



**ICABGEH-20**



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## PREFACE

This volume contains the papers presented at the **IV. International Congress on Domestic Animal Breeding Genetics and Husbandry 2020 (ICABGEH-20)** will be held online on August 12-14, 2020.

The ICABGEH-2020 Congress is organized by the Agricultural Faculty of Ondokuz Mayıs University and the Turkish Agricultural Engineers Association. ICABGEH-20 is the fourth international event of congress series with the participation of very popular invited speakers Dr. Maria DATTENA (AGRIS Sardegna), Dr. Roswanira Abdul WAHAB (Universiti Teknologi Malaysia), Dr. Zeynel CEBECI (Cukurova University) and Dr. Abdul CHAUDHRY (Newcastle University). This event is planned for brought together leading researchers, engineers, and scientists in the domain of animal science from around the world. It also provides opportunities for the delegates to exchange new ideas and application experiences as online, to establish business or research relations, and to find global partners for future collaboration.

The organizing committee is seriously planning and is already working towards enabling the Turkish and international animal science scientific community to meet the challenges and to move safely and successfully into the advanced information era. To this end, ICABGEH-2020 has been focused on recent developments, as far as research on animal science aiming at protecting the environment and food safety. Thus, ICABGEH-2020 has achieved its main twofold objective: Firstly, the presentation of current research works in the field of animal science, and secondly, connecting the animal science community.

**Prof. Dr. Hasan ONDER,**

Congress President

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## **NUTRITIONAL APPROACHES TO SUSTAIN RUMEN FUNCTION BUT MODIFY METHANE EMISSION**

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### **Abstract**

*Ruminant animals such as buffalo, cattle, goat and sheep are recognised for their value to supply high quality nutrients as meat and milk to sustain health and vitality of growing human population worldwide. However, these animals are criticised for their contribution to the environmental pollution, global warming and climate change. The nutrient wastage in the form of faeces, urine and gases from these animals is also considered as an energy loss causing an increased cost of production for a farm enterprise. Since most of these issues are related to rumen fermentation, it is imperative to develop strategies that not only optimise rumen function but also reduce nutrient wastage especially in the form of methane emission from these animals. In fact such nutritional approaches may also help us address some (e.g. food security and climate change) of the United Nations Sustainable Development Goals (SDG) which are agreed by member countries around the globe. This paper will introduce the importance of ruminant animal production in supplying high quality food to ensure food security for ever increasing human communities while reducing its impact on environmental pollution (e.g. methane) and climate change. A few examples of past and present studies involving dietary means to sustain rumen function, modify methane emission, promote food quality and yet maintain animal wellbeing will also be provided.*

**Key words:** Diets, Rumen function, Methane, Food security, Climate change

**EFFECTS OF SUGAR BEET PULP BASED TOTAL MIXED RATION ON GROWTH PERFORMANCE,  
BLOOD PROFILE AND HEALTH STATUS IN MALE NILI RAVI BUFFALO CALVES**

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**Abstract**

*Sugar beet pulp is comprised of highly digestible fiber that improves the growth rate in calves. The research was conducted to explore the effects of varying levels of sugar beet pulp (SBP) based total mixed ration (TMR) on growth performance and blood metabolites in buffalo male calves. Three TMRs were formulated based on quantity of SBP added i.e. 0% (TMR1), 15% (TMR2) 30% (TMR3) and assigned to three different groups (n = 6) of calves for a period of 60 days under a Completely Randomized statistical Design. Calves were offered iso-nitrogenous and iso-caloric ration @ 3% of BW/day on dry matter (DM) basis. Data regarding dry matter intake (DMI), body weight (BW) gain, nutrients digestibility, body condition scoring (BCS), feed efficiency (FE) and selected blood metabolites were collected. It was found that DMI, BW, BCS, blood glucose, blood urea nitrogen and digestibility of DM, crude protein and crude fiber were significantly higher ( $p < 0.05$ ) in calves fed with TMR3 compared to other groups. It was concluded that 30% SBP supplementation can enhance growth rate and nutrient digestibility of nutrients in Nili Ravi buffalo male calves.*

**Key words:** Buffalo, Sugar beet pulp, Performance

## THE EFFECTS OF OREGANO ESSENTIAL OIL AND CAPSICUM OLEORESIN ADDITION TO LAMB FEED ON MEAT MINERAL CONTENT

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### **Abstract**

*The aim of this study was to evaluate the effects of dietary oregano (*Oregano Onites L.*) essential oil and hot pepper oil (*capsicum oleoresin*) addition to lamb feed on meat mineral composition. In the experiment, thirty-six lambs aged 8 weeks old were randomly distributed to three dietary treatments, each with 12 lambs (6 male, 6 female). Three dietary treatment groups are control group without feed additives, Oregano group with 300 mg oregano essential oil/kg feed, Capsicum group with 300 mg feed capsicum oleoresin/kg feed. The experiment was maintained for 56 days. Lambs were fed lamb grower feed and freshwater was available ad-libitum during the experiment. At the end of the experiment, a total of 18 lambs (3 males and 3 females in each group) were randomly sampled for meat mineral concentration evaluations at 56d of age and slaughtered. Then, the left longissimus dorsi muscle of each left carcass from the end of the 12th costa was cut and removed, approximately 2.5 cm thick and analyzed. There were no effects of oregano and capsicum essential oils supplementation in concentrate feed on lamb meat macro mineral concentration as calcium, sodium, and magnesium ( $P \geq 0.05$ ). Although, 300 mg oregano essential oil supplementation increased significantly phosphorus and potassium macro mineral contents in the meat ( $P \leq 0.05$ ). Also, the oregano and capsicum essential oils supplementation decreased zinc concentration compared with the control group in the study ( $P \leq 0.05$ ). The addition of oregano and capsicum oil did no effect the iron and copper content of meat ( $P \geq 0.05$ ).*

**Key words:** *Lamb, Oregano essential oil, Capsicum oleoresin, Meat minerals*

### **INTRODUCTION**

Feed additives are used to improve growth performance in conventional animal production. The using them in animal feeds change some performance criteria of an animal such as the amount of yield, feed conversation ratio, daily weight gain, etc. and some product features e.g. carcass composition, nutritional value, stability, and shelf life of the meat (Kinnucan et al., 1997). Therefore antibiotics have long been used successfully in animal diets as growth promoters (Bampidis et al., 2005). However, their use has been prohibited in some countries, while in others has been subject to very strict control, due to the possible development of drug resistance in human pathogenic bacteria (CAFA, 1997). Also, consumers are demanding naturally raised, trusted, and natural meat products. Hence, new natural alternative feed additives are needed to reduce production costs and to make healthier and higher quality animal production by

eliminating consumer concerns, as well as not to decrease productivity. In recent years, aromatic plants and their extracts have received increased attention as potential alternatives to growth promoters.

It has been known for many years that essential oils and extracts have antimicrobial activity (Hammer et al., 1999) and many studies are showing that they are effective against pathogenic bacteria (Wallace 2004; Si et al., 2006). Essential oils affect and act less selectively against beneficial microorganisms such as *Lactobacillus spp.* and *Bifidobacteria spp.* in the flora of the farm animals digestive system compared to pathogens (Si et al. 2006).

Oregano (OEO) is an aromatic plant with a wide distribution throughout the Mediterranean area. OEO has antimicrobial and antioxidant properties, due to its natural phenolic components, mainly thymol and carvacrol (Xu et al., 2008). The active components (carvacrol and



thymol) of OEO are potent antimicrobials affecting populations of some fungi, bacteria and protozoa, this change in populations modifies ruminal fermentation, which is fundamental in the conversion of dietary nutrients to muscle tissue (Walsh et al., 2003; Veldhuizen et al., 2006). Specifically, there is evidence that carvacrol potentially decreases acetate concentrations, and it increases propionate and butyrate in sheep. Both are volatile fatty acids precursors of muscle and fat components in the animal (Koyuncu and Canpolat 2010).

Capsicum oleoresin is a compound that gives the hot pepper flavor and red color and is known as capsaicinoid. It was found in hot peppers (*Capsicum annum ssp.*) and is the main component of capsicum oil (10 to 15%) (Cardozo et al., 2004). The antioxidant activity of capsicum oleoresin varies depending on the amount of capsaicin (Viktorija et al., 2014). Capsicum oleoresin is effective in changing the pattern of dry matter intake (Rodriguez-Prado et al., 2012). Also, capsaicin oil seems to have very good potential especially in intensive fattening with low pH conditions (pH 5.5). Cardozo et al., (2005) demonstrated that in vitro system with rumen fluid from beef cattle fed a 10:90 ratio straw:concentrate diet at pH 5.5, capsicum oil reduced the ammonia N concentration, increased total VFA production and the propionate proportion, and reduced the acetate proportion, and acetate-to-propionate ratio. Meanwhile, capsicum oleoresin increased milk yield but did not any effect on milk fat concentration in dairy cattle (Harper et al., 2015). Therefore, there seems to be good potential for using capsaicin oil in fattening diets based on its effects on increasing DMI and potential effects on rumen microbial fermentation (Calsamiglia et al., 2007). Also, nutrition represents a significant aspect of the concentration of macro and micro elements of edible tissues of numerous species (Ribeiro et al., 2019). However, there were a very limited number of studies covering the effects of essential oils and herbal extracts on direct animals in vivo and their effects on carcass and meat quality of small ruminants.

This study aimed to evaluate the effects of dietary oregano essential oil and hot pepper oil (capsicum oleoresin) addition to lamb feed on meat mineral composition.

## **MATERIAL AND METHODS**

A total of 36 Menemen (illede France x Kivircik) male and female lambs, aged 8 weeks, were used for the study. At the beginning of the experiment, the mean body weight (BW) of male and female lambs for the 3 treatments was  $19.15 \pm 0.79$  and  $19.21 \pm 0.79$  kg, respectively. After being weighed, the animals were divided, according to live weight into three groups of 12 (six male and female) and housed in individual pens ( $2\text{m}^2/\text{lamb}$ ) for the duration of the experiment. The duration of animal was 56 days. The concentrate diets were prepared as the control (C) group without feed additives, oregano group (OEO) with 300 mg oregano essential oil/kg of feed, capsicum group (CEO) with 300 mg feed *capsicum oleoresin*/kg of feed. While lambs fed with concentrate diets and drinking water as *ad libitum*, alfalfa pellets was given as roughage with concentrate growth lamb feed that contains 10% alfalfa pellets.

Concentrate growing lamb mixed feed as a basic concentrate feed was prepared to a commercial feed factory. The ingredients and nutritional compositions of concentrate feeds are shown in Table 1. Oregano essential oil obtained by steam distillation from selected *O. onites ssp.* growing wild in Turkey was used in the study. The carvacrol and thymol contents, which are the most active compounds of oregano essential oil, were determined at 85.87% and 7.81% respectively (total 93.68%). Hot pepper oil obtained by steam distillation from selected *Capsicum annum L.* (Isot) was used in the study. Capsicum oil naturally produced from hot pepper contained 99% capsicum oleoresin was provided by commercial factory. All lambs were slaughtered at a commercial slaughterhouse at the end of the experiment.

Nutrient contents of feed ingredients and concentrate growing lamb feed were analyzed according to the methods reported in AOAC (1997). All samples were analyzed for dry matter (DM) (method 934.01), ash (method 942.05), crude protein (CP) (method 990.03), ether extract (EE) (method 920.39), crude fiber (CF) (method 962.09). The sugar content of the materials was determined by the Luff-Scroll method and the starch determination by the polarimetric method (AOAC, 1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were determined using the methods by (Van Soest et

al., 1991). Phosphorus (P) contents of the materials were read by spectrophotometer (model PE General TU-1880 Model Double Beam UV-V15) by calorimetric methods. Atomic absorption spectroscopy (Ultrospec 2100 pro UV/visible106 spectrophotometer) was used for determining calcium (Ca) concentration. The metabolisable energy content of the concentrate feed was calculated on chemical composition using a prediction equation (TSI-9610, 1991). Three male and three female lambs from each treatment group, weighed and slaughtered. After dressing and storing refrigerated for 24 h at 4 °C, carcasses were weighed and sectioned into two symmetric halves. Then, the left *longissimus dorsi* muscle of each left carcass from the end of the 12th costa was cut and removed, approximately 2.5 cm thick and analyzed for mineral concentration.

The elements calcium (Ca),phosphorus (P), potassium (K), magnesium (Mg), sodium (Na), iron (Fe), copper (Cu),and zinc (Zn)were measured with Perkin Elmer Optima 8000 ICP-OES. Prior to mineral analysis, the samples were homogenized. Approximately 0.5 g of the sample was accurately weighed into an acid washed TPFA digestion tube and 6 mL of HNO<sub>3</sub> (65%, w/v) and 2 mL H<sub>2</sub>O<sub>2</sub> (30%, w/v) were added (Nobrega et al., 2012). The microwave oven condition lines are 15 time and 110°C for both step 1 and step 2. The maximum total output of the microwave generator was 1450 W and the maximum pressure in the digestion tube was 45

bar. The digest was transferred into a 50 mL acid washed volumetric flask and the flask was filled up with demineralized water and stored in a polypropylene container. Each sample was decomposed into four replicates. Two water blanks were run with each batch of samples.

Measurements was carried out with a sequential, axially viewed Perkin Elmer Optima 8000 ICP-OES equipped with a main hard nebulizer, a glass cyclonic spray chamber and ICP Win Lab software Data System. The analyses were carried out under the following conditions: Rf power (W) 1450; Injector: Alumina 2 mm i.d.; Sample tubing: Standard 0.76 mm i.d.; Drain tubing: Standard 1.14 mm i.d.; Quartz torch: Single slot; Sample capillary: PTFE 1 mm i.d.; Sample vials: Polypropylene; Source equilibrium delay: 15 sec; Plasma viewing: Axial; Processing mode: Peak area; Gases: Argon and Nitrogen; Shear Gas: Air; Replicates: 3; Fe (nm): 259.943; Zn (nm): 213.857; Cu (nm): 324.757; Mn (nm): 257.610. The single component standards of Ca, P, K, Mg, Na, Fe, Cu, and Zn (each one with the content of 1000 ppm, Manchester New Hampshire).

The statistical analysis of the results included a one-way analysis of variance ANOVA using General Linear Models and Duncan's multiple range test, which were applied to the results using the SPSS 25 (IBM SPSS Statistics 25, 2016). The model included essential oil as main effects. Differences were considered to be significant based on the 0.05 level of probability

**Table 1.** The as a basic concentrate feed composition (g kg<sup>-1</sup>, as feed)

Ingredients	(g kg <sup>-1</sup> , as mixed)	Analyzed composition	(g kg <sup>-1</sup> ,as feed)
Barley	257.7	DM	896.9
Soybean hulls	124.1	Ash	72.7
Wheat brans	113.5	DP	165.0
Corn grain	103.1	EE	27.9
Wheat	103.1	CF	110.0
Alfa alfa pellets	103.1	Sugar	43.1
Soybean meal	102.5	Starch	275.9
Cotton meal	51.9	NDF	24.4
Soybean oil	5.2	ADF	139.0
Lime stone	23.6	Ca	12.0
Salt	5.7	P	4.0
Ammonium chloride	5.2	ME, kcal/kg	2600
Vitamin-mineral premix <sup>a</sup>	1.5		

<sup>a</sup> Premix contained per kg of concentrate: 11000 I.U. Vitamin A; 3 mg Vitamin B<sub>1</sub>; 5000 I.U. Vitamin D<sub>3</sub>; 0.069 mg 25-OH-D<sub>3</sub>; 8 mg Vitamin B<sub>2</sub>; 150 mg Vitamin E; 3 mg Vitamin K<sub>3</sub>; 4 mg Vitamin B<sub>6</sub>; 0.02 mg Vitamin B<sub>12</sub>; 60 mg Niacin; 15 mg D-Pantothenic; 2 mg Folic acid; 0.2 mg Biotin; 100 mg Vitamin C; 400 mg Co; 4000 mg Cu; 500 mg I; 5000 mg Fe; 500 mg Mn; 200 mg Se; 5000 mg Zn.

## RESULTS AND DISCUSSION

The effects of the OEO and CEO on macro and micromineral concentrations of the lamb meat were presented in Table 2 and Table 3. Concentrate diets containing essential oils were not statistically effective on macro minerals such as Ca, Na, and Mg concentrations in the lamb meat. But on the other hand; compared to the control group, oregano supplementation increased P content, while reducing capsicum significantly ( $P \leq 0.05$ ). At the same time, the addition of oregano essential oil in the concentrate growing lamb feed significantly increased the K content in meat compared to the control group while reducing capsicum oil ( $P \leq 0.05$ ). Ca concentration was the highest in control, but this difference was not found important significantly. Although there was seen biological difference among the dietary groups especially for Na concentration, these difference was not found significantly important. Na concentration in the OEO group was the highest ( $102 \mu\text{g mg}^{-1}$ ), while control and CEO had the same values (78, and  $79 \mu\text{g mg}^{-1}$  respectively).

As expected the meat tissue of the lamb carcasses contained the highest amounts of major elements. The concentration of major elements such as Ca, Na, and Mg except P and K mineral concentrations in the lamb meat was not influenced significantly with feeds adding oregano and capsicum essential oil. These findings regarding Ca, Na, and Mg contents in lamb meat were agreed with previous studies reported by researchers (Lin et al., 1989; G. Bellof et al., 2006; Hoffman et al., 2003; Reykdal et al., 2011). Unfortunately, there are no adequately published data about this issue of the effect of essential oils on the availability of macro elements in lamb meat. Compared with control, oregano essential oil supplementation increased the P concentration, while capsicum reduced. The control group is seen similar to both groups (oregano and capsicum) in terms of phosphorus content. Also, the phosphorus contents in the experimental groups are within the range found in the literature demonstrated by researchers (ARC 1980; Badiani et al., 1998; Karakök et al.,

2010). As known, phosphorus is an important element for many essential processes in the body. In combination with calcium, it is necessary for the formation of bones and teeth. Also, P is involved in the metabolism of fat, carbohydrate, and protein, and in the effective utilization of many of the B-group vitamins and in energy metabolism (Karakök et al., 2010). The biological reasons for these effects of essential oil supplementation on mineral metabolism are not apparent. Moreover, many factors such as stress and environmental can also influence the concentrations of blood and tissue minerals (McDowell et al., 1982). Because of that, mineral concentration in animal tissues can give an accurate indication of the change in the absorption and metabolism of dietary minerals (Abdelrahman and Hunaiti 2008). This finding suggests that oregano essential oil supplementation in the feed may increase the phosphorus metabolism in lambs, causing more phosphorus accumulation in meat.

In this study, potassium concentration in meat was significantly affected by oregano essential oil and capsicum essential oil supplementations. Potassium concentration was higher values for the OEO group and CEO group was same compared with the control, but all values fall within the normal adequate levels in the previously reported literature (ARC 1980; Bellof et al., 2006; Karakök et al., 2010). Potassium was the most abundant mineral in meat, with concentrations higher than 374 mg in fresh meat, regardless of gender or nutritional level. This was also observed for other mammalian species such as horses, cattle, buffalo, and avian species such as the ostrich (Riberio et al., 2010). The obtained results can be explained because this element is an intracellular ion crucial to allow muscle control, blood pressure, and also involved in nerve function (Riberio et al., 2010) and the level of under feeding was possibly not high enough to compromise this function. Hence it seems that oregano essential oil increased K metabolism in the lamb caused accumulation of K in the meat of animals; however, the mechanism needs to be further studied.

**Table 2.** Macro mineral content of lamb meat ( $\mu\text{g mg}^{-1}$ , in fresh meat)

Group	Ca	P	Na	K	Mg
Control	42±2	222 <sup>ab</sup> ±5	78±5	383 <sup>b</sup> ±10	36±1.0
Oregano	33±4	241 <sup>a</sup> ±7	102±9	434 <sup>a</sup> ±19	36±0.5
Capsicum	33±2	208 <sup>b</sup> ±8	79±6	374 <sup>b</sup> ±12	33±2.2
P	0.140	0.021	0.058	0.025	0.333

<sup>a, b</sup> The differences between means in the same row with different letters are important,  $P < 0.05$

Micro mineral contents for Fe and Cu in the lamb meat were not found statistically different among the groups in Table 3.  $P \leq 0.05$ . Although there were no differences among the dietary groups, Fe concentration was higher in the oregano group than control and lower in the capsicum group in the study. The Cu content in the lamb meat was higher in control than oregano and capsicum groups. In contrast, the mixed feeds with oregano and capsicum essential oil supplementations significantly decreased Zn concentration in the meat ( $P \leq 0.05$ ).

The meat and blood levels of minerals are important indicators of mineral status. Also, blood serum is frequently used for mineral assessment, because they are significantly correlated to the nutritional status of some trace elements (Levander, 1986). The adequate levels

of trace elements in lamb meat tissue must be mentioned for the discussion. The Fe and Cu concentration in the meat did not change significantly in the control and dietary treated groups. The meat microelement concentration of Fe and Cu remained within the normal reference range in the previously reported literature by (Reykdal et al., 2011; Riberio et al., 2020). In the present study high concentration of Zn was found to be significantly in the control group compared with the other groups ( $P \leq 0.05$ ). But, the concentrations of zinc of meats of oregano and capsicum groups were decreased according to control group. In the present study, it seems that oregano and capsicum essential oil adding to feeds cause the reducing accumulation of Zn in the meat of animals; however, the mechanism needs to be further studied.

**Table 3.** Micro mineral content of lamb meat ( $\mu\text{g mg}^{-1}$ , in fresh meat)

Group	Fe	Cu	Zn
Control	353±14	28±2	2235 <sup>a</sup> ±177
Oregano	400±45	24±1	1878 <sup>b</sup> ±26
Capsicum	346±14	22±1	1815 <sup>b</sup> ±79
P	0.371	0.057	0.040

<sup>a, b</sup> The differences between means in the same row with different letters are important,  $P < 0.05$

## CONCLUSIONS

It was concluded that supplementation of the oregano and capsicum essential oil at the level of 300 mg/kg in the mixed feeds did not have any adverse effects on the bioavailability of macro and microelements concentration in the lamb meat. Feed with oregano essential oil was significantly increased the phosphorus and potassium concentration of meat, however; feeds containing oregano and capsicum essential oils decreased the zinc concentration of meat. Further research is necessary to evaluate the mode of action and the effect of oregano and capsicum essential oil supplementations with different levels.

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## NUTRIGENOMIC APPROACHES IN POULTRY

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### **Abstract**

*The development of biotechnological applications in the last quarter of the century that we are in shows us that another way can be drawn in animal production. In light of the increasing knowledge in the field of molecular biology, it has been discovered that active and silent regions in the genome of the creature give different answers with their nutritional applications. Food applications have been shown to affect gene regions that determine criteria such as disease and yield in animals, and this molecular application is called "Nutrigenomics". Nutrigenomic; It is a concept that emerges with the combination of many branches of science such as proteomics, transcriptomics, bioinformatics, metabolic, genomics, and epigenetics. Thanks to this technological application, a more comprehensive expression of the mechanism of action of the ration components prepared in animal production on the genome activity is presented. In this way, the nutrients to be applied to the animal in every branch of animal production analysis for future generations can be discussed in more detail. Researches especially on poultry prove this. In this review, we will talk about how Nutrigenomic mechanisms work, what researches are done in poultry, and what approaches can be followed from now on.*

**Key words:** *Nutrigenomic, Poultry, Transgenomic, Proteomic, Metabolomic Molecular applications.*

### **INTRODUCTION**

With the development of molecular biology in recent years, researchers have gained knowledge and technology to determine the effect of the feed contents on the animal's genetic variation. The term "Nutrigenomics" was developed to describe the trend towards the individualized diet formulation in human nutrition research in the early 21<sup>st</sup> century. The concept of Nutrigenomics, first used by DellaPenna (1999), has been used to investigate the activity of nutrient contents in the genome. Nutrigenomics, nutrition-disease relationships, has focused on studies to determine the substances to be used in nutrition and the amount of use. Researches reveal the nutrient-genome or nutrient-proteome relationship using many disciplines to identify the basic nutrients or effects of feed components on intracellular activity and intracellular activity of the organism (Bordoni and Gabbianelli 2019). The feed contents can cause some changes that may affect the genome activity and genetic variation of the organism, which are usually caused by single nucleotide

polymorphism (SNP) (Long 2020). The SNPs also may affect translation activities, such as deletions in the genome, base additions, or the modification on the number of copies of nucleotides (Lyons et al., 1997). These mutations that have no effect on the proteome (silent mutations), may result some changes during transcription, translation, and post-transcriptional processes. Revealing the genomic information of the animals will contribute to understand the linkage between animal feeding and nutrigenomic applications in farm animals.

### **PRINCIPLES OF NUTRIGENOMIC MECHANISM**

The nutrients absorbed by the organisms can act as a signal to the cells in certain tissues. The biochemical signals are transmitted to the required mechanism through the receptors in the cell and can lead to a change in the level of expression on DNA or genes (Genomics) (Brody 1998). Transmission of any applied feed source to protein by mRNA translation and post-translation interactions (Proteomics) and the most recently produced proteins join the

intracellular metabolic pathway (Metabolomics) and affect intracellular traffic (Moody, 2001). To understand the effect of the nutrients applied in animal production on the genomic activity, it is necessary to know the transcriptomic, proteomic, and metabolomic disciplines to determine the effects of feeding practices.

### **Transcriptomic**

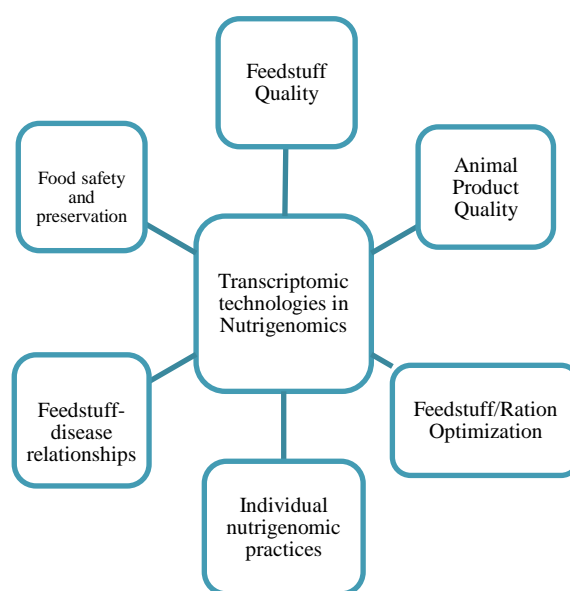
Transcriptomic is the discipline that involves the time from reading a gene region to coding (Corthésy-Thealoz, 2005). For a gene region to be expressed, enzymes that bind to the promoter region of the gene must be fully linked. Studies investigating the effect of nutrients used in animal production on enzymes involved in the transcription of gene regions that synthesize proteins have increased in recent years (Miller et al., 2014; Hettinga and Zhang 2018). Many methods have been developed to examine and determine the effects of nutrients and environmental factors such as heat, temperature, animal welfare on gene expression. Methods used to determine the level of expression of a gene region through mRNA; RT-qPCR (Reverse Transcription quantitative Polymerase Chain Reaction), SAGE (Serial Analysis of Gene expression), Northern Blotting and microarray technology. Through these methods, it is possible to observe the effects of the nutrient contents applied to animals' in the genome in a short time. The practices of transgenomic technologies in animal production are as shown in Figure 1.

### **Proteomic**

Proteomic is the discipline that studies all protein processes expressed in certain tissues and cells (Banks et al., 2000). It is one of the most complex discipline because of the difficulty for interpreting the information generated by synthesizing proteins and exposing different interactions to polypeptides that have the same amino acid sequence after translation (Pieroni et al., 2020). The main purpose of proteomics studies is to determine the protein structures, protein-substrate relationships and the effect of external factors such as food, temperature, and other environmental factors applied to the organism on protein expression and conformation (Gagaoua et al., 2020).

Post-translational modifications of a protein to be examined (different isoform structures, phosphorylation, glycolysis, and proteolytic

cleavages, etc.), as well as molecular weights, isoelectric points and different binding modes of mRNA can be determined (Pieroni et al., 2020). Methods such as mass spectrometry and 2D gel electrophoresis are used to determine the structure, the mass and the weights of proteins in the living organisms's proteome (Ohlendieck, 2011; Picard and Gagaoua, 2020).



**Figure 1.** Applications of transcriptomic technologies in animal production. Figure modified according to Zduńczyk and Pareek (2009).

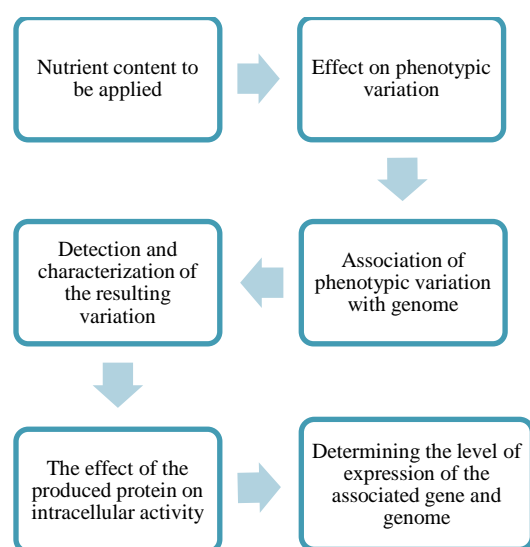
Nutrigenomic studies offer new research branches with new technologies for the future of animal production. Proteomic studies are the continuation of the transcriptome. A difference in the genome can cause the protein structure to affect the protein-substrate relationship.

### **Metabolomic**

Metabolomic is the science that studies gene-protein and protein-substrate relationships using high-tech methods such as nuclear magnetic resonance (NMR), gas chromatography (GC), mass spectrometry (MS), and high-performance liquid chromatography (HPLC) (Corthésy-Theulaz et al., 2005). While it is not precise with the information obtained in transcriptomics and proteomics, it can be interpreted about all possibilities, while studies in the metabolic field are an area in which all cellular activity is the simulation. Therefore, metabolic researches give more precise answers in the study of the living



thing's physiological response to stress factors, susceptibility or resistance to diseases, and reactions to the drugs to be administered. Nutrigenomic applications, selecting the correct nutritional content in animal production, the preparation of individual rations of animals, the determination of the genetic potential, of the organisms against disease diagnosis, and allows an analysis of the response to the drug (Nowacka-Woszek 2020). The nutrient content applied in animal production at the genome level and how it affects intracellular interactions are shown in Figure 2.



**Fig.2.** Nutrigenomic application steps (Cassar-Malek et al., 2008, adapted)

### Epigenetic Factors

Epigenetics focus on to explain how the molecular differences occurring around the DNA without any changes in the DNA sequence affect the activity and gene expression in the genome (Skinner et al., 2010; Guerreo-Bosagna & Skinner, 2012). Epigenetic mechanisms include DNA methylation, histone modification, non-coding RNA expression, chromatin structure changes, and RNA methylation (Mongan et al., 2019), which can cause some changes in gene expression in response to environmental changes. Although epigenetic mechanisms are similar in all living species, the emerging epigenomes of each species are significantly different. Epigenetic changes can be caused directly by environmental interventions during the basic development of an organism (Skinner

et al., 2010; Vandegehuchte & Janssen, 2014). It has been demonstrated that molecular differences caused by epigenetics can be transferred to future generations (Coolen et al., 2011). Many studies have been carried out in recent years to determine the epigenetic factors in farm animals. These studies focus on the epigenetic dynamics of nutrition and environmental interactions that affect gene expressions in the organism's genome, such as DNA methylation and histone modifications (Bordoni and Gabbianelli 2019). According to the studies on poultry feeding, epigenetic factors have the intergenerational epigenetic inheritance potential. However, most of the studies in this area have specifically studied DNA methylation or histone modification (Berghof et al., 2013).

A number of epigenetic reprogramming events take place in the early stages of embryo development in living organisms, including DNA methylation and histone modifications (Kafri et al. 1992).

Liu et al. (2015) investigated epigenetic models in chickens and hatched chicks in the early embryonic period. As a result of the research, DNA methylation and H3K9 acetylation levels decreased over time in liver cells, and demethylation, trimethylation, and acetylation of H3K9 were expressed depending on time and tissue. Epigenetic traces were relatively stable in hatched creatures and were found at lower levels. (Liu et al., 2015).

### FEEDSTUFF-GEN EXPRESSION LINKAGE IN POULTRY NUTRITION

#### Effects of Fatty Acids on Gene Expression

Transcription factors regulated with specific fatty acids have been identified in many organisms. These transcription factors in mammals are peroxisome proliferator-activated receptors (PPARa,  $-\beta / \delta$  and  $-\gamma$ ), estrogen receptors (ER), and sterol regulatory element-binding proteins (SREBP). Fatty acids in the rations are transferred to the cell nucleus after activation and regulate gene expression (Heuvel 2012).

The 7-days old *Gallus domesticus* chicks have been feed with 30%, 5%, and 10% T-3-enriched PUFA (Omega-3 Polyunsaturated Fatty Acid) (Roy et al., 2008). As a result of the research, positive directional regulation of genes encoding proteins involved in lipid oxidation has been

observed in living beings feed on omega-3 (Roy et al., 2008).

Flaxseed is rich in  $\alpha$ -linolenic acid and is used in broiler chicken rations to enrich tissues with n-3 fatty acids (FA). However, non-starch polysaccharides (NSP) in flaxseed reduce the digestibility of nutrients and limit the presence of n-3 FA. Adding carbohydrase enzymes to flaxseed-based rations can reduce the anti-nutritional effects of NSP. In this study, assuming that flaxseeds and enzyme supplements affect the expression of genes related to lipid metabolism in broiler liver and change their attachment-expression; the 0-day chicks have been feed for 42 days by adding flaxseed and enzyme at different dosages which were added to mixed eating. Flaxseed and enzyme-supplemented feeds have been found to reduce expression levels of positive regulated PPAR $\alpha$  target genes and CPT1A and ACOX1 genes related to De novo fatty acid synthesis. Briefly, flaxseed-based rations have been found to change the expression of genes involved in fatty acid lipid metabolism without affecting growth or production performance in chickens (Head et al., 2019).

#### **Effects of Minerals on Gene Expression**

Bivalent metals have a strong effect on gene expression. For example; in feeding, parental, or oral zinc cadmium application has been found to increase the rate of transcription of the metallothionein (MT) gene in the intestinal tissue (Ouellette et al., Page 122 1982). There are many studies on the investigation of the effect of minerals added to chickens ' rations on the transcription profile in gut cells. Rebel et al., (2006), observed that the mineral supplement added to the ration increased the mRNA levels of the genes associated with the intestinal cells, which are consistent with the number of cells in the villi and bowel movements of animals. It has been found that this increase in mRNA level may affect the level of expression of genes affecting animal feed digestion and the intestinal immune system (Rebel et al., 2006). Zinc acts as part of 'zinc finger motifs' that fix activator proteins to active segments of DNA. A suitable source of zinc is required to regulate the intracellular balance of pro-inflammatory enzymes such as cyclooxygenase-2 regulated by COX-2 gene (Fong et al. 2005). In a study of broiler chickens, the mRNA expression profile in the muscle tissue

of the trivalent chromium supplement added to the ration and in terms of its effect on protein synthesis, chromium added to the ration was found to be an essential element for the correct metabolism of nucleic acid, lipids, and carbohydrates (Pan et al., 2013).

The effect of three different forms of manganese (Mn) with doses of 100 ppm and 200 ppm was added to to 336-day-old commercial male broiler chickens' rations and the mRNA level of the MnSOD gene was investigated. Although Mn sources in breast meat have no effect on MnSOD mRNA expression level, Mn levels (100 and 200 ppm) were found to be effective (Lu et al. 2007). In thigh meat, both Mn sources and Mn doses were observed to have an effect on the level of mRNA expression of the MnSOD gene (Lu et al. 2007).

Moreover, another study investigated the effect of selenium (Se) on GSHpx, which is involved in Hsp70 and oxidative stress in turkeys (*Meleagris gallopavo*). As a result of the research, Hsp70 and GSHpx concentrations were suppressed in turkeys fed with Se fortified feed (Rivera et al. 2005).

It was thought that adding Zinc to the rations of parent stocks could reduce the mortality related to heat stress during the embryonic period of chicks (Zhu et al., 2017a). According to the findings of the study, an increase in antioxidant activity was observed in the liver tissues of embryos that related to mRNA and protein expression levels encoding metallothionein IV. Increased metallothionein IV mRNA expression has been found to result from a decrease in DNA methylation and an increase in histone 3 lysine 9 acetylations of the metallothionein IV promoter (Zhu et al., 2017a). In a similar study, it was discovered that Manganese addition to the ration protects the organism against heat stress during the embryonic period (Zhu et al, 2017b). In order to understand the ration contents to be applied correctly and to demonstrate epigenetic transgenerational inheritance in a chicken model, studies in this area should be focused.

#### **The Effect of Vitamins on Gene Expression**

Vitamin A is an essential fat-soluble nutrient that has functions in vision, reproduction, growth, mucous secretion, and epithelial tissues. Vitamin A is effective in the regulation process of retinol and retinoic acid. After binding to vitamin A

retinoic acid receptor, it stimulates cell differentiation (growth hormone, glycerolphosphate dehydrogenase, and leptin production) and transcription and translation of vitamin A sensitive genes (Duester 2000).

Vitamin E is a great source of antioxidants for biological systems. Vitamin E has been reported to protect cells involved in immune responses, such as lymphocytes, macrophages, and plasma cells, against oxidative damage and increase the function and proliferation of these cells (Puthongsiriporn et al., 2001).

Vitamin C, as a radical inhibitor, provides an electron to reactive free radicals, allowing them to stabilize (Deck 2018) and it can act in synergy with tocopherol by reconstructing tocopherol radicals (Makinen et al., 2001). It is known that the supplement in the ration, which contains a moderate level of vitamin C (200 to 250 mg/kg ration), has beneficial effects on the growth performance of chickens under heat stress (Imik et al., 2012). Zeferino et al. (2016) reported that vitamin C supplementation applied to the rations of chickens exposed to heat stress reduces the negative effects of heat stress on chickens.

In broiler chickens, the effect of different doses of vitamin D on genes associated with Tibial dyschondroplasia (TD), growth performance, and intestinal calcium homeostasis has been investigated. Hsiao (2018) observed that calbindin, -glucuronidase, TRPV6, and Na / Pi IIb cotransporter and homeostasis related genes mRNA levels increased in broilers fed 12-hour vitamin D-supplemented rations. It has also been found that vitamin D sources addition to the ration have a suppressive effect on TD (Hsiao 2018).

Another study was aimed to increase the meat quality and antioxidant capacity of broiler chickens by regulating the expression of the genes that regulate antioxidants of vitamin E. Therefore vitamin E was added to the ration; 240 one-day-old chicks were included in the 0, 100 mg/kg and 200 mg/kg vitamin E feeding program in addition to random ration (Niu, 2018). As a result of the experiment, parallel to the increase in vitamin E, the concentration of vitamin E in the blood serum and the development of the chest muscle has been increased. Total superoxide dismutase (T-SOD) and glutathione peroxidase (GSH-PX) activity increased in parallel with the increasement of

vitamin E in the ration, while malondialdehyde content decreased in contrast. The concentration of SOD and GSH-PX in the liver increased linearly with the increase in vitamin E. Consequently, it was reported that ration supplements with vitamin E may increased the lipid stability of chicken meat by increasing the AOE genes' expression in order to increase the antioxidant concentration in chickens (Niu, 2018).

#### **The Future of Nutrigenomic Applications**

Through the information and technologies developing in the field of molecular biology, the effect of nutrient contents to be applied in animal production at the genome level have become detectable. However, parallel to the number of studies in this field, it is necessary to understand the epigenetic mechanisms fully in order to understand food-yield or food-disease relationships. In order to investigate the activities of nutrigenomic applications in the genome and to understand what variation it will create in future generations, the nutrient components to be applied need to be studied in a complex way with other environmental factors. The discovery of the epigenetic mechanism would increase the reliability of the studies to be conducted in this field. Accordingly, it is inevitable that individual feeding methods will give more accurate results by considering the environmental factors to be applied to the animal thanks to the sharing of nutrigenomic and epigenetic studies. In the near future, it is expected that studies will aim to create an individual feeding program for the animal to reveal the genetic potential and the variation in its genome.

#### **CONCLUSION**

Nutrigenomic research has increased rapidly in recent years, but still more studies in farm animals and handling the applied nutrient ingredients together with epigenetic dynamics are needed to increase the reliability in this area. Although nutrigenomic studies applied in farm animals are higher than other model organisms in terms of breeding cost and labor, the results obtained from studies in this field are important for the future of animal production.

