

Figure 12. Type I error rates for Wilks'Lambda test statistic in g=5.

CONCLUSIONS

In this study, 54 design points were created for 10, 20 and 50 observations with 3, 4, 5 variable numbers 3, 5, 7, constant and increasing variance groups for each test statistic. The results of the Monte Carlo simulation run 10,000 times with each design and numbers are produced from Normal and Student-t distributions. Results are as follows. In Normal distribution in cases where the deviation from the Type I error rate deviates from the value of 0.05, it is mostly observed in the R test statistic followed by W and T statistics. W and T statistics were given close results in terms of the maximum bias. In the V statistic, the maximum deviation scenarios are less common than the other test statistics. This study suggests that the Pillai Trace statistic works well in the Normal Distribution. Other studies are that found the Pillai Trace test statistic to be reliable in the form of Olson (1974), Hopkins and Clay (1963), Holloway and Dunn (1967), Ito (1969), Seber (1984), Korin (1972) and Davis (1980,1982). The details of the test statistics which give the best results in constant and increasing variance cases with different sample sizes, group numbers and variable numbers according to the derivation when comparing the scenarios for both distributions are presented below.

In case of constant variance in Normal distribution;

When group number is 3, Pillai's Trace statistic,

When the group number is 4, the Pillai's Trace,

When the group number is 5, Wilks's Lambda statistic can be suggested.

However, in the case of constant variance, it can be said that Wilks' Lambda and Pillai's Trace gave better results regardless of the sample and variable numbers.

In case of increasing variance;

When the group number is 3, Pillai's Trace statistic

When the group number is 4, Wilks' Lambda statistic

When the group number is 5 Wilks' Lambda statistic, can be suggested.

However, it can be said that in general, the Wilks' Lambda and Pillai's Trace gave better results regardless of the sample and variant number.

In Student-t distribution, in the case of constant variance;

When the group number is 3, Hotelling Lawley statistic

When the group number is 4 Pillai's Trace statistic

When the group number is 5, Hotelling Lawley statistic can be suggested.

In the case of constant variance, it can be said that Hotelling Lawley and Pillai's Trace statistic gave better results regardless of the sample and variable numbers.

In case of increasing variance;

When group number is 3, 4, 5 Hotelling Lawley statistic can be suggested.

However, in the case of increasing variance, it can be said that Hotelling Lawley statistic in general is gave better result, regardless of the number and variety of statistics.

In general, when all the test statistics are examined, the Type I error ratios of the Pillai test statistic are the least deviating from the nominal α = 0.05 value, as in many studies. However, the theoretical distribution of this statistic is not known precisely. Using the Monte Carlo method, researchers can produce critical values at some Type I error rates and degrees of freedom, and they can present a comparative chart of the literature.

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THE EFFECTS OF DIFFERENT LIGHTING SCHEDULES ON GAIT DYNAMICS OF BROILER CHICKENS

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Abstract

This study was to evaluate the effects of different lighting schedules on the gait dynamics of broiler chickens. For this purpose, 500 male Ross 308 chicks were raised with four different lighting schedules (125 chicks in each group) until 42 day-olds. The lighting was implemented follow as: The first schedule was near continuous lighting (23 hours of light and 1 hour of dark), the second schedule was 8L:4D, the third schedule was 4L:2D and the fourth one was continuously 16L:8D. End of the 42 days, the 25 chickens were randomly selected from each group. Each bird was tested visually according to Bristol six-point system while they were walking in an open space. Gait score 3 (GS 3) was the most determined value in all groups. However, GS 0 and GS 5 were not observed in any groups. The 23L:1D and 4L:2D resulted in a higher rate of GS 4. A high rate of GS 1 was determined in 8L:4D schedule. Neither GS 1 nor GS 4 were observed in the 16L:8D group. According to the current study, intermittent lighting might be concluded to had positive effects on broiler gait dynamics in point of welfare. Nevertheless, it was suggested that intermittent lighting which included short dark periods (2 hours of dark) had an influence more unfavorable than the lighting schedule which including long dark periods (4 hours of light).

Key words: *broiler, gait score, lighting, photoperiod*

INTRODUCTION

The leg problems in animals are emerged depending on infectious or non-infectious origin. The main cause of non-infectious leg problems in broiler chickens is a fast growth rate. Increasing feed conversion by genetic selection in broilers has also led to increased appetite. Thus, increased body mass and breast muscle in modern broilers caused unbalance in their body and also brought leg problems together with this fact (Caplen et al., 2012). The 90% of slaughter-aged chickens show varying degrees of lameness and 30% of these lameness cases may vary from moderate to severe (Garner et al., 2002). Leg problems cause pain in chickens, denied access to feed and water, preventing escape from any threat and being a handicap their welfare. At the same time, it has also economic importance because it causes live weight and abattoir losses. On the other hand, it was reported that lighting schedule which including short light period instead of continuous or near continuous schedule might reduce leg problems in broilers (Gordon, 1994). “Bristol six-point gait scoring system” which is developed by Kestin et al. (1992) is the most utilized method in the evaluation of the leg problems in chickens (Caplen et al., 2012). The system is applied based on the observation of chickens walking and measure in a total of six levels between 0-5 (GS 0 = normal, GS 5 = lame). The improvement of rearing conditions and especially the regulation of lighting programs have an important place in researches in order to reduce leg problems in broiler flocks (Bessei, 2006). It has been stated that lighting programs which include certain dark periods may help to reduce feed intake and allowing broilers to rest (Duncan, 1998).

In this context, this study was conducted to investigate the effects of different photoperiod treatments on broiler gait dynamics.

MATERIALS AND METHODS

A total of 500 one-day old male Ross 308 chicks were used in this study. The 125 chicks were distributed to four environmentally independent pens with five repetitions. In each repetition had 25 chicks with the same initial body weight. The chicks were kept under 24 hours of light for the first week. Thereafter, experimental lighting was started as: The first treatment was continuously 23 hours of light:1 hour of dark (23L:1D), the second treatment was 4L:2D. The third photoperiod was 8L:4D and the fourth one was continuously 16L:8D. The chicks were raised standard commercial rearing conditions for 42 days. At the end of 42 days, 5 birds from each repetition, a total of

100 birds were randomly selected for gait observation. While the birds were roaming on the free-space, the data were collected according to Bristol six-point system. Chi-square test was applied for evaluation of data with IBM©SPSS21 package program.

RESULTS AND DISCUSSION

GS 0 and GS 5 were not observed in any groups. A large number of GS 3 was determined in all groups. According to Bristol six-point system, in GS 3, bird uses its wings for balance and support. Bird tends to lie down unless it is gently nudged. It cannot stand for more than 15 seconds without undisturbed and lies down after several serial steps. This score also similar to GS 2. The highest rate of GS 3 was in the 16L:8D schedule. GS 4 was found to be high in 23L:1D and 4L:2D whereas it was the lowest in 8L:4D. However, 16L:8D did not contain GS 4 value.

Table 1. Chi-square results of the effects of lighting schedules on gait score

Gait score	Groups				X^2	df	P value
	23L:1D$^{\mathbf{a}}$	8L:4D$^{\mathbf{ab}}$	4L:2D$^{\mathbf{a}}$	16L:8D$^{\mathbf{b}}$			
GS1	4% (1)	8% (2)	4% (1)	0	15.675	9	0.041
GS2	24% (6)	24% (6)	12% (3)	28% (7)			
GS3	44% (11)	60% (15)	56% (14)	72% (18)			
GS4	28% (7)	8% (2)	28% (7)	0			
N	25	25	25	25			

The different lowercase superscript indicates the difference between the groups.

Leg problems in broiler chickens are affecting modern broiler industry concerning health as well as welfare. It was stated that there are many challenges associated with determining the cause of leg fault in poultry and very little is understood about their gait (Paxton et al., 2013). Slowing the growth rate by reducing feed intake, implementing good management practices such as lighting programs, good litter or floor conditions, good air quality have been shown to reduce leg problems in broiler flocks. In this study, different lighting programs have been implemented which one is included 8 hours of dark concerning the welfare regulations in poultry. It was reported that shortening of the lighting period during the rearing phase had a benefit on leg problems (Gordon, 1994). In a study, contrary to this report, many lame birds had observed in short photoperiod (Sorensen et al., 1999). In this study, a large number of GS 3 was observed. This case may have been due to the fast growth rate of broilers so they had difficulty walking.

In conclusion, intermittent lighting has a positive effect on the gait of broilers. However, reducing the rapid growth rate along with lighting will be more beneficial on gait dynamics and leg problems.

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EVALUATION OF WEIGHT AND GROWTH RATES ON AWASSI SHEEP LAMBS

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Abstract

This study was conducted at Al-Fajij station using of 2262 records to evaluate some environmental factors affecting Awassi lambs body weight and growth rate in Jordan. Data were analyzed by SAS [2012] using general linear model (GLM). Least Square Means of body weight at birth [BW], weaning at 60 days [W60], 180 days age [W180] yearling age [W360], were 4.43±0.04, 18.07±0.21, 35.96±0.35, 59.71±0.94 [KG], respectively. Growth rates from birth to weaning [GR1], from weaning to 180 days of age [GR2], and from 180 days to yearling age [GR3], were 0.216±0.03, 0.141±0.03, 0.131±0.002 [KG/day], respectively. All studied traits were significantly affected [p<0.01] by year of production [YP] and interaction between Birth Type [BT] and YP. Sex of lamb [SL] had a highly significant effect [p<0.01] on BW, WW and GR1. The BW, WW and GR2 were significantly affected [p<0.01] by BT, and also W180 and GR2 were significantly affected by BT and age of ewe [AE], respectively. These results provided that lamb body weight and growth rate could be increased by better management of Awassi lambs under condition rearing in Jordan.

Key words: *Weight, Growth rate, Awassi lambs, Jordan*

A STUDY ON THE GENE CODING LINOLEATE ISOMERASE ENZYME IN RUMEN FUNGUS

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Abstract

Conjugated linoleic acid (CLA) is a collective mixture of isomers from linoleic acid that each of them has been identified to have biological activity but basically cis-9, trans-11 and trans-10, cis-12 isomers found to have physiological effects. These isomers mostly found within meat and dairy products derived from ruminant herbivores, in where, anaerobic gut fungi (AGF) inhabits naturally. In this study, CLA formation by AGF and possible roles of synthesised CLA on Salmonellosis were investigated. AGF, isolated from the feacal samples of ruminants, identified Neocallimastix sp using both morphological characteristics and ITS sequencing data. The preliminary findings showed that isolated AGF have ability to convert linoleic acid (LA) to CLA effectively.

Key words: *Rumen Fungi, Neocallimastix, Linoleat Isomerase.*

EVALUATION OF CATTLE ENTERPRISES IN NIĞDE PROVINCE I. CURRENT STATUS

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Abstract

Cattle meat is one of the priority food items of our country in terms of the healthy nutrition value and the healthy development of the society. The effect of changing economic and geographical conditions of the country on meat consumption amounts is important. The income level of the people has a direct impact on the consumption of cattle meat. However, fluctuations in cattle meat prices from time to time can turn red meat consumption in favor of chicken meat. Uncontrolled animal husbandry policies, animal husbandry demands and different forms of business are occasionally blocking the cattle breeding industry. Various studies on cattle livestock can be insufficient on bringing the sector vitality and different perspectives and it is necessary to keep the work on the subject updated frequently and regularly. For this purpose, it was aimed to determine the current situation by applying a questionnaire to the operator in Niğde region where mostly of 10 cattle (187 farms) and family management. As a result, % 93 of the enterprises has not the planning criteria and the rest were insufficient and unhealthy that have been detected.

Key words: *Niğde, cold climate, cattle breeding, cattle barns, cattle barn planning*

INTRODUCTION

Livestock sector in Turkey are among the sectors with potential, to meet the food needs, providing job opportunities to export a significant portion of the population and contribute significantly to the economy. Processing the existing potential in the sector and providing the sector with the opportunity to compete in international markets is directly proportional to increasing the quality and efficiency of the existing production. For this purpose, determination of the problems in the agriculture and animal husbandry sector and finding permanent and applicable solutions to these problems constitute one of the priority targets of our country.

In animal husbandry, local development projects and studies have been given importance even before modern animal husbandry. It is a sustainable development model that the researchers and producers in our country are trying to realize today is the local dynamics are prioritized. However, despite all the negative structuring and preventive factors of animal breeders, cattle breeding has always been a priority consumption choice and business line in our country. At the same time livestock; is important in terms of providing products to many different sectors in the field of industry.

The number of animals and livestock enterprises are increasing day by day and official data determine this situation by years. While our total bovine assets were around 10 million heads in 2002, this figure increased by 13.2% in 2017 and reached 16 million 105 thousand heads. The number of cattle among bovine increased by 13.2% to 15 million 944 thousand heads, while the number of water buffalos increased by 13.6% to 161 thousand 439 heads. Again according to 2017 data, approximately 8 million heads of cattle, 6.536.073 heads of hybrid cattle and 1.602.925 heads of domestic cattle were recorded. The number of milked animals was approximately 6 million for 2017 and milk production was 17,762,319 tons for the same year (Anonymous, 2018). The average milk production per milked animal was calculated as 3148 kg for 2017. While the number of cattle slaughtered in 2017 was 3,602,115, the amount of meat obtained from cattle was 987,482 kg (Anonymous, 2018a). While the amount of meat obtained from beef is approximately 1.2 million tons in 2015 and 2016, it is seen that this figure has decreased in 2017 (Anonymous, 2018a). It can be said that the amount of meat falling is the result of the problems that have been going on in cattle for years. Efficiency has decreased gradually with problems such as wrong policies in the field of animal husbandry, understanding of business without project and system and lack of solutions to the problems of animal producers.

Central Anatolia Region of Turkey's has a significant potential for animal husbandry agricultural and livestock sector in particular involves. Cattle breeding; It is a sector that can get high profit against the initial investment costs and at the same time provide employment with low costs. The main source of livelihood or additional income of a significant portion of the region's population is cattle. While the animals are mostly housed in the courtyards where families live under primitive conditions, it is seen that even the large capacity enterprises do not attention necessary project standards and occupational health issues. In order to eliminate unhealthy conditions, which are one of the main problems of animal husbandry, which are seen both in the study area and in general in bovine animal husbandry enterprises and to prevent unsuitable structuring, different shelter projects should be developed according to the types and capacities of the enterprises and the applications should be meticulously and periodically inspected. With irregularly or unconsciously established barn structure, both animal health and unit yield of animal may be reduced. This will adversely affect the profit of the grower and cause a decrease in his income.

Based on these factors, in our study, it was aimed to analyze the current situation of cattle holdings in Niğde province, to maximize the efficiency obtained from livestock activities and to provide an up-to-date perspective on the problems in cattle holdings.

MATERIALS AND METHODS

The main material of our study is the primary data obtained from the questionnaires applied to bovine breeders in Niğde and Merkez, Bor and Ulukışla districts. In order to realize the objectives of the study, a survey was conducted with the producers in the region and detailed observations were made during the site visits. In the study, statistical data of the state institutions and various literature information are included as secondary data.

As of 2017, the presence of bovine animals in the province of Niğde was determined as 147.911 heads. The number of cattle of Niğde province by districts is given in Table.1 (Anonymous, 2017).

Table 1. Cattle existence of district of niğde province's in 2017 (Anonymous, 2017).

District	Cattle Existence				
	Cattle	Hybrid Cattle	Domestic Cattle	Water Buffalo	Total
Merkez	75.620	4.921	98	19	80.658
Altunhisar	5.414	2.532	115	0	8.061
Bor	32.407	1.969	64	0	34.440
Çamardı	3.025	2.325	113	0	5.463
Çiftlik	11.459	1.480	31	0	12.970
Ulukışla	5.503	691	125	0	6.319
Total	133.428	13.918	546	19	147.911

In order to start the survey and field observations, first of all, information was collected from the related animal husbandry associations and official institutions about the animal husbandry and the operating pattern in the region. The survey was conducted face-to-face in 2017, when climatic conditions were favorable and during spring and autumn, when animal husbandry activities were intense. In the surveys conducted within the scope of the research, bovine enterprises that can represent the region were determined by preliminary survey studies and random sampling method. To the local producers; questions were asked about basic issues including cattle breeding activities, business information, business management, evaluation of products obtained from the business, waste assessment systems and animal health. In order to prevent misleading answers by the farm owners and/or workers, the address information of the business and the identity of the business owner was hidden.