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**THE EFFECTS OF FETAL NUTRITIONAL PROGRAMMING IN RUMINANTS ON OFFSPRING'S  
GROWTH, HEALTH AND PRODUCTIVITY**

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**Abstract**

*This scientific paper reviews some important conditions necessary to state an effect of maternal nutrition on offsprings' metabolism, performance, reproduction, health and also immune system development. The fetus completely relies on dam's nutrition supply for growth and development. The balanced and adequate maternal nutrition not only ensures placental and fetal development but also influences health and productivity during the postnatal period of animal. Late gestation is probably the most important gestation period but the first two trimesters of gestation should not be neglected. Nutrition during early pregnancy could have impacts on organ and tissue development whereas nutrition during last trimester of pregnancy affects on body growth and immunity of newborn. In ruminants, the fetus is sterile in uterus that colonisation with microorganisms starts after parturition. During pregnancy, the pregnant ruminant animals can not transfer her antibodies to the fetus due to the cotyledonary placenta, thus the newborn should receive the antibodies from colostrum, establishing a defence mechanism for the postnatal period. Colostrum provides passive immunity to ruminant animals after birth. The process of colostrumogenesis takes place several weeks before birth and its' chemical ingredients could be affected by pregnant animal nutrition. Maternal nutritional restriction in late gestation produce less or poor quality colostrum and therefore calves with lower birth weight, increased some disease which can negatively effect of longevity and also death loss. Moreover, nutrient deficiency in pregnancy has been demonsrated to impact the reproductive performance of dams and their progeny. Diets for pregnant animals should be balanced with regard to protein (amino acids), energy and micronutrients.*

**Key words:** Maternal nutrition, Young ruminant, Performance, Immunity, Health

## CONSUMER CONSCIOUSNESS ON BROILER NUTRITION WITH ANTIBIOTICS AND HORMONES AND ITS REFLECTION OF CHICKEN MEAT CONSUMPTION

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### **Abstract**

*This research has been carried out to determine the effects of the current misinformation (hormone, antibiotic, feed additives and growth of broiler chickens in a short period) on consumers' view of the poultry meat sector. In the study, a province (Istanbul), which reflects community the best in sociocultural and socioeconomic way was chosen and face to face survey method was applied to 384 people with a different demographic structure. According to the results of the study, in broiler chickens' feeding, 78.4% of the consumers think healthy feed was not used, 97.4% think GMO feeding was harmful for human health, 88.3% think hormones were used, 84.6% think antibiotics were used for treatment and 70.6% of consumers stated that rapid growth was due to use of antibiotics and hormones. When the demographic structure of consumers is taken into consideration, it is revealed that young consumers and consumers with high educational and income status rely more on scientific methods. As the consumer age increases, the proportion of those who believe that rapid growth is due to antibiotics and hormones increases. According to these results, it is determined that consumers need to be informed about how broiler chickens are fed and whether additives are healthy based on scientific data.*

**Key words:** Broiler meat, Consumer, Hormone, Antibiotic, Safe food, Healthy nutrition

### **INTRODUCTION**

Although it has never been used in poultry in many countries, the use of hormones in compound feeds was banned in the 1970s and antibiotic use in 2006 (FDA 2016; Ozturk, 2016). However, controversy continues regarding the use of feed additives in diets. Scientific researches on the extent to which feed additives used in animal feed can be transferred to chicken meat and their effects are closely monitored by consumers. In a study aimed at evaluating the attitudes of consumers towards food safety, the factors that most concern consumers are; GMO, artificial food color substances, meat, milk and poultry hormone and antibiotic residues, pesticide residues and food additives have been reported (Demir and Aydin, 2018; Yang, 2017; Karasu and Ozturk, 2020). It has been reported in the written and visual media that speculations on poultry meat are seriously frightening beyond the fact that consumers are directed to avoid

consuming poultry meat (Karadavut ve Taşkın, 2014). In many studies, it was reported that the incompatible news about the used the antibiotics and hormones in the broiler diets had a negative effect on chicken meat consumption (Topçu et al., 2015; Lust et al., 2018). Therefore, in this study, firstly, it was tried to determine how the consumers will react if hormone and antibiotics are used in the diet of broiler chickens. Secondly, it is aimed to investigate the level of consciousness of the consumer about hormones and antibiotics and whether they can be used in feeding.

### **MATERIALS AND METHODS**

In the survey, multiple questions were asked related to participants' subjective and objective knowledge of composition of diets, knowledge antibiotic and hormone use. A quantitative survey questionnaire was developed to achieve study objectives, and it was directed face-to-face by



single-stage random sampling method between March and June 2017 in Istanbul. The sample was predetermined in terms of age (all were 18 years or older), number of respondents (394) and locality of residence (urban). A questionnaire was prepared to measure consumers' demographic characteristics, general purchasing preferences and behaviors, and broiler feed and product quality perceptions (Figure 1). In addition to awareness of therapeutic antibiotics and growth promoting antibiotics, the awareness of consumers that hormone and promoting antibiotics are prohibited was investigated. Descriptive analysis and khi square ( $\chi^2$ ) tests were used in the analysis of the data obtained from the survey. All statistical analyzes were performed in SPSS 21.0 software program. The effects were considered significant if  $P < 0.05$ .

## RESULTS AND DISCUSSION

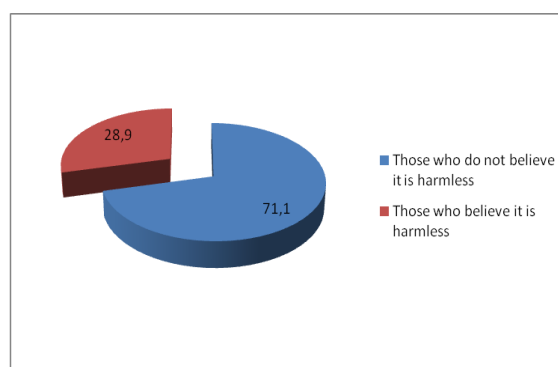
### The answers of questionnaires to demographic structure

Of the 384 consumers surveyed, 51.6% are between the ages of 18 and 45, 34.1% are between the ages of 46 and 60, and 14.3% are over the age of 60. When education levels are analyzed, 66.9% of consumers are university graduates, 24% are secondary school and high school graduates, 8.3% are primary school graduates and 0.8% are literate. 7.3% of consumers surveyed are between 0 - 500 USD, 26.3% between 500 - 1.000 USD, 40.4% between 1.000 - 1.500 USD per month, 20.8% between 1.500 - 3.000 USD and 5.2% stated that they earned more than 3.000 USD.

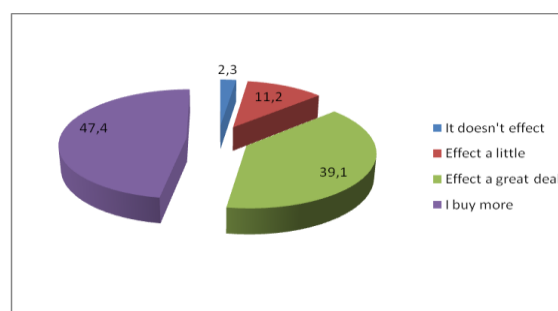
### Responds of questionnaires;

In our study, 78.4% of consumers surveyed stated that they think that broiler chickens are not fed with healthy feed, while only 21.6% say they think broilers are fed with healthy feed. While 73.4% of the consumers stated that they did not know about the compositions of broiler diets, 26.6% stated that they had knowledge. Do you think that hormones are used in feeding the broiler chickens? was asked to questionnaires. A big majority of the consumers (88.3%) stated that they think hormone is used in the feeding of broiler chickens and only 11.7% of them think that hormones are not used in production. If you know that the feed additives in diets are natural, how does this information affect your decision to buy or consume broiler meat? The question was

asked to consumers. While 67.7% of the consumers surveyed stated that they would affect a great deal, 20.3% effect little, and 4.4% never, and 7.6% would buy more.



**Figure 1.** Do you believe that feed additives are natural or harmless to human health?



**Figure 2.** Do you think that the use of antibiotics for therapeutic purposes in the feeding of broiler chickens is harmful to human health?

Despite the fact that added hormones are banned in production, it is asserted that hormones are used as additives and broiler meat is not safe food. Demir and Aydın (2018) reported that 70% of consumers' poultry meat was risky in line with the news regarding the use of hormones and antibiotics. Yang (2017) stated that the use of hormones negatively affects consumer preferences.

In a study conducted in Kars province, it was reported that as the education and income level of the consumers increased, the rate of those who think chicken meat is risky in terms of hormones and / or antibiotics increases. It has been reported that the news in the media is the most important factor triggering this decision (Demir and Aydın, 2018). In the United States, between 1994 and 2014, all news and articles written in two different national newspapers were examined to identify and compare the use

of antibiotics and hormones in poultry production. It has been determined that the content written over 20 years on the concerns of consumers about the use of antibiotics / hormones in poultry production, purposes of use and transparency (Edgar et al., 2017). Hormones act as growth promotants in animals for improved weight gain and feed efficiency before slaughter in meat industries. It is estimated that more than 90 percent of all U.S. feedlot cattle are injected with hormones to improve growth rates (USDA, 2013). Though hormone use is prohibited by EU and US federal regulations in poultry and swine production (USDA, 2015), other growth promotants (beta-agonists, such as Ractopamine and Zilpaterol), are also used in 60% to 80% of feedlot cattle in the U.S. (Penn State Extension, 2016). Hormones and antibiotics are never given to poultry. If growth hormones are fed to chickens with feed, they are destroyed in the digestive system and made ineffective. In order for the growth hormone to be successful, chickens had to be injected daily and several times a day. However, such an application is not possible logistically. Because it is not feasible and economical to catch thousands of chickens one by one and inject hormones several times. Antibiotics are used for therapeutic purposes in food producing animals by applying low concentrations to animal diets. The results of a survey show that the acceptability of antibiotic use was influenced by the participants having objective information about antibiotic use and antibiotic resistance (Gulab, 2018). Gulab, 2018 reported that attitudes towards animal welfare and demographic characteristics such as age, gender and race affect the acceptance conditions of antibiotic use in animals. It was also aimed to determine how the use of therapeutic antibiotics in the feeding of broiler chickens affects the purchasing decision of the consumers in this study. 11.2% of the surveyed consumers stated that they would never affect their purchasing decisions, 39.1% would tend to purchase less, 47.4% would not buy chicken meat and 2.3% would buy more (Figure 2). In this study, what do you think is the most important factor for broiler chickens to gain a live weight of 2.5-3.0 kg in a short period (42 days duration)? The question is directed to consumers. Only 14.1% of consumers who participated in the survey stated that the most important factors for

the broiler chickens to gain rapid live weight are the development of animals by scientific methods (breeding), feeding with balanced diets by all the nutrients and sheltering in suitable environments. 15.4% of consumers stated that broilers grew rapidly due to the feed additives added to the diets, while the vast majority of 70.6% stated that animals grew rapidly due to antibiotics and hormones.

## CONCLUSIONS

These findings obtained in our study show that false perceptions, which are common throughout the world and do not match the facts, continue to increase. This finding revealed that the effects of unfounded rhetoric and speculative news damaging the sector on consumers are quite large. In order to reduce the impact of these negative perceptions on consumers and to make a positive impact on purchasing decisions, it is necessary to provide satisfactory information by subject matter experts using appropriate communication channels and techniques.

## ACKNOWLEDGEMENTS

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## THE EFFECTS OF GENETICALLY MODIFIED FEEDS ON CONSUMERS' PREFERENCES IN BUYING BROILER MEAT

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### **Abstract**

*In this study, it was aimed to determine how the purchasing decisions of consumers change in case of feeding animals with genetically modified GM feeds. For this purpose, data was collected by surveying 384 subjects from the central districts of Istanbul province by face-to-face interview with one-step random sampling method. Descriptive analysis and chi-square tests were used to analyze the data obtained from the survey. Statistical analysis was performed using SPSS 21.0 software. It was determined that the food consumed by the animals was GM feeds in the preferences of the consumers to purchase chicken meat, which negatively affected the consumers. 2.9% of consumers who participated in the survey stated that feeding animals with GM feeds will not affect their purchasing decisions at all, 22.4% will affect less, and 74.7% will affect too much. According to the results of the research, feed consumption with GM affects the purchasing decisions of male consumers more negatively than female consumers and older consumers than younger consumers and they reduce the amount of chicken meat they buy. In addition, as the number of people in the families of the respondents' increases, the use of GM feed in animal feeding affects the purchase decision more. As the income status of consumers increases, the rate of affecting the purchase decision increases. Research results have shown that consumers of chicken meat have a deep suspicion that the use of GM feeds in animal feeding will negatively affect their health, and, if possible, they tend not to consume products obtained from animals fed GM feeds.*

**Key words:** Genetically modified (GM) feeds, Broiler meat, Consumer preference

### **INTRODUCTION**

Genetically modified (GM) organism is produced by copying and transferring the genetic properties in an organism to another organism that does not have these properties. Nowadays, the scientifically evaluated direct hazardous effects of GM food and feed on fauna and flora are contradictory; indeed, reviewing available data in the literature provides some evidence of GM human health and environmental risks. The possibility of horizontal gene transfer of GM organism related DNA to different species is not different from other DNA and is unlikely to raise health concerns (De Santis et al 2018). GM plants have become part of regular farming in many parts of the world for food and feed production. Poultry meat and meat products are an indisputable food in the human nutrition due to

its rich nutrient content. Today, broiler meat is the most important alternative to cover the animal protein deficit quickly and at low cost. The broiler industry is one of the fastest growing industries that can keep pace with the growing world population. For example, chicken meat production is 0.5 million tons in the 2000s in Turkey reached 2.5 million tons in 2019 and increased to 23 kg from 8.5 kg per capita (TUIK, 2019). Corn and soybean meal used as energy and protein source play an important role in this acceleration in broiler production. The fact that these two products are GM feeds causes the sector to be questioned about healthy food production among consumers. Consumer perceptions, attitudes and behaviors that do not rely on scientific data can seriously damage the

industry over time (Topçu et al., 2015; Öztürk, 2016).

In this study, it was aimed to determine the point of view of consumers by examining the use of Genetically Modified feeds that affect the decision process in purchasing chicken meat and which is met with concern.

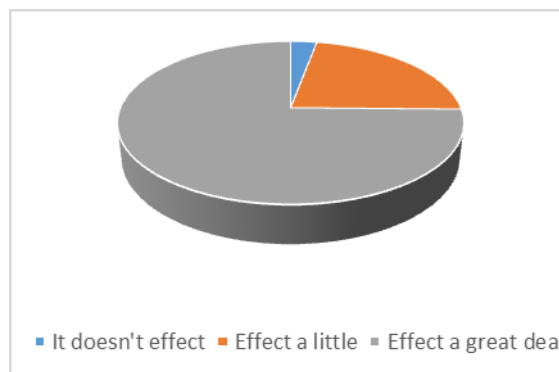
### MATERIALS AND METHODS

The data used in the study were collected in the central districts of Istanbul province in 2017 by interviewing 384 people face-to-face with a single-stage random sampling method and formed the main data of the study. In collecting data; a questionnaire form was prepared to determine the demographic characteristics of consumers and general purchasing preferences and behaviors and the survey studies were carried out by the researcher himself. In the analysis of the data obtained from the survey, descriptive analysis and khi square ( $\chi^2$ ) tests were used. Statistical analysis was performed using SPSS 21.0 software. The effects were considered significant if  $P < 00.5$ .

### RESULTS AND DISCUSSION

The family size for correspondents formed 50.3% family of 4 people, 16.9% family of 5 people, 12.5% family of 3 people, 11.7% family of 2 people, 7.6%'s consists of a family of 6 and 1% of 7 people. 40.4% of consumers monthly between 1.000 - 1.500 USD, 26.3% between 500 - 1.000 TL, 20.8% between 1.500 - 3.000 USD, 7.3% between 0 - 500 USD and 5.2% of them stated that they earned more than 3.000 USD. In addition to this information, 66.9% of consumers are university graduates, 24% are secondary school and high school graduates, 8.3% are primary school graduates and 0.8% are literate. The responses of consumers to the question about how GMO feed use information will affect their purchasing decisions in the feeding of broiler chickens are shown in Figure 1. While only 2.9% of consumers surveyed stated that using GM feed for feeding broiler chickens will not affect their purchasing decision, they stated that it would affect 22.4% little and 74.7% very much. 73.4% of the consumers who participated in the survey stated that they did not know about the content of the mixed feeds of the chickens they bought, and 78.4% of the consumers thought that broiler chickens were not fed with healthy feeds. The

vast majority of consumers (97.4%) stated that they think that using GMO feed in the feeding of broiler chickens is harmful to human health.



**Figure 1.** How does the use of GMO feed in the feeding of broiler chickens affect your purchase decision? Answers to the question.

According to the research findings, there is no significant relationship between the educational status of consumers participating in the survey and their purchasing decisions. However, the knowledge that GM feed is used in broiler feed shows that consumers act slightly differently according to their gender ( $P < 0.05$ ). However, in fact, both sexes reported that the use of GM feeds in feeding would affect purchasing decisions at very close ratios (versus 74% to 76%). In our study, feeding information with GM affected the purchasing decisions of young consumers less than older consumers. In other words, elderly consumers reduced the amount of chicken meat they bought if GM feeds were used in the diet. It has been determined that the level of education does not affect the willingness to purchase. As the number of family members increases, consumers' GM feed information affects their decisions to purchase chicken meat more ( $P < 0.05$ ). Considering the income situation, low and high income groups have less response in terms of purchasing behavior, while middle income groups have more serious reactions. In other words, knowing that GM feed is used, they stated that they will reduce the consumption of chicken meat at a higher rate than the low and high income groups. Discussions on the social, economic and political consequences regarding the safety of GM crop consumption in agriculture and food sectors continue increasingly. Some researchers report that laboratory animals fed diets containing GM

product toxic problems (Krimsky, 2015), while others report that GM-containing feeds have no safety issues (Delaney et al., 2018).

In our study, the rate of those who stated that the use of GM feeds in animal feeding would affect their purchasing desires was 22.4%, while the rate of those who reported that it would be very effective was 74.7%. Although consumers adopt biotechnological applications, it turns out that they are concerned about the products obtained from feeding with GM feeds and show negative attitudes. Yanpar et al. (2010) stated in their study that 10.1% of the surveyors stated that they did not see any inconvenience in GM product consumption, 95.4% of them should be labeled with GM products and 86.2% of them would not buy a product with GM label. Henderson (2018) reported that in the United States, consumers are willing to pay additional money to avoid chicken products fed with GM feed. Thus, it has been stated that there are two different market segments and the industry producers should produce chicken products without GM with a regular labeling system.

The vast majority of consumers imagine that hormones, antibiotics, GM feeds and some additives are used in animal nutrition and state that this situation is inconvenient for health (Ardebili and Rickertsen, 2020; Şengül and Zeybek 2020). In addition to this information, Şengül and Zeybek (2020) stated that older consumers are more concerned about whether chicken meat is healthy or not.

Consumer information about how GM is obtained and what effects it has on animal products when GM products are used in animal nutrition is either too inadequate or full of scientific facts and even false information (Wunderlich and Gatto, 2015). The vast majority of consumers receive information on GM food products from the media, internet and other news sources. Consumers worldwide are displaying limited understanding, misconceptions, and even unfamiliarity with GM food products. (Wunderlich and Gatto, 2015). In a study, only 8.7% of Turkish students approve genetic modification for improved nutritional content, compared with 68.2% who oppose modification for nutritional purposes and 22% who remain undecided (Turker et al. 2013).

Chicken meat, which is mostly referred by non-specialists, is referred to as unhealthy, risky and

hormonal food, and this information pollution created in the society causes serious damage to the poultry sector (Topçu et al., 2015; Öztürk, 2016). Since the broiler meat is strategic in meeting the protein needs of the increasing population cheaply (Öztürk, 2016), the decrease in consumption decreases the balanced nutrition opportunities of the general public. İnci et al. (2014) reported that the main factors negatively affecting the poultry meat consumption in Turkey are drugs or feed additives.

Consumer responses to food safety risks are affected by their demographic characteristics, such as gender, age, income, and education (Grobe et al. 1999; Kirk et al. 2002). Concerns about unnaturalness of GM foods could change quite rapidly given increased familiarity with these products and information about the similarities between GM techniques and conventional breeding. Ardebili and Rickertsen (2020)'s results suggest that the acceptance of GM foods is associated with attitudes towards naturalness, trust in public authorities, knowledge, and personality traits.

## CONCLUSIONS

The results of the research indicated that the vast majority of consumers do not prefer to buy meat that is fed with GM feed, and this affects their buying decisions. It is very important to act with scientific data so that the perceptions, attitudes and behaviors of consumers can be conscious and directed correctly. Accurate and adequate up-to-date information should be provided to manufacturers, consumers, regulatory agencies, governments, policy makers, researchers so that GM products can investigate potential risks in detail.

## ACKNOWLEDGEMENTS

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**IMPACT OF SUPPLEMENTATION OF MORINGA OLEIFERA AND LEUCAENA LEUCOCEPHALA TREE FODDER ON THE PRODUCTION OF INDIGENOUS GOATS IN MOZAMBIQUE**

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**Abstract**

*This study was conducted to assess the effect of supplementation with *Leucaena leucocephala* (LL), and *Moringa oleifera* (MO) tree leaves on growth and reproduction performance of indigenous goats in southern Mozambique. Fifty-six indigenous goats with an average age of 8 months and a body weight of  $17.6 \pm 4.0$  kg were randomly divided into seven treatment groups of 4 castrated males and 4 females each. Treatment 0 served as the control group (Co), and these animals only grazed on natural pasture without any supplementation. In addition to the natural pasture, three groups received 50 g (LL50), 75 g (LL75) and 100 g (LL100) of *L. leucocephala* dried leaves, respectively while groups 4 to 6, received 40 g (MO40), 60 g (MO60) and 80 g (MO80) of *M. oleifera* dried leaf meal, respectively. *Leucaena leucocephala* contained 23.7% crude protein (CP) and 11.1 MJ/kg DM of metabolizable energy (ME), while *M. oleifera* leaves contained 28.8% CP and 7.61 MJ/kg DM of ME. The study lasted for 16 months from July of 2015 to November of 2016. Compared to the control, treatment supplementation of the tree leaves, irrespective of level, had a significant effect ( $p < 0.05$ ) on the overall body weight gain and the final body weight of the bucks but did not significantly affected the does ( $p > 0.05$ ). No difference could be detected between the final body weight and overall average daily gain ( $p > 0.05$ ) based on the supplementation source (*Leucaena Leucocephala* versus *Moringa oleifera* dried leaf). Average daily gain (ADG), during the dry season, ranged from 7.85 to 10.4 g/head/day for goats fed LL leaves and from 7.92 to 13.3 g/head/day for goats fed MO and these values were higher ( $p < 0.05$ ) compared to values recorded for the control goats who lost weight with ADG ranging from -36.11 to -20.74 g/day. All female reproduction efficiency parameters measured such as birth rate, twinning rate, birth weight and weaning were significantly ( $p < 0.05$ ) higher in supplemented goats compared to the control goats. Body weights at birth and weaning weight of the offspring of supplemented goats were however not significantly ( $p > 0.05$ ) affected by supplementation. The highest survival rate (100%) was observed in goats supplemented with *Moringa oleifera* (MO40), while the lowest was recorded in goats supplemented with *Leucaena leucocephala* leaves (LL75). The results of this study suggest that *L. leucocephala* and *Moringa oleifera* tree leaves could be used as supplementation to goats to overcome the adverse effects of seasonal fluctuations in feed quality on growth and reproductive performance.*

**Key words:** *SheFodder trees, Growth, Reproductive, Smallholder, Goats, Supplementation*



**A MACROANATOMIC, MORPHOMETRIC AND COMPARATIVE INVESTIGATION ON SKELETAL SYSTEM OF THE GEESE GROWING IN KARS REGION II; SKELETON APPENDICULARE**

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**Abstract**

*The aim of this study was to comparatively, morphometrically and macroanatomically investigate skeleton appendiculare. A total of 24 goose cadavers were used. Scapula length was  $107.31 \pm 1.05$  mm in female geese; it was  $116.63 \pm 0.65$  mm in male geese. While the length of the os coracoideum was  $78.5 \pm 0.6$  mm in female geese; it was detected as  $87 \pm 0.8$  mm in male geese. The clavícula length was  $66.90 \pm 0.71$  mm in female geese and it was  $73.39 \pm 0.59$  mm in male geese and it was determined that both clavícula shaped furcula with a distinct curvature. While the length of the humerus in female geese was  $175.02 \pm 1.59$  mm; it was measured as  $191.28 \pm 1.44$  mm in male ones. While the mean ulna height was  $162.60 \pm 1.26$  mm in female geese; it was determined as  $178.84 \pm 0.83$  mm in male geese. The mean radius length was measured as  $154.20 \pm 1.63$  mm in female geese, it was  $169.75 \pm 1.31$  mm in male geese. It was detected that os carpi radiale was in a triangular shape, os carpi ulnare was in the form of a pipe. It was determined that the height of carpometacarpus was  $94.57 \pm 0.75$  mm in female geese;  $100.95 \pm 1.03$  mm was in male geese. Pelvis length was measured as  $154.27 \pm 1.81$  mm in female geese; it was  $169.91 \pm 1.68$  mm in male geese. While femur length was detected as  $86.85 \pm 0.98$  mm in female geese; it was  $93.87 \pm 1.12$  mm in male geese. While tibiotarsus length was  $160.94 \pm 1.88$  mm in female geese; it was  $174.20 \pm 1.28$  mm in male ones. While the tarsometatarsus length was  $93.15 \pm 1.19$  mm in female geese; it was measured as  $101.64 \pm 0.88$  mm in male geese. As a result, in this study, morphometric and morphologic values of examined bones of adult geese were determined and contributed to the elimination of the information in this area. It is thought that obtained findings will contribute in scientific research, evaluations of sexual dimorphism, zooarchaeological studies and operations on poultry animals.*

**Key words:** Anatomy, Goose, Kars, Poultry, Skeleton appendiculare

**INTRODUCTION**

One of the main issues of people today is the need for basic food sources. Food from animals is of high importance within this need. The largest source for increasing species diversity in food production is poultry animals. Among waterbirds, geese are at a different position with their species that are raised for multiple purposes and the diversity they provide in production. Among the commercially and economically important yield features of geese are their meat, liver, fat, feathers and eggs (Aslan, 2013). In addition, according to a study (Kılıç et al., 2018), intramedullary mature goose radius as the intramedullary pin was used in the treatment of femoral fractures in puppies and rabbits as organic osteosynthesis material. With this application, which was found to be clinically,

radiologically and histopathologically successful, a new usage area for goose bone emerged. Goose is the common name for the large species that constitutes the Anser strain of the Anatidae family from the Anseriformes tribe (Demirsoy, 1995; Tilki and Saatçi, 2013). In poultry, the locomotor system both contains the formations necessary for body balance and movement and reflects individual features. The skeleton constitutes the passive part of the locomotor system along with the joints and the skeletal muscles constitute the active part (Nickel et al., 1977). The most important feature of poultry is that they have pneumatic bones. These bones are in participation with the respiratory system through air sacs (sacci pneumatici). Non-flying poultry do not have pneumatized bones. There are three types of poultry bones: morphologically

compact bones, cancellous bones, and medullary bones (Dursun, 2007). In poultry, appendicular skeleton comprises of skeleton of the pectoral girdle, skeleton of the wing, skeleton of the pelvic girdle and skeleton of the pelvic limb. Poultry wing is equivalent to the front legs in mammals. Scapula, os coracoideum and clavícula (two clavícula merge form furcula) creates skeleton of the wing (cingulum membri thoracici) (Dursun, 2007). The wing is followed by these bones respectively; humerus, radius-ulna, carpus, metacarpus and ossa digiti (Nickel et al., 1977). Cingulum thoracic membrane of binding to the osseous in poultry while the body is in mammals muscle (Dyce et al., 1987, McLelland, 1990). Scapula and coracoid bones become smaller in good flightless birds, even ostrich was lost (Kuru, 1987). The bones (sacrum, coxa) that make up the ossa cinguli membri pelvici in the mammals are fused as well as the vertebrae lumbicalis (Nickel et al., 1977, König et al., 2016). Ossa membri pelvici consist of; femur, patella, tibiotarsus, fibula, ossa pedis, ossa tarsi, ossa digitorum pedis and phalanges (N.A.A., 1993, Dursun, 2007).

## **MATERIALS AND METHODS**

For this study, permission was obtained from Kars Provincial Directorate of Agriculture (dated 31.03.2017 and numbered E.791642) and KAÜ-HADYEK (KAÜ-HADYEK/2017-047). The cadavers supplied by breeders who butcher for food were brought to the laboratory of Kafkas University Faculty of Veterinary Science Anatomy Department and the study was conducted there.

A total of 24 goose cadavers; 12 of them female (1 old) and 12 male with average weights of  $3.25 \pm 0.15$  kg (female) –  $3.92 \pm 0.21$  kg (male) were used in the study. The weights of the goose cadavers were recorded with the help of digital precision balances (1g of unapproved sensitivity from 0-15 kg, and 2g from 15-30 kg, Baykon brand coded BCS21-6 MR). After the superficial muscles of 20 of the geese (10 female, 10 male) were dissected, the bones were revealed by maceration. After the superficial muscles of the geese whose bones are to be studied were dissected, boiling was performed for two hours in the water in which 10-15% sodium bicarbonate ( $\text{NaHCO}_3$ ) was added (Taşbaş and Tecirlioğlu, 1965). The bones were thoroughly cleaned after the cooling procedure and soaked in a 10% hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) solution for

two hours to whiten. After the last of the bones were thoroughly washed, they were left to dry in the sun (Taşbaş and tecirlioğlu, 1965; Mussa et al., 2015). Measurements were taken from all goose bones with the help of a digital caliper and measuring tape in accordance with the method laid out by DRIESH, 1976. Denomination was made in accordance with N.A.A. In order for pneumatic bones to be determined, 1 female and 1 male goose were injected acrylic (takilon), and 1 female and 1 male goose were injected a liquid rubber material (latex) colored with red fabric dye from the trachea for corrosion cast study. Each of the geese was injected with 120 ml of latex. They were then soaked in a 10% formaldehyde solution and dissected. The muscles were dissected and the pneumatized bones that the latex reached through air sacs were detected. Each of the geese was given 120 ml of an acrylic mixture containing 20% monomethyl-methacrylate and 80% plimetyl-methacrylate through the trachea. In order to ensure that this mixture solidified, cadavers were soaked in tap water for 24-48 hours. Then, examinations were made after the cadavers were soaked in a 30% potassium hydroxide (KOH) solution at a temperature of  $60^\circ\text{C}$  until the tissues were melted in order to make corrosion and cleaned. Thus, pneumatized bones were detected by means of both latex and acrylic. The mean and standard deviation values of all measurements and differences between genders were determined with the "independent samples t" test in the SPSS (version 20.0) packaged software.

## **RESULTS AND DISCUSSION**

### **Skeleton appendiculare**

Geese skeleton appendiculare were examined in 4 parts; ossa cinguli membri thoracici, ossa alae, ossa cinguli membri pelvici and ossa membri pelvici.

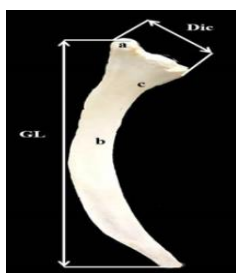
### **Ossa cinguli membri thoracici (Bones of the pectoral girdle)**

The wing bones was composed of scapula, os coracoideum and clavícula. Humerus, ossa antebrachii, ossa carpi, ossa metacarpalia and ossa digitorum manus were seen from the proximal to the distal to the wing.

### **Scapula (shoulder blade)**

Scapula was found to be curved in the middle and in the form of a sharper sword of the geese. The last vertebra cervicalis specialis (16-17)

corresponds to the level of the thickened front end; it was determined that it joins with os coracoideum and furcula to form a joint pit involving the caput humeri.



**Figure 1.** Measurements taken from scapula  
 GL: Greatest length, Dic: Greatest cranial diagonal, a: Acromion, facies articularis claviculae, b: Corpus scapulae, facies lateralis, c: Collum scapulae.

**Table 1.** Comparison of some parameters taken on scapula in male and female geese

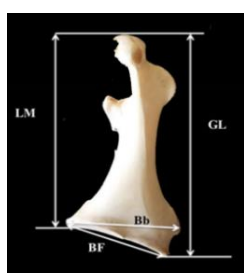
Parameter	Female (n=10)	Male (n=10)	P value
GL	107,31 ± 1,05	116,63 ± 0,65	<0,001
Dic	22,41 ± 0,46	22,43 ± 0,62	0,987

GL= Greatest length, Dic= Greatest cranial diagonal

In measurements taken (Figure 1), there is a significant difference in GL parameter in female and male geese; in male, the scapula length was found to be greater than that of females, while there was no significant difference in Dic parameter (P = 0.987) (Table 1).

#### Os coracoideum (corvine bone)

Os coracoideum; clavicle, scapula and humerus that articulates with the narrow end of cranial, and caudal end of which articulates with the sternum was found to be wide (Fig. 2). The strongest bone was found between the pectoral girdle bones. Saccus claviculae was found to pneumatize the os coracoideum.



**Figure 2.** Measurements taken from os coracoideum. GL= Greatest length, LM= Medial length, Bb= Greatest basal breadth, BF=Breadth of the facies articularis basalis.

**Table 2.** Comparison of some parameters on os coracoideum in male and female geese

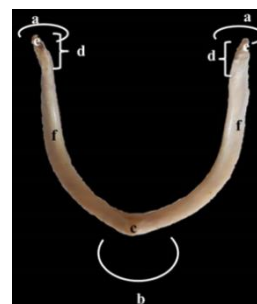
Parameter	Female (n=10)	Male (n=10)	P value
GL	78,5 ± 0,6	87 ± 0,8	<0,001
Lm	68,4 ± 0,6	75 ± 0,6	<0,001
Bb	32,76 ± 0,6	35,5 ± 0,4	0,001
BF	30,45 ± 0,4	33,6 ± 0,4	<0,001

GL= Greatest length, LM= Medial length, Bb= Greatest basal breadth, BF=Breadth of the facies articularis basalis

As shown in Figure 2, GL, Lm, Bb and BF values were statistically different in male and female geese (P <0.001). In male, these parameters were found to be higher than females (Table 2).

#### Clavicula (collar bone)

In the male and female geese, the clavicula was flat, while the cranial shaped curvature formed the furcula (Figure 3). The upper end of the clavicula was seen to be involved in the formation of the joint cavity of the art. humeri. It was determined that the lower end of the clavicula was formed in the synostosis on the middle plane with the other side and shaped the single bone called furcula. As seen in Table 3, the GL parameter was significantly different between male and female geese, it was longer in males than females. Clavicula was not found to be pneumatized.



**Figure 3.** Furcula (Clavicula dexter + Clavicula sinister). a: Extremitas omalis claviculae (Epicleidium, extremitas scapularis), b: Extremitas sternalis claviculae, c: Apophysis furculae (Hypocleideum, lamina interclavicularis, proc. interclavicularis), d: Facies articularis acroracoideae, e: Proc. acromialis, f: Scapus claviculae

**Table 3.** Comparison of clavicula lengths in male and female geese

Parameter	Female (n=10)	Male (n=10)	P value
GL	66,90 ± 0,71	73,39 ± 0,59	<0,001

GL= Greatest length

### Ossa alae (Bones of the wing)

Ossa alae was found to be composed of humerus, skeleton antebrachii, carpus, carpometacarpus and digiti (Figure 4).

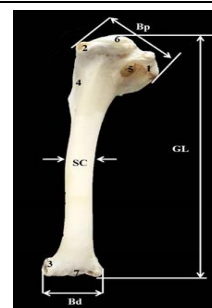


**Figure 4.** Ossa alae. 1: Humerus, 2: Radius, 3: Ulna, 4: Ossa carpi, 5: Carpometacarpale, 6: Os metacarpale minus, 7: Os metacarpale majus, 8: Digiti II, 9: Digiti IV, 10: phalanx I in Digiti III, 11: phalanx II in Digiti III.

### Skeleton brachii (Humerus, arm bone)

The humerus was the strongest bone in the wing. It was seen that there was an oval structure (caput humeri) on the upper end of the medial. In the dorsolateral of the caput, it was found that the bump was shaped as a tuberculum laterally. In the ventromedial of caput, the tuberculum mediale was found; crista tuberculi medialis was found to extend downwards from the tuberculum mediale. For. pneumaticum was seen in the mediodistal of tuberculum mediale. Trochlea humeri was detected at the distal end of the bone. It was found that the larger condylus ulnaris and smaller condylus radialis on trochlea. The trochlea had epicondylus ulnaris and epicondylus radialis (Figure 5). The length of the humerus in the geese was higher than the length of the antebrachium (radius + ulna). Saccus clavicularis was found to pneumatize the humerus.

GL, Bd and Bp values were statistically different in male and female geese ( $P < 0.001$ ). In other words, these parameters were higher in males than females. There was no significant difference between the sexes in terms of SC parameter ( $P = 0.146$ ) (Table 4).



**Figure 5.** Measurements taken from humerus. 1: Tuberculum ventrale, 2: Tuberculum dorsale, 3: Condylus dorsalis, 4: Crista deltopectoralis, 5: For. pneumaticipitalis, 6: Caput humeri, 7: Fossa olecrani, GL: Greatest length, Bp: Breadth of the proximal end from the tuberculum laterale or dorsale to the tuberculum mediale or ventrale without the crista lateralis, SC: Smallest breadth of the corpus, Bd: Greatest breadth of the distal end.

**Table 4.** Comparison of some parameters of humerus in male and female geese

Parameter	Female (n=10)	Male (n=10)	P value
GL	175,02 ± 1,59	191,28 ± 1,44	<0,001
Bp	37,73 ± 0,67	42,90 ± 0,79	<0,001
SC	11,84 ± 0,35	12,50 ± 0,27	0,148
Bd	25,56 ± 0,33	27,91 ± 0,40	<0,001

GL: Greatest length, Bp: Breadth of the proximal end from the tuberculum laterale or dorsale to the tuberculum mediale or ventrale without the crista lateralis, SC: Smallest breadth of the corpus, Bd: Greatest breadth of the distal end.

### Skeleton antebrachii (Radius-Ulna, Forearm bones)

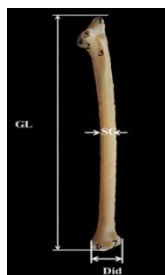
It was determined that the forearm skeleton consisted of thick ulna and thin radius (Figure 6-7). Radius and ulna were not found to be pneumatized.

### Ulna (Elbow bone)

Ulna was thicker and longer than the radius. Corpus ulna was almost flat and papilla remigalies were found to be the basis for the adherence of the feather. It was determined that condylus distalis at the bottom was in contact with the radius when performing the art. radioulnaris. It was determined that condylus distalis at the bottom was in contact with os carpi ulnare and os carpi radiale when performing the art. carpoulnaris (Figure 6).

As shown in Figure 6, GL, Dip, Bp and Did values were statistically different between male and female geese ( $P < 0.001$ ;  $P = 0.001$ ). In other

words, these parameters were higher in males than females. There was no significant difference between the sexes in terms of SC value ( $P = 0.11$ ) (Table 5).



**Figure 6.** Measurements taken from the ventral side of the ulna

1: Olecranon, 2: Incisura radialis, 3: Impressio brachialis, 4: Cotyla dorsalia, 5: Crista intercotylaris, 6: Incisura tuberculum carpalae, 7: Sulcus intercondylaris, GL: Greatest length, SC: Smallest breadth of the corpus, Did: Diagonal of the distal end.

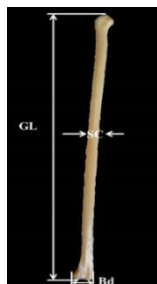
**Table 5.** Comparison of some parameters taken from ulna in male and female geese

Parameter	Female (n=10)	Male (n=10)	P value
GL	162,60 ± 1,26	178,84 ± 0,83	<0,001
Dip	21,40 ± 0,29	23,45 ± 0,40	0,001
Bp	10,53 ± 0,68	15,69 ± 0,24	<0,001
SC	8,72 ± 0,32	9,40 ± 0,24	0,111
Did	16,57 ± 0,23	18,81 ± 0,31	<0,001

GL: Greatest length, Dip: Greatest diagonal of the proximal end from the caudal border of the olecranon to the cranial border of the facies articularis lateralis (dorsalis), Bp: Greatest breadth of the proximal end from the facies articularis medialis (ventralis) to the facies articularis lateralis (dorsalis), SC: Smallest breadth of the corpus, Did: Diagonal of the distal end

### Radius (Rotary bone)

It was determined that the caput radii at the proximal end of the radius was articulated with the condylus radialis of the humerus.



**Figure 7.** Measurements taken over radius  
 GL: Greatest length, SC: Smallest breadth of the corpus, Bd: Breadth of the distal end

**Table 6.** Comparison of some parameters belonging to radius in female and male geese

Parameter	Female (n=10)	Male (n=10)	P value
GL	154,20 ± 1,63	169,75 ± 1,31	<0,001
SC	5,94 ± 0,21	6,28 ± 0,28	0,335
Bd	11,53 ± 0,24	12,69 ± 0,19	0,001

GL: Greatest length, SC: Smallest breadth of the corpus, Bd: Breadth of the distal end

As a result of the measurements (Figure 7), GL and Bd values were statistically different in male and female geese ( $P < 0.001$ ;  $P = 0.001$ ). In other words, these parameters were higher in males than females. There was no significant difference between the sexes in terms of SC value ( $P = 0.333$ ) (Table 6).

### Skeleton manus (Hand skeleton)

#### Ossa carpi (Hand wrist bones)

In the ossa carpi it was seen that only os carpi ulnare and os carpi radiale were on the antebrachial line. Os carpi radiale the two sides appeared to be a blunt edge as sharp as a plump triangle. Os carpi ulnare was found to be in the form of a pipe (Figure 8). The bones in the ossa carpi were not pneumatized.



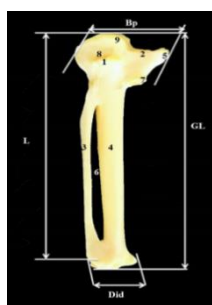
**Figure 8.** Os carpi ulnare and os carpi radiale  
 1: Os carpi ulnare (Os cuneiform), a: Crus breve, b: Crus longum, c: Incisura metacarpalis, d: Proc. muscularis, 2: Os carpi radiale (Os scapholunare)

#### Carpometacarpus (Hand wrist-comb bone)

It was observed that carpometacarpus was composed of a total of 3 bones shaped by the distal order of ossa carpi and metacarpus 2, 3 and 4. It was determined that os metacarpale majus was longer and stronger and os metacarpale minus was shorter and weaker. Os metacarpale alulare was seen as rudimentary. Extremitas proximalis carpometacarpi and extremitas distalis carpometacarpi had two ends. In the measurements taken (Figure 9), GL, L and Did values; male and female geese were found to be statistically different ( $P < 0.001$ ;  $P = 0.009$ ), while males in females carpometacarpus was determined to be higher than the length of these parameters. In addition, there was no significant

difference between the sexes in BP value ( $P = 0.23$ ) (Table 7).