Questions of surveys, including the subjects that farm owners avoid giving private information, were asked repeatedly in different ways in order to maximize accuracy and thus, multiple control opportunities were provided within the survey. Considering the educational status and differences of opinion of business owners and caregivers, care was taken to ensure that the questionnaire questions were simple and understandable.

The questionnaire consists of four main sections and thirteen different subsections. In order to be able to make a clear evaluation, possible questions were added to the questions and more specific answers were obtained with some constraints. These constraints have been achieved by making clear statements on factors such as duration, number, frequency and quantity.

The data obtained as a result of the surveys were calculated with the necessary procedures and interpreted with the contribution of the observations made in the research area.

Within the scope of the study, 187 cattle holdings were interviewed and 187 survey results were evaluated. In some questions, farm owners had left some of the questions as unanswered with saying that they are not sure or cannot remember. For such questions, evaluations were made as much as the number of answers received.

General Informations About Cattle Farming

86.1% of the surveyed enterprises was in the villages of the Central district, 10.2% are in the villages of Bor and 3.7% are in the villages of Ulukışla. Frequency percentages was the three largest villages; Yeşilgölcük (15.0%), Yıldıztepe (11.8%) and Alay and Bağlama (10.7%).

The smallest village was Yeniyildiz (3.7%). Information on the distribution of villages is given in Table 2.

Table 2. Distribution of surveyed enterprises

Location	Frequency	Percentage
Bağlama (Central)	20	10,7
Hasanköy (Central)	13	7,0
Hacıabdullah (Central))	9	4,8
Kiledere (Central)	11	5,9
Edikli (Central)	18	9,6
Sazlıca (Central)	10	5,3
Agacasar (Central)	10	5,3
Alay (Central)	20	10,7
Kılavuz (Bor)	19	10,2
Yıldıztepe (Central)	22	11,8
Yeniyıldız (Ulukışla)	7	3,7
Yeşilgölcük (Central)	28	15,0
Total	187	100

When the age information of the owners was examined, the 20-30 age range was found to be 8%, the 31-40 age range was 41.2%, the 41-50 age range was 31%, the 51-60 age range was 17.6% and the age above 61 was 2.1%. The 31-40 age range, with a frequency percentage of 41.2, is the highest age range. The fact that the owners are mostly young and middle aged can be considered as a situation that allows them to be open to innovation and development. The jobs that require intensive effort are done by men and the women in the farm are might involved in animal care, barn cleaning and milking.

Tutkun et al. (2017) interviewed 192 farm owners in their research on dairy cattle farming in Diyarbakır province and they found that 32% of farm owners were between 31-40 years old, 30% were between 41-50 years old rate of young and middle-aged people.

In our study, the experience of the farm owners participating in the survey is between 6-10 years (frequency 21.9%) and then 11-20 years (frequency 18.7%).

Özkan and Erkuş (2013) reported in their research that cattle owners in Bayburt have 20 years of work experience on cattle breeding.

Şahin and Yılmaz (2008) calculated the work experience of business owners engaged in agriculture and animal husbandry in Van province as 22.86 years and reported their average age as 40.48. Aydın (2011) determined in Kars that the pasture growers' work experience in this livestock subsector is 18.96 years on average.

In our study, 98.4% of the surveyed farm owners did not receive training on animal husbandry. Although the farm owners have not received any training in this field, the fact that they have nearly 20 years of work experience shows that these activities are learned in the family and carried out according to local methods.

In our study, 59 of the 101 farm owners livelihood was on farming, agriculture and animal husbandry (frequency% 31,6) when 42 of them were working on the only animal husbandry (frequency 17.1%). The remaining owners mentioned both animal husbandry and different trade branches as their main livelihoods.

Aksoy and Yavuz (2008) reported that 43.5% of the livestock producers in Erzurum made livestock at a level that would cover family expenses, 55.4% said that they made livestock for both housing expenses and employment and commercial purposes.

Kara et al. (2007) reported that in the Eastern Anatolia Region 89% of the enterprises worked in animal husbandry to meet their needs.

The education level of the farm owners is also important for the livestock sector. It is hoped that as the educational level increases, adaptation to the developing and changing sector conditions will increase and modern structuring will be targeted. However, the education rates obtained in our study did not meet the expectations. 65.78% of the farm owners are primary school graduates, 17.65% are secondary school graduates and 16.58% are high school graduates. In the study of Ünalan et al. (2013) reported in their research that 60% of the farm owners are primary school graduates in Niğde province.

Almost all of the surveyed farm owners stated that they do not intend to expand their livestock conditions or enterprises at rate of 98.4%. When we repeated the question with different questions, it was seen that 96% of them did not look positively on farm arrangements and regeneration practices. Main reasons for this situation was the high first investment expenses, high animal sales prices and low state subventions. At the same time, their low income levels cause them to be negative for innovation. 158 (84.5%) of the 166 operators participating in the survey and answering the question provided the additional budgets required for the enterprise by their own means. Conditions expressed as their own opportunities can be expressed in the form of mutual assistance within the family, financial support of the elders of the family and personal additional income sources. The remaining farm owners stated that they used bank loans.

These financial constraints were found to be worrisome in terms of possible livestock projects that could be implemented for the region. This situation brought to mind that there was a need to implement agricultural extension activities with the local people and operators.

Of the 187 farms surveyed, 161 were combined farms (frequency 86.1%). In small-scale family businesses, this situation occurs in the form of feeding both fattening and dairy cattle. 19 of the enterprises was dairy farms and only beef cattle in 7 of them.

When the types of stables of the respondents were examined, it was found that 38 enterprises were free open barns and 64 enterprises were closed barns with added stops; 85 enterprises were not found to be of any standard. It has been observed that such enterprises feed animals randomly in areas surrounded by a fence or wall and do not comply with a certain order. No free stall open barn system was found in the enterprises. Proportions of barn types are given in Figure 1.

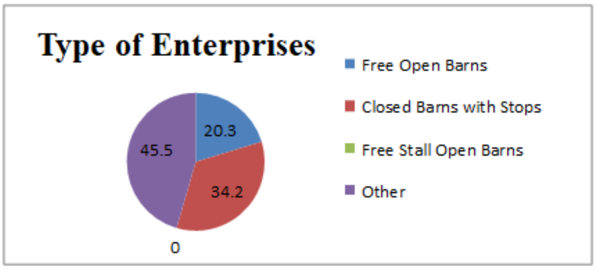

Figure 1. Barn types of enterprises

In the study of Elmaz et al. (2010) examined 677 bovine livestock enterprises in Burdur province and they determined 184 (27.2%) of these enterprises were semi open-free barns, 142 (20.9%) were closed-free barns and 351 (51.9%) were closed-fixed tied barns.

In the study carried out in Ergani district of Diyarbakır province, it was stated that all shelters in livestock enterprises are closed-tied barns and approximately 90% of them were made individually (Han and Bakır, 2011).

Tilki et al. (2013) examined the general structure of cattle holdings in the central and districts of Kars and stated that 326 (79.13%) of the holdings in the province are closed-stables. Among the reasons why this system is preferred, the first option is easy care and feeding of animals, this was followed by preferences made because it was "traditional" and "healthier than other systems".

Aydın et al. (2016) examined the shelter characteristics of cattle holdings in their study in Hınıs District of Erzurum Province and found that the most common barn type in Hınıs district was closed barn (51.5%). Tilki et al. (2013) stated that the rate of those who prefer closed tied stables in barns was 79.13%.

Tutkun et al. (2017) found that 3% of the shelters in 197 farms in Diyarbakır were open, 8% were semi-open and 89% were closed barns.

When the number of animals belonging to the enterprises is examined, it can be said that animal husbandry in the region is mostly composed of family type small enterprises. 77% of the enterprises are family-type enterprises with a maximum of 10 animals. The number of animals between 11 and 20 is 18% and the rate of enterprises with more than 30 animals is 5%. The question of animal breeds in the farm was answered as Black Pied. When the number of animals in the farms is examined in detail, 25% of the total number of animals is calf-heifer, 9% is bull and 27% is calf. The ratios of animal assets are given in Table 3.

Table 3. Number of animals

	Animal Existence (%)			
	1-10	11-20	30+	Overall Average(%)
Cow	32	9.54	3.21	38.7
Bull	8	-	-	9
Heifer-Calf	22	1.56	0.79	25
Beef	-	-	-	0.3
Calf	15	6.9	1	27

In the study conducted in the Eastern Mediterranean Region' cattle enterprises, it was found that was 71% dairy cattle, 49% native breed cattle and 19% culture hybrid cattle (Boz, 2013). In the study conducted to determine the structural characteristics of dairy cattle farms in Kayseri province, the ratio of cows was 62.9%, heifers 10% and calves were 20% in total animal existence (Şahin, 2001).

Tokmak et al. (2011), in their study in Niğde province, interviewed 100 dairy cattle owners and approximately 37% of the current animal existence was cows, 20% heifers, 20% male and female calves, and 23% male and female calves.

Feeding routines of animals also differ. Most farms (83.2%) graze their animals on pasture, but there is no specific pasture order. The remaining owners feed their animals both with feed from the factory and with feed from their own land.

Sahin and Yilmaz (2008), examined livestock enterprises in the province of Van and only 18.03% of the owners stated that they pay attention to grazing the pasture at operating capacity. In the same study; The most important problems in pasture use are reported as overgrazing, premature grazing and improper use of pasture lands.

The mismatch of standards values for enterprises can be explained by the fact that 86% of the enterprises are completely without project. Other businesses stated that they were connected to a project during the installation phase. However, when project resources are examined, only 3 out of 187 enterprises use state-controlled projects or projects drawn by engineer, the rest enterprises use as project resources that the suggestions of the elders (6%) and the implementation of the projects of environmental enterprises (8%).

The construction conditions of the enterprises and the status and characteristics of the internal structures are also examined. It is concluded that 86% of the enterprises do not have any infrastructure or drainage structure, and in those enterprises, evacuation and systems are very inadequate and unhealthy. This situation can be observed in and around the enterprise as scented water and fecal deposits, flies and intense odor. At the same time, this situation can lead to adverse health problems and shows that animals living in these conditions have more foot problems. 70% concrete, 18% soil and 12% partial concrete were used in the shelters floor.

68% briquettes were used as shelter building material. This rate is followed by 27% brick and 5% stone mesh. 69% of the roofs of the shelter was covered with sheet metal and the rest was covered with tile.

Tilki et al. (2013) reported that the most common building material for the barns in Kars is stone (39.81%) and reinforced concrete (35.44%). However, they observed that this building material was used with soil in some barns. It was found that the same situation was valid in reinforced concrete and wooden building materials and in many shelters these two building materials were used together.

The images of different structures within the enterprise are given in Figure 1.

Figure 1. Images of enterprises

Another important problem in livestock enterprises was the current status of the in-house auxiliary equipment compartments. Only 114 out of 187 firms were able to receive valid answers and classified them. The responses of the other 73 enterprises can be given as an example of builded without suitable project. In our study, the ratios of auxiliary equipments in the surveyed enterprises are given in Table 4.

This situation is not considered suitable both for the hygiene of animal products and for animal welfare. As Niğde is in a rainy climate region, the owners stated that they took the tool equipment into the barn according to the seasons and that the farm equipments was kept in the courtyard outside during the dry summer season. When the rates given in Table 4 are examined, it is seen that approximately 30% of the enterprises have animals' paddocks. However, it can be said that the padoxes are insufficient and small compared to the number of animals.

Table 4. Ratios for auxiliary equipments partitions and different warehouse

Enterprise	Auxiliary Equipment Section						
	Milking Room	Milk Room	Forage Room	Calf-Heifer Room	Padox	Waste Storage	Machine Equipment Division
Available (%) 114 entp.	9.3	0.8	19.3	12.8	29.8	0	28
	Alternative Answers						
Absent (%) 73 entp.	Storage in the basement of the house - Courtyard storage		Gathering Under Porch		Over Nylon Covered Outdoor Storage		Storage in the Barn
	63		9		11		17

In 77.8% of the enterprises with closed barn, the windows are small, the area and the number is insufficient. In the other stables, it was observed that there was sufficient window area and number, but due to their location, the air was made in such a way that the air could come into direct contact with the animals. This condition creates conditions that will adversely affect animal comfort especially in winter. Ventilation chimneys were seen in only 7 of the enterprises but it was observed that it was not sufficient in terms of project standards.

Tilki et al. (2013) found that there were no ventilation chimneys in the barn of 26 (6.31%) farms in which they conducted surveys in Kars and that there was only one ventilation chimney in 15 (3.64%) enterprises. Existing ventilation chimneys are generally found to be inadequate. It was reported that there were no windows in the stables belonging to 43 (10.44%) enterprises that participated in the survey, and there was only 1 chimneys in 23 (5.58%) enterprises.

Again, natural ventilation was preferred in almost all of the enterprises with closed barns. As a result of insufficient ventilation and lighting, the stables are dim and dark. Inadequate ventilation also causes another major problem; indoor humidity and dust problem is seen constantly. This high humidity, harmful gases and dust make breathing difficult for people working inside and animals. It can cause an increase in diseases as well as respiratory problems in animals.

An image of an enterprise that uses the inside of the barn at the same time as a storage area and an example of inadequate ventilation and lighting conditions (a cave-like barn) is given in Figure 2.

Figure 2. In-barn storage and insufficient situation (ventilation, lighting e.g).

RESULTS AND DISCUSSION

As a result of the research, serious deficiencies were determined both in terms of shelter planning and management in the existing stables in and around Niğde.

Achieving economic development and rural development in Niğde and similar cities depends on the profitability of the livestock sector and the utilization of animal products produced in the most economic way. Otherwise, the benefit will not be reflected to the producers and prevent them from developing in animal husbandry.

Most of the farm owners in the study area have not received any training in cattle breeding. They carry out their animal husbandry activities and enterprise management completely by traditional methods. Farm owners often see livestock as a source of low income. Providing the breeders in the province with practical technical information about animal husbandry will enable them to conduct business activities and animal husbandry practices more rationally, and this situation will reflect positively an increase in productivity.

It has been observed that the knowledge of the breeders in the region on some issues is insufficient and they are not open to development. For this reason, it was concluded that it would be beneficial to provide training in subjects such as shelter planning, animal nutrition and care health and hygiene knowledge in order to have more conscious breeders.

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DEVELOPMENT OF THE NEW PHOTOSENSITIVE DRUG FOR PHOTODYNAMIC THERAPY IN VETERINARY MEDICINE

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Abstract

Photodynamic Therapy (PDT) involves the use of photochemical reactions mediated through the interaction of photosensitizing drug, laser light and molecular oxygen. Molecular oxygen in the medium is transformed into singlet oxygen species by stimulating the photosensitive drug with visible light in a suitable wavelength region. Singlet oxygen formed by the interaction of drug and light in the environment shows an effect in the treatment of the damaged area. PDT method using different photosensitizers is used in clinical studies in veterinary medicine. Phthalocyanines having stable π-cojugated system are known to have very good singlet oxygen quantum yields. In this study, we synthesized the new water-soluble tetrasubstituted oxo-titanium(IV) phthalocyanine compound. This compound was synthesized in the presence of titanium(IV)butoxide of 2-methoxypyridine substituted phthalonitrile by the tetramerization reaction. As a result of the quaternization reaction of synthesized phthalocyanine, a water-soluble phthalocyanine compound was prepared. The fluorescent properties of the synthesized phthalocyanine compound were examined. As a result, it was observed that this compound had the potential to be used in PDT.