**2.3.4. Ossa digitorum manus (Hand finger bones):** It was determined that ossa digitorum manus consists of 3 fingers of different size and structure. Of these, 2 were formed from two phalanx. The third finger, the strongest finger, consisted of a total of 3 phalanx. The fourth finger is formed from a single. It was found that the finger was attached to the proc. alularis located medially at 1.5-2 cm below the proximal end of the carpometacarpus.



**Figure 9.** Measurements taken from carpometacarpus.

Did: Facies articularis digitalis major, 1: Proc. psiformis, 2: Trochlea carpalis, 3: Os metacarpale minus, 4: Os metacarpale majus, 5: Proc. extensorius, 6: Spatium intermetacarpale, 7: Proc. alularis, 8: Fossa infratrochlearis, 9: Trochlea carpalis, GL: Greatest length, L: Length of metacarpus II, Bp: Greatest breadth of the extremitas proximalis, Did: Greatest breadth of the extremitas distalis.

**Table 7.** Comparison of some parameters on carpometacarpus in female and male geese

Parameter	Female (n=10)	Male (n=10)	P value
GL	94,57 ± 0,75	100,95 ± 1,03	<0,001
L	93,50 ± 0,83	98,83 ± 0,82	<0,001
Bp	23,04 ± 0,25	24,74 ± 0,64	0,23
Did	12,98 ± 0,31	14,20 ± 0,26	0,009

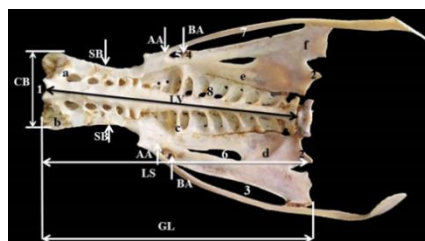
GL: Greatest length, L: Length of metacarpus II, Bp: Greatest breadth of the extremitas proximalis, Did: Greatest breadth of the extremitas distalis

### Ossa cinguli membri pelvici (Bones of the pelvic girdle)

#### Os coxae (Hip bone)

Synsacrum and ossa coxae were combined to form the goose pelvis. It was determined that each of the hip bones consisted of the fusion of three bones, namely os ilium, os pubis and os ischii. Os ischii and os ilii were determined to shape the acetabulum. Os ilium was the largest

bone involved in the formation of coxae. Ala preacetabularis ili, ala postacetabularis ili and corpus ilii were found to consist of three parts (Figure 10). Os ischii was composed of two parts: corpus ischii and ala ischii. Incisura acetabularis, pila ilioischiadica and antitrochanter on corpus ischii were detected. Os pubis consisted of two parts: corpus pubis and scapus pubis.



**Figure 10.** Pelvis'in ventral'den görünüşü.

LV: Sulcus ventralis synsacri, CB: Extremitas cranialis synsacri, 1: Margo iliocranialis, 2: Spina iliocaudalis, Proc. marginis caudalis, 3: Fenestra ischiopubica, 4: Antitrochanter, 5: For. obturatum, 6: For. ilioischiadicum, 7: Scapus pubis, 8: Forr. intertransversariae, a: Crista iliaca lateralis, b: Ala preacetabularis ilii, c: Proc. costalis, d: Fossa renalis, e: Ala postacetabularis ilii, f: Ala ischii, GL: Greatest length (without pubis), LS: Length from the cranial border of the ilia to the spinaeilio-caudales, LV: Length along the vertebrae, centrally, CB: Cranial breadth, SB: Smallest breadth of the partes glutaea, AA: Breadth between the borders of the acetabulum, measured at the narrowest part, BA: Breadth in the middle: breadth cross the two antitrochanter. In the measurements taken (Figure 10) GL, LS, LV, CB, SB and BE values in male and female geese was statistically different ( $P < 0.001$ ,  $P = 0.006$ ;  $P = 0.002$ ) were seen. In other words, these parameters were higher in males than females. In addition, there was no significant difference between the genders in AA and DIA values ( $P = 0,147$ ;  $P = 0,914$ ) (Table 8).

### Ossa membri pelvici (Bones of the pelvic limb):

#### Femur (thighbone)

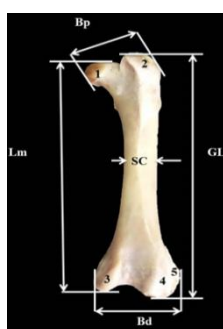
Caput femoris and trochanter major (femoris) were found to be almost the same level. Trochanter minor was found in the distomedial of collum femoris (Figure 11). Trochlea femoris was seen on the anterior aspect of the distal end of the femur. Behind the distal end of the femur was found 2 condylus. Condylus medialis with the tibia and the larger condylus lateralis tibia

and caput fibulae artifacts with the joint were determined. Femur was not pneumatized.

**Table 8.** Comparison of some parameters on pelvis in male and female geese

Parameter	Female (n=10)	Male (n=10)	P value
GL	154,27 ± 1,81	169,91 ± 1,68	<0,001
LS	151,44 ± 1,44	165,61 ± 1,76	<0,001
LV	137,24 ± 1,78	150,18 ± 2,47	<0,001
CB	35,31 ± 0,56	39,63 ± 0,52	<0,001
SB	26,92 ± 0,40	29,01 ± 0,53	0,006
AA	45,73 ± 1,74	48,62 ± 0,77	0,147
DIA	12,81 ± 0,32	12,87 ± 0,41	0,914
BA	50,85 ± 0,93	56,30 ± 1,20	0,002

GL: Greatest length (without pubis), LS: Length from the cranial border of the ilia to the spinaeiliocaudales, LV: Length along the vertebrae, centrally, CB: Cranial breadth, SB: Smallest breadth of the partes gluteae, AA: Breadth between the borders of the acetabulum, measured at the narrowest part, DIA: Diameter of one acetabulum: greatest distance including the labium acetabuli, BA: Breadth in the middle: breadth cross the two antitrochanter.



**Figure 11.** Measurements taken from femoris. 1: Caput femoris, 2: Trochanter major (femoris), 3: Condylus medialis, 4: Condylus lateralis, 5: Condylus fibularis, GL: Greatest length, Lm: Medial length, Bp: Greatest breadth of the proximal end, SC: Smallest breadth of the corpus, Bd: Greatest breadth of the distal end

As a result of the measurements (Figure 11), GL, Lm, Bp, Bd and Dd values were statistically different in male and female geese ( $P < 0.001$ ;  $P = 0.01$ ). In other words, these parameters were higher in males than females. In addition, there was no significant difference between Dp and SC values ( $P = 0.11$ ;  $P = 0.07$ ) (Table 9).

**Patella (Knee cap bone)**

The patella; mm. femorotibialis and m. iliotibialis were found to be a small sesame bone within the

common tendon and at the level of the trochlea femoris. The male and female geese were triangular.

**Table 9.** Comparison of some parameters taken on femur in male and female geese

Parameter	Female (n=10)	Male (n=10)	P value
GL	86,85 ± 0,98	93,87 ± 1,12	<0,001
Lm	82,26 ± 0,87	88,36 ± 1,01	<0,001
Bp	22,43 ± 0,26	24,66 ± 0,32	<0,001
Dp	17,00 ± 0,43	18,54 ± 0,33	0,11
SC	9,44 ± 0,13	10,09 ± 0,17	0,07
Bd	22,99 ± 0,35	24,94 ± 0,37	0,01
Dd	18,22 ± 0,32	20,05 ± 0,32	0,01

GL: Greatest length, Lm: Medial length, Bp: Greatest breadth of the proximal end, Dp: Greatest depth of the proximal end, SC: Smallest breadth of the corpus, Bd: Greatest breadth of the distal end, Dd: Greatest depth of the distal end

**Tibiotarsus (Foot-leg wrist bone)**

It was determined that the goose skeleton consisted of the strong tibiotarsus and weak fibula, and tibiotarsus was not pneumatized. It was found that tibiotarsus was approximately 2 times longer than femur.



**Figure 12.** Measurements taken from tibiotarsus and fibula. 0: Spina fibulae, 1: Proc. cnemialis, 2: Tuberculum centrale, 3: Caput fibulae, 4: Corpus fibulae, 5: Crista fibularis, 6: Condylus medialis femoralis, 6: Condylus lateralis femoralis, GL1: Greatest length of tibiotarsus, GL2: Greatest length of the fibula, La: Axial length, Dip: Greatest diagonal of the proximal end, Dd: Depth of the distal end.

As a result of the measurements (Figure 12), GL, La, Dip, Bd and Dd values were statistically different in male and female geese ( $P < 0.001$ ;  $P = 0.010$ ;  $P = 0.001$ ). In other words, these parameters were higher in males than females. In addition, there was no significant difference

between the sexes in terms of SC ( $P = 0.270$ ) (Table 10).

### Fibula (Calf bone)

It was observed that the fibula was proximal to the distal to a flat rod. It was determined that the tapered corpus of the fibula was progressively tapering to the distal length of the tibia. It was observed that the fibula was bounded by two spathum interosseum, proximal and distal along its length. In the measurements taken as shown in Figure 12, it was determined that there was no significant difference between females and males in fibula length ( $P = 0.189$ ).

**Table 10.** Comparison of some parameters of tibiotarsus in male and female geese

Parameter	Female (n=10)	Male (n=10)	P value
GL	160,94 ± 1,88	174,20 ± 1,28	<0,001
La	151,89 ± 1,87	167,64 ± 1,32	<0,001
Dip	28,36 ± 0,65	30,65 ± 0,45	0,010
SC	8,48 ± 1,64	8,20 ± 0,18	0,270
Bd	18,55 ± 0,25	20,02 ± 0,14	<0,001
Dd	17,47 ± 0,75	20,63 ± 0,32	0,001

GL: Greatest length of tibiotarsus, La: Axial length, Dip: Greatest diagonal of the proximal end, SC: Smallest breadth of the corpus, Bd: Greatest breadth of the distal end, Dd: Depth of the distal end.

### Ossa pedis (Foot bones)

#### Ossa tarsi (Ankle bones)

Tarsal bones were not found independently. The proximal row of ossa tarsi was found to be fused with the distal part of tibiotarsus and the distal row of ossa tarsi with the proximal metatarsus.

#### Tarsometatarsus (Foot wrist-comb)

Tarsometatarsus was a composite bone composed of os metatarsale II, os metatarsale III, os metatarsale IV and ossa tarsi. Os metatarsale I and V were absent. The first finger in the distal part of the os metatarsal I was smaller and did not fuse with them. Hypotarsus was found to constitute the proximal boundary of os metatarsale III. It was determined that there were 3 crista hypotharsis in the hypotarsus of geese and the longest of crista medialis hypotarsi. The distal end of the one-piece metatarsus was divided into 3 trochlea with a very distinctive 2 notches. It was determined that there was a bone spur on the facies medialis aspect of extremitas distalis tarsometatarsi in male and female geese. This formation was better developed in males.



**Figure 13.** Measurements taken by tarsometatarsus. 1: Eminentia intercotylaris, 2: Cotyla medialis, 3: Cotyla lateralis, 4: Sulcus extensorius, 5: Tuberositas m. tibialis cranialis, 6: Facies dorsalis, 7: For. Vasculare distale, 8: Trochlea metatarsi III, GL: Greatest length, Bp: Greatest breadth of the proximal end, SC: Smallest breadth of the corpus, Bd: Greatest breadth of the distal end

**Table 11.** Comparison of some parameters of tarsometatarsus in male and female geese

Parameter	Female (n=10)	Male (n=10)	P value
GL	93,15 ± 1,19	101,64 ± 0,88	<0,001
Bp	19,05 ± 0,35	20,93 ± 0,30	0,001
SC	8,77 ± 0,11	9,41 ± 0,11	0,001
Bd	21,48 ± 0,39	24,03 ± 0,32	<0,001

GL: Greatest length, Bp: Greatest breadth of the proximal end, SC: Smallest breadth of the corpus, Bd: Greatest breadth of the distal end.

The statistical data generated as a result of the measurements taken in Figure 13; GL, Bp, SC and Bd showed significant differences between male and female geese ( $P < 0.001$ ;  $P = 0.001$ ). In other words, all of these parameters were found to be significantly longer in males (Table 11).

### Ossa digitorum pedis (The foot toe bones)

There were four fingers on the goose leg. The first finger in the medial was caudal, while the 2nd, 3rd and 4th fingers were directed towards the cranial.

Phalanges (Finger bones): Two phalanx were found on the first finger, three on the second finger, four on the third and five on the fourth finger.

## DISCUSSION

While the length of the scapula was 61.8 mm (Özgel et al., 2002), it was measured as  $116.63 \pm 0.65$  mm in male geese and  $107.31 \pm 1.05$  mm in female geese. The diverticulum subscapulare of the saccus clavicularis in geese has been



reported to pneumatize the scapula (Onuk, 2008). In this study, it was determined that saccus clavicularis had pneumatized scapula. It has been reported that the length of the bald ibis os coracoideum is 46-51 mm (Özgel et al., 2002). The length of the bone was  $78.5 \pm 0.6$  mm in female geese and  $87 \pm 0.8$  mm in male geese. It is reported that there is no pneumatization in the os coracoideum (Hogg, 1984) in chicken, duck (Çevik Demirkan, 2002) and geese (Onuk, 2008) have been reported to be pneumatized. In the present study, it was determined that os coracoideum was pneumatized.

The forward curvature of the clavícula was less pronounced and stronger in the hen and rooster, and almost flat in the turkey (Gültekin, 1957). In male and female geese, the clavícula was found to be flat but with a distinct curvature, it was seen that two clavicles were joined together in synostosis. While clavícula was reported as a pneumatized bone in the duck (Çevik Demirkan, 2002), clavicle was not pneumatized in geese.

It has been reported that humerus is longer than antebrachium in chicken, domestic duck and quail (Çevik Demirkan, 2002). In pigeons, antebrachium was reported to be longer than humerus (Yıldız et al., 1998). In the study, it was found that humerus was longer than antebrachium in female and male geese. The length of the humerus is  $51,80 \pm 0,49$  mm in partridge,  $67,77 \pm 0,55$  mm in pheasant (Lök and Yalçın, 2007), in the long-legged buzzard 100-110 mm (Atalar et al., 2007), in goose 172,5 mm (Allison et al., 2006), 72.5 mm in the chicken, 87.4 mm in the domestic duck, 46.1 mm in the pigeon (Yıldız et al., 1998),  $95.84 \pm 1.63$  mm in the female ducks, 106 in the male ducks. (Çevik Demirkan, 2002). In this study, humerus length was  $175.02 \pm 1.59$  mm in female geese and  $191.28 \pm 1.44$  mm in male. The humerus length values we found were higher than those of the other birds, but similar to the Canadian geese. The humerus in the blue-headed parrot is reported to be pneumatized (McKibben and Harrison, 1986). It was reported that diverticulum humerale of saccus clavicularis in the geese had pneumatized humerus (Onuk, 2008). In our study, saccus clavicularis was found to pneumatize the humerus.

The average length of ulna is 29.7 mm in domestic quail, 29.4 mm in wild quail (Yaman, 1997),  $49.74 \pm 0.46$  mm in partridge,  $61.53 \pm 0.50$  mm in pheasants (Lök and Yalçın, 2007), 110-130

mm (Atalar et al., 2007) was reported as  $100.1 \pm 3.0$  mm (Charuta et al., 2005) in males of the domestic duck. In our study, the length of ulna was  $162,60 \pm 1,26$  mm in female geese and  $178,84 \pm 0,83$  mm in male geese. Papillae remigalis, which were the basis for the attachment of feathers to ulna, were clearly seen. While the curvature of corpus ulnae is less pronounced in chickens and ducks, it is stated that it is more prominent in goose and duck (Nickel et al., 1977). There was a slight curvature in the corpus ulnae. Mean radius length was  $44.54 \pm 0.44$  mm in partridge,  $56.09 \pm 0.47$  mm in pheasants (Lök and Yalçın, 2007), female Pekin duck  $94,9 \pm 2.1$  mm, male Pekin duck  $91.0 \pm 13.59$  mm (Charuta et al., 2005). In this study, the mean radius length was  $154.20 \pm 1.63$  mm in female geese and  $169.75 \pm 1.31$  mm in male geese. It was observed that the poultry species compared with them were extremely large. It was determined that the radius and ulna were equal in length (Rezk, 2015) in the cattle egret and that the ulna was long in radius. The largest width at the distal end was  $11.53 \pm 0.24$  mm in male geese and  $12.69 \pm 0.19$  mm in female geese, whereas Charuta et al., (2005) in the study of Peking duck in males  $9.4 \pm 0.6$  mm female  $9.0 \pm 0.3$  mm. It was reported that radius and ulna has not been pneumatized in the partridge, pheasant and long-legged buzzard from birds (King, 1957; Lök and Yalçın, 2007). Antebrachium was not pneumatized in geese.

It was reported that os carpi radiale is short and quadrangular in duck (Çevik Demirkan, 2002), it is almost rectangular in partridge and rectangular and bow tied with pheasants (Lök and Yalçın, 2007). In the study, it was observed that the two sides were shaped two blunt edge one sharp edge, pointed, triangular shaped. Os carpi radiale is generally similar to geometric shapes, but the reported figures and findings are not similar. Os carpi ulnare in duck (Çevik Demirkan, 2002), pheasant and partridge (Lök and Yalçın, 2007) V shaped, cattle egret (Rezk, 2015) U-shaped was stated. In this study, os carpi ulnare was seen as a pipe shaped. Os carpi ulnare's shape did not match the findings of other reported studies

Mean carpometacarpus length was  $29,94 \pm 0,31$  mm in partridge,  $34,82 \pm 0,26$  mm in pheasant (Lök and Yalçın, 2007), 59-71,2 mm long-legged buzzard (Atalar et al., 2007), male and female Peking duck in  $75.0 \pm 1.7$  -  $73.3 \pm 2.0$  mm

(Charuta et al., 2005). In the study, the length of carpometacarpus was determined as  $94.57 \pm 0.75$  mm in female geese and  $100.95 \pm 1.03$  mm in male geese. Carpometacarpus length was higher in geese compared to other studies.

It was observed that the apex pubis of the os pubis ended in bent to the ventromedian. It was reported that os pubis did not participate in the formation of acetabulum in chicken and duck (Dursun, 2007; Çevik Demirkan, 2002). It was observed that os pubis did not participate in acetabulum formation in geese. It has been reported that ilium, ischium and pubis to be pneumatized in the blue-headed parrot (McKibben and Harrison, 1986). Saccus abdominalis has been reported to pneumatize coxa (King, 1975). There was no indication of pneumatization of coxa in chickens (Hogg, 1984b). In the wild duck (Çevik Demirkan et al., 2006) and the goose (Onuk, 2008), it was stated that saccus abdominalis pneumatized to the synsacrum. In this study, it was found that synsacrum was pneumatized by saccus abdominalis.

The average length of the femur was reported as 81.5 mm (Allison et al., 2006) in Canadian geese,  $60.14 \pm 0.49$  mm in female ducks and  $60.92 \pm 0.93$  mm in male ducks (Çevik Demirkan, 2002). In this study, femur length was  $86.85 \pm 0.98$  mm in female geese and  $93.87 \pm 1.12$  mm in male geese. The length of the femur was similar to that of the Canada goose while it was higher than the other poultry. It was reported that there was no pneumatization in the femur in the hens (Hogg, 1984a). Diverticula femoralia of saccus abdominalis was reported to pneumatize the femur in geese (Onuk, 2008). In the study, it was seen that the femur was pneumatized by saccus abdominalis.

It is reported that the patella has a triangular appearance in the partridges, and in pheasants it resembles the talus of mammals (Başoğlu and Beşoluk, 2016), rectangular female ducks and square in shape male ducks (Çevik Demirkan, 2002). In our study, it was observed that patella was triangular in male and female geese.

The average length of tibiotarsus was measured as 150.5 mm (Allison et al., 2006) in Canadian geese,  $94.9 \pm 1.2$  in female ducks and  $102.5 \pm 0.94$  mm in male ducks (Çevik Demirkan, 2002). This study measured  $160.94 \pm 1.88$  mm in female geese and  $174.20 \pm 1.28$  mm in male. It has been reported that tibiotarsus in pigeons and chickens

is longer than 1/3 of femur from femur (Dursun, 2007). As reported in the literature (Nickel et al., 1977; Çevik Demirkan, 2002) tibiotarsus was found to be 50% longer than the femur. As in the duck (Çevik Demirkan, 2002), it was observed that the fibula was extended to the distal part of the tibia length and to limit the two spatium interosseum, proximal and distal along its length. In the study, it was determined that tarsometatarsus, which is the only one in the geese, was formed by the fusion of os metatarsale II, os metatarsale III (principal) and os metatarsale IV. In hypotarsus, it is reported that in some birds there is only one crista and sulcus, most birds have more than one crista and sulcus (N.A.A., 1993). In the geese 3 cristae hypotharsia (crista lateralis hypotarsi, crista intermedia hypotarsi and crista medialis hypotharsia) was found and crista medianoplantaris (crista medialis hypotarsia) to be the longest. In extremitas distalis tarsometatarsia, trochleas (trochlea metatarsi II, trochlea metatarsi III, trochlea metatarsi IV) belonging to each tarsometatarsus were seen. It was reported in some birds (N.A.A., 1993) that adjoined trochlea accessoria wasn't detected. Among the trochleas were the incisura intertrochlearis lateralis and the incisura intertrochlearis medialis. The plantar of the trochleas had a deep pit fossa supratrochlearis plantaris. Canalis interosseus distalis was not apparent, but for. vasculare distale was a big hole. The tallest bone of the tarsometatarsus was chicken and the shortest bone was reported as goose (Gültekin, 1966; N.A.A., 1993). However, in our study, we found that the tarsometatarsus length of larger other poultry tarsometatarsus (Zeffer and Norberg, 2003) because of this information is contrary. In most birds, tarsometatarsus is shorter than tibiotarsus; two bones were reported to have approximately equal length in the rain bird (King and McLelland, 1984). It was determined that tibiotarsus length was greater than tarsometatarsus in geese. Tarsometatarsus length; 38,5 mm in common buzzard, 45,6 mm in mallard duck, 18,9 mm in sparrow, 17,9 mm in finch, 104,8 mm in gold eagle, 78,3 mm in diver bird (Zeffer and Norberg, 2003). This length was  $93,15 \pm 1,19$  mm in female geese and it was  $101,64 \pm 0,88$  mm in male geese.

## CONCLUSIONS

Goose (*Anser anser domesticus*) is one of the poultry animals shown as most examples in veterinary anatomy teaching. It is also the geographical sign of our region. Therefore, the bones of adult geese were examined in our study. As a result, in this study, morphometric and morphologic values of examined bones of adult geese were determined and contributed to the elimination of the information in this area. It is thought that obtained findings will contribute in scientific research, evaluations of sexual dimorphism, zooarchaeological studies and operations on poultry animals.

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**PEDOBAROGRAPHIC EXAMINATION OF FORELIMB AND HINDLIMB IN GERMAN SHEPHERD  
DOGS**

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***Abstract***

*Purpose: In this study, Pedobarographic examination of a healthy German Dog was performed, temporo-spatial and kinetic walking values were obtained for the forelimb and hindlimb. Data of this method, which can be used in clinical examination for animals, were evaluated by making foot pressure maps. Material-Method: A clinically healthy German Shepherd Dog (8 years old, 30 kilograms) was used in the study. A pressure sensitive walking plate was used for pedobarographic measurements. This plate is 2 meters tall and 60 centimeters wide. The dog was carried out a total of 16 times on this plate. During walking, the forelimb and hindlimb kinetic data were recorded separately. Results: The dog was walked at 3.4 kilometers per hour. While walking, the forelimb applied an average of 239 Newtons, the hindlimb applied an average of 213 Newtons to the ground. When the pressure maps were examined, it was seen that the maximum force applied by the digital pads on the ground was more than the metapodial pads. Conclusion: During normal walking, kinetic data of the forelimb were higher than those of the hindlimb. In addition, it was seen that the data of each digital pads could be obtained by Pedobarographic method. This system is thought to be useful in the veterinary field.*

***Key words:*** Foot Pressure Map, Pedobarographic Analysis, Gait Analysis

## BIOMECHANICS OF CLAW IN BOVINE

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### **Abstract**

*The shape changes that occurs in the claw by the influence of the body weight while the cow's press their feet on the ground that is called ?claw biomechanics?. The digital cushion, the suspensory apparatus of the digit, the wall of the capsule ungula and the coronary cushion play an important role in occurring of biomechanical energy and distribution of pressure when the foot presses on the ground. Functionally, the biomechanical pressure is different on the fore and hind limbs. The flow of mechanical pressure is formed more in the lateral part of the claw on the hind limb. It is accepted that the fore limbs carry about 60% of the animal's weight. However, this ratio varies to 50%, for high-yielding dairy cows in peak lactation. The biomechanics of the claw has also been affected by the forced release of bioactive substances as a result of the exposure of dairy cows to high production densities and their removal from a natural life cycle. The transition to intensive dairy farming resulted in the redistribution of the mechanical pressure inside the claw. It is more correct to call this "mechanical pressure" rather than trauma. Irregular distribution of body weight to the claws causes disruption of the claw biomechanics, as a result, the corium unglae is traumatically affected, then the foot diseases is occurred. So it is important to ensure that the cattle foot can perform its normal function. For the prevent of foot diseases, the normal structure and function of the claw must be well known. For this reason, in recent years, many researchers have contributed to clarifying the structure and function of the cattle claws. In this paper, it was aimed to bring together the contributions of the researchers and create a subject integrity on how the nail can work.*

**Key words:** Biomechanics, Bovine, Claw

**INVESTIGATION OF THE ANATOMICAL STRUCTURE OF CERVIX UTERI, CORPUS UTERI AND  
CORNU UTERI IN RED FOXES (*Vulpes vulpes*)**

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**Abstract**

*The Red fox (*Vulpes vulpes*) is the largest of the true foxes and the most abundant wild member of the carnivora. This study aimed to determine the anatomical structure of the cervix uteri, corpus uteri and cornu uteri of the Red foxes. Animals that were taken to the Kafkas University Wildlife Rescue and Rehabilitation Center, Kars, because of various reasons, such as traffic accidents and firearm injuries, were used in this study. The uterus of four Red foxes of similar ages, which could not be rescued by the Center despite all interventions, were dissected. Measurements were taken from the cervix uteri, corpus uteri and right-left cornu uteri using digital callipers. The weights of each organ section were measured using a precision scale (min: 0.0001 g - max: 220 g). The average cervix uteri length, width, thickness and weight were  $11.54 \pm 1.56$  mm,  $4.46 \pm 0.52$  mm,  $5.18 \pm 0.08$  mm,  $1.18 \pm 0.04$  g, respectively. The mean length of the corpus uteri was  $20.68 \pm 3.06$  mm, width was  $2.88 \pm 0.50$  mm, thickness was  $2.22 \pm 0.19$  mm, and weight was  $0.90 \pm 0.01$  g. The mean the cornu uteri length, width, thickness and weight were  $79.85 \pm 0.86$  mm,  $4.85 \pm 0.79$  mm,  $4.33 \pm 0.18$  mm and weight  $2.33 \pm 0.12$  g, respectively. In conclusion, the Red foxes, was given information about the uterus of the female genital organs. We believe that the findings of this study may be useful for further studies on foxes and surgical operations.*

**Key words:** Anatomy, Uterus, Red fox, Wild animal

**INTRODUCTION**

The Red fox (*Vulpes vulpes*) is a mammal of 70-90 cm in height and 7-10 kg in weight in the Canidae family of carnivora. This species, which can be seen in many regions of the world, is also available in Turkey. There are species living on the continent of Europe, Asia, North Africa and America. It is a seasonal monogamous carnivorous with big ears and a long tail, famous for its intelligence and fraud (Larivière and Pasitschniak-Arts 1996). It has a 3-month gestation period and reaches sexual maturity at the age of 7-10 months (Anonymous 1). Reproductive activity of red foxes has been reported to vary depending on the abundance of food. In the years with rodent shortages, fewer litter size was seen and the reproductive efficiency decreased (Halvorsrud 2014). A study in red foxes showed an important relationship between ovulation rates and the size of the embryo sac. At the same time, the animal's age,

condition, population density, adult sex ratio and year of the population are also effective (Allen 1984). In carvivores, the uterus has a short corpus and a long, very narrow cornu. The size of the uterus varies according to breed, age, size of the animal, whether it is giving birth and when it is in the cycle (Kaymaz et al. 2013). In carnivores, the uterus is located in the abdominal cavity. It consists of three layers: uterine endometrium (tunica mucosa), myometrium (tunica muscularis) and perimetrium (tunica serosa the innermost endometrium is a two-layer muscle layer (external longitudinal and internal circular muscle layers) under the endometrium and the serous perimetrium enveloping the uterus from the outside (König and Liebich 2015). Cervix uteri is an organ that connects corpus uteri with vagina and has a short, thin channel in the middle. Its anatomy, length, and width differ among mammals. It differs in the junction of the vagina to uterus. Rodent has 2 cervix uteri since this

junction is limited. The corpus uteri, which follows cervix uteri, opens to the vagina through two separate channels (Bertram et al. 2019). This type of uterus is called uterine duplex. The uterus is called simplex, since there is more binding in humans and primates. Since most domestic mammals are between these two forms, the uterus is bicornis (König and Liebich 2015). Corpus uteri is the part of the uterus between the place where cornus started and the cervix uteri. The mean corpus uteri length is 10-30 mm in the bitches (Bahadır and Yıldız 2014), while 20-30 mm (Sission 1910, Sission and Grossman's 1975, Kaymaz et al. 2013) and 15 mm in queen (Bahadır and Yıldız 2014). Cornu uteri length is reported as 120-150 mm (Sission 1910, Sission and Grossman's 1975) or 100-140 mm (Kaymaz et al. 2013) and cornu uteri thickness is 8 mm (Kaymaz et al. 2013, Bahadır and Yıldız 2014). This study was carried out to examine the anatomical structure of the red fox (*Vulpes vulpes*) uterus, a member of the wildlife.

## MATERIALS AND METHODS

In this study, Kafkas University, the Wildlife Rescue and Rehabilitation Center (Kars, Turkey) brought causes such as traffic accidents and, gunshot wounds, but unrecoverable despite all the intervention four fox were used. This study was carried out after approval from the Ministry of Agriculture and Forestry, General Directorate of Nature Conservation and National Parks (21264211-288.04-E.115615). The study was carried out in the laboratory of Anatomy Department of Kafkas University Veterinary Faculty. The female genital system was dissected. Measurements were taken using a digital calliper. The weights of the organs were measured using precision scales.

## RESULTS AND DISCUSSION

Mean cervix uteri length was determined as  $11.54 \pm 3.13$ , width  $4.46 \pm 1.04$ , thickness  $5.18 \pm 0.17$ , weight  $1.18 \pm 0.09$  mm (Fig 1, Table 1).

**Table 1.** Selected red fox (RF) cervix uteri measurements

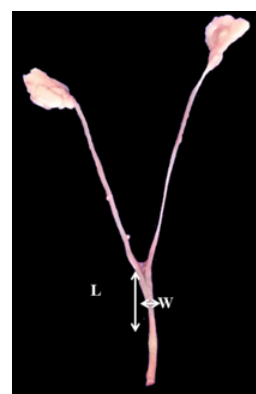
Measurements	RF1	RF2	RF3	RF4	Mean $\pm$ SE
Length of cervix uteri (mm)	8.00	11.35	11.20	15.62	$11.54 \pm 1.56$
Width of cervix uteri (mm)	3.25	4.90	4.05	5.66	$4.46 \pm 0.52$
Thickness of cervix uteri (mm)	4.93	5.30	5.27	5.21	$5.18 \pm 0.08$
Weight of cervix uteri (g)	1.08	1.29	1.15	1.21	$1.18 \pm 0.04$

RF: Red fox, SE: Standard error



**Figure 1.** Measurements taken from cervix uteri (W: Width of cervix uteri, L: Length of cervix uteri)

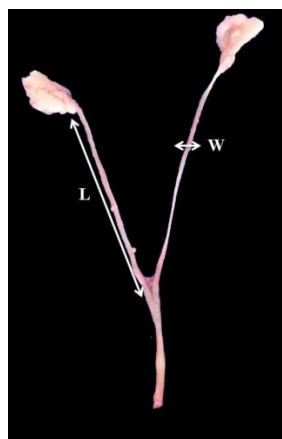
Mean corpus uteri length was determined as  $20.68 \pm 6.134$ , width  $2.88 \pm 1.00$ , thickness  $2.22 \pm 0.37$ , weight  $0.90 \pm 0.03$  mm (Fig 2, Table 2).



**Figure 2.** Measurements taken from corpus uteri (W: Width of corpus uteri, L: Length of corpus uteri)

Mean cornu uteri length was determined as  $79.85 \pm 1.72$ , width  $4.85 \pm 1.58$ , thickness  $4.33 \pm 0.37$ , weight  $2.33 \pm 2.24$  mm.





**Figure 3.** Measurements taken from cornu uteri (W: Width of cornu uteri, L: Length of cornu uteri)

### CONCLUSIONS

Red foxes are the main stone is one of the most important ecosystems. Global warming, hunting, the effect of chemicals harmful to nature, etc. severe winter conditions as well as the factors that makes it difficult the survival of foxes. In this sense, it is important to benefit and indirectly reduce threats to other species and balance the ecosystem. Our work may be important for the reproduction of this species, the number of which has been decreasing and that has become endangered. With the study, it was contributed to the elimination of the lack of information about the anatomical structures of the parts of the uterus, which is one of the red fox's female genital organs.

**Table 2.** Selected red fox (RF) corpus uteri measurements

Measurements	RF1	RF2	RF3	RF4	Mean ±SE
Length of corpus uteri (mm)	21.08	28.50	13.60	19.55	20.68± 3.06
Width of corpus uteri (mm)	1.78	4.15	3.06	2.52	2.88 ± 0.50
Thickness of corpus uteri (mm)	1.87	2.50	2.59	1.93	2.22 ±0.19
Weight of corpus uteri (g)	0.87	0.93	0.92	0.88	0.90 ± 0.01

RF: Red fox, SE: Standard error

**Table 3.** Selected red fox (RF) cornu uteri measurements

Measurement	FEMALE-1		FEMALE-2		FEMALE-3		FEMALE-4		Mean ± SE
	right	left	right	left	right	left	right	left	
Length of cornu uteri (mm)	82.65	80.82	83,60	78,07	80,50	77,05	78.32	77.81	79.85 ± 0.86
Mean Length of cornu uteri (mm)	81.73		80,83		78.77		78.06		
Width of cornu uteri (mm)	5.54	6.43	6,47	6,00	3,10	2,55	4.92	3.83	4.85 ± 0.79
Mean Width of cornu uteri (mm)	5.98		6.23		2.82		4.37		
Thickness of cornu uteri (mm)	3.94	4.02	5.40	3.40	4.26	3.99	4.72	4.91	4.33 ± 0.18
Mean Thickness of cornu uteri (mm)	3.98		4.4		4.12		4.81		
Weight of cornu uteri (g)	2.31	2.16	2.53	2.04	1.93	2.35	2.43	2.94	2.33 ± 0.12
Mean Weight of cornu uteri (g)	2.23		2.28		2.14		2.68		

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## DETECTION OF EHV-1 AND EHV-4 INFECTIONS IN RACE HORSES USING POLYMERASE CHAIN REACTION

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### **Abstract**

*The aim of this study was to detect and differentiate equine herpes virus type-1 (EHV-1) and type-4 (EHV-4) by polymerase chain reaction (PCR). The diagnostic sensitivity of the PCR was also examined. For this purpose, suspicious samples obtained from different breeding farms and racehorse hospitals were used. During this study, multiple organ and tissue samples of 26 aborted fetuses were collected and analyzed with PCR. In addition, 98 nasal swab specimens from racehorses aged 2 and above representing the respiratory symptoms of EHV infections were collected and examined by the PCR method. This study started with the optimization of PCR using EHV-1 (89c25p) and EHV-4 (TH20p) DNA, which are reference strains. It was observed that the gB common oligonucleotide primers and species-specific gC primers used in optimization matched the viral DNA specific for target EHV-1 and EHV-4. Finally 7 EHV-1 positives at tissues of aborted fetuses of 26 and 1 EHV-1 and 2 EHV-4 positives at nasal swab samples of 98 has been detected and differentiated by PCR. This was the first PCR study to detect and differentiate EHV-1 and EHV-4 in Turkey. Results of this study reveal the presence of EHV-1 and EHV-4 in Turkey. Especially the presence of EHV-4 virus has been shown for the first time. As a result of the study, detection of EHV-1 and EHV-4 genomic DNAs by PCR indicated that both viruses circulate between racehorse populations. In conclusion, we agreed that the PCR is a sensitive, economical and time-saving method in detecting and differentiating EHV-1 and EHV-4 infections.*

**Key words:** EHV-1, EHV-4, infection, Polymerase Chain Reaction (PCR)

### **INTRODUCTION**

Herpesviruses are in the Herpesviridae family and there are 3 subfamilies in this family: Alphaherpesvirinae, Gammaherpesvirinae and Betaherpesvirinae (Murphy et al., 1999; Newton et al., 2000; van Regenmortel et al., 2000). Herpesviruses have been isolated from many animal species, including humans, through studies performed to date. For example, in the equid herpesviruses equida family, felid herpesviruses in feline, canid herpesvirus in dogs, bovine herpesvirus in cattle, gallid herpesvirus (Marek's disease) poultry, Iguanid herpesvirus iguana, herpes simplex, herpesgenitalis, Epstein-Barr, virus, virus, herpes simplex, herpesgenitalis; they become (van Regenmortel et al., 2000). In other words, each virus has a host and the virus-host relationship varies according to natural life and laboratory conditions. The nuclear material of herpesviruses is DNA, double stranded, linear,

32-75 G + C mol 80-150X10, molecular weight (MW). These viruses have more than 20 structural polypeptides with molecular weights ranging from 12000-20000. Virion consists of 4 structural components and is 120-200 nm in size. The capsid is icosahedral and contains 150 hexameric and 12 pentameric capsomers (van Regenmortel et al., 2000).

Herpesviruses have 33 virion proteins, including 7 identified capsid proteins, 8 envelope glycoproteins and multiple tegument proteins (Crabb et al., 1993). Glycoproteins, which are located in the envelopes of alphaherpesviruses, play an important role in infectivity and pathogenicity, but also constitute the main antigenic structures for the immune response in the host animal (Packiarajah et al., 1998). That is, antibodies against EHV-1 and EHV-4 are largely related to glycoproteins on the envelope (Crabb et al., 1993; Telford et al., 1992; Tsujimura et al.,

2001). Therefore, envelope proteins are preferred in the development of recombinant subunit vaccines prepared with DNA technology (Packiarajah et al., 1998). The glycoproteins of EHV-1 are gp2, gp10, gC (gp13), gB (gp14), gD (gp18), and gp2, gp10, gC (gp13), gB (gp14), gD (gp18), gG sodium dodecyl sulfate-polyacrylamide gel are defined precisely by electrophoresis (SDS-PAGE) method (13). Of these proteins, glucoprotein (gD) is found in gene 72 and is a membrane glucoprotein, required for cell-to-cell fusion and virus entry in EHV-1. Csellner et al. (2000) explained that the mutant lacking gD did not infect cells in RK cell culture. Glucoprotein (gE) is found in gene 74 and is membrane glucoprotein. gE has an important function that provides viral virulence and is effective in cell-to-cell spread. It has been reported that it is not necessary to be present for viral replication, viral attachment and penetration in *in vitro* environments (Damiani et al., 2000 and 2000). Matsumura et al. (1998) as a result of their *in vivo* study using the KyA strain that lost at least 6 genes including the gE and gI genes in its genome; They reported that gE and gI genes play a crucial role in the virulence of EHV-1 and no clinical symptoms could be observed in 6 horses infected with mutant strains lacking these genes, and this strain was avirulent for horses. Us2 protein-gene 68 Us2 homologs is a membrane related protein found in EHV-1, EHV-4, HSV-2, CHV, BHV-1 and Marek virus, located in the subfamily *Alfaherpesvirinae*. Meindl et al. (1999) reported that EHV-1 Us2 protein supports virus-to-cell spread and *in-vivo* replication of the virus. In their study using the Us2-negative mutant strain with wild type or a normal strain, the researchers found that experimentally infected BALB/c mice decreased pathogenicity in the Us2-negative group, and viral penetration was delayed and smaller plaques were formed in cell cultures reported that it supports the non-essential virion component associated with the membrane. Cell cultures prepared from different tissues were used to produce herpesviruses. Viruses reproduce in fetal horse dermis, sheep-rabbit kidney, Vero, HeLa cells and produce cytopathic effect in these cultures (O'Callaghan et al., 1999).

The examination of genomic DNA of EHV-1 and EHV-4 with restricted endonuclease analysis indicated that both viruses genetically and

antigenically different from each other (Allen et al., 1986). These findings led to a new classification and both viruses have been renamed as EHV-1 (equine abortion virus) and EHV-4 (equine rhinopneumonitis virus). EHV-1 (Ab4 strain) has a double helix DNA molecule consisting of 150.223 kilobase pairs (kbp) long, a fixed long region (L) and a variable short region (S), and 2 ORF (open reading frame) in the region near the left terminal. (van Regenmortel et al., 2000). EHV-1 (equine abortion virus) primarily causes abortions in mares and often causes upper respiratory tract infection in young horses. In addition, it causes neurological diseases and exanthema in mares and external genital organs, to a lesser extent (Donaldson et al., 1998; Matsumura et al.; 1994, van Maanen et al.; 2000, Yilmaz et al., 1995). Abortion occurs as a result of infections occurring in mares in the last stages of pregnancy, and if abortion does not occur, neonatal pneumonitis is observed in foals born infected with EHV-1 (Drummer et al., 1995; Gilkerson et al., 1998). It has been reported that EHV-1 causes abortions in several cases in donkeys (Gupta et al., 2000).