Key words: *Photodynamic therapy, veterinary medicine, phthalocyanine, drug, titanium*

INTRODUCTION

Photodynamic therapy (PDT), a new and emerging technique for the treatment of cancer using photosensitizers, involves the use of photochemical reactions mediated through the interaction of photosensitizing drug, laser light and molecular oxygen [Sellera et al., 2016; Giuliano et al., 2007; Jinling et al., 2001]. The main factor of PDT is photosensitizing drugs. In treatment of PDT, the patient receives an injection of the photosensitizer, waits for a period of time and the tumor is illuminated by light with an appropriate wavelength, respectively. The waiting time after injection is to ensure that the photosensitizer is enriched in the tumor. The excited photosensitizer then causes a series of reactions and destroys the tumor cell. Compared with conventional treatments such as surgery and radiotherapy, PDT is a highly advantageous technique in that it selectively destroys the tumor and does not seriously damage healthy tissues [Giuliano et al., 2007; DVM et al., 2013; Jinling et al., 2001]. PDT is generally used in the treatment of tumors and infections localized in the living organism and PDT is a noninvasive, painless treatment. The photodynamic approach offer a positive way forward in animal healthcare with the added bonus that there is no downside in terms of communicated drug resistance seen over time with conventional antimicrobials. At the same time, PDT has been investigated in the treatment of skin tumors in veterinary medicine and can alternative treatment of infected lesions in animals [Sellera et al., 2016; DVM et al., 2013]. It is important for the applicability of the method that the drugs to be used in PDT are water soluble. Phthalocyanines have generally investigationed as photosensitizers for use in PDT. Phthalocyanines are synthetic substances of the class of porphyrin. Phthalocyanines having stable π-cojugated system are known to have very good singlet oxygen quantum yields. The quantum yield of singlet oxygen can be further increased, depending on the metal center and the substituted groups to bind to the phthalocyanine ring [Nyokong, 2007].

MATERIALS AND METHODS

Materials and Instruments

All reactions were carried out under argon atmosphere. All solvents were dried by molecular sieves or proper methods [Armarego et al., 2003]. 2-methoxypyridine substituted phthalonitrile derivative **1** as starting material was prepared according to the literature [Yabaş et al., 2019]. IR spectra were recorded on a PerkinElmer Spectrum100 FT-IR Spectrometer using ATR. UV-vis spectrum was recorded on a Shimadzu UV-1800 UV-vis spectrophotometer. ^{1}H NMR spectra were obtained by using JEOL Resonance ECZ400S 400 MHz spectrometer. A Agilent/Cary Eclipse G9800A fluorescence spectrophotometer was used for spectrofluorometric measurements. Melting points are determined with an Electrothermal 9100 digital melting point apparatus.

*Synthesis of 2-methoxypyridine tetrasubstituted oxo-titanium(IV) phthalocyanine (**2**)*

Mixtures of **1** and titanium(IV) butoxide ($Ti(OC_4H_9)_4$) in pentanol:DMF (1:1) was heated at 180^{o}C at the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) for 15h. After cooling, the green product mixture was precipitated by MeOH and filtered off. The green solid was washed with MeOH and diethylether, respectively. It also was dried. The solid was dissolved in THF and filtered. The solvent of THF solution was removed in the evaporator and the solid was dried. The dark green solid was soluble in THF, DMF and DMSO. Yield: 36%. Mp: >300^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6, 25 ^{o}C): δ= 8.7-7.4 (br m, Pc Ar-H, Py-H); 2.7 (s, OCH_3). UV-Vis (DMSO) λ_{max}/nm; 699, 638, 350. IR (ATR) υ (cm^{-1}) 3055; 1602; 1533;1486; 1012; 875; 820; 730; 705.

*Synthesis of water soluble quaternized 2-methoxypyridine tetrasubstituted oxo-stitanium(IV) phthalocyanine (**3**)*

Mixtures of **2** and CH_3I in THF was heated at 40^{o}C for 24h. After cooling, the precipitated green solid was filtered off. Then, it was washed with diethylether and dried. The dark green solid was soluble in H_2O, DMF and DMSO. Yield: 67%. Mp: >300^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6, 25 ^{o}C): δ= 8.3-7.0 (br m, Pc Ar-H, Py-H); 2.6 (s, OCH_3); 2.9 (s, N-CH_3). . UV-Vis (DMSO) λ_{max}/nm; 705, 641, 359. IR (ATR) υ (cm^{-1}) 3057-3041; 1604; 1534;1486; 1015; 877; 821; 731; 705.

RESULTS AND DISCUSSION

Titanium phthalocyanine compound **2** was synthesized as a result of tetramerization reaction of phthalonitrile derivative **1** with $Ti(OC_4H_9)_4$ in basic medium. As a result of quaternization reaction of compound **2**, water soluble titanium phthalocyanine compound **3** was synthesized (Figure 1).
The synthesized compounds were characterized by ^{1}H-NMR, UV-Vis, IR spectroscopies.

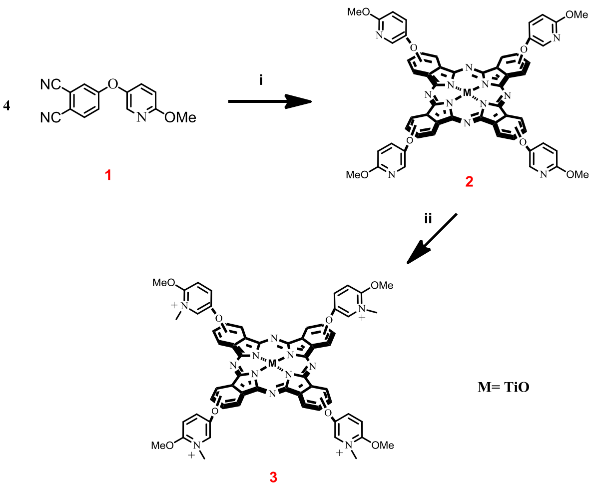

Figure 1: Synthesis of compounds **2** and **3**. (i) Ti(IV) butoxide, DBU; (ii) CH_3I.

The single Q-band which is characteristic for metallophthalocyanines, observed in the UV-Vis spectrum of compound **2**, confirms the formation of metallophthalocyanine.
In the IR spectrum of compound **2**, the disappearance of the C≡N peak at 2224 cm [Yabaş et al., 2019] of phthalonitrile derivative **1** confirms the formation of phthalocyanine by tetramerization reaction.

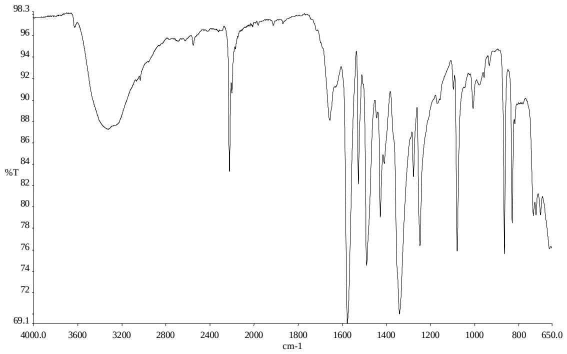

Figure 3: IR spectrum of compound **1** [Yabaş et al., 2019].

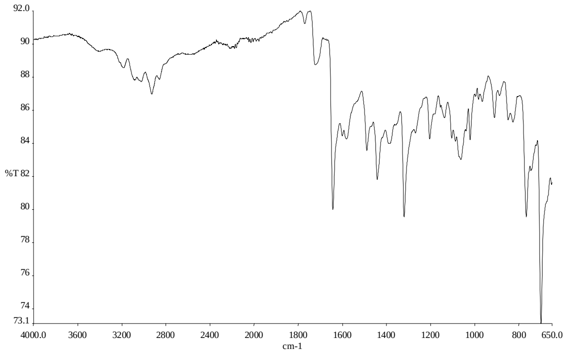

Figure 4: IR spectrum of compound **2**.

In the ^{1}H-NMR spectra of the synthesized compounds, the peaks and the integral ratios of the peaks are consistent with the desired structure.

Fluorescence quantum yield was calculated to determine the potential to used as a drug in PDT of the synthesized novel water-soluble phthalocyanine compound **3**.

The emission spectrum of compound **3** was measured to calculate fluorescence quantum yields (Figure 5). Fluorescence quantum yield of compound **3** was also calculated using the following equation (equation 1). Unsubstituted ZnPc was used as standard reference (Φ_F = 0.18 in DMSO) [Durmuş et al., 2008; Fery-Forgues et al., 1999; Maree et al., 2002].

$$\Phi_F = \Phi_F(\mathrm{Std}) \frac{F A_{\mathrm{Std}} \eta^2}{F_{\mathrm{Std}} A \eta^2_{\mathrm{Std}}} \qquad (1)$$

where F and F_{Std}: the areas under the fluorescence emission curves of the compound **3** and the standard, respectively. A and A_{Std}: the absorbance of the samples and standard at the excitation wavelength, respectively, η and η_{Std}: the refractive indexes of solvents used for compound **3** and standard, respectively. [Durmuş et al., 2008; Ogunsipe et al., 2004; Jacques et al., 1981]

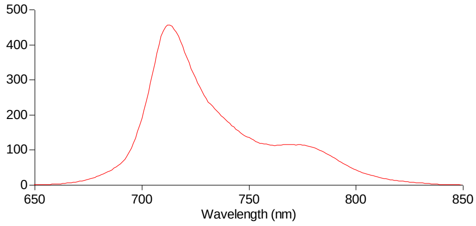

Figure 5: Emission spectrum of compound **3**.

As a result of the calculations, fluorescence quantum yield of compound **3** was found to be 0.29. This value is similar to that of typical metallophthalocyanines [Durmuş et al., 2007]. The fluorescence quantum yield for compound **3** is higher than the fluorescence quantum yield of unsubstituted ZnPc.

CONCLUSIONS

In this study, the new water soluble quaternized 2-methoxypyridine tetrasubstituted oxo-titanium monophthalocyanine **3** synthesized and characterized. The fluorescence properties of compound **3** were studied and the fluorescence quantum yield calculated. It was observed that the obtained new water soluble drug molecule had the potential to be used in PDT. In the following studies, it is planned to investigate the activity of this synthesized drug molecule for PDT in in vitro and in vivo environments.

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POTENTIAL USAGE OF RUMEN MICROORGANISMS

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Abstract

In ruminants the stomach has four compartments and rumen is the one of them. Rumen contains a complex microbial flora that consisting of 1010–11 cells/ml bacteria, 103–5 cells/ml fungi and 104–6 cells/ml protozoa. A vast range and quantity of enzymes were secreted by these microorganisms for degrade the polysaccharides such as cellulose, hemicellulose and lignin into soluble sugars and volatile fatty acids. Cellulose is a good raw biomaterial because of its reducible and reusable properties and many of agricultural wastes contain cellulose and cellulosic materials at highly concentrations.

The rumen microorganisms can use for many different areas. The methane, biogas or bioethanol production from agricultural wastes are the most encountered ways that using rumen microbiota. Moreover, novel treatment methods for paper industries, new discovered biosorbents from rumen could also be used for ecofriendly solutions. However, recycle of agricultural biomass by anaerobic digestion with rumen microorganisms or reusable newly and biotechnological materials such as cellulose nanofiber could be derivate from agricultural biomass with microbial flora of ruminants.

Keywords: *Rumen Microorganisms, Anaerobic Digestion, Biomass*

INTRODUCTION

In terrestrial plants, the majority of organic matter is found in insoluble polysaccharides. Mammals do not have enzymes to break down polysaccharides such as cellulose, but in ruminants, the microorganisms in the rumen, which is a special digestive organ, can break down these substances. They can break down cellulose and other polysaccharides with ruminants. They are of considerable economic importance as the cost of food to humans is largely dependent on these animals (Çökmüş, 2010; Alatas and Umucalılar, 2011; Ji et al, 2016).

In addition to cellulose, starch, lactate, succinate, pectin-degrading microorganisms, methanogenic anaerobes are also found in rumen. Microbial ecosystem of rumen contains different types of microorganisms such as bacteria (10^{10}-10^{11} cells/ml), archaea (10^{7}-10^{9} cells/ml), protozoa (10^{4}-10^{6} cells/ml), fungus (10^{3}-10^{5} zoospore/ml) and bacteriophage (10^{8}-10^{9}virus/ml). Most of these microorganisms are anaerobic and gram negative. The change of diet of the animal would be changed the microbiota in the rumen that might cause the animal to become ill or die. The fermentation products of saccharolytic rumen microflora also undergo microbial fermentation where volatile fatty acids and carbon dioxide and methane are produced (Çökmüş, 2010; Alatas and Umucalılar, 2011; Takizawa et al, 2018; Berhelot et al, 2019).

During the 9-12 hours that food stays in the rumen, cellulose is transformed by microorganisms into cellobiose, free glucose and volatile fatty acids, also carbon dioxide and methane from these substances. Moreover, microorganisms in the rumen synthesize essential amino acids and vitamins. Biochemical reactions taking place in the rumen occur with the participation of many microorganisms and therefore it is possible to use microorganisms from rumen microbiota in different industrial biotechnology areas (Çökmüş, 2010).

THE IMPORTANT AND EXISTENCE OF RUMEN MICROORGANISMS IN RUMINANTS

Ruminants have four-compartment stomach structure consisting of rumen, reticulum, omasum and abomasum. Although there are no microbial activity in the first 3 chambers of the stomach in ruminants, but the fourth chamber (abomasum) has the enzymic structure but also could not breakdown the structural plant material such aslignocellulosic material. Moreover, ın ruminants there are a relationship with microorganisms that contain cellulolytic and hemicellulolytic enzymes and could digest cellulose and lignin. The digestive system of animals is an ideal place for fermentation, and more than 60% of digestive activities have been gone in reticulorum. This fermentation process have importance for understanding the microbial transformation of lignocellulosic material and the environmental footprint of agricultural livestock and product quality. The microbial community structure of the rumen has great importance because of the symbiotic effect with multicellular microorganism as well for our everyday lives (Edward et al, 2008; Özel and Sarıçiçek, 2009; Jami and Mizrahi 2012).

Table 1. Types of ruminal microorganisms

Types	Domain	Number (per ml or g of ruminal contents)	Percentage of total microbial mass
Bacteria	Eubacteria	10^9–10^{11}	40–90
Methanogens	Archaea	10^5–10^8	2–4
Protozoa	Eukarya		0–60
Flagellates		10^2–10^3	
Ciliates		10^4–10^6	
Fungi	Eukarya	–	10
Bacteriophages	–	10^{11}–10^{12}	–

POTENTIAL USAGE OF RUMEN MICROORGANISMS In INDUSTRY

The potential applications of microorganisms that could found in rumen fluid are paper waste treatment, anaerobic digestion of organic wastes of conventional agricultural activities, renewable energy generation, bioethanol, biogas and methane production or producing newly synthetized nanomateirals such as cellulose nanofibers. The microbial consortium of rumen could also used for new biosorbent applications and environmental treatment, anaerobic bioconcervation or mine drainage tretament(Hu and Yu, 2005; Grebenet al, 2009; Yue et al, 2013; Takizawa et al, 2018; Xu et al, 2018; Nagler et al, 2019; Guo et al, 2019).

Paper industry generates too much wastes as sludge that contains organic fibers and ash, cellulose, hemicellulose and lignin residues and these polymers could be hydrolyze by bacteria with anaerobic digestion. The rumen fluids host a complex anaerobic microbial ecosystem and these bacteria have a wide range of digestion enzymes for lingocellulosic biomass. Takizawa et al used rumen-derived microorganisms in methane production from paper industry waste and found that they were more effective in converting waste into methane in 2018 and 2019 (Takizawa et al, 2018-2019).

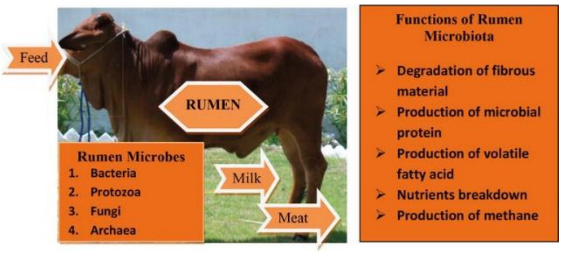

Figure 1. The function of rumen microbiota

Conventional agricultural and forestry activities could be generate renewable and sustainable biomass such as lignocellulosic material. This waste could be used for produce renewable energy by biological conversion methods with rumen microflora that could digest the agricultural wastes. Lignocellulose rich biomass could be treated by rumen microbial consortium that very effective and have a vast range of enzymatic capabilities. The pre-treated lignocellulose rich biomass could be used for production of biogas or methane or bioethanol (Hu and Yu, 2005; Jinet al, 2014; Nagler et al, 2019).

RESULTS

Rumen content is acidic due to the fermentation caused by bacteria and protozoa population in the rumen. Ruminants are able to degrade and use the fibrous feed offered, which will be converted as source of energy by the presence of colonized anaerobic microbiota in the rumen like bacteria, fungi, and protozoa (Chaucheyras-Durand and Ossa, 2014). Important cellulolytic (cellulose digesting) bacteria species include:Fibrobacter succinogenes, Ruminococcusalbus and Ruminococcusflavefaciens. The most common protozoa varieties which are contribute to the cellulose and starch digestion are Entodinium, Diplodinium and Isotricha. The main end products of fermentation are volatile fatty acids (VFAs) and microbial biomass, which are used by the host ruminant.

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THE EFFECTS OF VIBURNUM OPULUS L. FRUIT POWDER ON THE PERFORMANCE, EGG TRAITS AND EGG CHOLESTEROL OF LAYING JAPANESE QUAILS

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Abstract

This study was conducted to investigate the effects of cranberry (Gilaburu, Viburnum opulus L. fruit powder (CFP) on the performance, egg traits, egg cholesterol and blood serum parameters of laying Japanese quails. One hundred and thirty two female and forty-four male 30-weeks-old quails were allocated to four dietary treatments. Quails were caged three females and one male quails and fed diets supplemented with 0 (control), 5, 10, 15% CFP for 8 weeks. Each treatment comprised 11 replicates of quails. The addition of CFP did not significantly affect body weight, egg production, feed consumption, feed efficiency, egg weight, egg albumen weight, egg yolk weight, egg Haugh unit and breaking resistance. Adding 5 and 15 g/kg CFP to the laying quail diets decreased eggshell weight (P=0.000, cubic effect). The addition of 5 g/kg CFP to the diet resulted in significantly (P<0.01, linear and quadratic effect) better-yolk height as compared to control and other treatment groups. Egg yolk cholesterol concentration did not significantly affect with CFP supplementation (P>0.05). The results of this study demonstrated that CFP addition had a significant eggshell weight reducing and yolk height-increasing effect without adverse effects on performance and egg traits of laying quails.

Key words: *Viburnum opulus L., gilaburu, cranberry fruit powder, quail, egg traits, egg cholesterol*.

THE EFFECTS OF VIBURNUM OPULUS L. FRUIT POWDER ON THE SLAUGHTER TRAITS, BLOOD SERUM PARAMETERS AND GUT HEALTY OF JAPANESE QUAILS

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Abstract

This study was conducted to investigate the effects of cranberry (Gilaburu, Viburnum opulus L. fruit powder (CFP) on slaughter traits, blood serum parameters and gut healty of Japanese quails. One hundred and twenty, 30-weeks-old quails were allocated to four dietary treatments. Quails were caged female and male quails and fed diets supplemented with 0 (control), 5, 10, 15% CFP for 8 weeks. Each treatment comprised 15 replicates of quails. The addition of CFP did not significantly affect slaughter traits, internal and sex organs, meat quality, color and pH, GUT and microbiota, and serum parameters. The results of this study demonstrated that CFP addition had a significant effect of cold breast redness (P<0.01), thawing-cooking loss (P=0.000), wing weight, Warner-Bratzler shear force and glucose (P<0.05). As a result of the gilaburu supplement had a positive effect on the gut healty and blood serum parameter of glocuse.

Key words: *Viburnum opulus L., gilaburu, cranberry fruit powder, quail, slaughter traits.*

DETERMINATION OF MILK YIELD TRAITS FOR HOLSTEIN CATTLE RAISED IN KIRSEHIR

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Abstract

In this study, 4589 milk yield records of Holstein cows raised in 389 farms located in Kırşehir Cattle Breeders Association between 2007-2017 were used. Milk yield characteristics, 305 days of milk yield (DMY), lactation lenght (LL) and dry period (DP), and effects of calving year, parity, calving season, calving age and farm size on these factors were determined. Heritability and repeabilty for 305 DMY, LL and DP were estimated as 0,23, 0,07 and <0,01;0,23, 0,14 and 0,02, respectively. In addition, breeding value was calculated as -8.58 kg year. To conclude, it can be believed that milk yield traits and genetic parameter estimations calculated for Hostein cows raised in Kirsehir province were quide for breeding studies in future.

Key words: *Holstein dairy cattle, hertabilty, variance components, breeding values, genetic trend*

DETERMINATION OF MILK YIELD TRAITS FOR HOLSTEIN CATTLE RAISED IN KIRSEHIR

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Abstract

In this study, 4589 milk yield records of Holstein cows raised in 389 farms located in Kırşehir Cattle Breeders Association between 2007-2017 were used. Of milk yield characteristics, 305 days of milk yield (DMY), lactation lenght (LL) and dry period (DP), and effects of calving year, parity, calving season, calving age and farm size on these factors were determined. In present study, the overall mean of 305 DMY, LL and DP were calculated as 7350,5 ± 30,70 kg, 398,2 ± 1,61 days and 62,5 ± 17,02 days, respectively. Effects of calving year, parity, calving season and farm size on 305 DMY and LL were statically important ($p<0,01$). Effects of calving year and farm structure on DP were statically important ($P>0,05$), but effects of parity and calving season on its were not important. 305 DMY were increased and phenotypic trend was calculated 62.6 kg for years and statistically significant. To conclude, milk yield traits calculated for Hostein cows raised in Kırşehir. (This article carried out part of Murat KARAAGACs' MSc thesis in Kirsehir Ahi Evran University Institute of Natural Science and Department of Agricultural Biotechnology).

Key words: *Holstein Cattle, 305-day milk yield, lactation length, dry period.*

THE POSSIBILITY TO SYNCHRONIZATION OF OESTRUS BY APPLYING DARKNESS AT OUT-SEASON IN AKKARAMAN SHEEP

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Abstract

The aim of this study is to investigate the possibility to synchronization of oestrus by applying darkness at out-season in Akkaraman sheep. In this study, 3-4 aged were used 50 Akkaraman sheep as animal material. The study was conducted in April. Forty-eight hours prior to estrus synchronization application, intramuscular injection of 1 cc $PGF_{2\alpha}$ was performed to lutalyse the corpus luteum on the ovary in all ewes. 48 hours after the injection, vaginal implant device CIDR (0.30 g progesterone) in all sheep was used for return to the beginning of the follicular development (0.day). The sheep were randomly divided into two groups. A group of sheep (control; n = 25) were synchronized by using natural and synthetic hormones. The other group (darkness; n = 25) was exposed to darkness in a fully controlled sheep housing during 13 hours. For all animals was taken blood in a specific program and melatonin hormone concentrations were determined in Elisa device. At the end of darkness application, melatonin hormone concentration in darkness applied group (90.83 ± 29.27 pg / ml) was higher than control group (66.58 ± 21.05 pg / ml) ($P < 0.01$). At the end of the experiment, melatonin concentration was higher in the darkness applied group (89.30 ± 29.57 pg / ml) than the control group (54.44 ± 22.97 pg / ml) ($P < 0.01$). According to these results, it has been shown that melatonin hormone concentrations can be changed with the application of darkness in out-breeding season in sheep, and therefore heat synchronization can be realized.

Key words: *maternal age, placenta, cotyledon, fetal development, kid birth weight*

INTRODUCTION

Sheep is a breeding species depending on the season. However, they may show heat in tropical regions throughout the year and periodically (Jainudeen ve Hafez, 1983). In the northern hemisphere, including our country, sheep have seasonal breeding properties. In our country, heat is seen intensively in the last period of summer and autumn months.

The interactions between the hormones produced by the hypothalamus, anterior pituitary and ovaries play a role in the regulation of the estrus cycle (Roche, 1996; Hunter ve ark., 2004). These hormones are GnRH secreted from hypothalamus, FSH, LH, prolactin and oxytocin secreted from pituitary gland, estrogen secreted from ovaries and PGF2α secreted by uterine endometrium. In addition, the relationship between the uterus and ovariums is important because of the secretion of PGF2α, which causes the destruction of CL and stops the synthesis of progesterone hormone (Bartlewski, 2001; Davies, 2005). The effects of photoperiodism on seasonal reproductive activity in sheep and these effects are known to be mediated by the pineal gland and its hormone melatonin (Gökdal ve Baş, 1996). The pineal gland shows a rhythm of night and day. It also ensures the adaptation of the creature against light changes. Production and synthesis starts with darkness and ends with light. Light stimuli cause fluctuations in the amount of melatonin. The prolongation of the light period or the sudden emergence of light stops melatonin production (Neils, 1997; Yilmaz, 1999). For this reason, melatonin has been given a symbolic name as biochemical descriptor of darkness (Neils, 1997).

Melatonin hormone is a type of neuronal hormone synthesized from the pineal gland due to photoperiod. This mechanism takes place under the control of the nervous system. Melatonin released from the pineal gland is another hormone that is effective in the initiation of ostrus cycle (Zhdanova, 2005). Light signals from the retina are also reduced due to reduced daylight. As a result, the level of melatonin released from the pineal gland begins to increase (Zarazaga et al., 2010). As a result of photoperiodic changes, melatonin hormone is secreted by stimulation of suprachiasmatic nuclei which are light sensitive cells. The secreted melatonin hormone acts on the hypothalamus and reaches the anterior pituitary through the hypothalamus-pituitary portal veins and sinusoidal capillaries of the gonadotropin-releasing hormone produced in the neurosecretory cells. Afterwards, it stimulates gonadotropin cells and causes the secretion of FSH and LH which enables the development of follicles (Senger, 1995). Thus, seasonal reproductive activity begins. Starting with the hormone melatonin, GnRH, FSH and LH stimulate the release of estrogen hormone, progesterone and inhibin from the ovary. These secreted hormones control the release frequency of GnRH, FSH and LH by positive and negative feedback mechanisms (Ekiz, 2005).

There are synchronization practices that enable sheep to show heat during and outside the breeding season. In the synchronization of heat, progestagens, estrogens, PGF2α and its analogs, gonadotropins such as PMSG, Gn-RH, hCG, hormones such as melatonin and combinations therefore commonly used in practice (Akçapınar, 1994: Yaralı and Karaca 2004). However, there is a limited number of studies on the synchronization of heat by dark application, especially outside the breeding season. In this study, it was aimed to investigate the usability of dark application in out-season estrus synchronization in sheep.

MATERIALS AND METHODS

In this study, 50 heads of Akkaraman sheep were used. This study was carried out in April, which is considered as an out-season period. All experimental material was injected with 1 cc of $PGF_{2\alpha}$ intramuscular (IM) at the beginning of the experiment for the luteolysis of the corpus luteum. 48 hours after the injection, all sheep were subjected to heat synchronization using the vaginal implant device CIDR containing natural progesterone (0.30 g progesterone). The CIDR device was removed after 12 days. The animals were divided into two groups (control and darkness group) in equal numbers. Following this procedure, animals in the darkness group (n=25) were subjected to dark application for 13 hours (18:00-07:00) in a fully environmentally controlled shelter. Darkness application was continued for 12 days. The mating process was carried out for 4 days after the end of the darkness application. In the control group, after removal of the CIDR devicewas administered intramuscularly (IM) 600 IU PMSG per animal. 24 hours after PMSG application, mating was carried out for 4 days.

The change in melatonin hormone was determined by taking 8 ml of blood from the jugular vein with the help of vacutainer from all animals. In the control group, just before inserting the CIDR device, removed CIDR device (at the begining of mating) and at the ending of mating were taken the blood. In the darkness group, just before inserting the CIDR device, removed CIDR device (at the begining of darkness application), middle and end of darkness apllication and at the ending of mating were taken the blood. Blood samples were centrifuged at 400 rpm at +4 ° C for 10 minutes. The resulting plasma was then stored at -20 ° C until the day of analysis divided into 1 ml of tubes. Determination of melatonin concentrations in blood was carried out by ELISA (Competitive-ELISA).

All data obtained from the study were analyzed using SPSS 20.0 package program. The suitability of the data for variance analysis was evaluated with Levente variance homogeneity test.

RESULTS AND DISCUSSION

The changes in melatonin hormone concentrations in the groups (Darkness and Control groups) are given in Table 1 and Table 2.

Table 1. The changes in melatonin hormone concentrations in the control group (pg/ml).

Blood Samples	Melatonin Concantrations
CIDR Device Inserting	64.05±27.02
CIDR Removed (At the begin of mating)	66.58±21.05
At The End of Mating	54.44±22.97

There was no difference in melatonin hormone concentrations in the group (P> 0.05). In the sheep in the control group, melatonin concentrations increased up to the beginning of mating but there was a decrease after mating. It was determined that the concentration of melatonin was observed to be at a certain level in the synchronization of heat with synthetic hormones outside the breeding season. This may be due to the use of PMSG hormone in the synchronization application. Because PMSG has direct FSH and LH effect, it does not affect melatonin synthesis.

Table 2. The changes in melatonin hormone concentrations in the darkness group (pg/ml).

Blood Samples	Melatonin Concantrations
CIDR Device Inserting	74.67±23.639^{a}
CIDR Removed (At the begining of darkness apllication)	91.29±30.71^{ab}
Middle of The Darkness Apllication	105.54±43.40^{b}
End of The Darkness Apllication (At the begining of mating)	90.83±29.27^{ab}
Ending of The Mating	89.30±29.57^{ab}

Differences between the means indicated by different letters in the same column were significant (P <0.05).

Melatonin hormone concentration increased during darkness apllication. The highest melatonin concentration was reached at the end of darkness application. Melatonin hormone concentration started to decrease as a result of the begin of mating (the end of darkness application). Ravault and Chesneau (1999) reported that melatonin concentration increased until the end of dark application, but decreased after the begin of mating.

CONCLUSIONS

The results of this study showed that the concentration of melatonin hormone, which initiates the estrus cycle, can be increased when dark application is performed outside the reproductive season. On the other hand, it is seen that it can achieve success in heat synchronization without using natural and artificial hormones.

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HYGIENE CONDITIONS AND ANIMAL-ENVIRONMENT RELATIONS IN FARM ANIMALS BARNS

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Abstract

In all type farms, animal health and the environment which lives of animals represents a major component, besides feeding, genetics and management quality. A healthy animal can only be grown in healthy and hygienic conditions. Health and environmental hygiene are a can not be ignored feature of the all types of farm production process quality. Animal health and care can be solved by prevention applications, vaccination strategies, disease reduction precautions and control of diseases by or a risk identification and risk management approaches. Animal health - care management strategies and risk applications, several animal hygiene issues, have not been widely adopted and it is not known to most businesses yet. Appropriate animal health care takes into consideration not only the zootechnical and veterinary affairs, but also the disease aspects and environmental hygiene conditions, economic relevances and furthermore product-production process quality related features. Enterprise-level hygiene covering all farm units is an important indicator of welfare for farm animals and is mostly dependent on facilities, management strategies of enterprise owners, climate conditions, and the behavior of the animals. According to the information given above the objective of this study is to determine the associations between farm animals behaviors (cattles, sheep and chickens), barn hygiene, animal hygiene, environmental conditions and the qualities of experiencing in Turkey.

Key words: *Farm animals, animal barns, environment, hygiene conditions*

INTRODUCTION

Animal Hygiene is an interdisciplinary science which the part of the veterinary and zoology sciences and it is relying on knowledge of varying demands of animals, describes the preconditions of health preservation and investigates by adverse environmental effects. The expression also describes the multilateral effects of housing and nutrition on health, behavioural characteristics of farm animals, production economy and on the rural environment. Theoretical and practical knowledge of animal hygiene is based on nutrition, microbiology, animal physiology, husbandry and immunology. On this basis, animal hygiene apply the preventive veterinary measures with considering consequences. Animal hygiene also covers detailed with herds and health protection of them. At the same time it contributes to the better understanding of monitoring of performance, management and technique of feeding and watering, air quality and ventilation, technique of manure handling and hygiene, cleaning and disinfection, risk factors affecting animals, medicine and food inspection and state animal husbandry. From the information provided should to describe how should be the hygiene conditions of farm animals and to assess whether a relationship exists between hygiene and environmental conditions.

Cattle Barns

The hygienic status of barns surroundings and the various hygienic measures carried out in cattle barns are important because environmental hygiene can affect yield quality and quantity directly. The hygiene of dairy cows can be used as an determiner of animal welfare, as it provides information about the quality of life of the farm facilities and the quality of the animals (Hultgren and Bergsten, 2001). Most studies that have evaluated the hygiene of dairy cows were performed with animals housed in freestalls and confirmed that the cows' hygiene level is an important determiner of their welfare and that it is influenced by the characteristics and conditions of the facilities where they are lived (Nielsen et al.,1997; Hultgren and Bergsten, 2001; Zdanowicz et al., 2004; Zurbrigg et al., 2005; De Palo et al., 2006; Sant'Anna and Paranhos da Costa, 2011). Poor hygiene conditions in cows would be associated with an increased occurrence of disease such as environmental mastitis (Schreiner and Ruegg, 2003).