The most important transmission in EHV-1 and EHV-4 infections is through the aerosol route and the foals become infected in the first year of their life (Carvalho e al., 2000). However, EHV-1 and EHV-4 viruses also cause latent infection, and infected horses become porter and, in the event of the infection, these animals can easily transmit the factors to the susceptible (Carvalho et al., 2000). The abortions generally occurs in the final stage of pregnancy in mares in the Equine herpesvirus infections. The most important clinical findings are abortion and EHV-1 is mostly isolated from abortion cases in pregnant mares. Allen and Bryans (1986) reported that 98% of the herpesviral abortions were caused by EHV-1. Mares are usually in the last period of 7-11 months of pregnancy period and sudden developing abortion occurs with or without clinical symptoms (Gilkerson et al., 1998) and this occurs in the last 4 months of pregnancy by 95%. Abortion cases can be sporadic or turn into an epidemic that occurs within a few weeks. For example it was reported that 52 mare abort in the outbreak caused by EHV-1 at TJK Izmit pension (Yilmaz et al., 1995). The clinical symptoms of EHV-1 and EHV-4 in horses are similar. Findings were fever, coughing,

rhinotracheitis and tracheobronchitis (Bürki et al., 1989). In addition to these, a catarrhal runny nose, excessive lacrimation and the mandibular lymph nodes adenopathy is common (Walker et al., 2000). When the EHV-1 virus is located in the central nervous system, clinical signs such as shaking gait, weakening, imbalance and nodding are encountered in animals (Newton et al., 2000). The histopathological examination of the organs of the fetuses following the EHV-1-related abortion cases, necrotic lesions in the liver and lung and intranuclear inclusion body in the cells, and germinal center necrosis in the cells, and eosinophilic inclusion bodies in the cells, petechial hemorrhage in the brain, bronchiolitis, and pneumonia in the brain clear yellow fluid collection, pulmonary congestion and edema are observed (Murphy et al., 1999).

There is no drug treatment of infections caused by EHV-1 and EHV-4 in horses. Administration of antibiotics to horses with Rhinopneumonitis can prevent secondary infections from occurring and spreading, and this treatment continues for 4-6 days. In addition, it is recommended that horses be housed in a warm, quiet and isolated place, and laxative foods are given (Murphy et al., 1999). Hygienic measures must be taken to control the infection. Especially aborting mares should be taken to a completely isolated place and their contact with other horses should be stopped immediately. In addition, in order to protect pregnant mares from infection, vaccination and good care-feeding should be applied at different intervals during pregnancy and pregnant mares should be kept separate from other horses. If this process can not be applied, care should be taken to vaccinate all horses in the herd (Robinson et al., 1997). Again, in order to prevent the rapid spread of the infection in the population, importance should be given to stable hygiene and horses should be kept in well-ventilated shelters.

In the diagnosis of the infection, both direct and indirect methods are used to reveal the presence of antibodies formed at the end of the disease. In recent years, Polymerase Chain Reaction (PCR), which is stated to be more sensitive and practical by various researchers, has begun to be used in determining the disease. In our country, no source was found that the PCR technique was applied for the diagnosis of the disease. The aim of the study was the detection and

differentiation of EHV-1 and EHV-4 by polymerase chain reaction (PCR). Samplings were done from nasal swabs, various tissues and organs belonging to the sick horses and aborted fetuses. 2 years and upper elderly racehorses with fever and upper respiratory tract disorders, miscarried horses and aborted fetuses were studied during the study.

## **MATERIALS AND METHODS**

### **Sampling**

In the study, 26 aborted fetuses and 96 nasal swab samples from symptomatic horses obtained from different sources were used to determine the genomic DNA of EHV-1 and EHV-4 viruses. In the study, liver, lung and spleen belonging to aborted fetuses were used in the preparation of homogenized tissue emulsion at all samples. Additional tissues and organs such as kidney, heart, fetal membranes and umbilical cord were added to the homogenized tissue emulsion where sampling is available. 98 nasal swab samples taken from racehorse hospitals, 2 and older race horses were used in the study. The clinical findings seen in these animals are runny nose, cough, mild fever, nosebleeds, recurrent upper respiratory tract symptoms, and these symptoms have varied between animals.

### **Extracted EHV-1 and EHV-4 DNA**

Vacuum-dried EHV-1 89c25p strain (52) DNA and EHV-4 TH20p strain (16) DNA, ~50 µg each was obtained as a courtesy of Dr. Tomio Matsumura (Epizootic Research Center, Equine Research Institute, The Japanese Racing Association) was used for positive control and optimization of PCR stages in the study.

### **PCR Core Kit**

This kit was used for the amplification of DNA of viruses extracted from materials in the study with standard strain DNAs obtained from Japan in PCR and was supplied by Sigma (MB-345).

### **Mineral oil**

Mineral oil from Sigma was used to coat the surface to prevent evaporation on the PCR master mix (Fluka BioChemika-69794).

### **Ethidium bromide**

Ethidium bromide (Fluka BioChemika-46065), which is the product of Sigma Company, was used to provide the visibility of PCR products formed in electrophoresis under UV light. To prepare the Ethidium bromide (EtBr) solution, 1g EtBr was placed on a 100 ml glass cylinder,

adding 1ml of 95% ethanol on the magnetic stirrer for 5 minutes at low speed. It was kept for a while and dissolved and the solution was completed to 100 ml with distilled water.

#### **Agarose**

The agarose (A-9539) supplied by Sigma was used in the preparation of the 1% gel required for electrophoresis.

#### **DNA ladder (Marker)**

The DNA ladder (50-2000 bp) used to determine the molecular weights of the PCR product (DNA bands) formed in the gel as a result of PCR was obtained from Bio-Rad (170-8200).

#### **DNA extraction kit**

In the study, GenElute Mammalian Genomic DNA kit (Sigma MB-660/G1N70) was used for DNA extraction from aborted fetus organs, and InstaGene-Matrix (Bio-Rad 732-6030) DNA isolation kit was used for DNA extraction from nasal swab samples.

#### **Loading Buffer**

In the study, 10 X loading buffer used to load PCR products into the pits on the gel were obtained from Takara (A152).

#### **Taq Polymerase enzyme**

Taq DNA polymerase enzyme was used to combine with each complement of each DNA chain after DNA denaturation at 5u/μl while preparing the master-mix and was obtained from Sigma (MB-300 / D1806).

#### **10X TAE buffer**

The 10X TAE buffer used in the study was used to carry out genomic DNA in PCR products on the gel and to prepare the gel.

#### **10X TAE buffer preparation formula**

Tris Base 48.4 g

EDTA 7.4 g

Sodium acetate 16.4 g

Glyceal acetic acid 17 ml

Make up to 1000 ml with distilled water.

#### **Primers**

In order to determine genomic DNA of EHV-1 and EHV-4 viruses, synthetic oligonucleotide primer pairs, which show complete homology for both viruses and define short sequences selected from the gB gene, were prepared in Genosys and used in polymerase chain reaction.

5'-GGA TGC CAT GGA GGC ACT ACA-3 'forward

5'-GTT TGG CGG TGA CGT TGG AAG-3 'reverse

With these primers, 840 bp molecular weight product formations are expected to occur.

For EHV-1; Primers selected from the gC gene and designed as follows are used.

5'-GCG AGA TGT GGT TGC CTA ATC TCG-3 'forward

5'-GAG ACG GTA ACG CTG GTA CTG TTAA-3 'reverse

With these primers, product formation is expected to occur at a molecular weight of 649 bp.

For EHV-4; Primers selected from the gC gene and designed as follows are used.

5'-AGC CAC GAA CAA CTC AAC CGA TGT-3 'forward

5'-GAG ACG GTA ACG CTG GTA CTG TTAA-3 'reverse

With these primers, 507 bp molecular weight product formations are expected.

#### **Polymerase Chain Reaction**

Reconstitution of Reference EHV-1 and EHV-4 DNA; Matsumura's procedure was followed for reconstitution of vacuum dried DNA extracts of EHV-1 and EHV-4 strains from Japan. After the tubes were centrifuged, 100 μl of autoclaved double-distilled sterile distilled water was added to each. In this way, the rate in each tube reached a concentration of 0.5 μg / μl. For the DNA extract to be used in the experiments, the original solutions were prepared by diluting X100 with distilled water and a DNA dilution was prepared (10 μl of the original DNA solution was added and 990 μl of twice distilled sterile water was added). Diluted DNAs were divided into 10 pieces of 100 μl after this dilution process, which was carried out to protect the original solution against the risk of contamination that may occur during the studies, and was stored at -20 ° C with the original solution.

#### **Standardization of Polymerase Chain Reaction**

In the first stage of the study, the optimization of PCR was made by modifying the method reported by O'Keefe et al. (1991). In PCR studies, oligonucleotide primer pair selected from the gB gene and showing complete homology for both viruses, 50 pmol. It was used by diluting at the concentration specified in the reference.

#### **Optimum values for reference DNA.**

For this purpose, in order to determine the appropriate amount of DNA, template DNAs of the reference EHV-1 and EHV-4 viruses were subjected to separate reactions in different

quantities (1µl, 2µl, 3µl, 4µl and 5µl) in the same dilution.

For 100 µl PCR reaction; 10 µl 10X PCR buffer, 2µl dNTP mix, 2µl Primer gB/F

2µl Primary gB/R, 0.5 µl Taq DNA polymerase enzyme, 2 µl template DNA (average 10 ng), 81.5 µl dH<sub>2</sub>O, 50 µl of mineral oil was added.

Following these processes, the mixtures are 5 min at 94° C, 1 min (32 cycles) at 94 ° C, 32 min at 55° C, and 3 min at 72 ° C on PCR thermocycler (Biometra) device. 32 cycles amplification was performed and maintained at 4° C until electrophoresis. The PCR products obtained were then subjected to gel sided electrophoresis for 1 hour at 90 Volt electric current. Final product containing ethidium bromide was examined by transilluminator (Biometra) device under the U.V. light and photographed.

In addition, type specific oligonucleotide primer pairs designed by choosing among the gC gene of EHV-1 and EHV-4 from Dr. Matsumura, 50 pmol. It was diluted to a concentration and used in optimization studies. In order to determine the appropriate amount of DNA for this purpose, template DNAs from the reference EHV-1 and EHV-4 strains were subjected to separate reactions in different amounts (1µl, 2µl, 3µl, 4µl and 5µl) in the same dilution.

#### **Master-mix for gC 1 f / r primers Master-mix for gC 4 f / r primers.**

For 50 µl PCR reaction;

5 µl 10X PCR buffer, 1µl dNTP mix, 1µl Primer gC 1 F, 1µl Primer gC ¼ R, 0.25 µl Taq DNA polymerase enzyme, 1 µl template DNA, 40.75 µl dH<sub>2</sub>O, Added on 50 µl mineral oil. For 1 µl 50 µl PCR reaction; 5 µl 10X PCR buffer, 1µl dNTP mix, 1µl Primer gC 4 F

1µl Primer gC ¼ R, 0.25 µl Taq DNA polymerase enzyme, 1 µl template DNA, 40.75 µl dH<sub>2</sub>O, 50 50 µl of mineral oil was added.

Following these processes, amplification was performed in the PCR thermocycler (Biometra) device, 94 ° C 5 min, 94 ° C 75 sec (30 cycles), 60 ° C 90 sec (30 cycles) and kept at 4° C until electrophoresis. In this amplification process, Lawrence et al. (1993) and Matsumura's methods were followed.

The obtained PCR products were then subjected to electrophoresis for 25 minutes at 100 Volt electric current.

#### **DNA extraction from aborted fetal tissues**

For this purpose, the method recommended by the manufacturer of the kit was applied for DNA extraction from tissues. Different organs of each 26 aborted fetuses were sampled and recorded. Each fetal tissue was preserved in icy containers during the processes. Tissues were chopped with sterile scalpel and homogenized. 25 mg of this organ mixture was taken into sterile micro centrifuge tubes and homogenization of the tissues was provided using sterile toothpicks. Thereupon, 180 µl of dissolving solution (lysis buffer) and 20 µl of proteinase-K were added and vortexed and incubated until fully digested (3 hours) at 55 ° C, 200 µl of melting solution was added, vortexed and at 70° C. It was incubated for 10 minutes. Then 200 µl of ethanol was added and vortexed. The mixture was transferred to the holding column and centrifuged at 6500 rpm for 1 minute. The holding column was transferred to a new collector tube and 500 µl of wash solution was added onto it and centrifuged again at 6500 rpm for 1 min. After this procedure, the retention column was transferred back to a new collector tube and a second 500 µl wash solution was added. The holding column was dried by centrifuging at 12000 rpm for 3 minutes. The retention column was placed in a new collector tube and 200 µl of elution solution was placed on it. After centrifuging at 6500 rpm for 1 min, the pure DNA obtained by repeating the elution step in the same tube was used in PCR.

#### **DNA extraction from nasal swab samples**

For this purpose, the extraction method was applied as recommended by manufacturer Bio-Rad. Nasal discharge of suspicious animals were taken with sterile swabs and diluted in 2 ml of PBS, 30 sec at high speed. It was vortexed, and then the tissues adhered to the cotton were passed into the liquid by being crushed with sterile injector tips. The liquid in the tube was drawn with a syringe and taken into sterile sample collection containers and stored at -20° C until examination.

For testing, 200 µl of samples were taken into sterile tubes, centrifuged for 1 minute at 12000 rpm, the upper liquid was discarded, the sediment was reconstituted with 1 ml of PBS and the upper liquid was discarded. The sediment was suspended with 20 µl distilled water. 200 µl of InstaGene matrix was added thereto and

incubated at 56 ° C for 30 minutes; the mixture was vortexed for 10 seconds at high speed and incubated for 8 minutes at 100° C water bath, re-vortexed for 10 seconds, centrifuged at 12000 rpm for 3 minutes and 20 µl of the supernatant was used in PCR trials. The last step was repeated with each new PCR trial.

#### **PCR from extracted fetal organs DNA.**

For this purpose, suspect organs were examined using the primer pair of EHV-1 and EHV-4 DNA prepared from the gB gene region and showing full homology for both viruses. In the test, the method reported by O'Keefe et al. (1991) was modified and applied. Accordingly, the following procedure was performed for 100 µl of PCR reaction.

For 100 µl PCR reaction; 10 µl 10X PCR buffer, 2µl dNTP mix, 2µl Primer gB/F

2µl Primary gB/R, 0.5 µl Taq DNA polymerase enzyme, 2 µl template DNA, 81.5 µl dH<sub>2</sub>O, 50 µl of mineral oil was added.

Following these processes, the mixtures were amplified at the following temperature, time and cycles in the PCR thermocycler (Biometra) device. 32 cycles of; 94 ° C 5 min, 94 ° C 1min, 55 ° C 2 min, 72 ° C 3 min, stored at 4° C till electrophoresis.

The obtained PCR products were then subjected to gel electrophoresis treatment for 1 hour at 90 Volt electric current. Final product containing ethidium bromide was examined by transilluminator (Biometra) device under the U.V. light and photographed.

The same examples were also examined using type specific oligonucleotide primer pairs prepared from the gC gene region of EHV-1 and EHV-4 obtained from Dr. Matsumura. Amplification of the samples was attempted in the PCR thermocycler (Biometra) device at the following temperature, time and cycles. In this amplification process, Lawrence et al. (1993) and Matsumura's method was used.

#### **Master-mix for gC 1 up/com primers master-mix for gC 4 up/com primers**

For 50 µl PCR reaction; 5 µl 10X PCR buffer  
1µl dNTP mix, 1µl Primer gC 1 F, 1µl Primer gC ¼ R, 0.25 µl Taq DNA polymerase enzyme  
1 µl template DNA, 40.75 µl dH<sub>2</sub>O, Added on 50 µl mineral oil For 1 µl 50 µl PCR reaction;  
5 µl 10X PCR buffer, 1µl dNTP mix, 1µl Primer gC 4 F, 1µl Primer gC 1/4) R, 0.25 µl Taq DNA

polymerase enzyme, 1 µl template DNA, 40.75 µl dH<sub>2</sub>O, 50 50 µl of mineral oil was added.

Following these processes, the mixtures were amplified at the following temperature, time and cycles in the PCR thermocycler (Biometra) device; 1 cycle at 94 ° C 5 min, 94 ° C 75 sec (30 cycles), 60 ° C 90 sec (30 cycles), 72 ° C 90 sec (30 cycles), Stored at the 4° C till electrophoresis.

The obtained PCR products were then subjected to 25-minute horizontal gel electrophoresis treatment at 100 Volt electric current. Final product containing ethidium bromide was examined by transilluminator (Biometra) device under the U.V. light and photographed.

#### **PCR from nasal swab samples**

The collected samples were examined using gC oligonucleotide primer pairs of EHV-1 and EHV-4 from Dr. Matsumura. Amplification of the samples in the PCR thermocycle (Biometra) device was attempted at the following temperature, time and cycles. In this amplification process, Lawrence et al. (1993) and Matsumura's method was used.

#### **Master-mix for gC 1 up/com primers master-mix for gC 4 up/com primers**

For 50 µl PCR reaction; 5 µl 10X PCR buffer, 1µl dNTP mix, 1µl Primer gC 1 F, 1µl Primer gC ¼ R, 0.25 µl Taq DNA polymerase enzyme

1 µl template DNA, 40.75 µl dH<sub>2</sub>O, Added on 50 µl mineral oil for 1 µl 50 µl PCR reaction; 5 µl 10X PCR buffer, 1µl dNTP mix, 1µl Primer gC 4 F, 1µl Primer gC ¼ R, 0.25 µl Taq DNA polymerase enzyme, 1 µl template DNA, 40.75 µl dH<sub>2</sub>O, 50 50 µl of mineral oil was added.

Following these processes, the mixtures were amplified at the following temperature, time and cycles in the PCR thermocycler (Biometra) device for 30 cycles.

1 cycle at 94 ° C 5 min, 94 ° C 75 sec, 60 ° C 90 sec, 72 ° C 90 sec, stored at 4° C till electrophoresis.

The PCR products obtained were then subjected to 25-minute horizontal gel electrophoresis treatment at 100 Volt electric current. Final product containing ethidium bromide was examined by transilluminator (Biometra) device under the U.V. light and photographed.

## **RESULTS AND DISCUSSION**

### **PCR Results of Aborted Fetuses.**

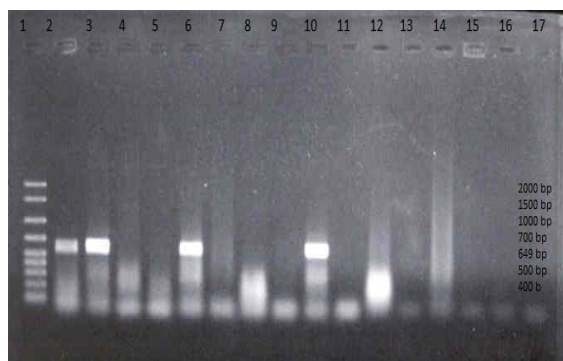
According to the PCR test results, genomic DNAs of EHV-1 extracted from tissue and organ



emulsions of aborted fetuses were identified in 7 of 26 samples, at 649 bp. With gC1 F and gC¼ R primers (Fetus samples No: 1, 2, 5, 6, 15,16) (Table 1 and Figure 1). Genomic DNA of EHV-4 could not be determined from 26 examined samples.

**Table 1.** Findings of PCR results.

Samples	Results
26 aborted fetal organs and tissues	<i>EHV-1</i> : 7 viral DNA were detected <i>EHV-4</i> : No viral DNA detected
98 nasal swab samples	<i>EHV-1</i> : 1 viral DNA were detected <i>EHV-4</i> : 2 viral DNA were detected



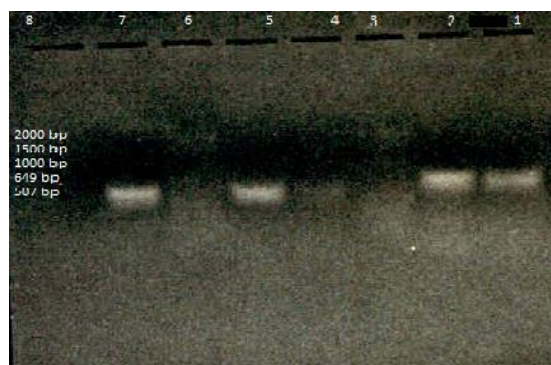
**Figure 1.** PCR results. EHV-1 genomic DNA detection from aborted foetuses at 649 bp. with gC1 F and gC¼ R primers by horizontal gel electrophoresis.

Lanes; 1- DNA ladder, 2- EHV-1 Positive control (89c25p/TH20p reference DNA 10 ng), 3,6 and 10 EHV-1 positive samples fetal DNA, 4,5,7,8,9,11,12 and 13 EHV-1 negative samples, 14- Negative control.

**PCR results from nasal swab samples.**

98 nasal swab samples from two years and elder symptomatic horses were collected and DNA extractions were done. PCR analyses were done by using gC primers specific for EHV-1 and EHV-4. Results; in 2 specimens (Sample No: 51 and 57) were found positive for EHV-4 and 96 samples were negative in contrast. 1 of 98 samples (Sample No: 55) were found positive for EHV-1 and 97 were negative in contrast. Genomic DNA was detected at 507 bp in PCR products belonging to samples found to be positive for

EHV-4 and at 649 bp in PCR products for EHV-1 (Table-1 and Figure 2).



**Figure 2.** PCR results. EHV-1 and EHV-4 genomic DNA detection from nasal swab samples at 649 bp. and 507 bp with gC1 F and gC¼ R primers by horizontal gel electrophoresis.

Lanes; 1- EHV-1 positive DNA, 2- EHV-1 Positive control gC1 f/r 89c25p reference DNA, 3 and 6 DNA ladder, 4 and 7 EHV-4 positive DNA, 5- Positive control TH20p reference DNA gC 4 f/r, 8- Negative control.

Herpesviruses are the leading factors that cause offspring in horses (Gilkerson et al., 1998; O'Callaghan et al., 1999). The studies conducted in many countries of the world (Matsumura et al., 1991; Murphy et al., 1999; van Maanen et al., 2000) showed that the majority of abortion cases were associated with EHV-1 and rarely due to EHV-4. It has been revealed by different researchers in the past years when EHV infections are seen in our country (Mengi et al., 1996; Yilmaz et al., 1995). In the diagnosis of EHV-1 and EHV-4 infections in horses, samples are taken from the organs of the fetus when nasal discharge, buffy coat and abortion occur. It is not always possible to obtain appropriate results in serological tests, in such cases some alternative tests that may help diagnosis should be put in place. PCR comes to the forefront as a fast and reliable diagnosis method as reported by researchers (Carvalho et al., 2000; Kennedy et al., 1996). In the study, extracts prepared by homogenizing the tissues belonging to different organs such as lung, liver, spleen, fetal membranes and kidneys of 26 aborted fetuses provided at certain times and from different studs were examined by polymerase chain reaction and 7 of the samples were found to carry the genomic DNA of EHV-1 and therefore it

was positively evaluated for EHV-1 infection. However, since the purpose of the thesis study is only for determining the genomic DNA of EHV-1 and EHV-4 viruses.

When the results of this study are combined with fetus-compatible pathologies and positive serological test findings of Yilmaz's (1971) that the isolated substance is neutralized by the positive control serum and presence of positive titer provided by complement fixation demonstrated that the presence of EHV -1 in Turkey determined by direct and indirect methods.

In the study, genomic DNAs of EHV-4 and EHV-1 were detected in two and one of the nasal swab samples, respectively. Since the purpose of the thesis is not to determine the condition of the infection, there is no comparison with this aspect, but considering that the DNAs of EHV-1 and EHV-4 used as positive control can be detected with appropriate results. It were concluded that the detection of EHV-1 and EHV-4 by PCR is an appropriate method, as reported by Sharma et al. (1992). In the study, it was observed that a 649 bp band was formed as a result of PCR examination of the emulsion prepared from organs such as lung, liver, kidney and spleen of 26 fetuses. When the results obtained were compared with other researchers' reports as the effectiveness of PCR, it was revealed that the test was a highly sensitive test, and that obtaining different bp results could vary depending on the primers and reaction values used. In addition, it has been observed that the test is a fast and reliable test that results in as little as 24 hours. Ballagi et.al, (1990) stated that there was a parallel between PCR and virus isolation results and that the test was economical, time-saving and reliable. Carvalho et. al. (2000) emphasized that EHV-1 genomic DNA was also detected from healthy-looking mare, fetus, foal and stallions, and aborted fetuses, and therefore a technique that reveals DNA with great sensitivity is required in detecting latent infection. Edington et. al. (1994) reported that by polymerase chain reaction, they identified 87.5% of genomic viral DNA in bronchial and trigeminal ganglia of slaughtered horses, and based on these results, EHV-1 and EHV-4 latent infection was common in the horse population and they mostly identified the virus in respiratory lymph nodules. Studies have shown

that many disease ethiological agents or genomic DNA of microorganism can be detected by polymerase chain reaction. In this study, the different primers of EHV-1 and EHV-4 from the aborted fetuses and nasal swab samples were differentiated by polymerase chain reaction and PCR product genomic DNAs of EHV-1 and EHV-4 viruses were revealed with this test.

### CONCLUSIONS

This is the first study of determination of EHV-1 and EHV-4 by PCR in Turkey. As a result, it is determined that both viruses are found in our country. Especially, the presence of EHV-4 virus in our country has been demonstrated for the first time. In addition, with this study, it has been demonstrated that it is possible to distinguish the both viruses as infectious agent by PCR in the differentiation of EHV-1 and EHV-4 from each other. In addition, it was concluded that the PCR method is economical and reliable and time consuming in the diagnosis of EHV-1 and EHV-4 infection.

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## NOVEL PARVOVIRUS IN HORSES WITH NEUROLOGIC AND RESPIRATORY DISEASES

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### **Abstract**

*Metagenomics was used to identify viral sequences in the plasma and CSF (cerebrospinal fluid) of 13 horses with unexplained neurological signs and in the plasma and respiratory swabs of 14 horses with unexplained respiratory signs. Equine hepacivirus and two copiparvoviruses (horse parvovirus-CSF and a novel parvovirus) were detected in plasma from neurological cases. Plasma from horses with respiratory signs contained the same two copiparvoviruses plus equine pegivirus D and respiratory swabs contained equine herpes virus 2 and 5. Based on genetic distances the novel copiparvovirus qualified as a member of a new parvovirus species we named Eqcopivirus. These samples plus another 41 plasma samples from healthy horses were tested by real-time PCRs for multiple equine parvoviruses and hepacivirus. Over half the samples tested were positive for one to three viruses with eqcopivirus DNA detected in 20.5%, equine hepacivirus RNA and equine parvovirus-H DNA in 16% each, and horse parvovirus-CSF DNA in 12% of horses. Comparing viral prevalence in plasma none of the now three genetically characterized equine parvoviruses (all in the copiparvovirus genus) was significantly associated with neurological and respiratory signs in this limited sampling.*

**Key words:** Parvoviridae, Eqcopivirus, Horse parvovirus-CSF, Equine hepacivirus, Equine parvovirus H, Bosavirus, Virome

### **INTRODUCTION**

*Parvoviridae* family first established in 1975 and heretofore, Parvoviruses have been reported almost all vertebrate clades also arthropods (Penzes, Soderlund-Venermo et al. 2020). *Parvoviridae* family members genome is a linear, non-segmented molecule of ssDNA, 4–6.3 kb in size and non-enveloped with two to four open reading frames (ORFs). Recently, *Parvoviridae* family has three different subfamily one of them is *Parvovirinae* which infected vertebrate host, other one *Densovirinae* which infected arthropod hosts and the recently added one *Hamaparvovirinae* which infected both invertebrate and vertebrate. A limited number of viral metagenomics studies have analyzed horse samples.

A 2013 study identified a novel flavivirus that was named Theiler's disease-associated virus (TDAV) from an outbreak of this liver disease transmitted through equine-origin tetanus anti-toxin (Chandriani, Skewes-Cox et al. 2013). Metagenomics studies of equine serum pools recently identified the Theiler's disease-associated virus (TDAV), Equine parvovirus-H,

equine hepacivirus (EqHV), Equine pegivirus (EPgV) plus unexpectedly the porcine Suid betaherpesvirus 2 (Epstein, Quan et al. 2010, Kapoor, Simmonds et al. 2011, Lyons, Kapoor et al. 2012, Kapoor, Simmonds et al. 2013, Lyons, Kapoor et al. 2014, Divers, Tennant et al. 2018, Paim, Weber et al. 2019). To further characterize the eukaryotic virome of horses and identify possible equine pathogens we used here a combination of viral metagenomics and real-time PCR to analyze plasma and cerebrospinal fluid (CSF) from 13 horses with unexplained neurological signs, plasma and respiratory swabs from 14 horses with unexplained respiratory signs, and plasma from 41 healthy horses, when necessary.

### **RESULTS AND DISCUSSION**

Virus metagenomics analysis of plasma, CSF and respiratory swabs from horses with unexplained neurological and respiratory signs showed the presence of three parvoviruses, one of which (bosavirus) was likely introduced by the addition of fetal bovine serum to the respiratory swabs transport medium. Additionally detected by

metagenomics were equine hepacivirus, equine pegivirus (Kapoor, Simmonds et al. 2013), and equine herpesvirus 2 and 5 (Table 1).

**Table 1.** Distribution of translated sequence reads with similarity E score of  $<10^{10}$  to known mammalian viral proteins.

Species	Total Reads	Equine hepacivirus	Pegivirus E	Equid gammaherpesvirus 2	Equid gammaherpesvirus 5	Horse parvovirus CSF	Novel parvovirus: Eqcopivirus	Bosavirus
Neuro Pool1 Plasma	2574940					1023	17058	
Neuro Pool4 CSF	909718							
Neuro Pool2 Plasma	1612738	7						
Neuro Pool5 CSF	904551							
Neuro Pool3 Plasma	2017788							
Neuro Pool6 CSF	1042257							
Respiratory Pool7 Plasma	1507523		317				36069	
Respiratory Pool10 Swab	619689							1719
Respiratory Pool8 Plasma	603438					1087	16355	
Respiratory Pool11 Swab	834507			120	25			880
Respiratory Pool9 Plasma	1520432							
Respiratory Pool12 Swab	392620							395

Another copiparvovirus (equine parvovirus-H) was also detected using real-time PCR likely due to its lower concentration as reflected by higher real-time PCR CT values. The presence of viral nucleic acids detected by PCR or HTS does not prove the presence of infectious viruses. Nonetheless, the repeated detection of viral genomes in nuclease-treated, presumably sterile samples such as plasma and CSF, and the typically rapid clearance of viral particles from the circulation by the liver support the possibility of active replication (Ramratnam, Bonhoeffer et al. 1999, Zhang, Dailey et al. 1999).

When the prevalence of the plasma-associated viruses was determined in individual samples using real-time PCR none showed a statistically higher rate of detection or higher viral loads as determined by Ct when comparing plasma from 13 neurological or 14 respiratory cases to plasma from 41 healthy horses. Only the new eqcopivirus

showed a trend toward a higher rate of detection in plasma from unexplained respiratory cases (30% versus 17%) ( $p = 0.443$ ).

CSF and respiratory swabs were available only from neurological or respiratory cases respectively. The rate of virus detection could therefore not be measured in these anatomical compartments in healthy animals. Horse parvovirus-CSF and eqcopivirus were also detected in 1/13 (different) CSF samples and in 3/13 (different) respiratory swabs each.

The initial characterization of horse parvovirus-CSF genome was also from a CSF sample from a different unexplained neurological case [20]. PCR previously showed EqPV-H DNA to be present in the plasma of 13% healthy horses in the US (Divers, Tennant et al. 2018) and 12% of healthy race horses in China (Lu, Sun et al. 2018), rates of viremia similar to that detected here in 6/41 (15%) of healthy horses. The Parvoviridae family

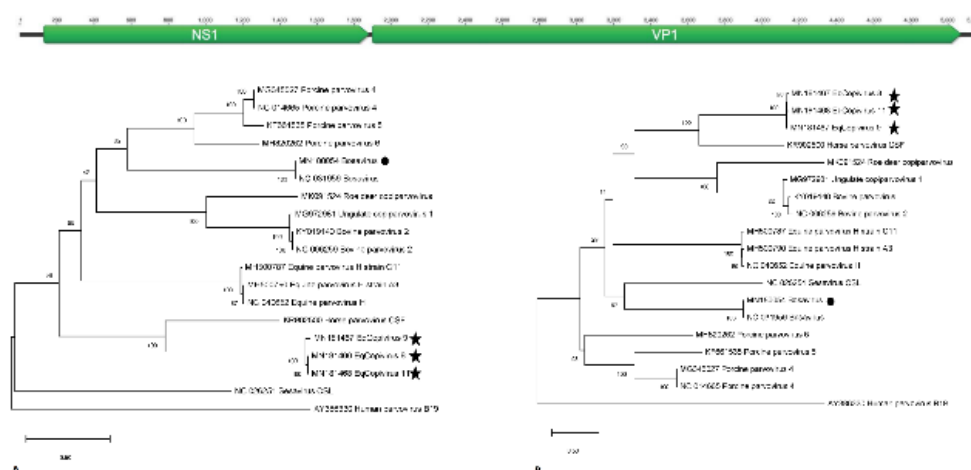
consists of non-enveloped, icosahedral, viruses (Mietzsch, Penzes et al. 2019).  
 with single stranded DNA genomes of 4 to 6 Kb

**Table 2.** Rate of real-time PCR detection for equine viruses and association with unexplained neurological or respiratory diseases using Fisher's exact test.

	Neuro Plasma <i>n</i> = 13	Neuro CSF <i>n</i> = 13	Respiratory Plasma <i>n</i> = 14	Respiratory Swab <i>n</i> = 13	Healthy Control Plasma <i>n</i> = 41	Total Samples <i>n</i> = 94	<i>P</i> Value = Plasma Neurological Versus Healthy	<i>P</i> Value = Plasma Respiratory Versus Healthy
Equine flavivirus	3 (23%)	0	0	2 (15%)	6 (15%)	11 (12%)	0.32	0.67
EqPV-H	2 (15%)	0	2 (15%)	0	7 (17%)	11 (12%)	1	1
Eqcopivirus	1 (7%)	1 (7%)	4 (30%)	3 (23%)	7 (17%)	15 (16%)	0.443	0.663
Horse parvovirus-CSF	1 (7%)	1 (7%)	1 (7%)	3 (23%)	2 (5%)	8 (8.5%)	1	1

Eight ICTV approved genera, including Copiparvovirus, are currently included in the Parvoviridae family. The eqcopivirus genome described here represents the third parvovirus species confirmed to infect horses by virtue of detection in multiple equine plasma, CSF, and

respiratory swab samples. That all parvoviruses identified to date in horses belong only to the copiparvovirus genus is somewhat unexpected and future studies are likely to expand the list of known equine parvoviruses (Figure 1).



**Figure 1.** A) Genome ORF (Open Reading Frame) structure of eqcopivirus genome. (B) Phylogenetic analysis of NS1 (left) and VP1 (right) proteins in the Copiparvovirus genus. Bootstrap values from a hundred replicate runs are shown. Symbols are used to highlight the new genomes described here

While a higher rate of eqcopivirus DNA detection was found in the plasma of horses with unexplained respiratory signs (30%) versus plasma from healthy horses (17%) that difference, based on the limited number of samples tested, was not statistically significant. Because CSF and respiratory swabs from healthy animals were not available only PCR results from plasma could be compared for disease association. Viruses resulting in neurological and respiratory disease may be expected to be

present in CSF or respiratory fluids respectively as were both horse parvovirus-CSF and eqcopivirus. The collection of CSF and respiratory swabs samples from healthy animals may be necessary to better test for possible associations between these copiparvoviruses and unexplained neurological and respiratory signs.

### CONCLUSIONS

The now repeated detection of horse parvovirus-CSF [20] (*n* = 2) and eqcopivirus (*n* = 1) in CSF of

horses indicates possible role for these viruses in these neurological signs which will require further studies. The availability of eqcopivirus genome sequences will now also allow the design of hybridization probes to determine whether infected cells can be identified in fixed brain or lung tissues from animals with unexplained neurological or respiratory signs.

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## GENETIC EVALUATION OF REPRODUCTIVE TRAITS IN TEDDY GOATS

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### **Abstract**

*Data available on 20455 kidding and performance records of 5545 Teddy goats and progeny of 406 sires maintained as separate flocks at three different locations Livestock Experiment Stations Rakh Ghulaman, Rakh Khariwala and Chak Katora (1971-2008) Punjab, Pakistan were analyzed for documenting reproductive traits and genetic sources which influence these traits. The data was analyzed using the GLM procedure (General Linear Models) of the Statistical Analysis Systems (SAS, 2004) to study the various reproductive traits. The genetic parameter estimation was done using REML procedure fitting an Individual Animal Model. The Least squares means for age at first service, age at first kidding, weight at first service, weight at first kidding, services per conception, service period, kidding interval, were  $245.65 \pm 0.73$  days,  $14.07 \pm 0.01$  kg,  $394.14 \pm 0.76$  days,  $18.06 \pm 0$  kg,  $1.24 \pm 0.004$ ,  $153.58 \pm 0.73$  days,  $327.53 \pm 1.12$  days, respectively. The percentage of single births was 43 percent, while multiple births were 57 percent. The sex ratio was 51:49 males and females. The heritability estimates for age at first service, weight at first service, age at first kidding, weight at first kidding, services per conception, service period and kidding interval were  $0.19 \pm 0.22$ ,  $0.21 \pm 0.01$ ,  $0.19 \pm 0.04$ ,  $0.20 \pm 0.04$ ,  $0.07 \pm 0.01$ ,  $0.06 \pm 0.05$  and  $0.05 \pm 0.03$ , respectively. The repeatability estimates for services per conception, service period and kidding interval were  $0.02 \pm 0.05$ ,  $0.01 \pm 0.04$  and  $0.05 \pm 0.03$ , respectively. It is envisaged from the present study that over the 34 years period selection remained ineffective to bring the desired changes and it will remain so if random use of breeding animals is practiced. The possible use of ineffective selection could be unavailability of efficient techniques for the evaluation of animals and incorrect performance recording etc. It is therefore, necessary to correct all these discrepancies by taking corrective measures as discussed above.*

**Key words:** Teddy goats, Genetic evaluation, Genetic traits, Pakistan

**RELATIONSHIP OF CIRCULATORY AND UTERINE CONCENTRATIONS OF ACUTE PHASE PROTEINS WITH SUBCLINICAL ENDOMETRITIS IN POSTPARTUM WATER BUFFALO, AND IMPACT ON FERTILITY**

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**Abstract**

*The concentration of acute phase proteins (APPs) indicates severity of inflammation. Haptoglobin (Hpt) and serum amyloid-A (SAA) are diagnostically most important APPs in ruminants. Our objective was to evaluate the effect of subclinical endometritis (SCE) on the circulatory and uterine profiles of Hpt and SAA in postpartum buffaloes diagnosed with SCE. We hypothesized that SCE would be reflected by differential Hpt and SAA concentrations. Based on cytobrush based endometrial cytology examination on day 21 postpartum (21dpp), buffaloes diagnosed with SCE ( $\geq 18\%$  polymorphonuclear, PMNs) were enrolled into SCE group ( $n=12$ , endometritic) and negative for SCE ( $<18\%$  PMNs) into control group ( $n=15$ , healthy). Uterine ultrasonographic examination and samplings (endometrial cytology, low volume uterine flush, blood) were carried out on 21 and 28 dpp. Buffaloes were artificially inseminated at observed spontaneous oestrus. The PMN% were higher ( $P=0.000$ ) in SCE group compared to control group on 21 dpp ( $39.82 \pm 4.56$  vs.  $11.62 \pm 0.96$ ) and 28 dpp ( $27.08 \pm 4.34$  vs.  $5.85 \pm 1.40$ ). Endometritic buffaloes had comparatively higher ( $P<0.05$ ) endometrial thickness on 21 dpp. Hpt concentrations in serum and uterus were higher on 21 dpp ( $P<0.01$ ) and 28 dpp ( $P<0.05$ ) in endometritic buffaloes and remained significantly higher in serum than in uterus at all the observations. Hpt concentrations increased significantly from 21 to 28 dpp in uterine flush of SCE group. Endometritic buffaloes had higher SAA concentrations in uterine flush ( $P<0.01$ ) and serum ( $P<0.05$  in serum, 21 dpp) on 21 and 28 dpp compared to the healthy counterparts. The serum (on 21 and 28 dpp) and uterine flush (on 28 dpp only) PGFM concentrations were significantly higher in buffaloes of control compared to SCE group. First postpartum overt oestrus was significantly delayed by SCE ( $75.58 \pm 4.7$  vs.  $60.7 \pm 2.9$  days). Lesser AIs/conception and higher overall pregnancy rate was recorded in healthy compared to the endometritic buffaloes. In conclusion, our results recorded the differential changes in uterine as well as circulatory Hpt and SAA concentrations along with reduced fertility in postpartum buffaloes due to SCE.*

**Key words:** Acute phase proteins, Buffalo, Fertility, Haptoglobin, Serum amyloid-A, Subclinical endometritis

**STRUCTURAL CONFORMATION, GENETIC STRUCTURE AND DIVERSITY AMONG SPATIALLY DISTRIBUTED NIGERIAN DWARF GOATS BASED ON MORPHOMETRIC CHARACTERS**

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**Abstract**

*Goat production and breeding in South-west Nigeria is largely under traditional system, and needs aggressive and State intervention for rapid improvement. To understand the body conformation, genetic structure and diversity among spatially distributed Nigerian dwarf goats, a survey was conducted between May 2015 and June 2017 in two States: Ekiti and Osun. Data were recorded on two hundred and twenty-seven (227) free-ranging and scavenging West African Dwarf (WAD) goats, consisting of 124 males and 103 females. Body traits measured included body weight (BWT), Heart girth (HGH), Diagonal trunk length (DTL), Height at the withers (HWT), Height at the rump (HRP), while body indices included Body Breadth Index (BBI), Body Depth Index (BDI), Body length Index (BLI), Trunk length index (Shape), Weight-Height Index (WHI), Overbuilding Index (OBI) and Leg Length Index (LLI). Data were subjected to analysis by randomized complete block design in factorial using General Linear Model Procedure (PROC GLM), ANOVA, Least square means procedures and Tukey HSD test, Principal component (PRINCOMP) analysis, Multiple linear regression (MLR) analysis by Statistical Analytical Systems software, Version 9.2, (SAS, 2010). K-means cluster analysis (K-CA) was conducted using SPSS software, Version 17.0. Significant ( $p < 0.05$ ) values between locations (BWT, DTL, TLI, OBI, BLI and WHI), between sexes for all traits and interaction of LOCxSEX (DTL, TLI, OBI, BDI, BBI, LLI and BLI) were obtained. K-Means cluster analysis (K-CA) identified five genetic clusters among WAD goats in the two States. The Squared Euclidean Distance measures between paired genetic-clusters 3/2, 5/1, 5/2, 5/4, 2/1, and 3/1 were 149.57, 135.50, 118.65, 107.64, 104.07 and 102.00 respectively. Pearson correlation,  $r$ , coefficients between 3/2, 4/3, 5/2, 4/1 were 0.744; 0.884, 0.906, and 0.983 respectively. Principal component loadings structure on PC1 revealed underlining structural conformation for sources investigated. Ekiti Does had body-trunk-girth structure while Osun Does possessed body-depth-length structure. Ekiti bucks possessed higher trunk structure while Osun bucks possessed heavier body-girth structure. Does revealed better girth-body-leg structure while Bucks indicated better weight-height-trunk structure. Between States, Ekiti goats were heavily built on weight-trunk-height structure; while Osun goats had better hearth girth structure. PC diversity biplot of PC1 against PC2 displayed most animals crowded at the origin, but few were placed far from origin indicating wider genetic diversity, net attributes and relationship among WAD goats in an environment. Multiple linear regression (MLR) equations by stepwise method revealed that Ekiti (HWT and TLI) and Osun (DTL, HRP and OBI) Does differ widely, Ekiti (BLI) and Osun bucks (BBI) differ, while both sexes recorded BWT and WHI common within sex. The general MLR equation regressed HWT, HGH, BDI, BLI and WHI as common traits to all WAD goats in the environment, although with very high VIF value of 81.26. All equations were significant at  $p < 0.0001$  with low SE: 0.570 – 2.936,  $R^2$ : 0.993 – 0.997, and DW statistics of 1.346 – 2.151. These results are useful information for improvement and selection programmes on WAD goats in South-west Nigeria and the Tropics.*

**Key words:** Diversity, Morphology, Metric traits, Osun and Ekiti States, PCA, Cluster analysis, Structural indices, West African Dwarf goats.