Several factors affect the cleanliness conditions of the cattles. These factors can be classifield as air humidity, type of housing, material and construction of the floors, stall dimensions, use and amount of bedding, manure consistency, maintenance of the floors and cleaning of the animals (Ruud et al., 2011; Hauge et al.,2012). Silage is one potential source of contamination in cattle barns (Driehuis, 2013). In Kymäläinen and Kuisma (2014)' study they observed that the feeding troughs and drinking bowls were rather highly contaminated due to the contact with animals and silage. In the study which the analysis of the water samples taken from the drinking waterlines of cattle barns, some values are observed to be much different from each other. The main and apparent causes of the differences are the current state of drinking water net in the enterprise and their possibility of being affected by different organic substances such as animal wastes. However, the secondary causes for drinking water pollution may be different, variable and be specific in any enterprises. Therefore, cattle barns hygiene conditions should be checked continuously and periodically, and necessary analyzes should be performed (Erkan Can, 2019). In the scope of our project where animal drinking water quality is investigated in Adana, Turkey, the images taken from cattle drinking water troughs are shown in Figure 1.

Figure 1. Cattle drinking water troughs

Biosecurity factor and control of infectious agents should be examined in farm management. Biosecurity is a series of management practices and an essential aspect which designed to minimize or prevent and control infectious disease agents in to a farm, for environment and human health.

Seudomanas spp. are graam negative bacteria which are widespread in the environment of the dairy cow (drinking troughs, teats, feed etc.) because they need few nutrients to multiply or grow and they can survive in the moist environments (Kirk and Mellenberger, 2001). Some pathogens (Klebsiella spp. and Enterobacter spp.) also populate soil, feed and if moisture in the environment of the cow increases populations of them also increase (Hogan, 2005).

Many studies shows that mastitis which caused by number of different bacteria is an inflammatory disorder which can be end in death and it is most widespread health problem in dairy cows. The risk of mastitis is depend on both animal and the environmental factors generating in farms. Causal organisms are generally classified as either environmental or contagious. The reservoir for contagious is the udder, whereas environmentaal pathogens are those that survive in the outer environment such as bedding and manure (Harmon, 1994). The most common contagious pathogens are listed Table 1.

Table 1. Mastitis causing agents (listed by method of transmission) (SCC:Somatic cell count)

Mastitis Pathogen	Type	Typical infection severity/pattern
Staphylococcus aureus	C	Prolonged elevated Scc, sub/clinical mastitis (SCM/CM) (De Haas et al., 2002).
Streptococcus agalactiae	C	SCM and CM (Harmon, 1994)
Corynebacterium bovis	C	SCM (Harmon, 1994; Honkanen-Buzalski et al., 1984)
Coliforms e.g. E.coli, Klebsiella spp., Enterobacter spp.	E	CM with SCC (De Haas et al., 2002; Harmon, 1994)
Pseudomonas spp.	E	SCM and CM (Harmon, 1994; Kirk and Bartlett, 1984)
Actinomyces pyogenes	E	CM (Harmon, 1994; Jonsson et al., 1991)
Serratia spp.	E-C	Chronic CM (Harmon, 1994; Hogan and Smith, 2003)
Streptococcus dysgalactiae, S.uberis, S.bovis	E	CM (Harmon, 1994; Smith et al., 1985)
Enterococcus faecium, E.faecalis	E	CM (Harmon, 1994; Petersson-Wolfe et al., 2007)
Coagulase-negative Staphylococci	SF	SCM (Davidson et al., 1992; Harmon, 1994)

C= Contagious, E= Environmental, SF= Skin flora opportunists

Evaluating how diseases are introduced to the differs for each disease. Ways that some diseases are spread are presented in Table 2 (BAMN, 2001).

Table 2. Transmission of key bovine pathogens

	Staphylococcus aureus	Johne's disease	BVD*	Salmonella spp.
Transmission via:		x	x	x
Fecal-oral				
Nasal secretions/saliva	?		x	x
Milk	x	x	x	x
In utero		x	x	x
Sexual			x	
Incubation period	Days-months	Years	5-10 days	1-4 days
Duration of clinical disease	Days-years	Weeks-months	2 weeks	1-7 days
Duration of shedding	Days-years	Months-years	10-14 days	Weeks-months
Survival in environment	?	Months-years	Up to 14 days	Months
Growth in environment	Yes	No	No	Yes

*BVD = Bovine Virus Diarrhea

Sant'Anna and Paranhos da Costa (2011) made a study were to describe how the hygiene conditions of dairy cows vary over time and to assess whether a relationship exists between hygiene and somatic cell count (SCC) in milk. They conducted monthly hygiene evaluations on lactating cows in 2 dairy farms for 9 consecutive months, totaling 3,554 evaluations from 545 animals. They observed that most critical months for cow hygiene were those with the greatest rainfall, when a reduction in the welfare of cows and higher SCC values. Also researchers suggested that the evaluation and control of dairy cow hygiene are useful in defining management strategies to reduce problems with milk and improve the welfare of the animals.

DeVries et all (2012) studied to determine the associations between dairy cow standing and lying behavior, barn hygiene, cow hygiene and the risk of experiencing elevated SCC on 69 lactating Holstein dairy cows were housed in a sand-bedded, freestall barn with a free cow traffic automatic milking system. Study results show that cow hygiene is affected by the standing and lying behavior of cows and by the cleanliness of the cow's environment. Researchers emphasize the need for cows to be provided clean lying and standing environments. The study results also show that frequent cleaning of barn alley floors will help improve cow hygiene.

Cattle barn environments not only separated areas hygiene but also include a combination of different rooms and levels of hygiene physically. For this reason hygiene conditions of cattle barns must be taken in to attention as whole farm. In Kymäläinen and Kuisma (2014)' study was tested the suitability of microbiological dipslide methods to measure the hygiene level of the environmental surfaces in a cattle barn. A total of 1112 measurements were carried out during five measurement days. End of the study they detected that the poorest hygiene level was observed in the barn and the second dirtiest in the washing room. The corridor and personnel rooms had the highest hygiene status. The office and personnel kitchen and the milk room were generally the next cleanest, depending on the evaluation criteria.

Environmental hygiene also refers to other environmental problems such as dust, bad smells, air pollutants which are caused by storage and utilization of animal residues. Air pollutants in cattle barns also affect the safety of the environment, as well as the welfare and performance of animals and workers (Hartung and Schulz, 2011).

In a research was conducted to determine the biosecurity conditions of cattle farms in Malatya, 172 breeders are interviewed by using the random sampling method in selected cattle farms in specific districts. When animal health and shelter hygiene conditions are evaluated, it is determined that several parameters are "acceptable", however, the others are considerably insufficient. The research stated that precautions should be taken to obtain profitability, sustainable production and public health immediately and controlling farms for biosecurity and vaccination programmers should efficiently be conducted and also administrative sanctions must be applied when needed (Köseman and Şeker, 2016).

According to study which conducted in 60 dairy herds study showed a correlation between dirty cattle presented for slaughter and animal dirtiness on-farm. Researchers emphasized that factors associated with dirty animals were, in ranked order, high air humidity, animal type (heifers and bulls/steers), housing (freestalls and pens without bedding), manure consistency and lack of efforts directed toward cleaning the animals throughout the year. Besides them, additional factors about dirty animals in the dirty herds were found as water leakage from drinking nipples/troughs into lying areas, bedding type, and feed type (Hauge et all., 2012).

Lamsal (2018) pointed out that revealed that prevalence of Subclinical mastitis was high with increasing hygiene score of rear body parts that is with increasing unhygienic condition. In the study, three main body parts of animal were marked with scores methodolgy. Udder was competitively cleaner than rear and flank regions. Mean hygiene score of cow was 2.4 with udder, lower leg and flank being 2.80, 2.42 and 2.64 respectively. Data analysis showed one of the strong reasons for subclinical mastitis was poor hygienic status of animal and housing system.

In the pictures given below are images on the related subject which from our studies in Turkey (Figure 2).

Figure 2. Unclean cattle barns

Sheep Husbandry

As a species extensively managed traditionally at least for some of the year, sheep have received relatively little attention from a welfare perspective. Sheep welfare can be considered from the frame of the animals biological functioning, the naturalness of the way in which they are kept. These different fields can be integrated by considering the animal and the environment (Dwyer, 2009).

Basic of the sheep environmental factors are extreme climatic conditions (extreme moisture, bad ventilation, draughts, and temperature extremes particularly at lambing and weaning), transportation and faulties during transportation, shearing and housing types. Sheep and goats share many health problems while there are some important differences between the species. Diseases of sheep and goats may not always be apparent in the early stages of ilness, so barn owners should regularly monitor their animals for signs of illness and should check environmental hygienic conditions. So many of the diseases that affect sheep can not be detected easily by external examination of the sheep and they occurs because of bad hygienic conditions. Sheep may well be infested with small numbers of sheep scab mites or lice. Sheep and goats are vulnerable to several serious diseases including; bluetongue, brucellosis (Brucella melitensis), foot and mouth disease, goat pox, lead poisoning etc.

Shepherds should pay particular attention to cleanliness and hygiene of equipment and pens during pregnancy and lambing. Attention to cleanliness and hygiene is also important in the lambing area. Lambing area floors should be dry and well-drained. Additionaly in all areas of sheep farms, dry, clean, comfortable conditions under foot should be provided to minimise footrot and hygiene problems (CRWL, 2000).

Purchasing replacements and mixing of sheep from several herds can bring new disease onto the farm. Most diseases of contagious are introduced into farms when new animals are added. If a closed herd is not possible, should be use an animal quarantine program. A useful quarantina program consists of a process and area that prevents co-mingling of animals for at least 30 days, including separate water supplies. Useful biosecurity applications and healty and hygienic environmental conditions are a vital part of keeping disease away from your animals and this situaitons will also protect the health of your workers and any members of the public who may visit your farm or consuming of sheep products. Restricting and controlling movements of people, vehicles and equipment into areas where your sheep or goats are kept, cleaning and disinfecting equipment and vehicles, protective clothing and footwearis are vital part of a good biosecurity programme.

Some diseases may be occur when sheep graze different plants such as cape weed, oats, canola, wild turnip or young plants which have high nitrate concentrations. Sheep should not be grazed with plants with high nitrate levels and care should be taken to the surrounding vegetation. In sheep farms or grazing areas, all fields and buildings should be kept clear of debris such as wire or plastic, which could be harmful to sheep. When sheep are outdoors in winter, and especially when fed on root crops, they should be allowed either to turn back to pasture or to a straw bedded space. This space gives a more comfortable lying area as well as limiting the build-up of mud or dung on the fleece (CRWL, 2000).

Conditions that favour spread of disease are by and large those conditions that also have a potential adverse effect on animal welfare such as poor hygiene, overcrowding, rapid changes in temperature or weather conditions and unsuitable shelter (Roger, 2008). In a study about sheep housing and hygiene conditions, the effects on the health and welfare of sheep are discussed with regard to free access to external areas, to exposure to solar radiation, to ventilation and light regimes, to indoor climate and hygiene and to noise. Results is provided about the relationship between poor housing conditions, sheep welfare and problems of udder health with increased risk of mastitis and reduced yield and quality of milk (Caroprese, 2008).

Although the studies investigating the relationship between hygiene and yield in sheep breeding are limited, studies conducted in this field have concluded that hygienic conditions affect yield positively. In a study made by Alexopoulos et al.(2011), purpose was to survey the raw ovine milk produced in sheep farms of Greece and to explore the role of various factors on its quality thus contributing on the research on improving milk safety. A total of 155 samples from 21 farms were analyzed for TBC (Total Bacterial Count) and half of them for the rest of bacteria. As their results show as an average wereb TBC: 5.48 log cfu/mL, SCC: 6.05 log cells/mL (Somatic Cell Count), coliforms: 4.49 log cfu/mL, S. aureus: 3.94 log cfu/mL, environmental streptococcal counts: 4.95 log cfu/mL and PIC: 5.7 log cfu/mL. Even if some of the results were in accordance with the standard values, some results were reported to be more than the required values. End of the study, researchers indicated that most of the factors concern simple sanitation practices which if properly adopted by the farmers have proven their value and ensure a better outcome of milk quality.

Poultry Rearing

In Turkey, poultry-meat products continue to be a major nutriment of human consumption. Due to it is more accessible than red meat, chicken meat can reach high consumption amounts. In the management of poultry houses a high degree of cleanliness, hygiene standards, clean and healthy water and feed should be the first care.

However, poultry production and slaughtering farms which in unsuitable hygiene conditions, pose a serious health hazard. The organisms in chicken meat, could be readily transmitted to man in the handling of raw poultry, either directly or via another food item. Disease is a major hazard in poultry houses, especially in large scale enterprises which has huge number of animals. Hygiene is a most important factor in disease prevention. Efficient farm management and suitable hygienic conditions can eliminate over 90% of all diseases and prevent disease and its spread among the flock. Hygiene, sanitation and biosecurity play a major role in any effective disease control programme and high yield for poultry production enterprises. Poor levels of biosecurity will lead to a higher prevalence of disease in the case of haemolytic Gallibacterium spp. and a higher risk of flocks getting infected with thermophilic Campylobacter spp. or Salmonella spp. (Gibbens et al., 2001; Liljebjelke et al., 2005; Bojesen et al., 2010; Osimani et al., 2017). In principle, the hygiene problems of poultry operation are similar to those experienced with other meat animals, but certain features make control of microbial contamination more difficult with poultry. Because of the rapid rate of processing, which reaches may more than thousands birds/h on some lines, conditions support the spread of microorganisms (Mead, 1995)

Gibbens et al. (2001) indicated that a standard hygiene protocol followed by all staff who entered in a broiler farm could reduce the risk of a flock getting infected with thermophilic Campylobacter spp. by 50%. This hygiene protocol included a strict process with boot dips before entering the poultry houses and farm specific clothing which also demonstrates the importance of a specific farm and poultry house hygiene lock in preventing (zoonotic) pathogens from entering a broiler house.

Van de Giessen et all (1998), studied on two Dutch broiler farms to understand transmission routes of Campylobacter spp. in broilers and possibilities for prevention of infections. Isolates of Campylobacter spp. were typed by using randomly amplified polymorphic DNA (RAPD) analysis. The datas indicate that broiler flocks become infected from environmental sources and according the other datas suggest that on one farm transmission of Campylobacter spp. occurred from cattle to broilers via the farmer’s footwear. The results indicate that the application of hygiene measures significantly reduced campylobacter infections of broiler flocks on both farms.

Folorunso et all (2014) subjected a study to a 7-day which involved the monitoring of poultry farm hygiene. They took samples from water troughs from deep litter and caged chicken water troughs (drinkers) in three different poultry farms. Drinkers were washed before filling with water on Day 1. For Days 3, 5 and 7 water was served without prior washing. End of the study they found that those left for 3, 5 and 7 days uncleaned had progressively high bacterial loads. Researchers suggesting that the flock of birds and the consumers of the eggs and meat from the chickens are at risk of bacterial infection unless strict farm hygiene is ensured through regular monitoring.

It be showed that an increased frequency of sanitizing the watering lines was associated with higher flock performance by Tablante et al. (2002). This result suggests that a obvious benefit from improving inner biosecurity might be an important incentive for poultry farmers to implement a higher standard of hygiene in the broiler house.

Deep litter and raised netting flooring systems in broiler houses were compared for their effects on air quality and bird health during an 8- week growing period and weekly measurements were taken of respirable dust, numbers of airborne microorganisms and concentrations of gaseous pollutants by Madelin and Wathes (2007). They empahised that respirable dust concentrations and numbers of airborne microorganisms were significantly higher in the litter rooms and birds on litter were observed to have a higher incidence of lung damage and more of the birds on litter had viable microorganisms present in the lungs at necropsy.

Poultry premises and farm buildings should conform with requirements for isolation from the environment and strict observance of principles of hygiene and disease prevention (e.g. restrictions on movement of staff and vehicles). A poultry site must be prepared regularly for the entry of each new group as; removal of birds, litter and manure; dry and wet cleaning; vector and rodent control; disinfection; fumigation. Special care should be exercised in the performance of sanitary procedures after a disease outbreak. Immediate disposal of dead and diseased birds is an serious and effective tool in preventing the spreading of any disease. Regular visual inspection and routine microbiological testing, is very effective in checking the efficacy of cleaning and disinfection (Meroz and Samberg, 1995).