## A VIEWPOINT TOWARD GOAT BREEDING IN IRAN: SITUATIONS, PROBLEMS AND APPROACHES

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### **Abstract**

*Goat and sheep breeding observe all together by rural and nomadic people as a traditional work for their livelihood and income earning in many arid and semi arid regions of Iran. Goats traditionally had a strong influence on the socio-economic life of human populations, especially in rural and less favored regions of the world. In these regions this livestock constitutes an important source of proteins by converting different natural resources of lower quality. Due to their high tolerance to heat stress goats can be survive and produce in the most marginal regions of the world. On the other hand goats when are managed well contribute on the preservation of the ecosystems and can be used as an ecological tool for controlling the noxious weeds, reducing the incidences of wildfire, improving the rangelands and wild life habitat. Goats are raised principally for their meat, milk, fibre and skin. Goat farming can be very suited to production with other livestock such as sheep and cattle on low-quality grazing land. The impact of this breed on the carpet and meat industries in the areas where it is farmed is large which make it attractive to study to attempt to understand and potentially improve production and production efficiency. The aim of this article was to study the present situation and the trends of goat production and rearing as an essential tool for sustainable livelihood of rural and nomadic people in arid and semi arid regions, with emphasizing on the cashmere-producing goats in South Khorasan province, east of Iran.*

**Key words:** *Goats, Breeding Structure, Raeini, Cashmere, South Khorasan, Iran*

### **INTRODUCTION**

Mahatma Gandhi consumed goat milk everyday for more than 30 years. 12,000 year old paintings of goats have been found on the walls of caves in Europe. Goats were the first animals domesticated by man in 10,000 B.C. (McKenzie-Jakes, 2007). Goats were the first animals to be used for milk by humans. There are over 210 breeds of goats in the world. There are approximately 450 million goats around the world (McKenzie-Jakes, 2007).

In the developing countries, goats make a very valuable contribution, especially to the poor in the rural areas. The importance of this valuable genetic resource is underestimated and its extent of contribution to the livelihood of the poor is inadequately understood. They are often neglected in comparison with cattle and sheep. Part of this attitude towards them can probably be due to recognition of their capability, rather any prejudice against them, as it is believed that goats are intelligent, independent, agile, and tolerant to many diseases and parasites and can

look after themselves much better than other livestock species (Abdel Aziz, 2010).

The geographical and ecological conditions of Iran are well-suited to small ruminant production. The relatively low cost of sheep and goat farming (local breeds – well adapted to their environment plus extensive free communal grazing areas) and the increasing demand for expensive organic products in domestic and regional export markets encourages nomads to shift to organic production Livestock organic production (Ansari-Renani, 2016).

Goats traditionally had a strong influence on the socio-economic life of human populations, especially in rural and less favored regions of the world. In these regions this livestock constitutes an important source of proteins by converting different natural resources of lower quality (Skapetas and Bampidis, 2016). Due to their high tolerance to heat stresses goats can be survive and produce in the most marginal regions of the world. On the other hand goats when are managed well contribute on the preservation of

the ecosystems and can be used as an ecological tool for controlling the noxious weeds, reducing the incidences of wildfire, improving the rangelands and wild life habitat (Skapetas and Bampidis, 2016).

Goats are remarkably agile and will climb trees to browse. As with other herbivores, the number of animals that a goat farmer can raise and sustain is dependent on the quality of the pasture. However, since goats will eat vegetation that most other domesticated livestock decline, they will subsist even on very poor land. Therefore, goat herds remain an important asset in regions with sparse and low quality vegetation (FAO, 2013).

Nowadays goats and sheep face serious environmental challenges (degradation of rangelands, competition for land use, less water availability etc.). On the other hand climatic changes creates additional difficulties on the small ruminant farming. So the needs of policy on more research, organization and extension are increased (Skapetas and Bampidis, 2016).

Agricultural sector accounts for about 1/3 of the Iranian GDP and 1/4 of the country workforce. More than 90% of the Iranian food requirements are produced in the country. Animal agriculture covers over 40% of the agricultural activities. More than 57% of the available animal units in the country are sheep and goats. Iranian goat and sheep industry is characterized by owned by small farmers, based on extensive grazing, highly influenced by the environmental variables; its increment rate is declining in comparison with the past decades because of urbanizations industrialization and low income. Iranian goats are not grouped well according to their products importance. More than 20 breed of goats have been recognized in Iran but the two typical breeds are Marghoz and Raeni goats which produce attractive and expensive mohair and Kashmir fiber (Mueller et al., 2015).

The overall reproduction performances of Iranian sheep and goats are lower than the exotic pure breeds. It seems the variability in environmental patterns such as low rainfall and feed shortage, uncertainty in farmers' income and market conditions will be the most important factors in pushing the compulsory transition in Iranian sheep and goat industry. This transition may have critical effects on the animal-based food security mainly red meat. Therefore, more

attention is required from the government and non-governmental organizations for handling this trend to the well-managed right direction (Abdel Aziz, 2010).

Similar to sheep, goats are kept for producing different products including, meat, milk, fiber, hide, etc. The two main breeds are known for their Mohair and Kashmir production (Figure 1). Hide or skin marketing mainly of small ruminants is an open and real ongoing market throughout the year in all Iranian cities. Nearly all of the produced skins are exported (Valizadeh, 2008).



**Figures 1.** Goats and sheep breeding all together in many rural areas of Iran. During author visiting from very disadvantaged villages near city of Sarbisheh, close to Afghanistan borders, with 295 Km distance to city of Birjand, center of South Khorasan province, and east of Iran. Their main employments are rearing of goats, sheeps and chicken, plus carpet weaving in their peasantry and traditional houses (Pictures by author. Autumn, 2017).

#### **MATERIALS AND METHODS**

In this study, statistical population includes all holdings with goat units' raising activities at the time of survey. Information was collected through face-to-face interviews with the rural people that their main employments are rearing of goats and sheeps or one of his/her household members. In this study, statistical unit is an

agricultural holding with livestock units' activity, which at least has two heads of small livestock (sheep and goats). The information is collected by interview in case of availability of the holders of goats and sheeps, otherwise, by interview with the local informants.

Also The data of goat number, goat milk, meat and raw skins etc. in the different continents and countries were taken from the FAO data base, Jihad Agriculture Organization of Iran, National Bureau Statistics of Iran etc.. These data are processed statistically and analyzed further in order to arrive in appropriate conclusions. Also author utilized scientific journals and sites, his experiences, observations, interviews, pictures etc.that gathered them during two decade work and visiting rural and nomadic regions in various parts of Iran, specially in main locations of research namely South Khorasan province, east of Iran and Chaharmahal and Bakhtiari Province and its situation in south west of Iran. (Figure 2).



**Figure 2.** Map of main locations of research namely South Khorasan province, east of Iran (A) and Chaharmahal and Bakhtiari Province in south west of Iran (B).

#### **WORLDWIDE TRENDS AND ORIENTATION OF RAISING GOATS**

The general trends of goat production systems are increasing size of farms, while reducing their number, decreasing pastoral practices in the milk production, enlarging the stocks of major milk producing breeds and increasing the number of projects for the conservation of local breeds (Chetroui, et al., 2014).

Currently worldwide, raising goats sector tends to become increasingly important for the

national economies, being even a factor of economic development, particularly for rural areas. In all countries, due to accelerated increasing of human population number, resorts to more efficient exploitation of animal resources, applying more efficient technologies for breeding and exploitation of zootechnical interest animals. Competitiveness and profitability of goat milk on the world market are closely linked with seasonal production, livestock and productivity.

Over two thirds of the goats are grown in tropical and subtropical areas of the world, but yields from these are much lower compared to that of goats in temperate regions. This is due to poor feeding conditions and exploitation of less productive breeds (Chetroui, et al., 2014).

#### **FIBRE PRODUCTION FROM GOATS**

Cashmere is the fine, undercoat fibre (down) of cashmere goats. Cashmere is a luxury fibre regarded as one of the softest and warmest animal fibre principally used for clothing. Main producing countries of cashmere are China and Mongolia (60-70%) and Iran and Afghanistan (20-30%) (Ansari-Renani et al., 2013).

Being expensive, cashmere necessarily have a market which is limited to wealthy consumers who buy luxury goods not only for its intrinsic qualities of appearance, softness, warmth, handle and comfort but also simply because they are rare and expensive.

Of the 25 million goats in Iran, 5 millions are cashmere producing and the remaining goats produce small quantities of cashmere. Exact quantity of cashmere production and export of Iran is not known but it can be estimated that 5 million cashmere goats produce about 2000 tons of cashmere annually. This quantity of cashmere is exported either as raw unshorn (70%) or processed (30%).

More than 90% of the Iranian cashmere is produced by Raeini and Birjandi goats in Kerman and South Khorasan provinces respectively (Ansari-Renani et al., 2013). In South Asia, cashmere is called "pashmina" (from Persian *pashmina*, "fine wool"). In the 18th and early 19th centuries, Kashmir (then called Cashmere by the British), had a thriving industry producing shawls from goat-hair imported from Tibet and Tartary through Ladakh. The shawls were introduced into Western Europe when the General in Chief of the

French campaign in Egypt (1799–1802) sent one to Paris. Since these shawls were produced in the upper Kashmir and Ladakh region, the wool came to be known as "cashmere".

The quality of Iranian cashmere being long and highly curved ranks third after China and Mongolia. At present no price differential is paid to the producers for fine cashmere, as a major portion of cashmere is exported with some added value through processing (Ansari-Renani et al., 2013).

A major portion of cashmere is exported without any added value through processing. As a result of the marketing system, Iranian producers do not achieve good prices and have little incentive to produce better quality cashmere (Ansari-Renani, 2018).

#### **HOUSING AND STOCKING RATE WITHIN NOMAD PASTORALISTS IN SOUTHERN IRAN**

Tethering of livestock is prohibited in organic farming. Basically, there was no tethering of any kind of livestock among nomads. When nomad livestock returned from grazing, adult and young animals were penned separately near the tent in circular-shaped pens made up of wood, fenced overnight and milked in the morning before being taken out for grazing (Ansari-Renani, 2016).

In organic farming, it is obligatory that ruminants should graze on pastures ('free-range') and not fed in stables as long as the animal, weather and pasture conditions are suitable. If grazing is not possible, a permanently accessible open-air run is obligatory. Free-moving stables with permanent access to open-air runs are the principle of ruminant keeping. Only with permanent summer pasture grazing is an outdoor run not necessary, as long as the animals are not tethered.

The nomad livestock were not fed in stables or in restricted areas, but moved and grazed freely in extensive open grazing areas. Nomad families used the northern highland rangelands in spring and summer for grazing and migrated to the warmer southern Persian Gulf provinces in autumn and winter (Figure 3).

The nomadic pastoralists had no fixed homesteads and covered great distances with their livestock following pasture availability throughout the seasons. The transhumant pastoralists followed a regular seasonal

movement between set areas. Their movement was vertical where pastures at high altitudes are used in summer and pastures in the lowlands are used in winter or horizontal in the surroundings. Consequently, the livestock density (stocking rate) in Baft varied throughout the year, with the highest number of livestock and people in summer (Ansari-Renani, 2016) (Figures 3).



**Figures 3.** Establishing a wind water pump for producing new, safe, cheap and renewable energy resources and extracting groundwater resources for goat and sheep herds of nomad people, for improving their life conditions plus watershed management and pasture planting activities by CSP (International project of Carbon Sequestration) near cities of Sarbisheh and Nehbandan in South Khorasan Province- East of Iran.

#### **RESULTS AND DISCUSSION**

Agricultural sector accounts for about 1/3 of the Iranian GDP and 1/4 of the country workforce. More than 90% of the Iranian food requirements are produced in the country. Animal agriculture covers over 40% of the agricultural activities. More than 57% of the available animal units in the country are sheep and goats (Bureau statistics of Iran, 2019).

Goats and sheep form the most important group of ruminants in Iran mainly in rural areas. More than 57% of the available animal units in the country are sheep and goats. Most of the sheep and goats keepers which are mainly small farmers regard this enterprise as a complementary enterprise to plants culture or horticulture (Valizadeh, 2008).

In developing countries, much of the milk produced by goats is for family consumption, but goat milk can also be further processed into a variety of marketable products. Marketing of goat milk and its products is still in its infancy. So

far, there have been no marketing efforts attempted on a broad scale (Abdel Aziz, 2010).

The development of a professional marketing system is part of the challenge to benefit from the fact that many people consuming dairy products prefer products from goats (Abdel Aziz, 2010).

The potential of goats for sustainable supply of milk and meat for human consumption is unquestioned, and their contribution to improved nutrition of rural people is likely to increase. At the same time, goat cheese consumption is likely to increase also in developed countries. This is attributed to the image of goat cheese being a product of natural farm conditions compared with milk and milk products from high yielding dairy cattle in large industrial farms. Regarding goat meat, rising living standards in some parts of the world and the migration of people preferring goat meat to the developed countries, have increased the demand for goat meat in these areas (Abdel Aziz, 2010).

Government programs to support goat farming should focus on research and education in the areas of breed improvement, farm management, and control of infectious diseases, milk collection, processing and marketing (Abdel Aziz, 2010).

In developed parts of the world goats are considered, usually, as specialty or exotic livestock, whereas in the developing countries, especially those in South - East Asia and Africa goats constitute the major source of meat production (Ivanovic et al 2016).

Goat meat is a good source of proteins and also has health benefits when is consumed in appropriate portions. In comparison of beef, have similar protein, lower fat, higher calcium, magnesium, potassium, similar iron and lower B<sub>12</sub> and folate contents. On the other hand goat meat contains low amount of saturated fatty acids and cholesterol and it is a healthier alternative compared to other types of red meat (Ivanovic et al 2016).

Goat meat contains low amounts of saturated fatty acids and cholesterol. It is considered to be a healthier alternative to other types of red meat. Leather from goat skin is used for bags, boots, gloves and other products that requires soft hide. Traditionally has been a preferable material for leather bookbinding. Untanned goat skins are

used in different countries as containers for water, kefir, wine etc. High quality goat skins are provided from Black Bengal breed in Bangladesh. On average every sheep or goat keeper has 38 and 25 heads of animals respectively.

Iranian sheep and goat industry is characterized by:

- 1) Owned by small farmers
  - 2) Based on extensive grazing
  - 3) Highly influenced by the environmental variables (rain fall, weather, feed supply, drought etc)
  - 4) Economic variability due to uncertainty in feed availability, weather, rainfall, market, export and import animal products mainly food materials.
  - 5) Its number or increment rate is declining in comparison with the past decades (because of urbanizations industrialization, low income, etc).
  - 6) Genetic structure and physiological characters of the most Iranian sheep and goats are not clear.
  - 7) No comprehensive standard investigation had been carried out on distinguishing different breeds of these animals. What is known as breed of sheep or goat is based on the apparent physical conformation and.
  - 8) All of the Iranian sheep breeds, except one (Zel breed) are fat-tail types.
  - 9) Although Iranian sheep and goats are grouped according to their main product, but generally they are kept for providing different products or sources of income including meat, milk, fiber and hide.
  - 10) These small ruminants are resistant to high level of inorganic minerals in feeds and forages.
  - 11) Iranian sheep and goat live and produce over a remarkable wide range of environments from the desert type dry and warm climate to the mountainous cold zones.
  - 12) Iranian sheep and goats appear in different color from white to the completely black and many classes between.
  - 13) Iranian sheep produce mainly coarse fiber which is suitable for Iranian carpet industry.
  - 14) Most of Iranian breeds are high-set animals which is a suitable character for grazing over the rocky and mountainous areas (Valizadeh, 2008).
- The main obstacles of Iranian agriculture which affects its animal agriculture as well as other agricultural disciplines can be outlined as follows:

A – Desertification



B – Deforestation

C – Water shortage

D – Erosion

E – Low efficiency and out-put

F – Mostly illiterate small farmers (Valizadeh, 2008).

Goats, especially dairy ones, are an ideal species for poverty reduction and economic development for the poor in developing countries. Several reasons make goats particularly attractive for poverty reduction and improvement of family food security and livelihood of the poor in developing countries:

1. Goats are easily acquired by the poor as they require modest starting capital.

2. They can easily be tended by the weak, women or children.

3. They provide people by valuable nutrients.

4. Many people cannot drink cow milk as they are allergic to it. Several studies indicated that people with cow's milk allergy could tolerate goat's milk.

5. The growing demand for goat meat presents an opportunity for goat fattening (Abdel Aziz, 2010).

## CONCLUSIONS

In the nomadic system of sheep and goat production, one objective was to achieve animals' wellbeing through animal welfare-oriented husbandry and appropriate use. Curtailing freedom of movement, sensory deprivation and unsocial ways of husbandry; not allowing any contact with animals of the same species, or forcing too close a contact were not permitted in the nomadic farming system (Ansari-Renani, 2016).

Husbandry management practices transport and slaughtering, management of livestock among nomads was a social process, and they did their utmost for the wellbeing of their animal and to avoid animal cruelty of any kind. In the nomadic system, there were no tail ducking, dehorning and tethering.

A country rich in indigenous animal genetic resources like Iran is very much suitable for adopting this farming system. Moreover, the nomadic farming system with well-diversified livestock populations in terms of species and breeds is ideal for organic livestock production. Although the nomadic type of livestock keeping provides an excellent and 'green' alternative to

industrial production, nomad pastoralists need to overcome some challenges and harness strengths and opportunities, while developing their capacity in terms of knowledge, skills, infrastructure, animal feeding, hygiene, sanitation, disease control and assured certified supply chain required for organic livestock production. Nomad farmers need to be oriented and educated about the organic standards and how to overcome the risks they might face in adoption of organic livestock standards. The livestock advisors should be trained and skilled in providing services in livestock management and permitted therapies in organic rearing systems. Research on the locally adaptable management and disease-preventive measures needs to be emphasized by the government and organic-promoting agencies as well as NGOs.

The potential needs to be recognized of Iranian nomad farmers to meet the requirements of organic livestock product demand, not only locally but also globally in the near future. Organic livestock production can be encouraged through research and development efforts, including establishment of model organic livestock farms, processing units, traceability tools and capacity-building measures (Ansari-Renani, 2016).

Goat farming systems are diverse, both intensive and semi-intensive and extensive, reflecting the ability of these animals to adapt to a wide range of environmental conditions. A simplified description of the different operating systems, based on eco-regional criteria, shows that both loose housing - feeding with forage crops or on cultivated pastures and grazing meadows, or on areas that are not part of the agriculture circuit are practiced (Chetroui, et al., 2014). The geographical and ecological conditions of Iran are well-suited to small ruminant production. The relatively low cost of sheep and goat farming (local breeds – well adapted to their environment plus extensive free communal grazing areas) and the increasing demand for expensive organic products in domestic and regional export markets encourages nomads to shift to organic production Livestock organic production (Ansari-Renani, 2016). The severe damage that goats have caused in some regions is usually associated with high stocking density and mismanagement. Heavy goat damage is usually localized (Abdel Aziz, 2010).

Goats have a good appetite for and the ability to utilize effectively many trees and shrubs not available or not palatable to sheep and cattle. Therefore, they can be more damaging to perennial vegetation and soil stability. This is greatly realized during drought in arid zones, as goats have a reputation for being good survivors. Clearly, goats require careful management to avoid irreversible damage to the vegetation (Abdel Aziz, 2010).

Socioeconomic and political stability, availability of veterinary services, and adequate infrastructure and logistic supports are essential for implementing effective control programs. Inadequate infrastructure in most of developing countries is one of the major elements that conflict with effective implementation of building herd immunity (Mirzaie et al., 2015).

There are still the great deal that are not well understood concerning the different aspects of sheep and goat husbandry, interactions between industrialization, urbanization and the trends of small ruminants production in Iran. This fact is wellknown that Iranian sheep and goat population will be decreased and changed dramatically with respect to their systems, locations, herd sizes and specialization in the future but at the same time the price of their products mainly meat will be increased. It seems the variability in environmental patterns such as low rainfall and feed shortage, uncertainty in farmers' income and market conditions will be the most important factors in pushing the compulsory transition in Iranian sheep and goat industry. This transition may have critical effects on the animal based food security mainly red meat from small ruminants which is popular for Iranian consumers. Therefore, more attention is required from the government and non-governmental organizations for handling this trend to the well-managed right direction (Valizadeh, 2008).

On average, 50% of sheep and goat population are kept under nomadic and semi-nomadic system and the remaining 50% are managed under composite system. Traditional shepherding and displacement of livestock by nomads is common countrywide. Nomads displace their herds between different provinces and within a particular province (Ansari-Renani et al., 2013).

Iran is one of the main producers and exporters of cashmere in the world, third after China and Mongolia. Of the 25 million goats in Iran 5 million are cashmere producing goats. Nomads play an important role in sheep and goats production mainly because they keep 58.5% of sheep and 39.7% of goat population of Iran. Approximately 70% of goats in Iran are of mixed breeds and their crosses, which are mainly kept for meat production, while other types are known for their cashmere (Raeini, Birjandi, Abadeh and Nadoushan), mohair (Markhoz), milk (Najdi) and meat (Tali, Adani and Native black) production (Ansari-Renani et al., 2013). More than 90% of Iranian cashmere is produced in the eastern part of the country mainly by two breeds of goat namely Raeini in Kerman and Birjandi (Baluchi) in South Khorasan provinces. However Raeini goats mainly kept by nomad farmers is the most important cashmere producing breed both in terms of population and volume of cashmere produced (Ansari-Renani et al., 2013). Converting extensive, range-based nomadic system to organic production could become economically attractive, if price premiums could be captured for organic meat and livestock products. Development of business models will definitely attract commercial interests and ensure that vulnerable nomadic communities receive attractive returns for their untapped treasure of organic principles. Systematic studies need to validate the animal husbandry practices of nomads with respect to organic certification, so that revision or improvement can be made wherever necessary. In this way, organic livestock products will have considerable potential for high-value niche markets (Ansari-Renani, 2016). Also, following facts must be considered for goat milk, meat and raw skin production:

- Goats continues to play a significant role in the human nutrition. Their number is increasing more rapidly in comparison with the sheep, especially in the less developed parts of the world, indicating an increased role of this livestock in food production.
- In the developing countries of the world, during the period 2000-2019 goat milk production was increased significantly.
- The same thing can be said and for the goat meat and raw skin production.

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**DETERMINATION OF GROWTH CURVE OF AKKARAMAN LAMBS RAISED IN ÇANKIRI REGION  
FROM BIRTH TO 18 MONTHS OF AGE**

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**Abstract**

*The aim of this study is to prepare the growth curve and to determine the development of the Akkaraman sheeps raised in the Çankırı province until the eighteen month of age at different periods in steppe conditions. The animal material of this study was consist of Akkaraman sheep which was grown in "Breeding of Akkaraman Çankırı Province Sub-Project" within the scope of "The National Project of Animal Breeding in Public" conducted by TAGEM. The study was carried out on a total of 208 lambs which born in January, February and March 2018 in 4 different farms. Data were analysed using General Linear model procedures of Minitab. The weights measured on sheeps were analysed by fitting effects of sex of lamb (female, male), type of birth (single, twin), months of birth (January, February, March), age of dam at birth (2, 3, 4, 5 with 6 and above years of age) and farms (1, 2, 3, 4). The statistical significances between the subgroups were determined with "Tukey Multiple Comparison" test. In this study, average of weight at birth, 3, 6 12 and 18 months were found 3.874 kg, 31.441 kg, 41.001 kg, 42.228 and 55.528 kg respectively. The gender effect was found statistically significant ( $p<0.05$ ) on live weight at 6, 12 and 18 months of age while it was not statistically significant on birth and 3 month of age. It was found statistically significant ( $p<0.05$ ) on live weight that the effect of type of birth at birth and 12 month of age, maternal age at 6 month of age and farms at birth, 3, 6 and 12 months of age. On the other hand the effect of months of birth on live weight was not found statistically significant at all periods examined in this study. As a result, although the differences between the farms in Akkaraman sheep up to 1 year of age were determined, it was concluded that the 18-month-old live weight reached the desired levels in all the farms.*

**Key words:** Akkaraman, Growth curve, Live weight

**MORPHOLOGICAL TRAITS AS INDICATORS OF SEXUAL DIMORPHISM IN SOUTH AFRICAN NON-DESCRIPT INDIGENOUS GOATS**

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**Abstract**

*Sexually dimorphism is the consequences from the combination of sex specific genes on the sex chromosomes and sex-specific gene expression in females and males. The study was conducted to investigate the effect of sex on body weight (BW) and morphological traits such as withers height (WH), body length (BL), rump length (RL), rump height (RH) and heart girth (HG). One hundred South African non-descript indigenous goats (does = 80 and bucks = 20) age one to four year were randomly selected. The results showed that statistically significant differences ( $P < 0.05$ ) exist between bucks and does in BW, WH, BL, RH and HG but no significant differences ( $P > 0.05$ ) observed in RL. Correlation findings revealed that BW had a positive highly statistical significant ( $P < 0.01$ ) correlation with WH ( $r = 0.70^{**}$ ), RH ( $r = 0.40^{**}$ ) and BL ( $r = 0.54^{**}$ ) in does while BW of bucks had a positive correlation with WH ( $r = 0.28^*$ ). In conclusion, this study suggests that sex had an effect on body weight and morphological traits in South African non-descript goats. Furthermore, sexual dimorphism was in favour of males (bucks) than females (does). Correlation findings suggest that by improving WH, RH and BL might result the improvement of BW in South African non-descript indigenous goats. Results of the current study suggest that morphological traits might be the indicators of sexual dimorphisms in South African non-descript goats.*

**Key words:** *Body length, Correlation, Heart girth, Rump height, Withers height*

**MODIFICATION OF FATTY ACID PROFILE IN MILK OF MORKARAMAN SHEEP FED WITH CORN  
AND WALNUT OIL**

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***Abstract***

*The aim of our study is to increase the CLA and the unsaturated fatty acid ratio of the total fatty acids in the milk of Morkaraman sheep to produce a more functional food with adding 75% corn and 25% walnut oil to the ration. In the study, 50 head Morkaraman sheep were used. The first group formed the control group and the other group (T) was given an additional 50g/day 75% corn and 25% walnut oil to the ration. The study lasted three weeks, two weeks adaptation time and one-week study time. While the CLA ratio was %1.25 in 100 mg milk fat in control group, it was %1.64 in treatment group ( $p < 0.001$ ). While the PUFA ratio in milk increased (%32.5) in the treatment group compared to the control group, the MUFA ratio decreased (%4.5). C18:2N6C ratio in milk fatty acid increased with 75% corn and 25% walnut oil (%47.2). However, the addition of 75% corn and 25% walnut oil to the ration had no statistical effect on C22:6n3 (DHA) and C20:5N3 (EPA) ratio. The atherogenic and thrombogenic index were similar in both groups. As a result of the study, although the CLA ratio in milk increased, the desired optimum increase rate for other fatty acids could not be achieved. Although we added 25% of walnut oil to the ration due to the high linolenic acid ratio, there was no increase in the milk MUFA and n-3 ratio. For this, it is recommended to carry out new studies with higher rate of walnut oil in the ration.*

***Key words:*** Conjugated linoleic acid, Fatty acid, Milk fat, Sheep

## TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES IN ANIMALS REARED IN TURKEY

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### **Abstract**

Transmissible spongiform encephalopathies (TSEs) or prion diseases are a family of rare progressive neurodegenerative disorders that affect both humans and animals. They are distinguished by long incubation periods, characteristic spongiform changes associated with neuronal loss, and a failure to induce inflammatory response. The causative agents of TSEs are prions. The term "prions" refers to abnormal, pathogenic agents that are transmissible and are able to induce abnormal folding of specific normal cellular proteins that are found most abundantly in the brain. The abnormal folding of the prion proteins leads to brain damage and the characteristic signs and symptoms of the disease. Prion diseases are usually rapidly progressive and always fatal. The accumulation of this abnormal prion protein in tissues results in Creutzfeld-Jakob disease and Gerstmann–Straussler–Scheinker syndrome in human, bovine spongiform encephalopathy (BSE) in cattle, scrapie in small ruminants and chronic wasting disease in deer. Previous studies showed that these diseases were associated with the polymorphism of prion protein gene. In Turkey, there are a lot of studies about TSE group diseases in cattle, sheep and goat. According to these studies, the cattle, sheep and goats reared in Turkey seem resistance to TSE group diseases.

**Keywords:** TSE, BSE, Scrapie, Prion, Turkish livestock animals

## CRISPR/CAS TOOLBOX

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### ***Abstract***

Previously in bacteria and archaea, then recently in bacteriophages, CRISPR/Cas systems were discovered as a defense mechanism against viruses. The ability of precise recognition and specific targeting of intruder viruses' nucleic acids by CRISPR/Cas systems, inspired the scientist to modify and use this system as a genomic tool to edit genome of multicellular organisms including human, plants, and animals. Soon after its discovery, a more compact and modified version of this system was engineered which includes only two components: an endonuclease enzyme (Cas protein), which is able to cut dsDNA, and a target specific guide RNA (gRNA) sequence, which can specifically recognize the region to be cut and direct the endonuclease enzyme to that region. When introduced into the host cell/organism, Cas enzyme and gRNA sequence create double strand breaks at the target region of host DNA which is eventually repaired by NHEJ, thereby possibly creating a mutation in the targeted genome. If the exons of a functional gene are targeted, the function of that gene is possibly disrupted (Gene Knock-out). If a nucleotide sequence sharing homology with targeted site is introduced with Cas and gRNA, that exogenous nucleotide sequence can be inserted into the host genome via homology-based repair mechanism (Gene Knock-in). Slight modification on the system create different CRISPR tools with different functions. A catalytically deactivated Cas protein (dCas), which is capable to be guided and bind to target DNA without cutting it, is fused to catalytic domains of transcriptional activator (CRISPR activator; CRISPRa) and/or repressor (CRISPR inhibitor; CRISPRi) to modify gene expression of targeted genes. CRISPR toolbox was expanded by recombining different enzymes/proteins to dCas such as epigenetic modifiers (to modify chromosomal state), fluorophore molecules (to visualize genomic loci). Base editing enzymes providing conversion from A:T to G:C, C:G to T:A were also recombined a mutated version of Cas which can create a single strand break (Nickase) to form a gene editor to correct single base mutations. Recently, a reverse transcriptase and a template RNA guide including version of Cas (Prime editing) was invented which can rewrite a new genomic information at the targeted site. Not only gene editing but also other handy molecular tools based on CRISPR system have been invented including chromosome labeling, clonal cell tracing, regulatory region identification, cis element identification etc. These are some but not all CRISPR tools developed by scientist within last 8 years and it seems to be improved more

***Key words:*** CRISPR/CAS, Toolbox, Genome editing



## STATUS OF SHEEP FARMING IN SAMSUN AGRICULTURAL ENTERPRISES

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### **Abstract**

*Samsun is a province with high agricultural potential with its highlands and plains. There are 120437 agricultural enterprises in Samsun province. Of these enterprises 46.25% (55697) are livestock enterprises. Sheep production occupies 2.91% of total agricultural holdings and 6.30% of livestock holdings. Some 108 sheep farms are managed with vegetative production and 3400 sheep farms are run together with other livestock farms. Sheep businesses have under 50 heads in 27.17%, 50-100 heads in 38.48%, 10-200 heads in 20.38%, 200-500 heads in 9.6%, and 500 heads in 0.31%. The animal stock in sheep farming ranges from 2 to 1004 heads, with an average of 58.33 heads. Karayaka breed is dominant throughout Samsun. However, there are also Bafra, Merino, Kıvrıkcık, Bafra x Karayaka hybrids genotypes. The main source of income in sheep farming is provided by the sale of non-breeding rams and sheep and male lambs as butchers. Male lambs are slaughtered for meat production at the ages of 3 to 5 months. The slaughter of lambs is concentrated in May and August. Generally, lambing is practiced once a year and the number of lambs obtained per breeding sheep is around 1.05. A family of 4 people dealing with sheep must have at least 100 breeding female animals to maintain normal living standards. Given the proportion of enterprises with less than 100 sheep in the province of Samsun (65.56%), the public authorities must provide adequate support to the breeders to increase the number of animals. This will not only improve family income and living standards, but also ensure that the need for red meat is met from our country's potential.*

**Key words:** Sheep, Samsun, Farm management

## THE RELATIONSHIP BETWEEN TWIN BIRTHS AND INSULIN-LIKE GROWTH FACTOR I GENE EXPRESSION LEVEL IN THE PLACENTAL TISSUE

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### **Abstract**

*Insulin-like growth factor I (IGF-I) is an important factor that promotes follicle and oocyte development, ovulation and embryonic development in many mammalian species. Additionally, the gene expression level of IGF-I, which is associated with cell proliferation, in placental tissue can directly affect placental and fetal growth and development. Therefore, the aim of this study was to determine the relationship between birth type, placental characteristics and IGF-I gene expression in the placental tissue of Bafra sheep breed. A total of 16 sheep, which have single (n=7) or twin (n=9) gestation and with at least gave birth to 2, has been used as experimental animals in the present study. Following lambing, birth type, live weight and sex of lambs and placental traits have been recorded. IGF-I gene expression level has been determined by real-time quantitative polymerase chain reaction. In the study, no difference was found between single and twin bearing sheep in terms of weights of placenta and various cotyledon characteristics, whereas total cotyledon weights were found to be lower in singleton births than those in twin births ( $P<0.05$ ). Similarly, the total and medium cotyledon number of Bafra sheep with single births was found to be lower than Bafra sheep with twin births ( $P<0.05$ ). Additionally, cotyledon efficiency of sheep gave birth to singleton was found to be lower than those of sheep gave birth to twins ( $P<0.05$ ). Although the birth type did not affect placental IGF-I gene expression level, sheep that gave birth to twins exhibited 0.905 times more gene expression than that to single births. The results of the study showed that birth type did not affect placental IGF-I gene expression level in Bafra sheep breed, but changed placental properties.*

**Key words:** IGF-I, Gene expression, Placenta, Twin births, Fetal Development

### **INTRODUCTION**

Insulin-Like Growth Factors I (IGF-I) shows its most important effects in reproductive processes, except for their role in cell metabolism, proliferation and differentiation. IGF-I is particularly important in fetal and postnatal development (Igwebuike, 2010). The IGF system has been reported in the uterus and placenta of the ewe (Stevenson et al., 1994; Reynolds et al., 1997). Watson et al. (1994) reported that the presence of mRNAs for IGF-I in sheep embryos throughout preimplantation development from single-cell stage to the hatched blastocyst (Watson et al., 1994). Similarly, Stevenson and Wathes (1996) shown that the expression of mRNA for IGF-1 was maximal in the mucosal layer of the oviduct within which early embryonic development takes place.

IGF-I play an important role in the division and proliferation of IGF-I granulosa cells and increase the capacity of FSH and LH to bind to their receptors in granulosa cells (Behl and Kaul 2002; Mazerbourg and Monget, 2018). It also shows that IGFs have a synergistic effect on the induction of granulosa cells by providing FSH regulation (Behl and Kaul 2002; Mazerbourg and Monget, 2018). IGF- I the most thoroughly evaluated amongst the various growth factors being investigated for their role in ovarian follicular dynamics in the mammals (Hastie and Haresign, 2006). Previous studies have shown that IGF-I regulates its effects on ovarian activity or follicular development either alone or in harmony with gonadotropins secreted from the pituitary gland (Behl and Kaul, 2002; Mazerbourg and Monget, 2018). In addition, IGF-I increases the activity of gonadotropin hormones

by affecting granulosa and teka cells in the ovarian follicles (Taketani et al., 2008).

IGF-I is an important factor that promotes follicle and oocyte development, ovulation and embryonic development in many mammalian species (Behl and Kaul 2002; Mazerbourg and Monget, 2018). Additionally, the gene expression level of IGF-I, which is associated with cell proliferation, in placental tissue, may affect placental and fetal growth and development (Igwebuike, 2010). Therefore, the aim of this study was to determine the relationship between birth type, placental characteristics and IGF-I gene expression in the placental tissue of Bafra sheep breed.

### **MATERIALS AND METHODS**

The experimental procedures were approved by the Local Animal Care and Ethics Committee of Ondokuz Mayıs University, Samsun, Turkey, ensuring compliance with EC Directive 86/609/EEC for animal experiments. A total of 16 sheep, which have single (n=7) or twin (n=9) gestation and with at least gave birth to 2, has been used as experimental animals in the present study. All ewes were pregnant by naturally mate using mixed multiple sires and housed under the same conditions.

Following lambing, birth type, live weight and sex of lambs were recorded within 12 h after parturition. Each ewe was left to deliver the placenta naturally and placentas were collected from single or twin gestations 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 cotyledon samples to be used in RNA isolation from the collected placenta were without delay isolated, weighed and stored -80 °C until analysis of the placental IGF-I gene expression. After than the total cotyledon numbers (TCN) and total cotyledon weights (TCW) of placental cotyledons dissected from the chorioallantois were also counted and determined. Diameter of cotyledon were measured with a digital compass and divided into three categories as small (<20 mm diameter), medium (20-30 mm diameter) and large (>30 mm diameter). Additionally, placental efficiency (PE; lamb BW / placental weight), cotyledon efficiency (CE; gram lamb BW / total

cotyledon surface area) and cotyledon density (CD; number of cotyledons / per gram placental weight) were calculated for each ewe.

Commercial RNA kit (NucleoSpin® RNA kit) was used for RNA isolation in cotyledon samples and the process was made as recommended by the manufacturer of the commercial kit. After genomic DNA was eliminated by digestion with DNase I (Thermo Scientific, Waltham, USA), the RNA quality and quantity was determined using NanoDrop 2000 (Thermo Scientific, Waltham, USA), all RNA samples showed an A260/A280 values within the range of 2.01 to 2.08 and A260/A230 values above 2. Commercial cDNA kit (BIORAD iScript cDNA, 1708890) and Thermal Cycler (BIORAD) device were used for cDNA synthesis and the analysis was done as recommended by the manufacturer of the commercial kit. Primer and reference gene base sequences in the 5' and 3' directions used in Real-Time PCR are shown in Table 1.

In detail, the PCR was carried out in a reaction system of total volume of 50 µL containing 25 µL premix Taq™, 17.5 µL 0.1% DEPC water, 2.5 µL forward primers (10 µmol/L), 2.5 µL reverse primers (10 µmol/L) and 2.5 µL cDNA template. PCR procedure were carried out as follows: 98 °C for 4 min, followed by 32 cycles of 98 °C for 40 s, 60 °C for 40 s, 65°C for 30 s, and then 90 °C extension for 10 min, finally 4 °C to terminate the reaction. Relative quantification of all transcripts was performed by qRT-PCR using the real-time PCR system. Real-time quantitative PCRs were run with SYBR Premix Ex Taq™ II. The reaction system was in a total volume of 10 µL containing 5 µL 2 × SYBR Premix Ex Taq II, 0.4 µL forward primer (10 µmol/L), 0.4 µL reverse primer (10 µmol/L), 0.2 µL 50 × ROX Reference Dye, 3 µL 0.1% DEPC water and 1 µL template cDNA. PCR amplification was carried out as follows: a denaturation of 98 °C for 30 s, followed by 40 cycles of 98 °C for 5 s, specific annealing temperature 60°C for 30 s. The  $2^{-\Delta\Delta Ct}$  method was used to analyze the mRNA expression levels.

**Table 1.** Primer and reference gene base sequences in the 5' and 3' directions used in Real-Time PCR

Serial No	Oligonucleotide Name	Base Sequence 5'-3'
20191003-79	Primerpair 1-F	ATGGGCATTTCCCAATGA
20191003-80	Primerpair 1-R	GCAATCTACCAACTCCAGGGT
20191003-81	Primerpair 1-exon 2-F	TCATCTTCCTCCTGGGTCCTT
20191003-82	Primerpair 1-exon 2-R	GCTACTCACACACCTTGTTGC
20191003-83	GAPDH-F	GCAAGTTCCACGGCACAG
20191003-84	GAPDH-R	TCAGCACCAGCATCACCC

The effects of placental characteristics and IGF-I gene expression level on birth type were analyzed using a completely randomized design by the General Linear Model (GLM) procedure of the SPSS package program. Significant differences between means were tested using Duncan's test and results were computed as mean  $\pm$  s.e.m. Statistical significance was considered at  $P < 0.05$  and  $P < 0.01$ .

## RESULTS AND DISCUSSION

Sheep placenta has multiple cotyledonal structures that consist of maternal and fetal tissues and provide the circulation between dam and offspring (Igwebuike, 2010). The cotyledons on the sheep placenta are structures randomly distributed on the placental surface and positioned separately from each other (Igwebuike, 2010). The structures formed by the fetal-induced cotyledons on the sheep placenta by settling on the caruncula of the uterus endometrium are called placentomes (Redmer et al., 2004). This structure carries out all circulation activities between dam and offspring. Therefore, determination of cotyledon numbers on the placenta after birth is an indication of the number of placenta occurring during gestation, but also an indicator of the circulation rate between offspring and dam. The binding of fetal originated cotyledons to the uterus in sheep is 25-30 of pregnancy (Redmer et al., 2004). Growth restrictions (insufficient nutrition, abnormal environmental conditions and abnormal endocrinal activity, etc.) that occur between the days may affect the final cotyledon number of the placenta (Wathes et al., 1998; Redmer et al., 2004). Restrictive interventions in the last period of pregnancy may affect the morphology and size of cotyledons rather than the number of cotyledons (Vatnick et al., 1991). In the current study, various differences were detected between the placental characteristics of Bafra sheep that gave birth to single and twin (Table 2). Total

cotyledon number and medium cotyledon number were found to be statistically significant between the birth type groups, and the total and middle cotyledon number of single-birth Bafra sheep was found to be lower than that of twin-birth Bafra sheep ( $P < 0.05$ ). However, there was no statistical difference between the two experimental groups in terms of placental weight, large cotyledon number and small cotyledon number. Additionally, there was a difference in terms of various efficiency features of placenta and cotyledon of Bafra lambs with single or twin birth type (Table 3). The cotyledon efficiency of single bearing sheep was found to be lower than the cotyledon activity of twin sheep ( $P < 0.05$ ), but there were no significant difference was found in terms of placental efficiency and cotyledon activity density. The results obtained show that lambs born single and twin in Bafra sheep have different placental features, so it is thought that this difference may be due to the type of birth.

Previous studies have shown that the nutritional level of the mother affects the placental development and lamb birth weight in the middle period of pregnancy when placental development and growth occur in sheep (Sen et al., 2013). This situation shows how important placental functions are in order to continue fetal growth in the last period of pregnancy. It has been reported to be one of the main factors regulating fetal and placental growth due to the effects of IGF-I on cell proliferation and activity in mammals (Igwebuike, 2010).

**Table 2.** Placental components of Bafra lambs with single or twin birth type

Traits	Birth type	
	Single	Twin
PW (g)	320.53 ± 40.09	326.00 ± 55.00
TCW (g)	94.75 ± 8.61 <sup>a</sup>	135.60 ± 15.30 <sup>b</sup>
TCN	30.86 ± 3.74 <sup>b</sup>	41.78 ± 7.50 <sup>a</sup>
SCN	16.00 ± 3.54	19.00 ± 4.33
MCN	8.00 ± 0.62 <sup>b</sup>	14.56 ± 3.70 <sup>a</sup>
LCN	7.57 ± 1.02	8.33 ± 1.61

<sup>a,b</sup> Different superscript letters in the same line indicate a significant difference ( $p < 0.05$ )

PW = plasental weight, TCW = total cotyledon weight, TCN = total average cotyledon number, SCN = small cotyledon number, MCN = medium cotyledon number, LCN = large cotyledon number

**Table 3.** Various efficiency features of placenta and cotyledon of Bafra lambs with single or twin birth type

Traits	Birth type	
	Single	Twin
PE	14.03 ± 1.75	30.70 ± 7.75
CE	17.84 ± 1.35 <sup>b</sup>	30.86 ± 4.66 <sup>a</sup>
CD	0.107 ± 0.017	0.154 ± 0.029

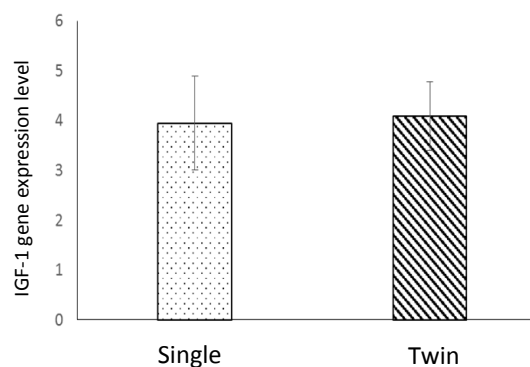
<sup>a,b</sup> Different superscript letters in the same line indicate a significant difference ( $p < 0.05$ ).

PE = plasental efficiency. CE = cotyledon efficiency. CD = cotyledon density

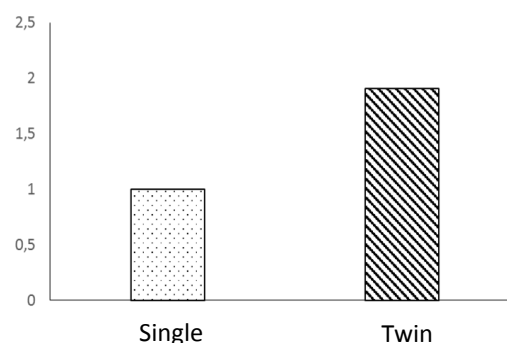
Some studies have reported that the regional production center of insulin-like growth factor family and its member IGF-I in sheep are placenta and uterus (Stevenson et al., 1994, Reynolds et al., 1997). Watson et al. (1994) reported that IGF-I gene expression level can be effective on embryonic development and placentation. Although it has been stated that IGF-I plays an important role in the regulation of fetal growth, there is not much information about the importance of this factor in many births and postnatal periods.

In the current study, the relationship between the differences in the type of delivery in the Bafra breed sheep with the placental features and IGF-I gene expression level was investigated. Although the IGF-I gene expression levels in the placental tissue of Bafra breed sheep with different birth

type showed similarity (single;  $3.95 \pm 0.941$  and twin  $4.09 \pm 0.70$ , Figure 1), twin-bearing sheep showed 0,905 times more gene expression than those of single-bearing (Figure 2). Although the Bafra breed sheep used in the experiment were exposed to single or twin births in the previous breeding season, in the same enterprise and similar breeding conditions (care, feeding, etc.), it is thought that the differences in birth patterns are due to differences in placental characteristics.



**Figure 1.** Gene expression levels of insulin-like growth factor I (IGF-I) in single and twin breeding Bafra sheep



**Figure 2.** IGF-I gene expression fold change of Bafra sheep breeds depending on the single and twin birth patterns

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## THE ROLE OF SMALL RUMINANTS IN THE EPIDEMIOLOGY OF FOOT-AND-MOUTH DISEASE IN TURKEY

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### **Abstract**

*Foot-and-mouth disease (FMD) is an acute viral infection of cloven-hoofed animals. It has got seven serotypes (A, O, C, Asia 1, and SAT-1, 2, 3), and three of them have been seen in Turkey (A, O, Asia 1). It was shown that serotype A and Asia 1 are exotic strains that enter mostly from the eastern borders of Turkey. Furthermore, Asia 1 and A were not seen since 2015 and 2018, respectively. As a combat strategy, large ruminants all over the country and small ruminants in the Thrace region are vaccinated. In this study, the positive samples submitted to the FMD (ŞAP) Institute between the years 2012 and 2018 were examined. Positive samples detected with primers specific to the 3D region and serotyping was done with type-specific primers by using rt-PCR and qrt-PCR. Pearson chi-square test was used for the evaluation of the relationship between the serotypes and the species, in the SPSS version 23. During seven years, positive samples received from large and small ruminants were 88.3% and 11.7%, respectively (Asia 1 samples were not included). Of these samples, 48.8% obtained as serotype A, 43.8% serotype O, and 7.4% untyped. Serotype A detected in 53.9% of large ruminants and 10.1% in small ruminants. In large ruminants, serotype O found in a ratio of 39.1% while it was 79.6% in small ruminants. A significant relationship observed between the species and the serotypes ( $p < 0.05$ ). In conclusion, it would be beneficial to examine the relationship between animal species and serotype sensitivity at the molecular and genetic levels.*

**Key words:** *Foot-and-mouth disease, Small ruminant, Serotype O*

## ESTIMATING THE NONPARAMETRIC CONFIDENCE INTERVAL FOR CORRELATION COEFFICIENT ON ANIMAL DATA

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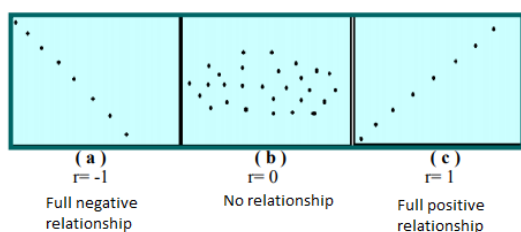
### **Abstract**

Correlation coefficient is widely used in the all areas of science to establish the degree and direction of two variables. Confidence interval is a special form of estimating a certain parameter. With use of this method, a whole interval of acceptable values for the parameter is given instead of a single value, together with a likelihood that the real (unknown) value of the parameter will be in the interval. The confidence interval is based on the observations from a sample, and hence differs from sample to sample. In this study, nonparametric confidence interval estimation for Pearson correlation coefficient were shown using an animal data set.

**Key words:** Correlation coefficient, Nonparametric confidence interval, Animal

### **INTRODUCTION**

Correlation is a statistical method that reveals the direction and degree of the relationship between variables. The measure of the relationship between the two variables is called the correlation coefficient (Arkin and Colton, 1939). The correlation coefficient is denoted by the small "r" and takes the value between -1 and +1 ( $-1 \leq r \leq +1$ ). If r value takes values close to -1, it is determined that there is a negative relationship between variables, and if it takes values close to +1, there is a positive relationship between variables. If the r value is close to 0, it means that there is no relationship between the two variables (Figure 1) (URL1).



**Figure 1.** Scatter plots of extreme correlation coefficients.

In Figure 1; (a) the decrease in the other depending on the increase of one of the variables is a linear relationship, (b) there is no relationship between the two variables and (c)

the increase in one of the variables due to the increase in the other is the linear relationship.

General comments regarding the strength of the correlation coefficient are given below (URL1);

- 0.00-0.25 Very poor relationship,
- 0.26-0.49 Weak relationship,
- 0.50-0.69 Moderate relationship,
- 0.70-0.89 High relationship,
- 0.90-1.00 Very high relationship.

The Pearson correlation coefficient can be calculated using the estimator;

$$r = \frac{n \sum xy - (\sum x)(\sum y)}{\sqrt{n(\sum x^2) - (\sum x)^2} \sqrt{n(\sum y^2) - (\sum y)^2}}$$

Correlation coefficients vary depending on the characteristics of the variables under investigation.

Correlation coefficients used to determine the relationship between classifiable qualitative variables;

- Phi coefficient,
- Cramer V coefficient,
- Ordinary coefficient,
- Lambda coefficient.

Correlation coefficients used to determine the relationship between sortable qualitative variables;

- Spearman correlation coefficient,



- Gamma coefficient,
- Kendall's tau-b coefficient,
- Kendal's tau-c coefficient,
- Somer's d coefficient.

Correlation coefficients used in determining the relationship between discrete / continuous qualitative variables;

- Pearson correlation coefficient (if both variables show normal distribution),
- Spearman correlation coefficient (if at least one of the variables is not normally distributed).

Correlation coefficients used to determine the relationship between a classifiable qualitative variable and a discrete / continuous quantitative variable;

- Double series correlation coefficients,
- Point double series correlation coefficients.

Correlation coefficient used to determine the relationship between a sortable qualitative variable and a discrete / continuous quantitative variable;

- Multiple series correlation coefficient (UR1).

For the animal science as in all the other branches of the science the correlation coefficient (CC) is a basic relation statistics. In many studies confidence interval of the correlation coefficient is ignored. But interval estimation is very essential for statistical prediction.

Recent studies have shown that imprecise estimates of correlation coefficients can result in a series of problems, including an increase in multicollinearity in multivariable regression analyses, as well as overestimating direct effects and increasing noise in path analysis. In this sense, it is essential for experimental planning to ensure sufficient n to estimate correlation coefficients with an acceptable level of precision (Olivoto et al., 2018).

In this study, nonparametric and parametric 95% confidence interval (CI) for correlation coefficient were compared with using goat kid growth data.

## MATERIALS AND METHODS

This study was carried out at the private dairy goat farm in Bafra province of Samsun, Turkey (40°31'N, 36°53'E and 650 m above the sea level). Data was collected from 82 Saanen kids from birth (W0) to six month of age (W6).

The well-known parametric confidence interval of Pearson correlation can be calculated as (Arkin and Colton, 1939);

$$CI = t_{n-2, \alpha/2} \cdot S_r$$
$$S_r = \sqrt{\frac{1-r^2}{n-2}}$$

The nonparametric confidence interval of Pearson correlation can be calculated as (Olivoto et al., 2018; Olivoto, 2019);

$$CI = 0.45304^r * 2.25152 * r^{0.50089}$$

## RESULTS AND DISCUSSION

From birth (W0) to six month of age (W6) of Saanen kids monthly live weight, the minimum correlation coefficient was obtained as 0.7239 between W0 and W3, and the maximum was obtained as 0.9902 between W4 and W5 (Table 1).

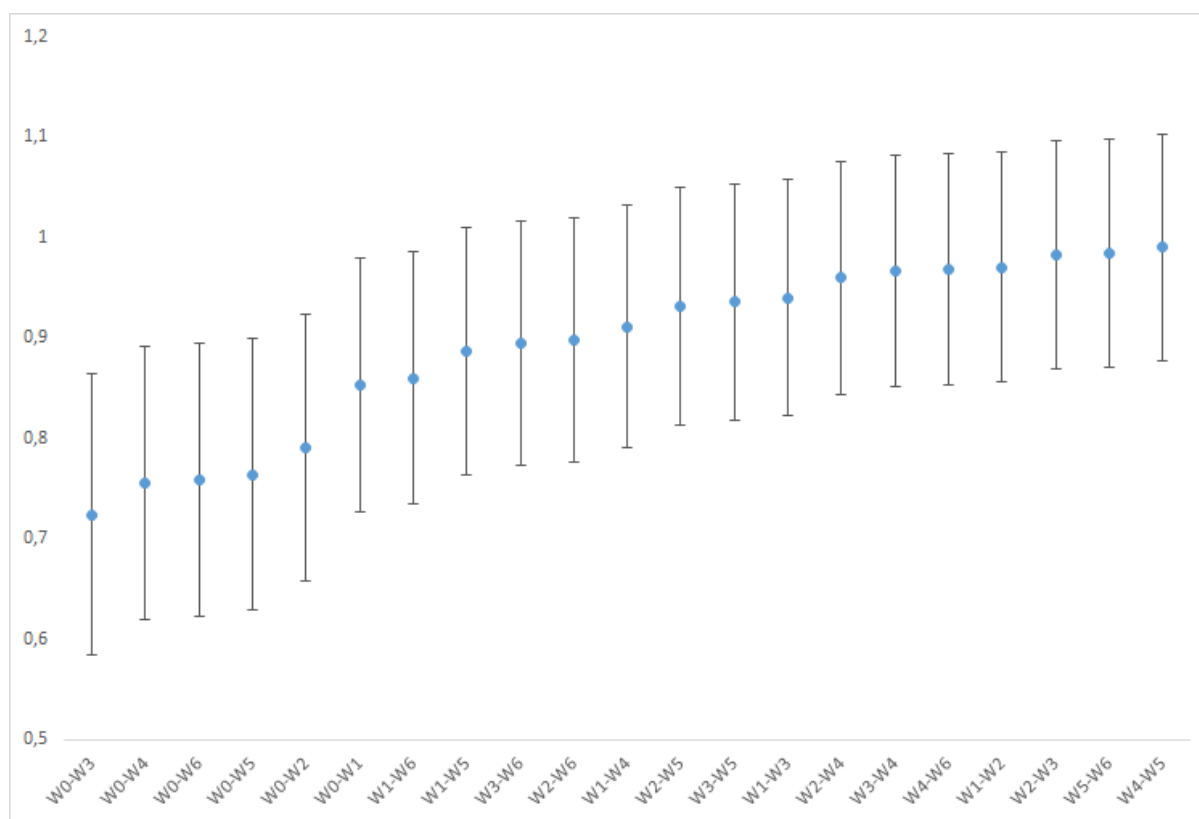
The correlation coefficients showed high relations as expected for live weights. To demonstrate the confidence intervals correlation coefficients was sorted ascending. Correlation coefficients and its nonparametric 95% confidence intervals were given in Figure 2. Correlation coefficients and its parametric 95% confidence intervals were given in Figure 3.

The range of nonparametric confidence intervals was 0.026542 when the range of parametric confidence intervals was 0.102553. It means that nonparametric confidence intervals was so close to each other when parametric confidence intervals was not. With the increasing correlation coefficient parametric confidence intervals decreased but nonparametric confidence intervals. Its underlying reason is the estimator of parametric CI when the CC increases the CI decreases because of the standard error of correlation coefficient as seen in Figure 3.

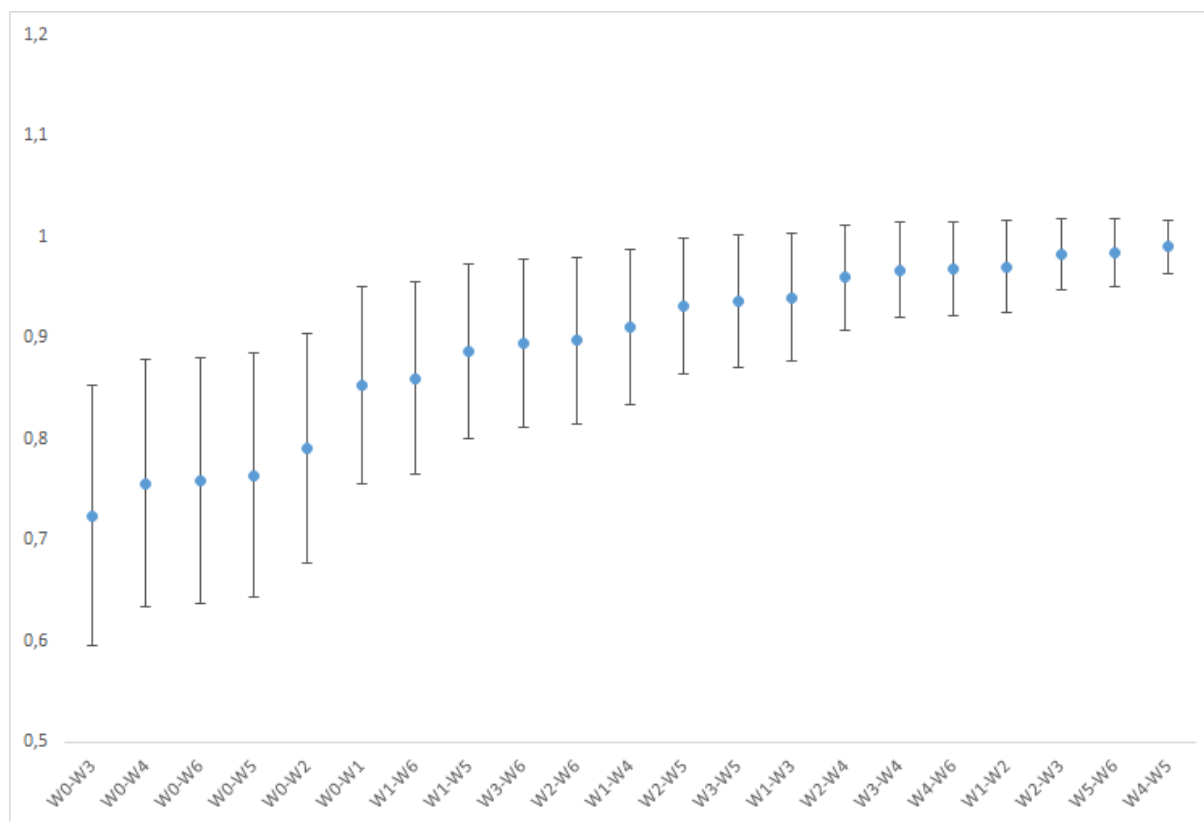
The nonparametric confidence interval for Pearson correlation coefficient is more reliable than parametric confidence interval. Use of nonparametric confidence interval can be preferred instead of parametric confidence interval for animal studies.

**Table1.** Pearson correlation coefficients for monthly live weights of Saanen kids

	W1	W2	W3	W4	W5	W6
W0	0.8527	0.7908	0.7239	0.7558	0.7641	0.7588
W1		0.9705	0.9402	0.9112	0.8867	0.8600
W2			0.9827	0.9599	0.9317	0.8972
W3				0.9671	0.9356	0.8948
W4					0.9902	0.9686
W5						0.9842



**Figure 2.** Correlation coefficients and its nonparametric 95% confidence intervals.



**Figure 3.** Correlation coefficients and its parametric 95% confidence intervals.

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**EXAMINATION OF THE RELATIONSHIPS BETWEEN EGG INTERNAL AND EXTERNAL QUALITY TRAITS WITH THE STRUCTURAL EQUATION MODEL**

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**Abstract**

*Egg quality is one of the factors that affect both incubation efficiency and consumption. Quality for eggs is examined in two parts, internal and external quality. This study aims to determine the structural relationships between the egg internal and external quality features. For this, 114 eggs obtained from 24 weeks old Lohmann Brown chickens commercially grown at the Ondokuz Mayıs University Research Farm were examined. In the study, egg weight, egg length, egg width and shell weight measurements were examined as external quality features. Also, albumen width, albumen height, yolk diameter, yolk height, and yolk weight measurements were used as internal quality features. Structural equation modeling, one of the multivariate statistical methods, was used to determine the relationships between internal and external quality features. The analysis of the data was carried out with the LISREL package. According to the results, it has been determined that the variables that are important in determining the external quality of eggs are shell weight (0.86), egg width (0.73) and egg length (0.54). When the variables explaining the internal quality features were examined, it was determined that the yolk weight (0.72), yolk diameter (0.53), albumen width (0.51), albumen length (0.40) and yolk height (0.27) were important. It was determined that the relationship between egg external quality and internal quality was 0.96 and external quality explained the internal quality by 91%. When the model fit criteria are examined, it is determined that the model shows a perfect fit according to  $\chi^2/sd$  (1.66) criterion and acceptable fit to RMSEA (0.076) criterion. Also, the model was found to be statistically significant ( $p=0,024$ ). As a result of this study, it is thought that evaluating the internal and external quality characteristics according to the important quality criteria obtained for the egg will contribute to increase the quality of the eggs and produce quality hatching eggs. Also, the determination of such models for eggs obtained from other chicken breeds will contribute to the literature.*

**Key words:** Structural equation modelling, Egg quality

## **PREDICTIVE MODELING OF MULTIVARIATE ADAPTIVE REGRESSION SPLINES: AN R TUTORIAL**

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### **Abstract**

*Multivariate Adaptive Regression Splines (MARS) algorithm, as a modified form of classification and regression trees (CART), is a nonparametric regression technique that aids to describe linear, nonlinear and interaction effects between sets of dependent and independent variables for classification and regression type solutions. An alternative to Response Surface Method (RSM) specified in the solution of optimization problems, MARS makes no assumptions about not only capturing functional relationships between response and independent variables but also providing distributional assumptions of the handled variables. The present study deals with applied predictive modeling of MARS data mining algorithm for regression type problems. A special R script file was designed for the MARS predictive modeling with various tuning parameters i.e. degree and nprune in earth package of R studio. All the computations for building MARS predictive models were performed by considering the smallest GCV value. Several goodness of fit criteria that measure predictive capabilities of the MARS models built here were calculated using ehaGoF, a new R package accessible at CRAN for regression type problems. A ten-fold cross-validation and training/testing approaches were adopted to determine whether overfitting problem was encountered in MARS modeling. In conclusion, it could be suggested that an R script tutorial specified here for MARS models producing good results may be a significant reference for next similar studies.*

**Key words:** MARS, Data mining, Cross-validation, Training set, Testing set, Regression

## USE OF DISCRIMINANT ANALYSIS TO DETERMINE THE FRESHNESS OF EGG

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### **Abstract**

*The freshness of the egg is important for both incubation and human consumption. Determination of its freshness without harming the egg is quite difficult with classical methods. Discriminant analysis is a method that can be used to differentiate groups from each other, to make explanations and predictions. In this study, 50 eggs were photographed as experimental material for 29 days and the obtained images were used as data. It is aimed to determine how many days the eggs were, by using the values obtained from the red (R), green (G), blue (B) measurements that compose each photograph. According to the results, true classification rate was estimated as 52.32%. Even if the canonical discriminant functions were statistically significant ( $P < 0.01$ ), true classification rate showed that discriminant analysis was insufficient to determine the how many days the eggs were. Since this situation cannot be explained mathematically, it can be said that artificial intelligence techniques can be used for this purpose.*

**Key words:** Egg, Freshness, Discriminant

### **INTRODUCTION**

One of the problems frequently encountered in multivariate analysis is classification problems. Discriminant analysis is used to determine which dependent classes the data belong to using independent quantitative observation values. This classification is the problem of determining which group it belongs to or from which group by examining the  $p$  number features of any individual.

Multivariate statistical methods have been developed in order to explain the relationships between variables where the properties of the results are affected by a large number of variables (Özdamar, 2004). One of the methods that determine the relationships between metric independent variables and categorical dependent variables is discriminant analysis (Alpar, 2017).

Image processing technique, which is one of the techniques used for classification purposes, is one of the important techniques that enables the analysis of images transferred to the computer by means of scanners, cameras etc (Demirbaş and Dursun, 2007). With image processing technologies, productivity increases while production costs decrease (Chen et al., 2010).

Eggs are one of the most important, affordable and nutritious products in human nutrition. The quality of fresh eggs decreases over time. However, the number of storage days is one of the factors that affect egg quality (Abdel-Nour, 2011).

When the egg is broken on a smooth flat surface, the yolk is usually in a central position surrounded by thick albumin (Karoui et al., 2006; Robinson and Monsey, 1972; Wells and Norris, 1987). Various methods are used to evaluate egg quality. These methods are divided into two groups: methods with and without eggs breaking. The Haugh unit (HU) is the most commonly used method for measuring egg quality by breaking the egg (Haugh, 1937). With the breaking of the eggs, the individual freshness of the eggs is measured. However, it is economically important for the industrial and consumer to determine the freshness of the eggs without breaking them. Various studies have been conducted to measure the freshness of eggs without breaking them (Aboonajmi et al., 2014; Aboonajmi and Najafabadi, 2014; Abdel-Nour, 2011; Karoui et al., 2008).

The aim of this study is to determine the freshness of the eggs stored in the refrigerator

for 29 days, using image processing methods and discriminant analysis method without damaging the egg.

### MATERIALS AND METHODS

White eggs were obtained from a commercial enterprise in the experiment. 50 eggs laid on the same day were used as trial materials. In order to transfer the egg photos to the computer, Canon 550D camera, which was fixed with tripod, was used. The photos taken were recorded at 18 MP size and fixed ISO value. The optimizations applied were carried out using the MATLAB program.

#### Acquisition of Images

Eggs are kept in the refrigerator and taken out at the same time only when photographs are taken and put in the refrigerator at the same time. The pointed parts of the eggs are placed in the device with the face down. Fixed camera was used with the help of constant light amount and tripod.

In the photographs obtained, the dirty spots on the black parts of the background of the eggs were cleaned with the help of image processing program (Figure 1). The cleaned photos were cut in 2000X2000 pixel size (Figure 2).



**Figure 1.** Cleaned image.

The images obtained with the help of MATLAB program have been reduced from 2000X2000 pixels to 300X300 pixels.



**Figure 2.** Cut image.

#### Creating Raw Data

Each photograph size defined in the program consists of 270000 numbers in the size of 300x300x3. While generating 300X300 (90000) aspect data, RGB (red, green, blue) value for each value constitutes the X3 part of the data.

- Data is printed from the top down as 90000X3 with the help of the program.
- Determinants of the data were taken.
- The data are multiplied by their raw state and determinants.
- The data obtained were obtained as a 3X3 matrix.

$$\begin{vmatrix} R^2 & RG & RB \\ RG & G^2 & GB \\ RB & GB & B^2 \end{vmatrix}$$

The resulting matrix;

- The obtained 3x3 matrix has been turned into a single column and brought to a length of 1x9.

Each photo obtained is composed of 9 numbers. 1450 photos were collected for 29 days from 50 eggs. The input matrix was created in the size of 9X1450 (13050 numbers).

#### Creating the Target Matrix

29 days to be estimated against the input data are determined as targets. The target matrix is composed of 1 row of 1450 columns as opposed to the input matrix of 1450 columns. One day value is entered as a target in the 1x9 matrix that constitutes each photograph.

### Discriminant Analysis

Discriminant analysis was first introduced by Ronald A. Fisher in 1936 (Albayrak, 2006). It was used especially in classification problems and in various statistical studies with Fisher introducing discriminant analysis.

The aim of the discriminant analysis is to assign the units to the group they belong to by minimizing the classification of the error and determining the main masses of the units. Discriminant analysis is a method used to determine the separation of variables into groups. It is tried to find functions that will allow the units to be assigned to their real groups by taking a certain number of features. It is aimed to assign individuals to their main groups by determining the groups they belong to by minimizing the wrong classification while performing this function.

The characteristics used to separate the groups are called discriminant variables. In short, discriminant analysis is the process of revealing the differences of two or more groups by discriminant variables. It is a broad concept that includes several statistical approaches that are closely interrelated (Klecka, 1980; Akgül and Çevik, 2003).

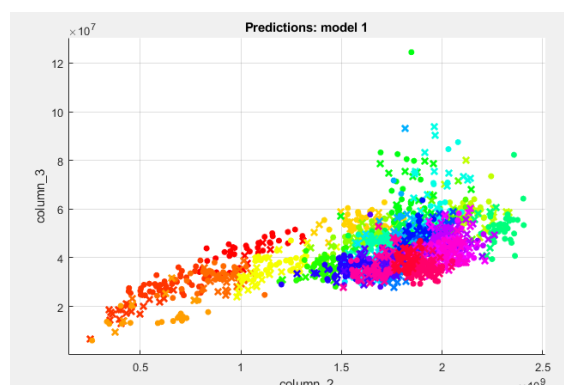
### RESULTS AND DISCUSSION

Discriminant analysis is a powerful separation method. However, the wide use of the analysis is limited because it is difficult to meet its assumptions. The fact that our study topic is egg freshness, the color values of squares totals and correlations constitute the data source has increased the variation width in the data very much. The increased absolute distance between the data distorts the possible normal distribution of the data. For this reason, the item of "no relation between data" in the assumptions of discriminant analysis was violated. Another assumption of discriminant analysis, the multivariate normality compatibility of the data was tested separately according to Mardia, Henze-Zirkler and Doornik-Hansen tests (Arslan et al., 2020) and it was determined that the data did not fit into the multivariate normal distribution ( $p < 0.05$ ). (Arslan et al., 2020). The variance and covariance equality of the data, which is another discriminant analysis assumption, was determined that the data calculated with the Box-M test did not comply

with the covariance equation assumption ( $p < 0.05$ ).

It has been determined that the preliminary assumptions required for the discriminate analysis do not meet the necessary conditions to achieve the intended objectives in our study.

In other words, it is determined that Discriminant analysis cannot be applied in this study data, and when applied, the results will not be reliable. According to the results obtained from the discriminant analysis, the correct classification rate in the discrimination of egg freshness (days) was determined as 52.3% (Figure 3).



**Figure 3.** Discrimination of the age of eggs.

In the Canonical Discriminant functions, all coefficients are determined as very close to zero. Findings reveal that discriminant analysis cannot be used safely in order to determine egg freshness. This problem, which cannot be solved linearly, should be evaluated as an evidence that it should be solved with artificial intelligence techniques.

### CONCLUSION

In order to determine the freshness of the eggs, it was determined that the data obtained from the image processing method did not meet the discriminant analysis assumptions. Despite not assuming assumptions, it was determined that eggs were classified as 52.3% correctly as a result of discriminant analysis. It has been determined that the values obtained from image processing cannot be used by discriminant analysis. Further development of the data needs to be included in the analysis.

In order to determine the freshness of eggs, it can be useful to determine their effectiveness by using various optimization methods and to try



new hybrid algorithms created by hybridizing these algorithms.

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**PARAMETER ESTIMATION OF FACTORS AFFECTING MILK YIELD IN A MULTIPLE COLLINEARITY  
BY BAYESIAN REGRESSION METHODS**

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**Abstract**

*Regression analysis determines the relationship model between dependent and independent variables. In this study, the body weight, milking time, milk yield and environmental factors obtained from 61 dairy cattle were used for internal and external temperatures. In this study, Bayes Regression method was used to estimate milk yield parameters in case of multiple connections. According to the results, it was seen that Bayes method can be applied successfully in the field of animal husbandry. It is thought that the use of this study for dairy cattle in other agricultural areas will be useful for better evaluation of the data obtained.*

**Key words:** Bayesian regression, Multiple collinearity, Milk yield

**INTRODUCTION**

The main purpose in cattle breeding is to achieve the highest yield in the most economical way as in other species. The level of yield is determined by two main factors, the animal's genetic structure and environmental conditions. These two basic elements need to be considered together in order to maximize the manufactured product (Jensen, 2001).

Friesian breed in the numerical sense in that culture comes first race of cattle bred in Turkey. However, as in other breeds, the yield levels of these breeds of cattle are largely affected by environmental conditions as well as their genetic structure. As a matter of fact, different levels of efficiency are obtained in different regions of our country and under different operating conditions. This shows to some extent the level of regions and businesses in cattle breeding (Erdem et al. 2007).

Recently, 305-day milk yield (P305) was replaced by test-day milk yield (Ferreira et al., 2002), with the latter approach showing several advantages: a) it permits the removal of environmental variation in phenotypic data on milk yield, since test-day milk yield considers the specific environmental effects for each production record, which is not possible when P305 data are used (Visscher and Goddard, 1995); b) it grants a more accurate evaluation of cows, due to the use of a larger number of records per cow, as

compared to the same records fitted to P305 (Rekaya et al., 1999); c) it is not affected by the accuracy of the different prediction methods for P305 (Rekaya et al., 1999), because it permits the use of part lactation information, without the need for adjusted factors and/or lactation prediction; d) it facilitates the genetic evaluation of lactation persistency (Jensen, 2001); e) it permits a more accurate estimation of the genetic and permanent environmental effects that affect milk yield.

**MATERIALS AND METHODS**

The milk yield and records of 61 head of Simmental, Holstein and eastern Anatolian red cows, which were grown in 3 private agricultural enterprises within the borders of Erzurum, between January and March 2020 constituted the research material. In the research, internal temperature (IT), external temperature (ET), live weight (LW) and milking period (MP) were emphasized as milk yield characteristics.

**Bayesian Methods**

Bayes' theorem has recently been used in decision making techniques. Since the aim is to minimize the risk of making wrong decisions as much as possible, it would make sense to participate in the decision-making process in personal experience and knowledge (Vila 2000).

Bayesian method is;

$$P(B|A) = \frac{P(BA)}{P(A)}$$

In the same way, we use the bayes theorem when finding conditional probability density functions. Here for probability density functions for  $\theta$  and  $y$  (Zellner, 1971),

$$f(\theta | y) = \frac{f(\theta) f(y | \theta)}{f(y)}$$

Using the Bayesian estimator;

$$\hat{\beta}_b = (\sigma^{-2}(X'X)\beta + \sigma^{-2}A_0\beta_0) (\sigma^{-2}(X'X) + \sigma^{-2}A_0)^{-1}$$

The analysis to be made depending on various assumptions about the average of the

preliminary distribution to be defined for the parameters also varies (Leamer, 1973).

## RESULTS AND DISCUSSION

Since the VIF values are greater than 10, there is multiple linear connection in the data.

**Table 1.** Multiple linear connections between parameters

	ET	IT	MP	LW	VIF
ET	1	0.935	0.197	0.710	138.46
IT		1	0.157	0.694	102.58
MP			1	0.142	36.15
LW				1	195.75

**Table 2.** Estimated parameter values according to Least Squares and Bayesian method and sum of error squares

Parameter	Estimate values					
	Simmental		Holstein		EAR	
	LS	Bayesian	LS	Bayesian	LS	Bayesian
a	5313.738	5311.575	5364.142	5360.244	5326.648	5313.986
b (ET)	1.758	1.751	1.658	1.647	1.708	1.698
c (IT)	37.470	37.578	36.363	36.912	36.812	37.014
d (MP)	123.134	122.143	125.452	123.550	124.286	124.584
e (LW)	-0.119	-0.117	-0.112	-0.117	-0.118	-0.115
R <sup>2</sup>	0.908	0.919	0.921	0.929	0.916	0.918
	Sum of Error Squares					
LS	1672.756	1789.812	1842.816	1835.352	1714.785	1666.815
Bayesian	1692.874	1795.312	1866.620	1846.544	1726.387	1669.182

In the study, the values obtained by the Least Squares (LS) and Bayes Approach (BA) of the milk yield model and the Sum of Error Squares of the parameters are shown in Table 2. Since the table is analyzed, it is observed that there are differences according to the methods in terms of parameter values and error square sum values. The "a" parameter, which shows the highest asymptote value that the varieties take values between 5364.142 and 5311.575 in its method, while in the Bayesian method it has values between 5360.244 and 5311.575. When the asymptot values were analyzed, the highest value was observed in the same type with 5364.142 values in the LS method and 5360,244 in the Bayes method. This variety was followed for Simmental by 5313.738 in LS method and 5311.575 in Bayesian method. The highest asymptot value was observed in Holstein with the values of 5364,142 in the LS method and 5360,244 in the Bayes method.

Since the bend point parameter "e" is examined, it takes values between -0.112 and -0.119, in the LS method, and between -0.119 and -0.117 in the Bayesian method. While the lowest bending point parameter value is observed in Simmental with the values of -0.119 and -0.117 in the highest value is in both methods.

Additionally; the explanatory variance (R<sup>2</sup>) values, which shows the highest value takes between 0.929 and 0.921 in its method, while in the Bayesian method it has values between 0.929 and 0.919. This value was followed for Simmental by 0.908 in LS method and 0.919 in Bayesian method. The highest R<sup>2</sup> value was observed in Holstein with the values of 0.921 in the LS method and 0.929 in the Bayes method.

According to the results, it was seen that Bayes method can be applied successfully in the field of animal husbandry. The use of this study for dairy cattle in other areas of agriculture may be

beneficial for better evaluation of the data obtained.