RESULTS AND DISCUSSION

Nowadays, many important developments and legal regulations have been applied about animal hygiene conditions and animal welfare situations in European Union member countries. New approaches related to animal hygiene in farm animals should became topical subjects in Turkey as parallel to developments in the world.

Animal welfare and hygiene standards in Turkey criteria should become a legal obligation. Existing relevant legislations, regulations and laws should be reviewed and developed or rearranged. Necessary investigations should be carried out meticulously and sanctions should be applied. Hygiene and disease prevention precautions should aim at (FAO and OIE, 2010):

- Maintaining the hygiene and safety of all facilities in farms.
- Preventing contact between healthy animals and potentially infected animals.
- Provide the all workers health on the farm and the implementation of hygienic working procedures.
- Reducing contact between livestock and professional or other visitors, and taking all hygienic measures necessary to minimize the possible introduction of pathogens and contaminants (e.g vehicles).
- Ensuring overall health of livestock through good nutrition, providing pure drinking water and reducing stress.
- Keeping records of animal populations and making observations and analyses periodically in facilities/on farms.

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POSTER PRESENTATIONS

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POSTER PRESENTATIONS

PRION PROTEIN GENE POLYMORPHISM IN EIGHT SHEEP BREEDS OF ALGERIA

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Abstract

A total of 213 Algerian sheep from eight breeds (Ouled Djellal, Rembi, Hamra, Berbere, Barbarine, Sidaou, Taadmit and Tazegzawt) with no clinical manifestation of scrapie were analysed to identify the genetic variability of sheep PRNP at codons 136, 154 and 171. Sequencing of the entire coding sequence of PRNP showed four main alleles (ARQ, ARR, AHQ and ARH) based on codons 136, 154 and 171 with different frequencies among the investigated breeds. Moreover, 14 additional nonsynonymous polymorphisms (Q101R, N103K, M112T, A116P, M137I, L141F, I142M, H143R, N146S, R151G, Y172D, N176K, H180Y and S240P) as well as two synonymous polymorphisms at codons 231 and 237 were found in the PRNP gene. Interestingly, the N103K, M137I and I142M polymorphisms were not described in sheep. The ARQ, ARR and ARH haplotypes were present in all breeds with a highest frequency of ARQ in Barbarine. The ARH was absent in Barbarine breed and the VRQ haplotype was absent in all Algerian breeds studied. The ARQ and ARR alleles were the most common with frequencies ranging from 30 to 65% and from 8 to 26%, respectively, in different breeds. These results represent the first study on PRNP variability in Algerian sheep and may serve as a basis for the development of breeding programmes to render national sheep breeds resistant to scrapie.

Key words: *Algeria, Polymorphism, PRNP, Sheep breeds.*

STUDY OF THE MSTN GENE IN SHEEP OF CAUCASIAN MERINO AND ASCANIAN MERINO BREEDS IN BULGARIA

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Abstract

The MSTN gene encoding the myostatin protein, which is a growth factor-transforming beta protein, inhibits the skeletal muscle growth. Decreasing of its activity leads to an increase in the formation of skeletal muscle. In sheep, myostatin affects the muscles and the thickness of fat. The aim of this study is to determine the frequency of occurrence of the MSTN gene alleles in the breeds Ascanian merino and Caucasian merino. Methods: The 337 bp fragment of exon 3 of the sheep MSTN gene was examined by PCR-RFLP analysis using the restriction endonuclease Hae III. Results: In all investigated animals from both breeds were determined only the wild allele m and the homozygous genotype mm. Conclusions: In present study it may be concluded that exon 3 of MSTN sheep gene is found to be monomorphic for the both herds of Merino breeds - Ascanian and Caucasian reared in Northeastern Bulgaria.

***Key words:** sheep breeds, MSTN gene, PCR-RFLP analysis*

INTRODUCTION

Nowadays, sheep meat users have high demands on the quality and especially the tenderness of the meat. The candidate genes for meat traits and gene assisted selection both are in great help of sheep breeders. Molecular genetic researches have pointed out a discovery of several genes which affect some quantitative traits, such as meat production (Deykin et al., 2016). There are many published articles on different genes associated with meat traits in sheep and one of them is myostatin gene (Shafey et al., 2014).

Myostatin is a growth factor-transforming beta protein that blocks the growth of skeletal muscle. It is mainly produced in the muscles, circulating in the blood and affecting the muscle tissue. Inhibition of myostatin expression leads to an increase in muscle mass while its increased level leads to degeneration processes combined with incomplete regeneration, extensive fibrosis and fat replacement over time. Animals with neutralized endogenous myostatin have increased body weight and muscle mass as a result of increased fiber cross-section and total muscle mass. Myostatin protects myoblasts from apoptosis. Measurements of myostatin plasma levels indicate that increased levels of myostatin may result in muscle mass loss during pathological conditions or aging processes. In sheep, myostatin affects the muscle formation and the thickness of the fat (McPherron et al., 1997; Shafey et al., 2014).

MSTN gene or GDF8 gene is located at the end of the long arm (2q32.2 locus) of chromosome 2 of the sheep genome (Ovis aries). Structurally, this gene consists of three exons and two introns. In a number of sheep breeds (Romney, Suffolk, Lincoln, Dorset, etc.) polymorphism was found in exon III of this gene, which is associated with muscle hypertrophy (Bellinge et al., 2005; Nakev et al, 2013). GDF8 is a member of super-family covering a large number of factors affecting growth and differentiation of tissues in embryonic and post-embryonic development (Hickford et al., 2009). Mutations in the myostatin gene in sheep have a significant role in muscle growth (Boman, Vage 2009; Dhakad et al., 2017). Variation in the MSTN gene has been associated with a double muscling first reported in Belgian Texel sheep (Clop et al., 2006), later in Australian Texel (Kijas et al., 2007), in Charollais (Hadjipavlou et al., 2008) and other. MSTN g+6223G>A is now used as a commercial gene-marker for sheep breeding selection (Han et al., 2010). The aim of this study is to determine the frequency of occurrence of the MSTN gene alleles in the merino breeds - Ascanian and Caucasian.

MATERIALS AND METHODS

The present study was carried out in the DNA laboratory part of Faculty of Agronomy at the University of Forestry. The objects included were adult sheep from two Merino breeds - Ascanian (31 animals) and Caucasian (30 animals) reared in Northeastern Bulgaria. Approximately 3 mL of peripheral blood was collected from V. jugularis in vacuum tubes, containing EDTA. DNA was extracted by manual commercial kit for DNA purification according to the manufacturer's instruction (QIAamp DNA Blood Mini Kit Qiagen). The DNA concentration of each sample was determined by spectrophotometer Biodrop and tested by agarose electrophoresis on 1 % agarose gel.

PCR amplification reactions were carried out in total volume of 10 µl containing 4 µl DNA, 5 µl Red Taq Polymerase Master Mix (VWR) and 0,4 µl of each primer – forward and reverse (Bioneer) by using thermocycler QB-96 (Quanta Biotech).

For genotyping the MSTN locus was used PCR amplification according to Dehnavi et al. (2012). The primer set used for the amplification of studied region of exon 3 of chromosome 2 of sheep genome is F: 5'- CCG GAG AGA CTT TGG GCT TGA - 3' and R: 5'- TCA TGA GCA CCC ACA GCG GTC - 3'. After PCR amplification were obtained PCR products with expected lengths of 337 bp.

The conditions of the PCR amplification process were used according to Dehnavi et al. (2012). The digestion reactions were carried out in 10 µl final volume, containing 6 µl PCR product and 4 µl restriction enzymes HaeIII. PCR products were incubated at 37˚C for overnight (up to 15 h). The fragment sizes were determined using GeneRuler 50 bp (Sigma) supplied with 1 mL 6xDNA Loading dye. The obtained restriction products were tested on 2% agarose gel in 1xTBE buffer, stained with GelRed (Biotium) and visualized under UV - transilluminator UVP.

RESULTS AND DISCUSSION

The quality of extracted genomic DNA samples was tested on 1% agarose gel and it was visualized under UV light (Figure 1).

After PCR amplification of exon 3 of MSTN sheep gene were obtained fragments with size 337 bp (Figure 2). After digestion with specific endonuclease HaeIII it was detected only wild genotype mm (Figure 3) and only wild allele m in animals of both breeds. Mutant allele M and heterozygous genotypes Mm and mutant genotype MM were not detected in the two herds of sheep.

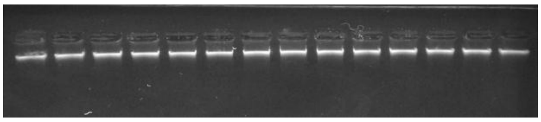

Figure 1. Testing of extracted DNA samples on 1% agarose gel with agarose electrophoresis

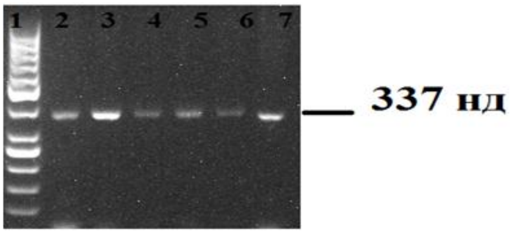

Figure 2. Testing of obtained PCR fragment after PCR amplification on 2% agarose gel for MSTN gene

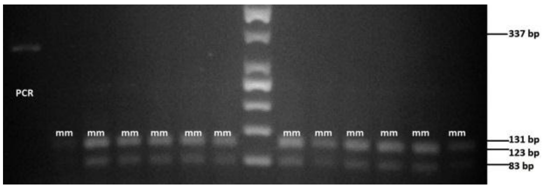

Figure 3. Restriction analysis of amplified product of MSTN gene with *HaeIII* restriction enzyme on 2% agarose gel electrophoresis

The third exon of MSTN locus was found to be monomorphic in the two Merino breeds - Ascanian and Caucasian grown in Bulgaria.

After digestion with HaeIII restriction enzyme other researchers detected two alleles – mutant allele M and wild allele m and three genotypes respectively: mutant genotype MM - one fragment with length 337 bp, heterozygous genotype Mm - four fragments: 337 bp, 131 bp, 123 bp and 83 bp and wild genotype mm – three fragments: 131 bp, 123 bp and 83 bp (Soufy et al., 2009; Dehnavi et al., 2012; Jamshidi et al., 2014).

The results in present study are in agreement with other studies. In our previous investigations – 121 adult sheep of Synthetic

Population Bulgarian Milk, 32 rams of Northeast Bulgarian Merino, 22 ewes and 3 rams from Karakachan breed, 30 adult sheep from Il de France sheep, 35 adult sheep from Karnobat Merino, we found similar results and all studied sheep were carried the genotype mm (Georgieva et al., 2015; Dimitrova et al., 2016; Bozhilova-Sakova et al., 2016; Bozhilova-Sakova and Dimitrova, 2017; Dimitrova et al., 2017). In Zel sheep (Dehnavi et al., 2012) it was reported polymorphism in intron 2, but intron 1 and exon 3 were monomorphic too. In Egypt Elkorshy et al. (2013) studied five Egyptian and Saudi sheep breeds and in all of them the exon 3 of MSTN locus was reported as monomorphic. In Iran Azzari et al. (2012) reported similar results in 110 native Dalagh sheep.

On the contrary, in Iranian Mehraban's sheep Jamshidi et al. (2014) detected two genotypes of exon 3 in MSTN gene – genotype Mm and genotype mm with frequencies 0,053 and 0,947, respectively. Soufy et al. (2009) observed polymorphism for exon 3 in Sanjabi sheep in Iran. They determined all three possible genotypes – MM, Mm and mm with frequencies 0,02, 0,01 and 0,97, respectively and the estimated allele frequencies were: for allele M 0,03 and for allele m 0,97.

CONCLUSIONS

In present study the exon 3 of MSTN sheep gene is found to be monomorphic for the both herds of Merino breeds - Ascanian and Caucasian, reared in Northeastern Bulgaria. It was detected only the allele m and the genotype mm, respectively with frequency 1.00.

ACKNOWLEDGEMENTS

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GENETIC DIVERSITY IN COMMERCIAL PURE CHICKEN LINES IN TURKEY

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Abstract

Genetic diversity provides an economic basis for protecting and conserving biodiversity. The identification and conservation of the current genetic diversity of chicken pure lines in Turkey are necessary not only to provide for today's demand for production, but also to respond to prospective breeding programmes in the future. In Turkey, four commercial brown layer lines (Rhode Island Red I, Rhode Island Red II, Barred Rock I, Barred Rock II) and four commercial white layer lines (Black Line, Brown Line, Blue Line, Maroon Line) were imported from Canada in 1995 and were used in selection studies based on various characteristics. In this study, a total of 200 chickens from these eight commercial layer lines reared in Ankara Poultry Research Station were genotyped by using microsatellite markers. The results obtained in this study showed that there was high level of inbreeding among chicken lines studied. There was not significant deviation from Hardy-Weinberg equilibrium in the lines. According to discriminant analyses, the chicken lines studied were divided into 2 main groups. Our results will provide reliable basic information for the future studies about Marker Assisted Selection program of Turkish commercial pure chicken lines and of domestic chickens.

Key words: *Genetic diversity, Turkish commercial chickens, genetic markers.*

EVALUATION OF LITTER WEIGHT AND MILK YIELD ON AWASSI EWES

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Abstract

This study was focused on Awassi ewes using of 2866 records at Al-Fajij station in Jordan. Objectives were to investigate environmental factors affecting milk yield (MY), Litter birthweight (LBW), Litter weaning weight (LWW) and lactation period (LP). Data were analyzed according to General Linear Model (GLM) by SAS (2012). Results showed that Least Squares Means (LSM) of MY, LBW, LWW and LP were 87.60±2.37, 6.44±0.05, 27.38±0.35 (kg) and 129.27±1.85 (day). LBW was highly significant affected ($P<0.01$) by year of lambing (YL), parity (PR), type of birth (TB), litter sex (LS), (YL×TB) but was insignificant effect ($p>0.05$) by line of ewe (LE) and age of ewe at lambing (AE). LWW was highly significant affected ($p<0.01$) by the same factors affecting LBW and was significant ($p<0.05$) by TB. YL had a highly significant effect on MY and LP. The (TB×YL) had a high significant ($p<0.01$) and significant ($p<0.05$) effect on MY and LP, respectively. LS had significant effect ($p<0.01$) on LP. It was concluded that better management might increase the litter weight and milk yield of Awassi ewes in Jordan.

Key words: *Litter weight, Milk, Awassi ewes, Jordan.*

BIOACTIVE PEPTIDES DERIVED FROM KORAT CHICKEN BREAST MUSCLE SUBJECTED TO VARIOUS THERMAL TREATMENTS FOLLOWED BY SIMULATED GASTROINTESTINAL DIGESTION

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Abstract

Chicken breast is a great source of nutrition with high protein content and low-fat content. In addition, muscle protein could be a good source of bioactive peptides after gastrointestinal (GI) digestion. Korat crossbred chicken (KC) is a new crossbreed between the male line of Thai indigenous chickens and the female line of commercial boiler chicken. Breast of KC was cooked under various conditions, namely heating at 70 °C for 30 min (H-0.5) and 24 h (H-24), autoclaving at 121°C for 15 min (AC-15) and 60 min (AC-60). All samples were subjected to in vitro simulated digestion by GI enzymes. Bioactivity of the digest was determined with antihypertension (an angiotensin-converting enzyme (ACE) inhibitory activity and antioxidant activities (ABTS radical scavenging activity, metal chelating activity, ferric-reducing antioxidant power (FRAP), and cytoprotective effect against cellular oxidative stress). The highest protein digestibility, ACE inhibitory activity, and metal-chelating activity were found in samples cooked by mild thermal treatment (H-0.5). The H-0.5 treatment also resulted in digest with the highest proportion of small peptides (<1 kDa). While the digest of AC-15 exhibited the highest FRAP and cytoprotective effect against tert-butyl hydroperoxide (TBHP)-induced oxidative damage of HepG2 cells and it contained the highest ratio of large peptides (>5 kDa). Therefore, KC breast heated at H-0.5 could serve as a source of ACE inhibitory peptides, while AC-15 could serve as a source of antioxidant peptides upon GI digestion.