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**DETERMINATION OF EGG YOLK BY MULTINOMIAL LOGISTIC REGRESSION ANALYSIS USING  
SOME EXTERNAL QUALITY FEATURES IN EGGS**

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***Abstract***

The aim of this study is to reveal the relationship between some egg external quality features and egg yolk, which is one of the egg internal quality features, by using multinomial logistic regression analysis. For this purpose, the external quality features of the egg; L values (mass, medium, pointed), a values (mass, medium, pointed), b values (mass, medium, pointed), egg weight and shape index properties are used and by applying factor analysis to these features, 11 variables are transformed into a 3-factor structure, with less variables, the data structure has become explained. Multinomial regression analysis was applied to estimate the properties of egg yolk with 3 factors obtained. The validity of the last model created as a result of the analysis was found to be statistically significant by calculating with the maximum likelihood estimator ( $P < 0.01$ ). Odds ratios of the variables that constitutive of model were obtained and according to the reference category chosen, it was observed that factor 3 determined the egg yolk category number 6 based on odds ratios. In addition, it was found that factor 1 was the factor that determined 8 and 9 of the egg yolk categories. In the light of this information obtained as a result of this study, it is thought that egg yolk, which is one of the egg internal quality parameters, can be determined with the egg external quality features, and the evaluation and evaluation of the relationship between them from a statistical perspective will contribute to the literature

***Key words:*** *Multinomial logistic regression, Factor analysis, Egg quality, Egg weight*

**THE EFFECTS OF INSUFFICIENT MANAGEMENT AND NUTRITION ON REPRODUCTIVE PERFORMANCE IN DAIRY COWS: STRUGGLE BETWEEN MODERN AND TRADITIONAL BREEDING**

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**Abstract**

*In today's world, milk has an important place in meeting animal protein needs. The milk in consumption is mostly obtained from breeding (high yielding and healthy) cows. In the Aegean region, cattle breeding are carried out by preferring Holstein breed mostly in different sized and semi-closed dairy farms. In Mugla province, there exists totally 120 thousand milking cows. However, "traditional breeding" (Inadequate maintenance-feeding) methods are mostly used in dairy enterprises. Therefore, the desired level of animal welfare (health, yield) and income cannot be achieved in dairy farms. Oestrus (heat), which is normally observed year-round in cows, is short-term (average 18 hours) and ovulation (ovum-release) occurs after oestrus. Therefore, oestrus cannot be detected (suboestrus) in approximately 50% of cows, especially in large enterprises. In addition, while 50% conception related to artificial insemination is obtained all over the world, this rate may decrease down to 25% as a result of insufficient management and feeding, especially in large enterprises with high milk yield. Moreover, fertility (calf yield) decreases by about 2% with every 5 litres of milk increase per cow. On the other hand, when the same breeding male (bull line) is used for a long time, abnormalities or other yield losses (abortus) due to inbreeding within the herd may be inevitable. Even due to long-term inadequate management and feeding, the reproductive process may be stopped completely (anoestrus) with a decrease in milk yield. In our country, cattle breeding are mostly performed by low education-level breeders in enterprises with insufficient shelter and feeding conditions, by the low level of forage (straw, dry grass) and insufficient silage and / or concentrated feedstuff. In the East, small-scale (semi-primitive) enterprises, where most low-yielding indigenous/hybrid breeds are dealing with degraded animal health rather than the yield (milk, calf). In the West, in medium/large sized enterprises, the minimum animal welfare level (normal lactation and annual calf yield) that can meet the demand for high milk (and calf) yields is not met. Undoubtedly, ensuring animal welfare and productivity is possible, along with the ideal level of management-feeding, through adequate and regular medical (Veterinary) services and breeding bull (artificial insemination with frozen semen). Therefore, in order to overcome the economic difficulties in the dairy sector; modern animal husbandry requires solutions to individual (insufficient professional knowledge, awareness, experience as well as team approach), animal (reproductive problems, low productivity), nutritional (insufficient or one-way feeding) and managerial (traditional approach) problems. In near future, as the first step in the effective struggle against traditional animal health and husbandry culture in the field, modern education (mainly for vet surgeons, vet students, caretakers and breeders), surveillance, survey and publication studies should be focused on.*

**Key words:** Dairy cows, Nutrition, Reproduction

**ANIMAL BREEDING STUDIES IN ATATÜRK AND REPUBLICAN ERAS**

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### **Abstract**

*In the present article, the studies of animal breeding (especially horse, cattle, sheep, and goat) carried out in Anatolia during Mustafa Kemal ATATÜRK (1881-1938) and in the Republican period (between 1923-2020) were tried to be summarized. In short, the total development struggle and civilization journey from the 1900s to the present has reached its goal in the fields of humanization, agricultural competence and animal breeding.*

**Key words:** *Animal, Breeding, Anatolia, Atatürk, Republic*

### **INTRODUCTION**

His military genius and great reformers (1881-1938), after World War I (1914-1918), especially Çanakkale Victory (1915) and then the War of Independence (1919-1923) as all have been won by Mustafa Kemal ATATÜRK who founded the new state thanks to the Republic of Turkey (1923). Over the past 15 years after being elected as the first President, he has lost millions (18 million dead) and left behind (12 million alive) war-weary, uneducated (literacy rate: 10% in 1923; 97% in 2019) and unqualified people (now only 13% unemployed, 2019) but led major reforms in Anatolia (geography, the cradle of civilizations), whose citizens remained. However, as stated by the visionary leader ATATÜRK, it was impossible to reach the ultimate national target 'modern civilizations' level (1919) only by military heroism. Consequently, the great contribution to the spirit of mobilization in all areas of life (social and cultural) and in all other sectors (economy and industry as well as maritime, agricultural and animal production (livestock-breeding)) was a national imperative for both ultimate human health and animal welfare.

### **ANIMAL BREEDING STUDIES**

In the Republican era (after 1923), major livestock policies; i) Updating of the Ottoman Legislation (Veterinary educational institutions in Istanbul and Ankara; now 32 Veterinary and 45 Agricultural Faculty and 202 Universities), ii) Animal Health Protection (vaccines) and Disease Struggle (epidemics in 20 years from 1919 to

1939) 40% animal death caused by the minimization) and iii) Livestock (mainly in Karacabey-Bursa and Lalahan-Ankara) and Production (from 17 million in 1923 to approximately 50 million in 1938; emergence). Indeed, animal husbandry mainly met the basic needs of food (animal protein) and transportation (on the battlefield / farm). Livestock (initially AI-artificial insemination, recently ET-embryo transfer and cloning) not only increased its efficiency (meat, milk, eggs, wool) but also provided effective fight against infectious diseases (Brucellosis, Tuberculosis, Bovine Plague, FMD- Foot and Mouth Disease) in the field. To this end, the Izmir Economic Congress (February 17-March 4, 1923) held in many economic sectors (banking, stock market, coal mining, railways, cooperatives, agricultural education), animal breeding (first AI in horse and sheep, 1926) and breeding (cattle, horse, buffalo, sheep, goat, gazelle, dog, cat, chicken, goose, fish, honey bee) in many cities (Bursa, Ankara, Konya, Afyonkarahisar, Şanlıurfa, Kars, Ardahan, Ordu, Sivas, Van, Denizli, Muğla) brought major modern reforms.

During the war years and the Republican period, cattle (mostly domestic and cross-bred) were used for food and transportation. However, the horses were bred for horseback riding (Arab and British purebred) and transportation (domestic) (Bursa, Eskişehir, Malatya breeding farms), sheep and goats (mostly domestic) were used for food (domestic) and wool/mohair (Merinos, Angora). In animal history, the first AI trials (dog) were

started by L. Spallanzani (Italy) in the 1780s, while the freezing of the bull semen was first performed by R. Foote (USA, 1936). At the beginning of the 1900s, Russia and Turkey, were the first the two countries to adopt the cattle breeding (mainly Brown Swiss, Simmental, Holstein and Jersey) (Lalahan, 1957). In our country, breeding trials in sheep (fine wool Merino) and cattle showed a partial increase in the late 1920s, especially in the Marmara region. But the 1929 World Economic Crisis (originating from the USA, spread to England and Germany) and II. World War (1939-1945) led to local/global outbreaks and stagnations (about 50% attenuation). Thus, reproductive (mainly AI) trials remained at very low rates until the late 1950s. As in developed countries, pioneering local production activities in Turkey have been increasing since the late 1960s due to global prosperity gained momentum again (2 million

doses of bull semen production in 2018, 3 million AI applied and 9 million female bovine available). In our country, AI was preferred especially for the production of dairy cows (Holstein breed) in the West (Thrace and Aegean), whereas the Brown-Swiss (combined) breed is used mostly in the East. Undoubtedly, AI applications in cows have been very common in America (Holstein) and Europe (Holstein, Brown-Swiss, Simmental) for a long time (about 75% success rate in AI).

In the field, unlike many other livestock species (*e.g.* horse and sheep) AI practices in cows (Figure 1) are the most widely used in animal production techniques so far. Unlike the AI, special production techniques indoors (natural mating under strict control) are also quite common in other domesticated animals, such as in goats (Figure 2) and cats (Figure 3).



**Figure 1.** Calves from Artificial Insemination in cows (personal collection, Erzurum).



**Figure 2.** ANGORA Goats, special production (by Professor Recai KULAKSIZ, Balıkesir University, Balıkesir-TR).

**Figure 3.** VAN Cat (special production) (by Professor Barış Atalay USLU, Burdur Mehmet Akif Ersoy University, Burdur-TR).

## DISCUSSION

In the present article, the studies of animal breeding (especially horse, cattle, sheep, goat) carried out in Anatolia during ATATÜRK (1881-1938) and in the Republican period (between 1923-2020) were summarized shortly.

As a result of wars and epidemics across the country for many years, only large scale (more than half) deaths and injuries have not been observed in Anatolian people, most of whom were tired of war. In the same period, the numbers of sheep and goats were reduced by almost half, similar to humans. However, a





civilization and developmental movement, which sets an example to the whole world, has been initiated, especially with the great leadership and guidance of ATATÜRK (especially between 1923-1938). Our commanders and administrators, led by ATATÜRK, have reached the goal of the nation-owned total existence struggle and the great sacrifices of all professionals (mainly Veterinarians and Agricultural Engineers) in the agricultural and animal husbandry sectors. Anatolian people have been wanted to be completely destroyed since the early 1900s through long wars and occupations. However, with the social solidarity and great sacrifices maintained until the 100<sup>th</sup> anniversary of our Republic (2023), these people never gave up agriculture and animal husbandry in order to fight for civilization and development and to meet their basic life expenses. The struggle for national existence (about 8 times increase in the number of people; from 10 million to 80 million) has been largely won. Our social civilization journey has not only progressed with great developmental movements in a style that surprised the whole world before the 100<sup>th</sup> anniversary of the Republic (between 1923-2023), but in major sectors (education, health, informatics, energy, transportation, defence, mining), agriculture and animal husbandry) has become a model country. On the other hand, in the same period, total population of national livestock (mainly cattle, sheep and goats) has increased significantly (at least 4 times increase; from about 15 million to 70 million). Moreover, as a result of animal breeding studies (in cattle, sheep and goats), which were tried to carry out persistently with the involuntary interruptions during the past century, domestic animal breeds were transformed into relatively high-yielding animal breeds. Therefore, significant increases have been achieved in all yield characteristics such as meat, milk and wool per individual animal of these species which are grown mainly for food purposes.

#### CONCLUSION

As a result; the national existence struggle initiated by the great leader ATATÜRK during the First World War (1914-1918) gained great stability and momentum, especially with the declaration of the Republic (1923) after the Çanakkale Victory (1915) and the War of

Independence (1919-1923). In short, the total developmental struggle and civilization journey that has started since the early 1900s has reached its goal in the fields of both modernization and agricultural competence and animal breeding. Obviously, sustainable breeding/production (thanks to optimum feeding/maintenance) was achieved in animal husbandry in the periods of ATATÜRK and the Republic (roughly between 1920-2020) and the strategies followed by the State authorities were quite successful.

However, in order to sustain the momentum and progress gained at the point reached in animal breeding, it is imperative that this kind of national solidarity is maintained without interruption, by taking care of all the work together in administrative, social and scientific fields. Of course, effective vaccines and cutting-edge technologies (USG, ET, cloning, transgenic/genomic, gene banking, private production) are also essential to keep up-to-date.

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**COMPARATIVE ANALYSIS OF INDIVIDUAL LACTATION CURVE MODELS IN SOME CATTLE BREEDS**

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**Abstract**

350 lactation records of Brown Swiss, Jersey and Holstein dairy cattle were applied to Wood, reverse polynomial, Wilmink, logarithmic quadratic, quadratic, logarithmic linear, Cobby and Le Du, Cappio-Borlino, Grossman, parabolic exponential and Guo-Salve models and individual lactation curves were obtained. For each race belonging to these curves, the mean square error, determination coefficient, corrected determination coefficient, Akaike information criterion, Bayesian information criterion and Durbin-Watson autocorrelation values were calculated and used to compare the models. As a result of the study, it was determined that Wood, Cobby and Le Du and Cappio-Borlino models gave the best results in Brown Swiss ( $HKO=0.925\pm 0.005$ ,  $R^2=0.994\pm 0.003$ ,  $\bar{R}^2=0.991\pm 0.004$ ,  $AIC=-9.79\pm 0.2$ ,  $BIC=-8.88\pm 0.2$ ,  $DW=2.21\pm 0.1$ ). Jersey ( $HKO=0.925\pm 0.001$ ,  $R^2=0.997\pm 0.001$ ,  $\bar{R}^2=0.990\pm 0.001$ ,  $AIC=-9.606\pm 0.1$ ,  $BIC=-8.699\pm 0.2$ ,  $DW=2.11\pm 0.1$ ) and Holstein ( $HKO=0.925\pm 0.001$ ,  $R^2=0.999\pm 0.001$ ,  $\bar{R}^2=0.998\pm 0.001$ ,  $AIC=-7.116\pm 1.1$ ,  $BIC=-6.208\pm 0.1$ ,  $DW=2.01\pm 0.2$ ) cattle. The worst results are the quadratic model in Jersey cattle ( $HKO=0.421\pm 0.035$ ,  $R^2=0.963\pm 0.003$ ,  $\bar{R}^2=0.953\pm 0.004$ ,  $AIC=-5.426\pm 1.2$ ,  $BIC=-4.518\pm 1.3$ ,  $DW=1.71\pm 0.3$ ) and Wilmink model in Brown Swiss ( $HKO=1.084\pm 0.099$ ,  $R^2=0.930\pm 0.009$ ,  $AIC=1.13\pm 0.6$ ,  $BIC=2.046\pm 0.1$ ,  $DW=2.37\pm 0.6$ ) and Holstein ( $HKO=1.330\pm 0.102$ ,  $R^2=0.929\pm 0.008$ ,  $\bar{R}^2=0.908\pm 0.010$ ,  $AIC=0.897\pm 2.3$ ,  $BIC=1.892\pm 0.7$ ,  $DW=2.47\pm 0.1$ ) cattle.

**Key words:** Lactation curve, Individual modeling, Cattle

**FATTENING PERFORMANCES AND SLAUGHTER TRAITS OF HOLSTEIN AND SIMMENTAL CATTLE  
REARED IN A COMMERCIAL BEEF ENTERPRISE IN ISPARTA PROVINCE OF TURKEY**

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***Abstract***

*Study was carried out in order to determine the fattening and some slaughter traits of Holstein and Simmental male cattle in a commercial beef enterprise in Isparta province in 2016. In the study, a total of 22 male cattle, including equal number of (11) Holstein and Simmental cattle slaughtered in different periods were used. While initial age and live weights were detected as 397.00 d and 532.18 kg for Holstein and 328.40 d and 418.40 kg for Simmental cattle, respectively, the fattening periods were 112.00 d and 139.00 d for Holstein and Simmental cattle. In the study, while the live weights of Holstein and Simmental cattle were 657.20 kg and 608.00 kg, respectively at the end of the fattening periods, average daily live weight gains of these breeds were detected as 1110 g and 1360 g during this period. In addition, the average feed conversion ratios during fattening were 6.97 and 7.11 respectively for Simmental and Holstein cattle. In the study, average hot carcass weight and dressing percentage of Holstein cattle were 355.72 kg and 54.13%. The same values for Simmental breed were 337.81 kg and 55.54%, respectively. It was thought that the findings of this study were important in terms of exhibition the fattening performances of different breeds in a same commercial beef enterprise for different periods of year. Also, the results indicating that Simmental had relatively higher values of both fattening performance and slaughter characteristics than Holstein cattle.*

***Key words:*** Holstein cattle, Fattening, Slaughter traits, Simmental

## **SURVIVAL RATE OF POLISH HOLSTEIN-FRIESIAN COWS TO SECOND, THIRD AND FOURTH LACTATION IN CONVENTIONAL AND AUTOMATIC MILKING SYSTEMS**

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### **Abstract**

*The aim of the study was to evaluate the influence of the conversion from conventional (CMS) to automatic (AMS) milking system on survival rate of dairy cows. A total of 6 361 Polish Holstein-Friesian cows in the second (SL2), third (SL3) and fourth (SL4) lactation as well as culling reasons were taken into consideration. The cows were born between 2002 and 2015 and calved between 2004 and 2018. All data for the survival analysis including culling reasons in 17 herds before and after the change from CMS to AMS were extracted from the SYMLEK official milk recording system. While analysing SL3 and SL4 only cows that calved for the second time were considered. They had a complete information on milk performance in the first full lactation. Cow's survival in SL2, SL3 and SL4 was analysed by multiple logistic regression, the following effects were taken into consideration: milking system (MS), age at first calving (AFC), first calving season (CS), milk yield for full first lactation (MY), course of the first delivery (CE), the birth of a dead calf in the first pregnancy and herd. The reasons for culling (udder diseases, low fertility (infertility and reproductive disorders), locomotor diseases, low milk yield, other diseases (metabolic, digestive and respiratory diseases), accidents and chance events) were analysed by the 2 test in the second, third and fourth lactation separately as well as in the first three lactations combined. A preliminary statistical analysis of the effect of MS, AFC, DC on SL3 and SL4 was carried out. A highly significant influence of H and MS x H interaction on SL4 was noted. Cows milked in AMS were characterised by a lower survival rate to second and third lactation compared to cows milked in CMS (respectively lower by 27.8% in SL2 and 31.0% in SL3). By analysing the data combined for the first three lactations it can be noted that after AMS introduction culling rate increased for the following reasons: locomotor diseases (by 0.85 p.p.), low milk yield (1.36 p.p.) and other diseases (3.01 p.p.). Also, it was also observed that the automation of milking reduced culling due to udder diseases by 0.37 p.p., low fertility by 3.24 p.p., and accidents and chance events by 1.60 p.p.*

**Key words:** Dairy cattle, Survival, Reasons for culling, Automatic milking system

**RETROSPECTIVE STUDY ABOUT THE TRANSFORMATION OF DAIRY CATTLE POPULATION IN  
TURKEY (1991-2019) AND POSSIBLE METABOLIC AND REPRODUCTIVE PROBLEMS**

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***Abstract***

*The present study analysed the transformation of dairy cattle population and changes in yearly milk production (1991 – 2019) in Turkey. In this study, metabolic and reproductive diseases frequently seen in high milk yielding dairy cows (HYDC) and problems causing low milk yield production were investigated. The number of dairy cows, which were culled and sent to slaughter was investigated for last 3 years in Turkey. On the other side, cost of the most common metabolic diseases by region has been analysed by a phone survey (40 vets were asked in different regions). Dairy cow population was 6.118.997 in 1991 (HYDC pure breed: 10,6% and traditional Indigenous dairy breed: 55,3%). Dairy cow population reached 6.580.753 in 2019 (7,5 % increase compared to 1991) which consisted pure breed HYDC of 49,4% and traditional dairy breed of 8,9%. This is a transformation in dairy cattle population in 28 years. Yearly total milk production increased by 141,2% from 8.616.412 tons (1991) to 20.782.374 tons (2019). Yearly per lactation milk production increased from 1.4 tons (1991), to 1.65 tons (2000) and to 3.16 tons (2019) which means 7,5% and 124,3% increase respectively. The growth of the number of HYDC pure breed and their total yearly milk production was 399,3% (650.759 – 3.249.002) and 555,6% (1.913.438 tons to 12.301.080 tons) in 28 years respectively. Numerous metabolic and reproductive diseases can often be observed in HYDCs due to genetic reasons as well as nutritional and maintenance mistakes during the transition period and dry period which can cause loss of yield. The cost of these metabolic diseases for the farmers was between 150-1200 TL per case. Many of the animals were sent to compulsory slaughter and culled because of these diseases. Indeed, a total of 738. 681 dairy cows has been sent to slaughter throughout the last 3 years.*

***Key words:*** Dairy cow, High milk yield, Metabolic diseases

## PRINCIPAL COMPONENT ANALYSIS (PCA) OF BODY MEASUREMENTS AND BODY INDICES IN THE PASUNDAN COWS

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### **Abstract**

*Principal component analysis (PCA) is important for describing the body conformation in livestock. Total of seven body measurements and thirteen body indices parameters from 144 heads of Pasundan cow (average 3 years age) at West Java Province of Indonesia were used in this study for PCA analysis. The body measurements in this study consisted of withers height (WH), body length (BL), and chest girth (CG), chest width (CW), rump height (RH), rump width (RW) and rump length (RL). Therefore the body indices in this study consisted of height slope (HS), width slope (WS), body index (BI), area index (AI), rump length index (RLI), conformation index (CI), length index (LI), body ratio (BR), proportionality (Pr), thoracic development (TD), pelvic index (PI), transverse pelvic (TP) and longitudinal pelvic (LP). The highest of Pearson's coefficient of correlations (r) value in body measurements was showed between WH and RH (0.93). Hence, the highest r value in body indices was showed between WS and TP (0.86). The PCA for body measurements and body measurements was revealed two factors of PC1 (WH, BL, RH) and PC2 (CG, CW, RW, RL) that explained about 73.36% of the total variation. Meanwhile, the PCA for body indices was revealed four factors of PC1 (BI, AI, RLI, LI, Pr, LP), PC2 (CI, TD), PC3 (WS, PI, TP) and PC4 (HS, BR) that explained about 89.38% of the total variation. It was concluded that the seven body measurements is important for describing body conformation of Pasundan cows such as body size (PC1) and body shape (PC2).*

**Keywords:** *Body measurements, Body indices, Body conformation, Pasundan cows, PCA*

**SOMATIC CELL COUNT IN GOAT MILK: ASPECTS RELATED TO MILK COMPOSITION AND DAIRY  
PRODUCT QUALITY**

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***Abstract***

Demand for dairy goat products is rising in both traditional and new markets. Goat milk and products increasingly are preferred for their health and nutritional benefits, including greater digestibility and lipid metabolism, in addition to their taste, compared to cow milk. Dairy goat production systems are developing towards the intensive level in the world, which causes problems in udder health and milk quality associated with increased milk yield per goat. Control of raw milk obtained for processing is one of the important issues facing the dairy industry today, and one of the most important parameters affecting raw milk quality is the somatic cell count (SCC). The monitoring of SCC is a valid tool for estimating the magnitude of milk yield and content losses in evaluation of cow milk. However, goat milk SCC is affected by numerous factors other than intramammary infection and there exists a confusion on the relevance of SCC as a limiting factor in milk yield. In many developed countries, legal restrictions have been imposed on goat milk SCC. The effects of SCC on raw milk and dairy product quality constitute an important place in forming the basis of these legal limitations. However, references concerning the relationship between SCC and milk contents are unclear in dairy goats. Positive, negative or no relationship was described between fat, total protein, total dry matter and lactose content and SCC. Similarly, there are conflicting results regarding the whey protein, sodium, chloride and potassium content of milk. The variation of milk contents has been associated with a dilution or concentration effect in variable milk volumes. Depending on the product type, high SCC affects important quality parameters such as yield, texture and sensory properties in dairy products produced from goat milk both during the manufacture and the storage stages. Overall, there is a need to continue studying in greater depth the relationships between SCC in goat milk and milk composition and dairy product quality.

***Key words:*** Dairy goat, Dairy product quality, Milk composition, Somatic cell count



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

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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*

**DETERMINATION OF GROWTH CURVE AND EFFECTS OF SOME FACTORS ON LIVE WEIGHT AND CHEST GIRTH BETWEEN BIRTH AND 24 MONTHS OF AGE IN YERLI KARA CATTLE**

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**Abstract**

*Yerli Kara Cattle is locally adapted breed with the widest living range in Turkey. This domestic breed has been conserving as In-Situ and Ex-Situ/In-Vivo within the scope of the "Project of Conservation Of Domestic Animal Genetic Resources". Live weight and chest girth measurements are the important parameters indicating growth and development in cattle breeding. The aim of this study is to research the effect of some factors such as gender, birth year, season of the birth and parity on live weight(LW) and chest girth(CG) between birth and 24 months of age and also to determine the correlation between live weight and chest girth in Yerli Kara Cattle. The animal material of this study was consist of 83 Yerli Kara Cattle, borned between the year 2015-2018, in the herd of Ex-Situ In-Vivo conservation of Genetic resources at Lalahan International Center for Livestock Research and Training. General Linear model procedures of Minitab were used to analyse the data. The statistical significances between the subgroups were determined with "Tukey Multiple Comparison Test". The relationship between live weight and chest girth was determined by "Pearson Correlation". The effect of gender at birth, 6 and 24 months of age, the effect of season of birth at 6 months of age, the effect of birth year at 3 and 6 months of age and the effect of parity at birth was found statistically significant( $P<0.05$ ) on LW. When we look at the same effects on CG, the effect of gender at birth and 24 months of age, the effect of birth year at 6 and 12 months of age and the effect of parity at birth was found statistically significant ( $P<0.05$ ) while it was not found statistically significant at any period examined in this study that the effect of season of birth on CG. On the other hand the relationship between LW and CG was found positive and statistically significant ( $P<0.01$ ) at all periods examined. The highest correlation coefficient ( $R^2 =0,876$ ) between LW and CG was found at birth. As a result of analysis it can be said that Yerli Kara cattle have reached the maturity after 3rd parity and therefore give birth to bigger calves after reaching the maturity. Furthermore high correlation coefficient between LW and CG shows that we can estimate the birth weight of calves with high accuracy using chest girth value with the help of a simple tape measure in Yerli Kara Cattle.*

**Key words:** Yerli Kara cattle, Growth curve, Live weight, Locally adapted breed

## CALF SEX AS A PREDICTOR OF CALVING EASE IN SUSSEX HEIFERS

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### **Abstract**

*The aim of this study was to evaluate the incidence of calving ease in Sussex heifers due to calf sex during parturition. A total number of fifty-one first calf Sussex heifers at 24 months old, weighing approximately 350 kg were used for this study. A number of two, two-year-old bulls, weighing approximately 800 kg were used for mating the fifty-one heifers with a bull to cow ratio of (1:30 and 1:21). The fertility of bulls was assessed by a private veterinarian before the mating season. Calving ease was scored as follows: 1 = no assistance during parturition (normal), 2 = heifer assistance as gently pull, 3 = heifer assistance as hard pull, 4 = heifer cannot calf, 5 = heifer calved a dead calf, and 6 = heifer calf with abnormal position. A total of fifty-one calves (male =21, female =30) were born during the study. The results showed a statistical significant ( $df = 2$ ,  $\chi^2 = 7.89$ ,  $p < 0.05$ ) between calf sex on incidence of calving ease. Normal parturition ( $n = 33$ ) was recorded as the higher incidence with 73% of female calves and 27% of male calves, followed by gently pull of calves ( $n = 11$ ) with 73% of male calves and 23% of female calves, and the lowest incidence was observed on hard pull of calves with 57% of male calves and 43% of female calves. Spearman correlation was employed between calving ease scores and calf sex, and the results showed a negative statistical correlation ( $r = -0.355$ ) at  $p < 0.01$ . The results of this study suggest that the chances of a heifer to experience dystocia were more when a male calf was born compared to female calves. Therefore, during parturition of Sussex heifers a close supervision might be given to heifers pregnant with male than female calves.*

**Key words:** Calf sex, Calving ease, Correlation, Sussex heifers

## **CORRELATION BETWEEN SOMATIC CELL COUNT AND DRINKING WATER CONSUMPTION OF A HUNGARIAN DAIRY FARM**

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### **Abstract**

*Milk production is major sector of animal husbandry all over the world. It serves as a good example for analysing water use of different locations, technologies and management practices. Average lactation of all Hungarian dairy farm was 2.1 in 2018. Useful lifetime has been increased since then. One of the biggest aim of Holstein-Friesian breeders is to keep their animals in production as long as possible. However, optimal timing of culling is important as well. The main reasons for culling are reproduction disorder, udder problems, metabolic diseases, lameness and low milk yield. The most critical point of milk production is mastitis, which is described by somatic cell count (SCC). Four groups were generated based on SCC; 1. Group: SCC<100 000, 2. group: 101 000-400 000, 3. group 401 000-1 000 000, 4. group SCC> 100 000. Drinking water consumption of these groups for producing 1 kg milk was assessed. Average daily milk production in investigated farm was 28.16 kg in 1. Group, 24.72 kg in 2. Group, was 24.31 kg in 3. Group and 23.61 kg in 4. Group. Daily average (liter/animal) drinking water consumption in different age groups during investigated period was calculated. Lactating cows have the highest drinking water demand (88+13.6 liter). Washing of milking and cooling systems can only apply drinking water quality. Teat preparation and cleaning solution affects water use as well. As a result of regression analysis, there was tight correlation between milk quantity and drinking water consumption.*

**Key words:** *Holstein Friesian, Drinking water consumption, Somatic cell count*

## EGG YOLKS FROM DIFFERENT FOWL SPECIES IN EXTENDER AFFECT CRYOPRESERVATION AND FERTILITY OF BUFFALO BULL SPERMATOZOA

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### **Abstract**

*Buffalo sperm more sensitive to cold shock and/or cryo-injuries and therefore, a better cryoprotectant is required for its preservation. Egg yolks from different avian species are used as croprotectants in semen extenders, however, differences in their composition lead to varying effects on freezability and fertility of semen. In our preliminary studies, compared to chicken egg yolk, we have reported an improvement in post-thaw quality of buffalo sperm using Duck, Quail, Turkey and Pigeon egg yolk. The present study was aimed to investigate the effect of egg yolk from Guinea fowl and Red Jungle fowl on the freezability, enzyme leakage and fertility of buffalo sperm. Semen collected from 6 Nili Ravi buffalo bulls (6 replicates) was cryopreserved with TCEY extender containing 5%, 10%, 15% and 20% egg yolk from Ginea fowl or Red jungle fowl or 20 % Chicken egg yolk. Post-thaw sperm quality was assessed in terms of sperm motility, plasma membrane integrity, liveability, viability, chromatin damage and release of intracellular enzymes; Glutamic oxaloacetic transaminase (GOT) and Lactate dehydrogenase (LDH). The data on post-thaw sperm quality were analyzed by analysis of variance and Least Significant Difference Test was applied to compare the treatment means. The results have shown that Guinea fowl egg yolk in the extender at 5, 10, 15 and 20% did not affect ( $P \geq 0.05$ ) post-thaw sperm motility, plasma membrane integrity, livability and viability. However chromatin damage was less ( $P \leq 0.05$ ) and GOT leakage was significantly lower ( $P \leq 0.05$ ) in extenders having 15 and 20% Guinea fowl egg yolk, whereas LDH release was similar ( $P \geq 0.05$ ) to controls having 20% Chicken egg yolk. Red jungle fowl egg yolk at 15 and 20% in extender significantly improved ( $P \leq 0.05$ ) post-thaw sperm motility and plasma membrane integrity and significantly reduced ( $P \leq 0.05$ ) the GOT and LDH leakage compared to controls. Moreover, sperm livability and viability was higher ( $P \leq 0.05$ ) in extender having 15 % Red Jungle fowl egg yolk and chromatin damage was lower ( $P \leq 0.05$ ) with 20 % Red Jungle fowl egg yolk, compared to controls. To assess the fertility rate, buffalo semen cryopreserved in extenders containing 15% Guinea fowl egg yolk or 15% Red jungle fowl egg yolk or 20% Chicken egg yolk was used to inseminate a total of 600 animals (200 inseminations per extender). Pregnancy was diagnosed by rectal palpation 60 days post-insemination. The data on fertility rate were analyzed by Chi square test. Post AI, fertility rates in buffaloes were not affected ( $P \geq 0.05$ ) by Guinea fowl egg yolk (15%) in extender, however, these were significantly higher ( $P \leq 0.05$ ) with Red Jungle fowl egg yolk (15%) compared to Chicken egg yolk (20%). In conclusion, Red Jungle fowl egg yolk (15%) in the semen extender improves the post-thaw sperm quality, reduces intracellular enzyme leakage and gives better in vivo fertility in buffalo, and can be suggested to be used in semen extenders an alternative to routinely used Chicken egg yolk.*

**Key words:** Guinea fowl, Red jungle fowl, Lactate dehydrogenase; Glutamic oxaloacetic transaminase, Buffalo sperm

**INVESTIGATION OF CONTAMINATION RATE OF SARCOCYST IN RASHT SLAUGHTERED CATTLE  
BY DIGESTIVE METHOD AND COMPARISON OF RESULTS WITH ABATTOIR STATISTICS**

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**Abstract**

*Sarcocystis is an obligatory intracellular protozoan parasite which can cause digestive disturbances in infected patients. Also, can cause important economic loss and disease in livestock. This study aimed to determine the incidence of Sarcocystis sp. Infection in slaughtered Cattle at the abattoir of Rasht, Iran by digestive method. During October 2015 to March 2016, 202 slaughtered Cattle in Rasht industrial abattoir (Rasht, Iran), were investigated for the presence of Macroscopic Sarcocystis by direct observation. Each of the investigated livestock was classified into groups according to the age, sex and infected muscle tissue. All tissue samples were sectioned to 2-3 mm slices and observed carefully for probable macroscopic cysts. The sections were then examined by the peptic digestion method. Our study showed that the digestion method is useful to identify infected samples. According to the results, Any of Cattle were diagnosed as being infested with macroscopic cysts. But there was a high frequency of Microscopic Sarcocystis infection in livestock slaughtered in this area was seen. All of the cattle were diagnosed as positive infested Sarcocystis species by the digestion method. The data analysis indicated that there is a statistically significant difference between age groups, the infection rate increased with age ( $p < 0.05$ ). The infection rate was independent of sex and difference between males and females was not significant ( $p > 0.05$ ). Also, there was a significant difference between prevalence of Sarcocystis infection in different examined muscles ( $p < 0.05$ ) and most microcysts were diagnosed esophageal (99.01%) and skeletal muscles (98.02%) for cattle.*

**Key words:** Sarcocystosis, Digestive method, Macrocyt, Microcyt, Rasht, Ruminants, Cattle

**INTRODUCTION**

Sarcocystis is an obligatory intracellular protozoan parasite which can affect humans and animals. The distribution of the parasite is worldwide and has been reported by many investigators from different parts of the world. Life cycle of the parasite consists of an intermediated host (man or herbivores animals) and definitive host (man and carnivores animals). Carnivorous such as canine and feline family, infect environment via faeces by passing 200million oocyst during infection period (Nourollahi-Fard et al., 2009; Latif et al., 1999; Fayer, 2004). Humans acquire infection by eating raw and under cooked beef, pork or mincemeat containing schizonts of Sarcocystis hominis and S.suihominis. The prevalence of sarcocystosis in slaughtered food animals has been investigated in many studies in different parts of the world

(Ginawi et al., 1997; Pena et al., 2001; Savini et al., 1992; Beyazit et al., 2007) and Iran (Valinezhad et al., 2008; Daryani et al., 2006; Atashparvar et al., 2001; Razavi et al., 2003; Arshad et al., 2007) using different methods which indicate the infection of 3.5 % to 100%. The clinical signs of intestinal sarcocystosis in human include digestive system disturbances such as nausea, vomiting, and diarrhea (Velásquez et al., 2008), especially in immunocompromised patients (Prayson et al., 2008). Li and Robinson (2006) in a human experimental infection model showed that abdominal distension, watery diarrhea, vomiting, chilling and fever, dizziness, headache, joint and muscle ache, epigastralgia and anorexia, appear five hours after ingestion. Unsporized sporocysts were found in the faeces 10 days after infection and sporocysts appeared on the 12th day. Muscular sarcocystosis in human is

caused by *S. lindemanni*. The infection is induced by ingestion of oocysts which passed through faeces of infected dogs (Rahdar and Salehi, 2011).

This article, has pursued three main objectives: determining the rate of pathogens in meat of cattle in Rasht of Iran by using peptic digestion method and macroscopic method, determining the percent of infection in muscle tissues and the comparison of results with abattoir statistics and the application of results in subsequent studies, including prevention, treatment, and control.

### MATERIALS AND METHODS

This study was conducted in Rasht, Iran. This city is known as a cattle livestock region and each year transfers thousands of cattle to other parts of Iran. During October 2015 to March 2016, 202 cattle carcasses were examined and Each of investigated cattle were classified into groups according to the age (<1, 1-2, 2-3, >3), and sex. Ages of the investigated animals were assessed by visual inspection of teeth. Thereafter, heart, skeletal, esophageal and diaphragmatic muscles of them were selected using naked eye inspection for macroscopic Sarcocystis, and digestion method, for detection of Sarcocystis bradyzoites to identification microscopic types of parasite.

Study design: Sarcocystis (macrocyts) were investigated in meat by direct observation. The chopped meat samples of each cattle were inspected for the presence of macroscopic Sarcocystis. Then approximately 100gr of the chopped meat was sampled for further study. In the laboratory, all tissue samples were sectioned to 2-3 mm slices and observed carefully for probable macroscopic cysts; any such cysts was removed. The sections were then examined by the digestion method.

Peptic digestion method: In this method, 100mL of digestion medium (2.5 g pepsin 700 FIP U g<sup>-1</sup> [Merck] and 10 mL hydrochloric acid in 1 L phosphate-buffered saline) was added to 50gr of each homogenized tissue sample and placed in a shaking water bath at 37°C for 30 min. The suspension was then centrifuged for 10 min at 1500 g and a precipitate smear was prepared, fixed with absolute methanol stained with Giemsa and examined by light microscopy at x100 and x400 for the presence of free bradyzoites (Shekarforoush et al., 2005).

The data analysis: Collected data were analyzed by the computer software Excel, ver.2013 and SPSS, ver.19 for Windows (SPSS Inc., Chicago, IL, USA) and chi-square  $\chi^2$  test was run to compare relative frequency of infection between different groups of sex, age and organs. Differences were considered significant when  $p < 0.05$ .

### RESULTS AND DISCUSSION

According to the results, any of Cattle (0.00 %) were diagnosed as being infected with macroscopic cysts. All (100.00%) of cattle were diagnosed as positive for Sarcocystis species by the digestion method (The sample of microscopic cysts was shown in Figure 1). The infection rate increased with age ( $p < 0.05$ ) and it was independent in terms of sex, although this difference was not significant ( $p > 0.05$ ).

In respect of Sarcocystis length, it was ranged from 3-8 mm. The overall prevalence of Sarcocystis infection in different examined muscles of 202 cattle based on detecting of bradyzoites was 100.00% by peptic digestion. Macroscopic method showed, including 98.02% (198 of 202) in skeletal, 69.31% (140 of 202) in diaphragm, 62.38% (126 of 202) in heart and most microcysts were diagnosed in esophageal (99.01%).

**Table 1.** The overall prevalence of Sarcocystis infection in different examined muscles in slaughtered cattle at Rasht slaughterhouse, Iran

	Heart No (%)	Skeletal No (%)	Esophageal No (%)	Diaphragmatic No (%)
Ma	0(0.00) <sup>a</sup>	0(0.00) <sup>a</sup>	0(0.00) <sup>a</sup>	0(0.00) <sup>a</sup>
Di	126(62.38) <sup>b</sup>	198(98.02) <sup>b</sup>	200(99.01) <sup>b</sup>	140(69.31) <sup>b</sup>

<sup>a,b</sup>, Different superscript letters in the same column indicate significant difference ( $P < 0.05$ ).

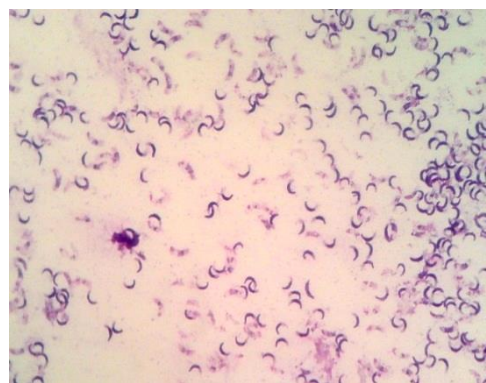
Ma = Macroscopic Method Di = Digestive method.

Sarcocystiosis caused by different Sarcocystis species is known as a protozoal infection with worldwide distribution in many species of

animals and human (Dubey et al., 1989). According to the results of present study, any of Cattle (0.00 %) were diagnosed as being infected

with macroscopic method, while All (100.00%) of cattle were diagnosed as positive for *Sarcocystis* by the digestion method. The absence of macroscopic cysts in this study may be due to a lower frequency of *S. capraefelis* in the Rasht area, perhaps due to the lower probability of pastures being contaminated by cat faeces than dog faeces since dogs are used to shepherd cattle. The data analysis indicated that there was a statistically significant difference between the results of two methods and the digestion method was most able and useful to diagnose *Sarcocystis* ( $P < 0.05$ ). Furthermore, Jacobs et al (1960) suggested that digestion techniques would be a profitable method to use in studying *Sarcocystis* studies. They were unable to find cysts by microscopic examination while 26.66% were positive by digestion method (Jacobs et al., 1960).

The prevalence of *Sarcocystis* in slaughtered animals has been investigated in many studies in different parts of the world including Iran (Ginawi et al., 1997; Pena et al., 2001; Savini et al., 1992; Beyazit et al., 2007; Valinezhad et al., 2008; Daryani et al., 2006; Atashparvar et al., 2001; Razavi et al., 2003; Arshad et al., 2007), indicating contamination rate of 3.5 % up to 100%. This study indicated that cattle of the Rasht were infected with *Sarcocystis* in considerable, indicating that the environment is high contaminated with different species of this parasite. *Sarcocystis* occurs either microscopic or macroscopic in striated muscles and sometimes in unstrained muscles (Dubey et al., 1989). In this study, the esophagus, heart, diaphragm and skeleton muscles were used as investigations have shown these organs to be the most common sites for *Sarcocystis* infection in some animals such as sheep, goat, cattle, camel and buffalo (Abo-Shehada, 1996; Beyazit et al., 2007). Several researchers such as Kudi et al. (1991) and Singh et al. (1992) reported the esophagus to be the predilection site for microscopic cysts; this is not in accordance with the findings of the present study (Kudi et al., 1991; Singh et al., 1992).