Key words: *triploid, striped catfish, Pangasianodon hypophthalmus.*

EFFECT OF MILK PREBIOTICS ON PRODUCTIVE PERFORMANCE AND HEMATOLOGICAL TRAITS OF GOAT KIDS

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Abstract

The objectives of this study was to determine an optimal dose of inulin supplementation to enhance future investigating the effects on parameters associated with performance, immune modulation, or health status in goat kids. The number of Saanen kids (n=20), during the experimental period, were given only milk on days 1-30, thereafter, days 31-75, they were fed with concentrate and roughage, and amount of milk decreased until weaned, and days 76-90, goats were only concentrate and roughage or each. There were five dietary treatments groups; control diet (T1), inulin extracted supplemented 2% (T2) and 4% (T3), and commercial inulin supplemented 2% (T4) and 4% (T5) of diet (DM), respectively. The results showed that final body weight and average daily gains were significantly difference (p<0.05) and feed intake were highly significantly difference (p<0.01), specifically the supplementation of inulin at 2% (T2). In addition, it was found that there was an increase fecal score, total VFA and propionic acid (C3) and lowing ratios of C2:C3 were significantly difference (p<0.01), specifically the supplementation of inulin at 2%. Furthermore, were highly of Lactic acid bacteria, percentages of phagocyte activity, index of phagocyte activity, lowing methane, appearance digestibility and nitrogen absorption significantly difference (p<0.05), among treatments.

Key words: *milk prebiotics, productive performance, hematological traits, goat.*

SOLID-STATE FERMENTATION COULD BE ALTERED THE NUTRITIVE VALUES AND AMINO ACID COMPOSITION OF OLIVE LEAF (Olea europaea L.) *

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Abstract

In this study, the effects of solid-state fermentation with Aspergillus niger on the nutritional values and amino acid composition of olive leaf (Olea europaea L.) were determined. For this purpose, olive leaf samples belonging to Gemlik cultivar were divided into 2 treatment groups for use before and after fermentation. The treatment groups were formed before the fermentation of the olive leaf (OL) group and the group (fermented OL) fermented with A. niger (ATCC 9142). Crude protein (CP), ether extract (EE), ash, crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) contents and amino acid composition of the samples taken from 5 different batches before and after fermentation were analyzed. The CP, ash, CF, ADF, NDF, and ADL of fermented OL were higher (P<0.05) than those of OL. After fermentation, aspartic acid, glutamic acid, glycine, alanine, and tryptophan contents of fermented OL were increased. As a result, nutrient values and amino acid composition of the olive leaf could be altered by solid-state fermentation with A. niger. However, in vivo experiments should be performed to determine the effects on animals. This research was supported by The Scientific and Technological Research Council of Turkey (TUBITAK, project number: TOVAG-117R039).

Key words: *solid state fermentation, olive leaf, Olea europaea L., Aspergillus niger.*

CHARACTERIZATION OF A VASA HOMOLOG IN SNAKESKIN GOURAMI TRICHOGASTER PECTORALIS AND ITS EXPRESSION IN GONADS

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Abstract

The vasa gene has been demonstrated to specifically express in germ cell lineage which therefore has been widely used as germline marker for reproductive development in a number of fish. Therefore, in this study, vasa gene was cloned and characterized as Tpe-vasa in snakeskin gourami, Trichogaster pectoralis. The full-length cDNA of 2,419 bp of Tpe-vasa contained 117 bp of a 5′-UTR, 1,950 bp of an ORF encoding a putative 649 amino acids, and 352 bp of a 3′-UTR. Tpe-vasa comprised of predicted region that shared consensus motifs with the vasa genes in teleosts, including arginine- and glycine- rich repeats, ATPase motifs, and DEAD box. Phylogenetic tree was constructed and demonstrated that Tpe-vasa was clustered in the vasa family. Using reverse transcription polymerase chain reaction, Tpe-vasa mRNA was specifically expressed in gonads. By in situ hybridization, using antisense RNA probe, the positive signal of Tpe-vasa mRNA was detectable in various stages of germ cells of mature gonad whereas no signal was observed when sense RNA probe was used. This finding indicated that the Tpe-vasa could be used as germline marker for development of gonad in snakeskin gourami.

Key words: *gene expression, gonad, Trichogaster pectoralis.*

CHICKEN BLOOD AS A SOURCE OF DIPEPTIDYL PEPTIDASE-IV (DPP-IV) INHIBITORY PEPTIDES

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Abstract

Protein hydrolysates are of interest for the food industry because of their beneficial effects for the food products and for human health. Protein hydrolysates are produced by breaking down the proteins to peptides with has bioactivity, such as DPP-IV inhibitors are promising new therapies for type 2 diabetes. Chicken blood is one of the important coproducts from chicken slaughterhouses. Despite its high protein content, it is currently underutilized. The objective of this study was to produce chicken blood hydrolysate possessing DPP-IV inhibitory activity. Protein hydrolysates were prepared from whole chicken blood, plasma, or red blood cell by Alcalase, Pepsin, Protamex, and Papain. The Alcalase-hydrolyzed red blood cell (A-BC) exhibited the highest DPP-IV inhibitory activity. The degree of hydrolysis of A-BC increased when hydrolysis time and enzyme concentration. Hydrolysis time of 6 h and 4% Alcalase produced the highest DPP-IV inhibitory activity and protein recovery. However, protein hydrolysate would likely be modified upon gastrointestinal (GI) digestion, thus, the effects of in vitro GI digestion of A-BC was investigated. The A-BC was further hydrolyzed by gastrointestinal (GI) proteases, and the DPP-IV activity of A-BC digest increased compared to the parent hydrolysate. Therefore, red blood cell is a potential source for producing protein hydrolysates with DPP-IV inhibitory activity.

Key words: *Chicken blood, protein hydrolysate, DPP-IV inhibitory activity, Alcalase, gastrointestinal digestion.*

INVESTIGATION OF ABSG2 GENE IN THREE FINE FLEECE SHEEP BREEDS IN BULGARIA

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Abstract

The present study was conducted in order to investigate and identify the polymorphism in ABCG2 gene which is associated with milk production in three breeds, raised in Bulgaria. Blood samples were taken from 96 animals of Askanian Merino, Caucasian Merino and Karnobat Merino sheep breeds. Genomic DNA was extracted, and genotypes were estimated by means of PCR amplification using specific set of two primers. After PCR amplification and electrophoretic analysis two alleles and three genotypes were revealed. The presence of allele with deletion of 35 bp (-) and genotype -/- was higher in Askanian Merino animals than the two others investigated breeds. The highest H_o *was observed in Karnobat Merino – 0.371. In Caucasian Merino and Askanian Merino there was statistically significant results p>0.01.*

Key words: *sheep breeds, ABCG2 gene, PCR, polymorphism*

INTRODUCTION

Sheep farming is important for agriculture in our country, especially in the mountain regions of the country. It is a reliable source of income for farmers by selling milk, wool and animals. This production has additional advantages as it requires less investment and labor than other animals. In Bulgaria the production of sheep milk is important due to its use in the preparation of traditional products such as yoghurt, white cheese, yellow cheese and others. In the case of dairy sheep breeds of milk revenues are within 36-42% of the total ones (Stancheva, Staykova, 2009; Slavova et al., 2015). Sheep milk is mainly produced by multi-purpose breeds with low-to-medium milk yields and only several sheep breeds have been developed specifically for milk production. In last decade candidate genes associated with milk production in sheep are searched for increasing milk yield. One of the candidate genes associated with milk production is ABCG2 (Kijas et al., 2012; Gutiérrez-Gil et al., 2014; Deykin et al., 2016; Clark et al., 2017; Xu and Li, 2017).

The membrane-associated protein encoded by the ABCG2 gene is included in the superfamily of the ATP-associated cassette (ABC) transporters responsible for the transfer of various molecules through the cell membranes (Higgins, 1992, Wu et al., 2008). The ABCG2 (A) allele decreases milk yield and increases protein and fat concentration of cow milk (Ron et al., 2006). In goat ABCG2 gene expression is a function of lactation stage and parallel to goat lactation curve (Wu et al., 2008). The ABCG2 gene is located on the chromosome 6 (NC_040257.1) of the Ovis aries genome. It has 20 exons and it is expressed in some tissues, including the mammary gland. The level of expression get increased during the lactation period in some domestic animals (Al-Mamun et al., 2015). The link between the mutation found in this gene and milk yield, protein and fat percentage and somatic cell count (SCC) is reported in dairy cows (Cohen-Zinder et al., 2005; Mani et al., 2009). According to Gutiérrez-Gil et al. (2014) the ABCG2 gene influences milk yield in sheep. A mutation representing an insertion/deletion of 35 bp was established in the intron 5 of sheep ABCG2 gene (Árnyasi et al., 2013; Oner al., 2014). Hofmannová et al. (2018) examined this area in terms of somatic cell count (SCC) in the milk of healthy dairy sheep and established a certain association with the study trait. This gene is in great interest because of its relationship to milk production in sheep, but more profound research is needed for its impact on different sheep breeds.

The aim of this study is to determine the frequency of occurrence of the ABCG2 gene alleles in three fine fleece sheep breeds – Ascanian, Caucasian and Karnobat.

MATERIALS AND METHODS

In this study a total of 96 sheep from 3 breeds were investigated for polymorphisms located in ABCG2 gene. Blood samples were collected from 30 animals of Caucasian Merino, 31 animals of Askanian Merino and 35 animals of Karnobat Merino sheep breeds. Blood was taken from *v. jugularis* in 3 ml vacuum tubes containing EDTA. The investigation was carried out in the Laboratory of Genetics of Agronomy Faculty, the University of Forestry.

DNA extraction

The blood samples were stored at –20°C until DNA extraction. Genomic DNA was extracted using manual purification kits QIAamp DNA Blood Mini Kit (Qiagen) according to the instructions provided in the manual. The DNA concentration of each sample was determined by spectrophotometer Biodrop. The quality of the obtained DNA was about 10–12 ng. DNA was tested using gel monitoring on 1% agarose (Healthcare) gel prepared with TBE buffer (Thermo).

PCR amplification

PCR amplifications were carried out in total volumes of 10 µl, containing 4 µl DNA template, 0.2 µl dd H2O, 0.4 µl of each primer (Sigma) and 5 µl of 2×(1.5 mM MgCl2) Red Taq DNA Polymerase Master mix (VWR, Int., Belgium).

For genotyping the ABCG2 locus was used only PCR amplification according to Oner et al. (2014). The primer set used for the amplification of studied region of Intron 5 of Chromosome 6 of sheep genome is F: 5’GCCTCTTCTCCCATACGTC3’ and R: 5’AAACCAGTTGTGGGCTCATC3’.

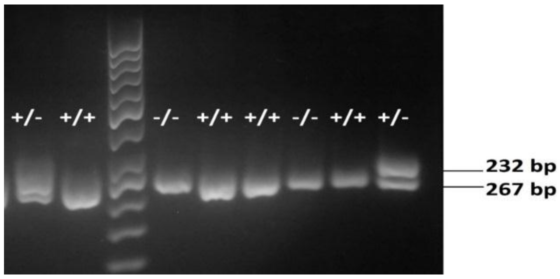
PCR amplification was carried out in total volume of 10 µl, containing 4 µl DNA template, 0.2 µl dd H2O, 0.4 µl of each primer (Bioneer) and 5 µl of 2×(1.5 mM MgCl2) Red Taq DNA Polymerase Master mix (VWR, Int., Belgium).

The conditions of the PCR amplification process were used according to Oner et al. (2014). The size of PCR products for ABCG2 gene were determined on 2,5 – 3 % agarose gel using GeneRuler™ Ladder, 50 bp (Thermo) supplied with 1 ml 6xDNA Loading dye and 10000x RedGel TM Nucleic Acid Stain (Biotium). The obtained PCR products were visualized under UV light.

RESULTS AND DISCUSSION

After agarose electrophoresis two alleles and three genotypes for ABCG2 were revealed. The length of the PCR product with deletion is 232 bp and 267 bp without deletion. The animals of the investigated breeds were found to be polymorphic by this region. Three genotypes were observed: +/+, +/- and -/- (Figure 1).

Figure 1. Amplified fragments of ABCG2 gene



The established frequencies are presented in Table 1 and 2.

Table 1. Allele frequencies.

Breed	n	Allele frequencies	
		+	-
CM	30	0.38	0.62
AM	31	0.24	0.76
KM	35	0.39	0.61

Table 2. Genotype frequencies.

Breed	Genotype frequencies					
	Observed			Expected		
	+/+	+/-	-/-	+/+	+/-	-/-
CM	0.27	0.23	0.50	0.14	0.47	0.39
AM	0.16	0.16	0.68	0.06	0.36	0.58
KM	0.2	0.37	0.43	0.15	0.48	0.37

According to the result it can be concluded that the presence of fragments with deletion was higher in all breeds. The frequency of allele ”-“ is highest in Askanian Merino sheep breeds - 0.76 and the frequency of genotype -/- is also highest in animals of the same breed - 0.68. Frequency of heterozygous genotype +/- (0.37) is highest in Karnobat Merino sheep breed and

H_o is also highest in the same breed – 0.371. The coefficient χ^2 is approximately equal in Caucasian Merino and Askanian Merino sheep breeds, where p<0.01 and the results are statistically significant. In Karnobat Merino sheep breed the value of χ^2 is lower with p>0.05 (Table 3).

Table 3. Heterozygosity, F_{is} and χ^2.

Breed	Heterozygosity		F_{is}	χ^2
	H_o	H_e		
CM	0.233	0.471	0.505	8.25^{**}
AM	0.161	0.365	0.559	8.27^{**}
KM	0.371	0.476	0.221	1.45^{*}

**p<0.01 – significant
* p>0.05 – nonsignificant

In our previous study (Bozhilova-Sakova et al., 2019) in animals of Askanian Merino, Karnobat Merino, Northeast Bulgarian Merino and Il de France sheep breeds there was no genetic diversity in this gene. All tested samples were with genotypes -/- which showed presence of deletion in studied region. The animals of two other breeds Caucasian Merino and Karakachan were found to be polymorphic by this region and three genotypes were observed: +/+, +/- and -/-. Oner et al. (2014) reported the "–" allele as predominant and its frequency differed from 0.50-0.65. On the contrary the "+"allele was found as prevailing in other study (Árnyasi et al., 2013). Hofmannová et al. (2018) identifies all three genotypes in the study of sheep from breeds Lacaune and East Friesian. In breed Lacaune is established predominantly allele with deletion (0.694), whereas in East Friesian chiefly occurs allele without deletion (0.784).

CONCLUSIONS

In conclusion it could be said that the results showed polymorphism in ABSG2 locus in investigated sheep from Caucasian Merino, Askanian Merino and Karnobat Merino breeds. Three different genotypes (+/+, +/- and -/-) were observed.

ACKNOWLEDGEMENTS

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CRISPR/CAS9 TECHNOLOGY IN LIVESTOCK ANIMALS

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Abstract

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) system has its origin on an adaptive immune system from prokaryotes which against exogenic elements in short fragments of viral DNA. This system naturally existing in the Streptococcus pyogenes type II CRISPR locus which consists of Cas9 nuclease and a sgRNA (single-guided RNA). Genome editing tools in livestock animals can provide important advantages compared to classical breeding. Given the high efficiency and low cost of genome editing tools, CRISPR/Cas9 system provides more advances in conferring resistance livestock animals to different diseases and improving some economic products such as meat, milk, eggs, wool, mohair and leather. CRISPR/ Cas9 system has been successfully adapted to almost all livestock animals such as chickens, pigs, cattle, sheep and goats. The most common example of this system used in animals is the genetic knock-out (KO) of the myostatin gene (MSTN) as MSTN is an important gene involved in the regulation of embryonic muscle cells and adult muscle development. With the aid of CRISPR/Cas9 system, the MSTN-KO phenotype was successfully induced in cattle, pigs, sheep and goats.

Key words: *CRISPR/Cas9, genome editing, livestock animals.*

PRODUCT DEVELOPMENT AND CONSUMER ATTITUDES TOWARDS GOAT MILK SOAP

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Abstract

This research aims to: 1) develop soap products from goat milk 2) study consumer attitudes towards goat milk soap products In Lampang. Province To use the information in the analysis of operational planning to generate sales As well as being a guideline for adjusting the marketing strategies of entrepreneurs The researcher used the marketing mix factors (4Ps) and the concept of demography as a conceptual framework in this study. Research tools Including questionnaires The sample group consisted of 400 samples and the statistics used in data analysis were percentage, mean, standard deviation. The results showed that 1) each step of production is used as handmade, using natural ingredients 100% . Component is a gentle formula soap, not irritating to the skin. Clean thoroughly Can be used on both face and body Remove wrinkles, dark spots that are ingrained. 2) Consumers΄ attitude towards goat milk soap products at a high level by marketing mix affecting the decision to buy goat milk soap products In Lampang Province , include 1) Products : The goat milk soap products that must be suitable for safe cleaning. There are a variety of formulas suitable for skin conditions. There is a proven, reliable result. And does not cause allergies or irritating to the skin, respectively 2) Price : The price that purchase must be appropriate to the quality and quantity of the product. 3) Place of distribution Must be comfortable, spacious and modern 4) Marketing promotion. The sample group gives importance to various advertising media. And want to reduce the price of products on various occasions respectively.

Key words: *goat milk, soap.*

EVALUATION OF MIXED CASSAVA PULP AND NAPIER PACKING GRASS FOR USE AS FEEDSTUFF IN LAYING HENS

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Abstract

Cassava pulp is a by-product generated from the cassava starch factory. This pulp contains 53.55% starch, 2.83% ash, 1.98% crude protein, 13.59% crude fiber and 0.13% ether extract on a dry matter basis. Since cassava pulp still remains high in starch content, therefore the pulp has widely used as an alternative energy source in animal diets. However, the fresh pulp also contains high moisture content approximately 70-80%, which can cause a problem of drying in the raining season. The combination of fresh cassava pulp and dried Napier Pakchong grass may help to resolve this problem. Therefore, the objective of this study was to evaluate the effects of mixed cassava pulp and Napier Packhong grass (MCN) on productive performance, cholesterol content and fatty acid profile of egg yolk in laying hens. A total of 180 ISA Brown, 67-week-old, were randomly distributed to 5 dietary treatments: one control and 4 MCN diets at levels of 5, 10, 15 and 20% with four replications of nine birds each (three birds/cage) using Completely Randomized Design (CRD). The experimental diets were formulated to include a similar level of ME and CP, respectively. Fibrolytic enzymes were added into all MCN diets to prevent the negative effect of high fiber content. Feed and water were provided ad libitum throughout the experimental period (8 weeks). The results showed that diets containing MCN up to 20% did not have negative effects on productive performance and plasma triglyceride of laying hens compared to control diet ($P>0.05$). Egg yolk cholesterol decreased linearly as increasing MCN in diets ($P<0.05$), a significant reduction was found in 20% MCN (107.22 mg cholesterol/whole egg) compared to control (142. mg cholesterol/egg). Moreover, MCN diets can alter the fatty acid profile in egg yolk, with higher production of omega 6, omega 9, MUFA and PUFA fatty acids than the control ($P<0.05$). Thus, dietary MCN can lower egg yolk cholesterol and alter fatty acid profile without having a negative effect on productive performance of laying hens.

Key words: *cassava pulp, Napier Packing grass, laying hen, productive performance, cholesterol.*

LOCAL SOYBEAN MEAL FEEDING EFFECT ON THE FATTENING PIG PERFORMANCE

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Abstract

Soybean processing products are the main sources of protein in the pig's diets over the world. The aim of this study was to determine the effect of feeding local and imported soya products to fattening pigs by feeding alone as the source of protein and examining its impact on the quality of pork. The experiment were conducted on 40 pigs in two groups (20 in each). At the start of trial piglets were 84 days old. The 1^{rst} group received the imported soybean cake, 2 nd local farm grown in Latvia soybean processing product mixed in the compound feed. During the study pigs were weighed three times at 84,140 and 190 days at age. Feed consumption was calculated. At the end of the study all pigs were slaughtered and determined the carcass traits. At the first growing period from 84 to 140 days of age, pigs daily gain was of 6.0% higher in 2nd group without significant differences. Also were not found the significant differences between piglet's daily gain of groups in the trial second period (140 to 190days), but daily gain in 2nd group was by 2.9% higher. In the all experimental (84 to 190 days) period in 1^{rst} group was 0.779 ±0.096 kg and 2nd 0.822 ± 0.103 kg by 5.5% higher in 2nd group without significant differences. Feed consumption per kg of liveweight and the pig carcass traits were similar for both groups. According to the classification of pork, all pig carcasses were evaluated by the "S" class. The results suggest that soybean grown and processed in Latvia is equivalent to imported soybean and gives good pig growth rates without reducing the quality of pork.

Keywords: *fattening pig, soybean meal, liveweight, pork quality*

INTRODUCTION

Soybean meal is a widely available feed for pigs and rich in protein. It has high biological value and digestibility. Soy products are also a significant source of energy in diets fed to pigs and soybean meal contains as much digestible and metabolizable energy as corn (Stein et.al., 2013). Soybeans are cultivated for their oil content, because protein is of good quality, and because they complement the protein of cereals so well that this blend has become the ideal feed for most pig diets worldwide. Soybean meal represents two-thirds of total world output in protein feedstuffs (Oil -World 2010). It is estimated that soybean accounts 85% of the protein supplements fed to pigs (Cortamira et.al., 2000). In the Europian Union (EU) soybean meal is the major oil seeed meal consumed in animal nutrition, but large percentage of the used soybean is imported. The production of soybean in Europe is low, compared to other oil seeds (Panagiota Florou-Paneri et.al., 2014).The main producers are USA, China, Argentina and Brazil (Oil -World 2010). Whilst it is an excellent vegetable protein feed for pigs, there are growing concerns about the environmental impact of its use, particularly due to the long transport distances (Smith et.al.,2013, Fredenburgh, 2013), and because the pig owners in Latvia have interest for local grown soybean. Significant differences exist in the composition and nutritive value of soybean meal from different origin. The chemical composition, protein quality and nutritive value of commercial soybean meal depended on the country of origin of the beans. Soybean meal samples from the European Union and United States had the greatest content of protein and the least amount of fiber compared with soybean meal from India, Brazil, Argentina, and China (Wang et. al., 2011). Average content of crude protein was 480, 505, and 488 g kg (as-fed) for Argentinean, Brazilian, and US soybean meal batches, respectively, and was greater for Brazilian soybean meal ($P \leq 0.05$) compared with the other 2 origins. Contents of most amino acids were greater ($P \leq 0.05$) in Brazilian compared with Argentinean soybean meal batches. Amino acid contents in USA soybean meal batches ranged between those from Argentina and Brazil (Goerke et.al., 2012). The aim of this study was to determine the effect of feeding local and imported soybean products to fattening pigs by feeding alone as the source of protein and examining its impact on the quality of pork.

MATERIALS AND METHODS

A total of 40 local Yorkshire× Landrace growing-finishing pigs with initial body weight 25 kg were selected from a commercial pig herd and used in the experiment. Pigs were allocated to trial in 1rst and 2nd groups for both grower and finisher periods, balanced for body weight and sex. They were placed by 20 pigs per pen (10 female and 10castrated male). Pigs were housed on concrete floors with shavings and access to drinking water at all times. A 2-hole feeder and nipple drinker were installed in each pen. Ambient room temperature in the commercial animal house used ranged between 18 and 22°C. The dietary treatments were formulated in a dose response feeding trial (Table 1). The 1rst group diets included imported soybean meal, but in the 2nd group diets soybean meal which made at the farm. Other ingredients were kept constant and included barley, wheat, fish meal, canola or soybean oil, salt and trace element vitamin premix, phytase, depending of pig liveweight and age. Diets formulated to be isoenergetic for metabolizable energy (ME) and with the similar crude protein (CP) content (Table 1.) Each diets was available on an ad libitum basis to pens with 20 pigs per pen for period from weaning age till end of trial.

Table1. Nutrients of diets for growing and fattening periods,% in DM

	1 rst group			2 nd group		
	Pigs liveweights (kg)					
	20-40	40-65	65-105	20-40	40-65	65-105
DM	88.6	88.5	89.1	88.7	89.0	90.0
CP	20.6	18.4	15.9	19.9	18.3	15.7
CF	3.33	3.07	5.2	4.85	4.71	4.49
Fat	3.36	2.71	2.65	2.98	3.69	3.72
Ca	0.99	0.9	1.19	1.14	1.12	1.12
P	0.51	0.48	0.51	0.54	0.54	0.57

The bodyweight (BW) for individual pigs and pen feed intake recorded to assess average daily gain (LWG) and average daily feed intake. The finisher pigs at the end of experiment, slaughtered at a commercial slaughter house. Hot carcass weights were obtained and backfat depth was measured at a specific site [i.e., the head of the last rib, 6 cm from the mid back line (P2), using a probe (Introscope Optimal Probe; SFK, Kolding, Denmark)]. For each individual pig, the percentage of lean meat (% Lean) was calculated as: 66.6708 – 0.3493×P2 (Latvia reg.of the Cabinet of Ministers Nr. 307.) and muscle eye area with the planimeter. One side of carcases was divided into fractions for determination ham.

Diets and ingredients were milled through a 1-mm screen before analysis. The chemical content of feed samples were determined in Scientific laboratory of Agronomic analysis of Latvia. The dry matter (DM), crude protein (CP), crude fibre (CF), fat, ash, Ca and P contents were analysed based on standard methodology, respectively DM with ISO 6496: 1999, CP with LVS EN ISO 5983-2: 2009, Ca with LVS EN ISO 6969: 2002, P with ISO 6491: 1998, CF with ISO 5498: 1981, fat ISO 6492: 1999, ash with ISO 5984: 2002/Cor1: 2005. The metabolizable energy (ME) were calculated and was from 13.3 till 13.5 MJ kg^{-1} (Mc Donald et al., 2002). All data were statistically processed to determine the differences between protein sources. Statistical analysis was performed according to the General linear Model procedure of SAS/STAT 9.22 software package (2010). Most data was reported as arithmetic means with the pooled SEM. The treatment means were compared using Student's t-test. Statements of statistical significance were based upon $P < 0.05$.

RESULTS AND DISCUSSION

Good feed is necessary for growth, body maintenance and production of meat, it contains sufficient energy, protein, minerals and vitamins. In table 2 are shown pig liveweights changes per experiment.

Table 2. Pigs liveweights changes (n=20)

Days	1 rst group		2 nd group	
	BW, kg^{-1}	SE±	BW, kg^{-1}	SE±
84	25.6	0.31	25.5	0.23
140	66.6	2.74	68.9	1.79
190	108.3	2.90	111.9	2.79

There is a little difference of pigs liveweights which fed rations containing imported or local soybean meal. It can be expain, that all rations were well-balanced and provided pigs with necessary nutrients. In the other research also are explained that is possibility of replaycing soybean meal with a mixture of legumes seeds (lupine seeds, rapseed) and got a high pig daily liveweightgain(Hanczakowska &Swiatkiewicz, 2014). At the beginning of the our trial, the pig start liveweights did not show any essential differences between groups, respectively in 1^{rst} group was 25.6±0.31 and in 2nd 25.5±0.23 kg (Table 2), at the age of 140 days the 2nd group pigs were heavier by 3.5% also without significant differences. At the final, the pigs which fed soybean meal grown in Latvia, showed by 3.3% higher liveweight than pigs which fed mixed feed with imported soybean meal. Daily liveweight gain were for pigs at 84 to 140 days 0.743±0.049 kg and 0.788±0.031 kg, but 2nd group showed by 6.0% higher results. The daily gain indices in fattening period from 140 to 190 days in 2nd group also was higher 0.877±0.032, but only by 2.9%. In all period of investigation (from 84 to 190 days) the 2nd group had also by 5.5% higher results (Table 3.)

Table 3. Pigs daily liveweights gains (n=40)

Weighing periods, days	1^{rst} group		2nd group	
	LWG, kg	SE±	LWG, kg	SE±
84-140	0.743	0.049	0.788	0.031
140-190	0.853	0.050	0.877	0.032
84-190	0.779	0.096	0.822	0.103

There were no significant diferences on pigs average daily gain, inclusion imported or local soybean meal.

Table 4. Carcass traits(n=40)

Indices	Control group	Trial group
BW, kg	108.3± 2.9	111.9±2.79
CW, kg	78.5±6.09	82.1±6.47
LC, cm	103.2±3.63	104.3±4.89
P2, mm	11.0±3.0	9.6±2.5
LM, %	61.6±0.8	62.0±0.72
MEA, cm^2	62.4±10.05	68.2±14.93
HW, kg	8.02±1.23	8.65±0.57

The table 4. shows the slaughter measures:carcass weight (CW) backfat (P2), lean meat (LM), the length of carcass (LC), muscle-eye area (MEA)and ham weight (HW). Dietary treatments did not significantly affect carcass traits (Table 4). According to the classification of pork, all pig carcasses of both groups were evaluated by the “S” class. Feed consumption per kg of liveweight gain were similar for both groups, 2.39 and 2.34 kg respectively.

CONCLUSIONS

In conclusion, the results suggest that soybean grown and processed in Latvia is equivalent to imported soybean and gives good pig growth rates without reducing the quality of pork.

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EXTRACELLULAR ENZYMES OF BACILLUS SUBTILIS K-C3 AND THE POTENTIAL TO USE AS A STARTER CULTURE IN FERMENTED SHRIMP

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Abstract

The growth and production of the extracellular enzymes, protease, lipase, and chitinase by Bacillus subtilis K-C3, isolated from commercial fermented shrimp (Kapi), were determined. This strain was able to grow and produce enzymes effectively at 25-35 degree Celsius and pH 6-8 in the presence of 10% NaCl. Changes in enzyme activities in Kapi, without inoculation and inoculated with B. subtilis K-C3 at the levels of 10^2, 10^4, and 10^6 CFU per gram of dry weight sample during Kapi production, were monitored. For Kapi without inoculation, B. subtilis K-C3 was present after the drying process and remained throughout all processes as established by the PCR-DGGE technique. For all the inoculated samples, B. subtilis K-C3 bands were detected at all stages of Kapi processing, indicating its survival during the entire process. All the inoculated samples exhibited higher proteolytic, lipolytic, and chitinolytic activities, compared to the control. Therefore, B. subtilis K-C3 could be used as a starter culture to enhance the fermentation of Kapi.

Key words: *Bacillus subtilis, shrimp paste, inoculation.*

PRODUCTION OF TRIPLOID STRIPED CATFISH (PANGASIANODON HYPOPHTHALMUS)

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Abstract

This study aimed to determine the optimum process to produce triploid striped catfish. Different temperatures and duration of treatment for heat-shock and cold-shock were employed to investigate the optimum method to produce triploid fish. Our results showed that cold-shock at 7.5 degree Celsius for 30 min was the optimum process to obtain 90 % triploid fish with 35.34 % of hatching rate. The treated larvae had 20 % of survival rate at 7 days post fertilization. Characterization of red blood cells (RBC) was conducted to determine triploidization. The RBC of triploid fish has significantly larger nuclear sizes. The result of flow cytometry of RBC was showed two discrete peak of DNA content in which the DNA content of RBC of triploid fish was approximately 1.5 times of that of diploid fish. Hematological study was demonstrated that there were no significantly different between triploid and diploid fish. Therefore, our results demonstrated optimum process to produce triploid fish which will be further investigates for their growth performance and gonadal development.

Key words: *triploid, striped catfish, Pangasianodon hypophthalmus.*

MODELING OF EGG PRODUCTION CURVES IN POULTRY

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Abstract

Modeling of egg yields has become popular in recent years. Modeling of egg yields is important in terms of revealing the economic efficiency of the enterprise. Egg production in poultry is generally determined by egg production curves using nonlinear mathematical models. In this study, Brown Egg Layer yields were used (between 18-59 weeks). Gamma, McNally, Cubic Spline and Logistic models were used in the study. In the comparison of the models, coefficient of determination, mean square error and Durbin-Watson values were taken into consideration. As a result of the study, the best model was determined as Cubic Spline (Coefficient of determination = 0.992, mean square error = 75417.4, Durbin-Watson = 2.01).

Key words: *Egg Production, Modeling, Poultry.*

Author Index

Nationality of Participants

Country	Number of Authors
Algeria	9
Bulgaria	8
Czech Republic	1
Germany	6
Indenasia	8
Italy	4
Jordan	4
Kazakhstan	3
Latvia	2
Pakistan	5
Thailand	18
Turkey	91
UK	2