**Figure 1.** *Sarcocystis* spp. zoites in meat after digestion x100.

Due to 100% microcystic infection in the studied cattle, unfortunately, we were not able to compare the relationship between infection and animal age. But, several researchers reported higher infection rates in older animals: (Seneviratna et al., 1975; Hussein and Warrag, 1985; Singh et al., 1992; Abo-Shahdad, 1996; Shekarforoush et al., 2005). Also no significant differences were observed between the infection rates of microscopic cysts in male and female. This finding was in accordance with those reported by Haddadzadeh et al. (2004) and Ghorbanpoor et al. (2007) in water buffalo, Abo-Shahdad (1996) in sheep and goat. Furthermore, as reported by Shekarforoush et al. (2005) also stated that there was no significant difference between the infection rates in different sex.

## CONCLUSIONS

The results of the present study imply that the method used in slaughterhouses (observing macrocysts) is not very effective. However, due to its high accuracy and low cost, the digestive method has a high potential to replace this traditional method.

## ACKNOWLEDGEMENTS

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## VIROLOGICAL AND PATHOLOGICAL INVESTIGATION OF PAPILOMATOSIS IN A SHEEP

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### **Abstract**

*Papillomaviruses (PVs) are a diverse group of small, non-enveloped, circular, double-stranded DNA viruses that are known to infect a wide variety of host-species in which they cause proliferation of the stratified squamous epithelium of the skin or mucosa. In ruminants, numerous PVs induce hyperplastic, benign lesions of cutaneous and mucosal epithelia. Papillomatosis is rarely seen in sheep. Especially, skin papillomatosis in animals is an important health problem causing economic losses. Because skin quality deteriorates. Also, it causes some disorders in animals according to different parts of the body. For example, papillomatosis cases in the digital region can cause lameness in animals. The aim of this study was virological and pathological investigation of papillomatosis in a sheep with skin papillomatosis in the digital region. For the pathological examination, tissue samples from the lesions were fixed in 10% neutral buffered formalin, embedded in paraffin wax, cut at 5 µm, and stained with hematoxylin and eosin (HxE). For virological investigation PCR technique used FAP59/64 and MY09/11 degenerate primers (L1 gene). Pathological results: Macroscopically, papillary style, large ulcerative lesions was detected in the digital and interdigital region of the anterior extremities. In microscopic examination, epidermal hyperplasia, hyperkeratosis, acanthosis and vacuolar degeneration were detected. In addition, inflammatory cell infiltration and bleeding was observed, in which neutrophil leukocytes were predominant. Virological results: the sample was detected as positive only FAP59/64 primer pair. This study first determination of papillomavirus in a sheep skin papillomatosis on the digital and interdigital region in Turkey. Furthermore, it is essential to identify the PV types in different animal species and investigate their prevalence/distribution, clinical consequences, for the development of prophylactic and/or therapeutic procedures and determine interspecies transmission potential and evolution of PVs.*

**Key words:** Histopatholgy, Skin papillomatosis, Sheep, PCR

**RNA-SEQ DATA USAGE FOR IDENTIFICATION OF MICRORNAs ASSOCIATED WITH LAMBING RATE IN IRANIAN INDIGENOUS SHEEP**

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**Abstract**

*The main objective of animal breeding is maximizing the profit of the herd. This goal is achieved by improving the genetic value of the herd by appropriate selection methods. The profitability of sheep breeding is most influenced by the litter size. The number of offspring per parturition depends on many factors, among which the ovulation rate is so important. MicroRNAs are single-stranded RNA molecules with approximately 22 nucleotides length that have been produced from endogenous hairpin-shaped transcripts. The microRNAs act as guide molecules in post transcriptional gene regulation by binding to the target genes usually in 3' UTR area. MicroRNAs are almost involved in all biological processes of the ovary. In this study, the transcriptome data of ovarian tissue of Iranian Shall and Sangsari sheep, having a different lambing rate, were used to identify the microRNAs with differential expression associated with lambing rate. The results obtained from differential gene expression analysis by using RNA-seq data were identified in total 19 microRNAs with significant differential expression, that include 14 over expressed and 5 down expressed microRNAs. Mirbase database investigation revealed that none of these microRNAs were previously reported in sheep, but seven microRNAs in cattle (including bta-mir-2904-2, bta-mir-1281, bta-mir-1843, bta-mir-2887-2, bta-mir-1842, bta-mir-1247 and bta-mir-4657) and three microRNAs in both cattle and goat (including chi-mir-324, bta-mir-324, chi-mir-186, bta-mir-186, chi-mir-197, bta-mir-197) were previously identified. The results of this study suggest that using RNA-seq data analysis, in addition to identifying the differential expressed protein coding genes; it is possible to examine the expression of a number of other non -coding regulatory RNAs in specific microRNAs.*

**Key words:** Ovarian tissue, RNA-seq data, Lambing rate, microRNA

**CAPRINE MUCOPOLYSACCHARIDOSIS IIID (MPS IIID)**

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***Abstract***

The mucopolysaccharidoses (MPS) are heritable lysosomal storage diseases (LSD) where the functional deficiency of any 1 of 11 lysosomal enzymes needed to degrade glycosaminoglycans (GAGs), formerly referred to as mucopolysaccharides. Depending on the lack of activity in any of these enzymes disrupts the degradation of GAGs and results in an accumulation of GAGs in lysosomes. Lysosomal accumulation of GAGs eventually leads to the cell, tissue, and organ dysfunction. One group of these diseases is known as a Mucopolysaccharidosis type III (MPS III) or Sanfilippo syndrome. MPS III is an autosomal recessive disorder including four subtypes (A-D) characterized by the inability to one of the four enzymes involved in lysosomal degradation of heparan sulfate (HS), a GAGs. Animal models for this syndrome including feline, canine, murine, and caprine have been described and caprine MPS IIID represents the only animal analog of human MPS IIID. Therefore goats used as a model studying the human disease. Among affected goats, phenotypic variation in MPS IIID disease expression with mild and severe forms has been reported. However similar to human phenotype the affected goat showed delayed motor development, growth retardation, and accumulation of gangliosides in the central nervous system. Caprine MPS IIID is caused by a deficiency in N-acetylglucosamine-6-sulfatase (G6S) activity. Caprine G6S's cDNA has been cloned and sequenced and the cDNA defect in caprine MPS IIID has been determined. The molecular base for this disorder is a nonsense mutation at nucleotide 322 (C→T) results in the change of the arginine codon to a stop codon. This mutation in addition to leads to stop enzyme function also introduces a recognition site for AluI that will enable carrier detection. Recently, a new silent mutation has been identified at nucleotide 354 caprine cDNA.

***Key words:*** Goat, Lysosomal storage, N-acetylglucosamine-6-sulfatase, Genetic disorder

## TRANSGENIC FISH IN AQUACULTURE

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### **Abstract**

*It has been more than a century since the first use of the term 'biotechnology' by the Hungarian scientist Karl Ereky. It is a multidisciplinary science, which uses knowledge of biological sciences and advanced technologies to obtain new and useful products for the benefit of humanity. Application of modern biotechnology range widely, and researchers can create new transgenic organisms by transferring genetic material from organism to organism across species, class, etc. The rapid advances in biotechnology have occurred in many areas such as agriculture, animal sciences, food, medicine, environment, etc. especially in the last 30 years. Today, transgenic varieties of many living things such as plants, animals and microorganisms have been developed and started to be used commercially over the world. Although it is quite difficult to create transgenic animals and the success of rates is about 10%, fish is considerably preferred because female fish give eggs in the millions and fertilized fish eggs develop outside the body of fish, affecting the success in the experimental studies. Transgenic fish have considerable potential in aquaculture, and the research of transgenic fish have been mainly focused on a) improving the characteristics of commercially important species, b) developing fish as bioreactors to produce important molecules such as proteins, enzymes, etc., c) using as an indicator to detect the presence of toxic substances in aquatic environments, d) functional studies of biomedical research and functional genomics. The first transgenic fish were produced in China in 1985, using the fertilized eggs of gold fish (*Carassius auratus* L.). Since that time, some species of fish (trout, catfish, salmon, tilapia etc.) have been genetically modified. Studies on transgenic salmon started in the mid-1980, and the first transgenic salmon was obtained in 1989. It is the first transgenic animal for human consumption approved by FDA in 2015. Moreover, GloFish, the first ornamental fluorescent fish, were created in 1999 and have been sold in the pet shops. Although, genetically modified fish have the potential benefit of high-quality animal protein for consumers, there has been a great deal of public controversy surrounding of the use of them. They are possibly considered to impose a threat to the human health, ecosystems, welfare of animal, and also ethics and religious issues. The potential benefits and risks of transgenic fish are briefly discussed in this presentation.*

**Key words:** *Biotechnology, Genetically modified fish, Glofish, Ecosystem*

**IN-SILICO FUNCTIONAL AND STRUCTURAL ANALYSIS OF AMINO ACID SUBSTITUTIONS OF  
COENZYME Q10A IN CATTLE**

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**Abstract**

*Coenzyme Q-binding protein (COQ10 homolog A) is a member of COQ10 (ubiquinone) family, a component of the electron transport chain present primarily in the mitochondria in most eukaryotic cells. It is multifunctional in nature and has been associated with high rate of fertility, development of embryos and enhancement of muscle strength. However, there is scarce information on in-depth and efficient bioinformatics analysis of genome data in cattle for proper comprehension of the genetic basis of many reproduction and disease traits. The present study, therefore, aimed at screening non-synonymous single nucleotide polymorphisms (nsSNPs) of COQ10A gene in cattle for possible deleterious mutations that may have structural and functional implications. A total of 56 SNPs with complete genome information comprising 43 non-synonymous, 11 synonymous, 1 stop codon and 1 frameshift variant were retrieved from Ensembl genome database (Bos Taurus). Using PROVEAN, SIFT, PolyPhen-2, PANTHER and SNAP2 computational tools, 19 nsSNPs were found to be harmful to COQ10A protein. However, further confirmatory analyses revealed that substitutions R86P and R87Q were more highly detrimental to protein structural stability and biochemical function. These two genetic variants were found in highly conserved region and ligand binding sites (PYV and 1TXCB03, respectively) of high biological importance. Both variants were markedly different from the native protein in terms of total free energy, secondary structure prediction, model quality and physicochemical parameters. Conclusively, when R86P and R87Q deleterious variants are validated using wet lab experimental protocols, they could be important biological markers for reproductive disorder and disease detection and therapy in cattle.*

**Key words:** COQ10, Structural analysis, Cattle

**THE EFFECTS OF SEASON ON QUALITY OF QUEEN THAT REARED FROM COMMERCIAL QUEEN  
REARERS IN MERSIN**

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***Abstract***

*A honey queen bee is the source of all hereditary features that her own colony. The sustainability of the colony depends on the characteristics of the queens and mated drones. In this study, some quality parameters were investigated by taking queen samples from different businesses that are commercially sold queens in Mersin province (between 15 April, 1 May, 15 May and 30 May 2017). Result of the study, the effect of the season on some quality parameters of queen bees was determined. Effect of the season on the body weight of queens and stored spermatozoa was statistically significant ( $p < 0.01$ ).*

***Key words:*** Apiculture, Queen Bee, Season, Quality

**THE MALAYSIAN STINGLESS BEE (HETEROTRIGONA ITAMA) HONEY MICROBIOME,  
NUTRITIONAL CONTENT AND ITS RAPID AUTHENTICATION**

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**Abstract**

*The Heterotrigona itama is the most domesticated stingless bees in Meliponiculture and is a highly valued food dietary complement. H. itama bee honey is distinctively sweet with characteristic strong acidic taste, flowering aroma and considerably more watery. Market value of the Malaysian stingless bee honey can reach a premium US100/kg, costing twice the Apis bee honey. While the microbiome varies from one region to another, the lactic acid bacteria (LAB), Fructobacillus and Lactobacillaceae forms the major colonizing bacterial species in H. itama honey. The microbes are deposited from the gastrointestinal tracts of bees into the honey, which then act as probiotic. The consumption of LAB has been proven useful against pathogenic healthcare-associated pathogens viz. Pseudomonas aeruginosa, Staphylococcus aureus and Escherichia coli, as well as exhibit good antagonistic activity against multi-drug resistance bacteria. Like most honeys, the nutritional content of the H. itama honey mainly comprises of carbohydrates (sugars), organic acids, proteins, and minerals. The appreciable presence of gluconic acid in the honey originates from glucose-oxidase secreted by the bees that metabolizes glucose, whereas the protein and amino acids come from pollen and the salivary glands of bees. Because of the high price that H. itama honey fetches, this commodity is often subjected to adulteration by unscrupulous racketeers. This is damaging the reputation of Meliponiculture, as well as to the health of consumers. Within a specific geographical origin, the cost-effectiveness and availability of the sugars or sweetener are the decisive factors for their selection as adulterants. Aside from deliberate feeding with commercial sugars, the blending of expensive (pure and rare) honey with a cheaper one also constitutes honey adulteration. Commonplace honey adulterants are high fructose corn syrup, corn sugar syrup, inverted sugar syrup and cane sugar syrup, all of which are either added into the authentic honey or are indirectly fed to the bees. Technology into the detection of adulterated H. itama honey based on chromatographic and spectroscopic methods are rather laborious as they require pre-treatments and lengthy preparation time. A more rapid approach which integrates Fourier transform infrared spectroscopy attenuated total reflectance (FTIR-ATR) spectrometry or laser-breakdown spectroscopy with chemometrics may prove more expedient. Both methods allow the rapid analysis of raw H. itama honey without sample pre-treatment and can be made portable. It is important that rapid authentication technology is made available in assisting standard quality checks on H. itama honey sold on the market, in addition to ensure quality products reaching the consumers and safeguard the reputations of manufacturers.*

**Key words:** Heterotrigona itama, Nutritional, Lactic acid bacteria, Microbiome, Honey adulteration



## USE OF PROCRUSTES ANALYSIS FOR HONEY SENSORY ANALYSIS

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### **Abstract**

*The taste, smell and color of honey is very important for both honey producers and consumers. The rate of sugar in the taste of honey is related to the type of sugar and the ratio of these sugars to each other. The smell of honey varies according to the region where the source of the nectar is taken and the source of the plant. In this study, sensory analysis of honey varieties to be examined were investigated by mechanical, thermal, visual, acoustic, chemical and electrical stimuli. The obtained results are classified by Procrustes analysis method. Idiogrid software was used for this analysis. In the data structure required for Procrustes analysis, each individual must have evaluated the same object. However, individuals do not have to evaluate the same feature or the same number of features. Generalized Procrustes analysis is a very effective analysis method in cases where individuals evaluate different features. In this study, the honey obtained from the bees at the Ondokuz Mayıs University Faculty of Agriculture, Apiculture Unit was used. These honeys were evaluated separately as extracted and honeycomb. 20 panelists were used in the study, and these panelists looked at the taste, smell, viscosity, melting in the mouth and aroma in the throat. Panelists scored these features between 1 and 5 points. Results showed that 92% compliance was obtained for honeycomb, while 89% compliance was obtained for extracted honey. For honeycomb, color, taste and smell have emerged as the most important features. For extracted honey, color, taste and viscosity have emerged as the most important features.*

**Key words:** Honey, Procrustes analysis, Sensory analysis

## CONJOINT ANALYSIS AND APPLICATIONS IN AGRICULTURE

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### **Abstract**

*Conjoint analysis is a method used by researchers to predict what kind of decisions consumers will make about products using a survey. The main idea is to calculate different properties of a product for any purchase decision and determine the importance level of properties. With this information, the characteristics of the products or services that are very important can be targeted and future production can be designed. Subconsciously, one person may be more price sensitive, when the other may be more characteristic oriented. Sensing which features are significant and which are insignificant is the goal of the conjoint analysis. The data obtained via surveys are then converted into numbers and managed by statistical analysis. There are two commonly used methods of conjoint analysis: Choice-based Conjoint Analysis and Adaptive Conjoint Analysis. The first one is the eminent combination format. Consumers are presented a number of suggestion cards and requested to choose the highly suited ones for them to purchase. The aim of the study is to present theoretical information about the conjoint analysis and to introduce applications in the field of agriculture.*

**Keywords:** *Choice probability, Orthogonal plan, Preference model, Multinomial-logit model*

**INVESTIGATION OF THE EFFECTS ON COLONY DEVELOPMENT OF HONEY BEES (*Apis mellifera* L.) FEEDING WITH BEET SUGAR AND CORN SYRUP**

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**Abstract**

*This research has examined the effects of two different feedings (beet sugar syrup and corn syrup) on the number of adult-brood bee population on frames, the adult -brood surface area and the number of dead bees. The 18 colonies included in the study were randomly divided into 3 different groups with 6 colonies in each group (Group A: Beet sugar syrup, Group B: Corn syrup, Group C: Control). There are three different measurement periods (February, March, April). As a result of the research, there was a significant difference between groups and periods in terms of the adult-brood surface area and the number of adult-brood bee population on frames ( $P < 0.05$ ). When the number of adult bees examined, the deaths were higher in honey bees feeding with corn syrup compared to beet sugar syrup. In conclusion, supplemental feeding is necessary for the bee colonies under Cukurova region conditions. However, the beekeepers must behave very carefully in the selection of the supplemental feeding material to be applied to the colonies.*

**Key words:** honeybee, *Apis mellifera* L., feeding, colony

**INTRODUCTION**

Honey bees are insects that meet all of the nutrients necessary to survive from nature and evaluate the flowing nectar and pollen sources best (Kumova and Korkmaz, 1998).

Because of global warming, climate change, diseases and environmental factors in recent years, the decreasing plant resources, which are important nutrients, affect the behavior and physiology of bees and cause significant losses in bee populations. (De la Rúa et al., 2009; Vaudo et al., 2015; Kumova, 2016).

Honey bees consume large amounts of carbohydrates such as nectar, honey and sugar syrup. If there is not enough honey left in the colonies in the winter months, the bees are starved and thousands of colonies are disappeared every year. In order to prevent winter losses, there should be enough honey in the colonies or supplemental feeding should be made in the seasons suitable for the colonies. (Haydak, 1970; Standifer et al., 1977; Skowronek, 1979; Winston 1987; Brodschneider and Crailsheim, 2010).

In colonies, supplemental feeding should be done on time and by the technique. It has been demonstrated by the studies that supplemental

feeding increases the colony population, honey and wax (Free and Racey, 1968; Skowronek, 1979; Kumova et al., 1993; Kumova, 2000; Somerville, 2005; Neupane and Thapa, 2005; Nicolson and Human, 2008; Carrillo et al., 2015; Abou-Shaara, 2017).

Today, honey, sucrose syrup, corn syrup, beet sugar syrup, cane sugar syrup, high fructose corn syrup, and syrups prepared from various fruits are widely used in the supplemental feeding of colonies (Sammataro and Weiss, 2013; Mirjanic et al., 2013; Johnson et al., 2014; Gameda, 2014).

However, in recent years, it has been observed that various products applied in supplemental feeding have effects on bee health and honey quality. No supplemental feeding should be made in natural and quality honey production (Somerville, 2014; Jacques, 2008; Makawi et al., 2009; LeBlanc et al., 2009; Zirbes et al., 2013).

This research was carried out in order to reveal the effects of beet sugar syrup and corn syrup, which are used by the beekeeper in the colony feeding from early spring season to nectar flow, on the colony development.

## MATERIALS AND METHODS

This study carried out in the University of Cukurova, Faculty of Agriculture, Department of Animal Science, and Beekeeping Business under Cukurova region conditions from February 26 to April 22 in 2016.

18 honey bee (*Apis mellifera* L.) colonies were used as a material. The queen bee of the colonies was built from a FI hybrid (*Apis ligustica* x *Apis carnica*) from the same colony in 2015. The risk of disease was eliminated by struggling against Varroa in early spring (February 1-15). Honeycombs of the previous year were used since bee colonies have different features in terms of honeycomb processing (Doğaroğlu, 1982).

The Langstroth 10-frame hive were used in the experiment. Bee colonies were randomly selected as four frames of bees and two frames of broods bees at the beginning of the study (26/02/2016). The 18 colonies included in the study were randomly divided into 3 different groups with 6 colonies in each group. (Group A: Beet sugar syrup, Group B: Corn syrup, Group C: Control).

The beet sugar syrup (sucrose 30-36%, glucose 27-30%, fructose 37-40%) was given to the Group A as 1 liter per week. The corn syrup (fructose 55.6%, glucose 39.6%) was given to the Group B as 1 liter per week. No feeding was applied to the Group C bee colonies during the research. Colonies A and B were fed twice a week for 9 weeks (Kumova, 2000).

The adult bee and brood surface areas of the colonies included in the study during the trial were determined with the Puchta method (Fresnaye and Lensky, 1961). The dead bees were counted once a week between March 4 and April 22, 2016.

The number of adult bee population on frames, the number of brood bee population on frames, the adult surface area, the brood surface area and the number of dead bees were tested in the randomized complete block design with 6 replications. The mathematical model of randomized complete block design with replications is  $Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ijk}$   $i=1,2,\dots,t, j=1,2,\dots,b$  and  $k=1,2,\dots,r$ . Where  $\mu$  is the mean effect,  $\alpha_i$  is the  $i^{\text{th}}$  different diets and control applications effect,  $\beta_j$  is the  $j^{\text{th}}$  period effect,  $(\alpha\beta)_{ij}$  is interaction effect and  $e_{ij}$  is the term of error (Montgomery, 2001). Significant differences between means were tested using Duncan's test. Statistical analyses were calculated by using SPSS 22.0 V. package program.

## RESULTS AND DISCUSSION

### The effect of feeding on the brood surface area

One of the main measures of colony development is determining the size of the brood surface area in the colony. During the period of the study (February-April 2016), the brood surface area increased continuously in all colonies (Table 1).

**Table 1.** The brood surface area (cm<sup>2</sup>/colony)

Groups	Measurement Periods ( $\bar{X} \pm S_{\bar{X}}$ )			$(\bar{X} \pm S_{\bar{X}})$
	February <sup>c</sup>	March <sup>b</sup>	April <sup>a</sup>	
Group A <sup>a</sup>	2439.16±877.95	4579.16±811.07	18016.66±1207.10	8345.00±965.37
Group B <sup>a</sup>	2037.50±198.92	4180.00±420.34	18066.66±1317.93	8094.72±645.73
Group C <sup>b</sup>	1745.83±264.37	3012.50±226.82	10447.50±544.73	5068.61±345.30
$(\bar{X} \pm S_{\bar{X}})$	2074.16±447.08	3923.88±486.07	15510.27±1023.25	7169.44±652.13

<sup>a,b</sup>. Different superscript letters in the same column and line indicate significant difference (P<0.05).

When the difference between the periods has examined, it is seen that the best period is April with an average of 15510.27±1023.25 (P <0.05). When the difference between the groups has examined, it is seen that the best groups are Group A (beet sugar syrup) with an average of 8345.00±965.37 and Group B (corn syrup) with an average of 8094.72±645.73 (P<0.05). Periods x

group interaction have found to be statistically significant (P<0.05).

The Group A and B are very different in terms of the brood surface area compared to the control group. The brood surface area increased by feeding with beet sugar syrup and corn syrup. Similarly, Zmarlicki and Marcinkowski (1979) indicated that the brood surface area increased 55% by feeding with sugar syrup.

**The effect of feeding on the adult surface area**

The effect of supplemental feeding on the adult surface area under the conditions of Çukurova

between February and April 2016 is given in Table 2.

**Table 2.** The adult surface area (cm<sup>2</sup>/colony)

Groups	Measurement Periods ( $\bar{X} \pm S_{\bar{X}}$ )			$(\bar{X} \pm S_{\bar{X}})$
	February <sup>c</sup>	March <sup>b</sup>	April <sup>a</sup>	
Group A <sup>a</sup>	2850.00±385.35	5808.33±736.140	21500.00±1190.58	10052.77±770.69
Group B <sup>a</sup>	3450.00±276.58	5558.33±462.13	20145.00±1408.82	9717.77±715.84
Group C <sup>b</sup>	1745.83±264.37	3012.50±226.82	10447.50±544.73	5068.61±345.30
$(\bar{X} \pm S_{\bar{X}})$	2681.94±308.76	4793.05±475.03	17364.16±1048.04	8279.70±610.61

<sup>a,b</sup>. Different superscript letters in the same column and line indicate significant difference (P<0.05).

When the difference between the periods has examined, it is seen that the best period is April with an average of 17364.16±1048.04 (P<0.05). When the difference between the groups has examined, it is seen that the best groups are Group A (beet sugar syrup) with an average of 10052.77±770.69 and Group B (corn syrup) with an average of 9717.77±715.84 (P<0.05). Periods x group interaction have found to be statistically significant (P<0.05).

The Group A and B are very different in terms of the adult surface area compared to the control

group. The adult surface area increased by feeding with beet sugar syrup and corn syrup. Similarly, Zmarlicki and Marcinkowski (1979) indicated that the adult surface area increased 36% by feeding with sugar syrup.

**The effect of feeding on the number of brood bee population on frames**

During the period of the study (February-April 2016), the number of brood bee population on frames increased continuously in all colonies (Table 3).

**Table 3.** The number of brood bee population on frames (number/colony)

Groups	Measurement Periods ( $\bar{X} \pm S_{\bar{X}}$ )			$(\bar{X} \pm S_{\bar{X}})$
	February <sup>c</sup>	March <sup>b</sup>	April <sup>a</sup>	
Group A <sup>a</sup>	2.00±0.00	3.16±0.54	6.33±0.21	3.83±0.25
Group B <sup>a</sup>	2.00±0.00	4.00±0.36	6.66±0.21	4.22±0.19
Group C <sup>b</sup>	2.00±0.00	3.66±0.33	4.16±0.16	3.27±0.16
$(\bar{X} \pm S_{\bar{X}})$	2.00±0.00	3.60±0.41	5.71±0.19	3.77±0.20

<sup>a,b</sup>. Different superscript letters in the same column and line indicate significant difference (P<0.05).

When the difference between the periods has examined, it is seen that the best period is April with an average of 5.71±0.19 (P<0.05). When the difference between the groups has examined, it is seen that the best groups are Group A (beet sugar syrup) with an average of 3.83±0.25 and Group B (corn syrup) with an average of 4.22±0.19 (P<0.05). Periods x group interaction have found to be statistically significant (P<0.05). The Beet Sugar and Corn Syrup were found to be effective on bee colonies. The Group A and B are

different in terms of the adult surface area compared to the control group.

**The effect of feeding on the number of adult bee population on frames**

During the period of the study (February-April 2016), the number of adult bee population on frames showed a continuous increase, but the number of adult bee population on frames in the A and B Group colonies fed was higher than the Control Group (Table 4).

**Table 4.** The number of adult bee population on frames (number/colony)

Groups	Measurement Periods ( $\bar{X} \pm S_{\bar{X}}$ )			$(\bar{X} \pm S_{\bar{X}})$
	February <sup>c</sup>	March <sup>b</sup>	April <sup>a</sup>	
Group A <sup>a</sup>	4.00±0.00	5.00±0.44	8.16±0.30	5.72±0.24
Group B <sup>a</sup>	4.00±0.00	5.66±0.21	8.00±0.36	5.88±0.19
Group C <sup>b</sup>	4.00±0.00	5.00±0.25	5.50±0.22	4.83±0.15
$(\bar{X} \pm S_{\bar{X}})$	4.00±0.00	5.22±0.30	7.22±0.29	5.48±0.19

<sup>a,b</sup>. Different superscript letters in the same column and line indicate significant difference ( $P < 0.05$ ).

When the difference between the periods has examined, it is seen that the best period is April with an average of  $7.22 \pm 0.29$  ( $P < 0.05$ ). When the difference between the groups has examined, it is seen that the best groups are Group A (beet sugar syrup) with an average of  $5.72 \pm 0.24$  and Group B (corn syrup) with an average of  $5.88 \pm 0.19$  ( $P < 0.05$ ). Periods x group interaction have found to be statistically significant ( $P < 0.05$ ). The number of adult bees were determined as  $10.0 \pm 1.3$  (number/colony) in sucrose syrup fed colonies, and  $7.5 \pm 0.16$  (number/colony) in corn syrup fed colonies between November 2008 and

April 2009 by Sammataro and Weiss (2013). In this research, the number of adult bee population on frames was lower than reported by Sammataro and Weiss. The number of adult bee population on frames can be differed depending on the year, season, and application date (Ruiz-Matute et al., 2010; Sammataro and Weiss, 2013).

#### **The effect of feeding on the number of dead bees**

The number of dead bees were evaluated once a week for 8 weeks in March-April 2016 (one week after April 26).

**Table 5.** The number of dead bees (number/colony)

Groups	Measurement Periods ( $\bar{X} \pm S_{\bar{X}}$ )		$(\bar{X} \pm S_{\bar{X}})$
	March	April	
Group A <sup>ab</sup>	19.16±2.91	20.50±3.40	19.83±3.15
Group B <sup>a</sup>	24.70±4.15	23.16±1.30	23.93±2.72
Group C <sup>c</sup>	17.16±4.45	14.50±2.39	15.83±3.42
$(\bar{X} \pm S_{\bar{X}})$	20.34±3.83	19.38±2.36	19.86±3.09

<sup>a,b</sup>. Different superscript letters in the same column indicate significant difference ( $P < 0.05$ ).

It observed that there are more bee deaths in Group B bee colonies fed with corn syrup compared to Group A colonies fed with beet sugar syrup ( $P < 0.05$ ). The period has not found to be significant statistically ( $P > 0.05$ ).

#### **CONCLUSIONS**

In conclusion, the results of this research has been demonstrated that supplemental feeding is necessary for the bee colonies to enter the main nectar flow from the early spring period with a stronger colony population under Cukurova region conditions. However, the beekeepers must behave very carefully in the selection of the supplemental feeding material to be applied to the colonies.

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## THE EXPRESSION PATTERNS OF C-FOS AND C-JUN GENES INDUCED BY FEEDING WITH PLANT EXTRACT, FATTY ACID COMBINATION-SUPPLEMENTED DIET IN STRESS-EXPOSED RATS

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### **Abstract**

*C-fos and c-jun are two important immediate-early genes that are rapidly expressed in cells after specific stimulations. Their products, C-Fos and C-Jun, can form dimer complexes, which serve as transcription factors by binding to the activator protein-1 site of target genes in a variety of neurons. C-Fos and c-Jun proteins have been considered to be valuable markers for the identification of neuronal activation and thus have been used to map functionally related neuronal pathways in the Central Nerve System (CNS) in response to peripheral sensory neuronal activation. In this in vivo experimental design, we hypothesized that the expression levels of these two important proto-oncogenes, c-fos and c-jun, may vary depending on different feeding regimes with plant extracts and fatty acid combination supplementations, Miarom-L (0.2 mL/L/day) and Livervital (2 mL/L/day), which were commercially produced by MIAVIT (GmbH, Essen, Germany), in comparison via corresponding control groups. A total of 48, male, and healthy Sprague-Dawley rats were used in the analyses. The rats were aged about 7–8 weeks and their average body weight was 150–200 g. They were allocated to six experimental groups as follows: I. Control group fed a standard chow diet; II. Stress group fed a standard chow diet; III. Stress group fed a diet supplemented with Miarom-L; IV. Stress group fed a diet supplemented with Livervital; V. Control group fed a diet supplemented with Miarom-L, no stress; and VI. Control group fed a diet supplemented with Livervital, no stress. Stress groups were exposed to two additional stresses including housing alone stress and crowded environment stress. Brain samples were obtained from euthanized rats in order to evaluate gene expression alterations. All procedures performed were approved by Bursa Uludag University Animal Experimentation Local Ethics Committee (App. No: 2018-07/01). RNA extraction was performed using TRIzol (Life Technologies, UK) cDNA samples were obtained by reverse transcription of the total RNA using the Universal cDNA Synthesis Kit, (Exiqon, Vedbaek, Denmark) and were analyzed on a Roche Lightcycler 480 using the Exilent SYBR Green Master Mix (Exiqon). All analyzes were calculated automatically by Roche LightCycler 480 software. Regarding the c-fos gene, altered expressions were observed with respect to experimental subgroups. Down-regulation of the c-fos in "Stress + Miarom-L" and "Stress + Livervital" groups when compared to corresponding control groups was interesting. Concerning the c-jun gene, promising results were obtained in the experimental design. Significant changes in the expression of the c-jun gene were observed. Remarkably up-regulation of the gene was determined in "Stress + Miarom-L", "Livervital", and "Stress+Livervital" groups. These results indicate a potential effect of feed-additives on gene expressions which may play important roles in the biology of stress mechanisms. One possible explanation for the present result may be through epigenetic modifications. However, further molecular studies are needed to confirm the present results and to achieve novel perspectives on the molecular biology of stress dynamics in mammals. Taken together, this study gives valuable results on the association between environmental factors (such as feed-additives) and gene expression levels (c-fos and c-jun) under stress conditions. There is still plenty of room for a better understanding of the genetic basis of stress dynamics based on CNS, especially for epigenetic alterations.*

**Key words:** C-fos, C-jun, Stress, Gene expression, Feed additives



**ANALYSIS OF SINGLE NUCLEOTIDE POLYMORPHISMS OF PRL, GH AND PIT-1 GENES IN THE POPULATIONS OF ALATAU, BLACK-PIED AND HOLDSTEIN BREEDS OF CATTLE IN THE KYRGYZ REPUBLIC**

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**Abstract**

*Cattle breeding is the main sector of livestock farming in Kyrgyzstan and the main tasks of this industry are to increase the milk and meat productivity and reproductive qualities of animals. Achieving this goal largely depends on selective breeding. In modern conditions animal breeding process is based on the use of molecular markers and, therefore, it is necessary to know the genetic characteristics of animal populations for targeted breeding. Objective: To study the frequency distribution of genotypes and alleles of single nucleotide polymorphisms of the PRL, GH, and PIT-1 genes in populations of the Alatau (n = 59), Black-Pied (n = 27) and Holdstein (n = 13) cattle breeds from Kyrgyzstan and analyze phylogenetic relationships with a number of other cattle breeds raised in other countries of the world. According to the results of molecular genetic analysis in cows of the studied breeds two alleles, PIT-1A and PIT-1B, were detected at the HinfI locus of the PIT-1 gene. The PIT-1B allele (69.5%) and the PIT-1AB genotype (47.5%) are most common in animals of the Alatau and Black-Pied (the PIT-1B allele - 72.2%, and the PIT-1AB genotype - 55.6%) breeds, in Holstein breed the PIT-1B allele (76.9%) and the PIT-1BB genotype (53.8%) are common. Regarding the RsaI polymorphism of the PRL gene, it was shown that among the cows of the Alatau, Black-Pied and Holdstein breeds, animals with the PRLAA genotype prevailed - 57.6%, 59.3% and 61.5%, respectively. Individuals with the PRLBB genotype were found with a low frequency - in the range of 1.69-7.69%. The GHLL allele in the AHI polymorphism of the GH gene among the studied cattle breeds was major and ranged from 83.1-98.1%. The most common allele and genotype are the GHLL allele (83.1%) and the GHLL genotype (66.1%) in animals of the Alatau breed, the GHLL allele (98.1%) and the GHLL genotype (96.3%) in the Black-Pied breed, the GHLL (92.3%) and the GHLL genotype (84.6%) in the Holstein. When analyzing combined carriage at three polymorphic loci, the greatest diversity among the identified genotypes was noted for the Alatau cattle breed' 12 genetic profiles. Of these, for four: PIT-1AB / PRLAA / GHLL, PIT-1AB / PRLAB / GHLL, PIT-1BB / PRLAA / GHLL and PIT-1BB / PRLAB / GHLL, the combined frequency was 64.40%. For the Black-Pied cattle, the share of these genetic profiles was 84.61%, for Holstein - 96.30%. A comparative assessment of the FST value among 41 other cattle breeds in comparison with individuals of the Alatau, Holstein and Black-Pied breeds raised on the territory of Kyrgyzstan made it possible to determine their location on the graph of the principal components. It is shown that the greatest differences in relation to other breeds are noted for the Mongolian, N'Dama and Ogaden breeds. The smallest differences with respect to other breeds were noted for the Wenshan, Boran, Hanwoo, Kazakh, and Lingnan breeds - the FST values did not exceed 0.026.*

**Key words:** Cattle, Gene, Prolactin (PRL), Growth hormone (GH), Pituitary-specific transcription factor 1 (PIT-1), Phylogenetic analysis, Kyrgyz Republic

**GENETIC VARIABILITY OF FABP4 C.328 G>A (RS110652478) POLYMORPHISM AND ITS ASSOCIATION WITH SLAUGHTER WEIGHT IN ABERDEEN ANGUS BULLS IMPORTED INTO TURKEY**

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**Abstract**

*In the last few decades, the main focus on animal breeding in many countries has gradually changed from conventional phenotype-based evaluation to molecular genetic analysis. These molecular techniques are rather confidential and effective for selecting animals with superior performance in specific desirable traits. The aim of the study was to determine the genotypic distribution of the c.328 G>A (rs110652478) polymorphism in the bovine fatty acid-binding protein 4 (FABP4) gene and to determine its effect on slaughter weight in Aberdeen Angus Bulls imported into Turkey. A total of 61 Aberdeen Angus bulls that were randomly selected from Tabiat Agricultural Farm located in Bursa province were used in this study. Bulls were imported from Brazil to Turkey and were housed for fattening in semi-open pens for approximately nine months with the same management procedures. Bulls were slaughtered at a commercial slaughter facility according to standard practices, and at the same time, blood samples were obtained from flowing blood. For each animal, genomic DNA was extracted from whole blood samples (~4mL) using the phenol-chloroform extraction method. The amount (ng/?L) and purity (260 nm/280 nm absorbance ratio) of extracted DNA samples assessed by the NanoDrop spectrophotometer. Genotyping of the FABP4 c.328 G>A (rs110652478) polymorphism, located in exon 3, was performed by polymerase chain reaction-restriction fragment length polymorphism method. Allele and genotype frequencies were calculated by the standard allele counting method and the Hardy-Weinberg equilibrium was tested by comparing expected and observed genotype frequencies using a chi-square test. Indices of genetic diversity including gene homozygosity ( $H_o$ ), gene heterozygosity ( $H_e$ ), effective allele numbers ( $N_e$ ), and the polymorphism information content (PIC) were calculated, on the basis of allele frequencies. The least-squares method as applied in a GLM procedure of Minitab software v19.1.1 was used to test the association between FABP4 c.328 G>A and slaughter weight. Results revealed that the most frequent genotype was GG (49.18%) and the minor allele frequency (A allele) was 0.33. The genotype distribution was in agreement with Hardy Weinberg equilibrium ( $P>0.05$ ).  $H_e$  value was 0.44 ( $H_o>50%$ ) and the PIC was 0.34, which suggests a mildly informative genetic marker for the tested population.  $N_e$  value, which expresses the effectiveness of loci allele impact in populations, was 1.83 in the present study. The moderately low levels of genetic variability observed in this study can be caused as a result of eventual inbreeding. Association analysis indicated that the effect of FABP4 c.328 G>A polymorphism on slaughter weight was not statistically significant ( $P>0.05$ ). However, there was a tendency for this trait ( $P<0.1$ ), and animals with the heterozygous genotype seemed to have higher slaughter weight ( $587.40\pm 10.80$  kg) compared to alternative homozygous genotypes ( $574.11\pm 15.60$  kg and  $558.41\pm 1.84$  kg for AA and GG genotypes, respectively). It is worth noting that the sample size in this study was rather limited because crossbreds and the animals with missing information were excluded from the genetic analyses to prevent unreliable results. Thus, studies performed with larger populations may be needed for confirmation. It is important to emphasize that analysis FABP4 c.328 G>A may provide valuable information not only for meat quality but also for meat quantity in beef cattle.*

**Key words:** Genetic variability, Angus, Polymorphism

## OXIDATIVE STATUS AND MOLECULAR ACTIVITY IN MILK DURING DIFFERENT LACTATION STAGES IN GOATS

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### **Abstract**

*The aim of this study was to investigate Malondialdehyde (MDA) levels of milk and expression levels of COX-2 (Cyclooxygenase 2) and NFE2L2 (Nuclear Factor Erythroid 2-Related Factor 2) genes from milk according to lactation stages. Determining the relationship between MDA levels and targeted genes was also an important goal. The study was conducted with 15 healthy goats aged 4-5 years old. Under sterile conditions, 100 mL morning milking samples were collected from mastitis-free animals at three different stages (First month of Lactation- Early lactation stage (EL), Fourth Month of Lactation- Mid-lactation stage (ML) and Last Month of Lactation- Late lactation stage (LL)). Milk MDA levels were determined spectrophotometrically at 532 nm. On the other hand, total RNA was isolated from milk somatic cell according to Trizol method. To determine the expression levels of COX-2 and NRF2 genes, G6PD gene was used as internal control. Gene expression results were calculated as fold change by 2<sup>-Ct</sup> method. MDA levels of samples in different lactation stages (EL, ML and LL) were 5.23±0.32; 10.55±0.98 and 8.48±0.39 nmol/mL, respectively. ML samples MDA levels were significantly higher than EL and LL (P<0.01). Also, isolated total RNA's of samples were appropriate for qPCR application (A260/280=1.80±0.01; Concentration: 171.67±18.08 ng/μL). While no significant difference were found between EL and LL samples, COX-2 gene expression levels of ML samples were upregulated almost 3 folds compared to the EL samples (P<0.05). There was no significant differences in terms of NFE2L2 gene expression. Oxidative status effects the milk and dairy products quality. COX-2 is one of the most important marker on oxidative stress. The results show that more molecular study is needed for understanding the milk secretion mechanism during lactation period.*

**Key words:** Goat, MDA levels of Milk, Gene expression, COX-2, NFE2L2

## THE ROLE OF TOLL-LIKE RECEPTORS (TLRS) IN ANTIVIRAL MECHANISM OF CHICKENS

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### **Abstract**

Poultry, which is an important part of human nutrition, have been affected by avian influenza virus, that it affected avian health and causes worrying economic losses, has different variants (H5N1, H5N2, H1N1, H7N7, H7N1 H3N8 and H7N3). Highly pathogenic avian influenza (HPAI) bird flu, which is highly pathogenic in chickens, negatively affects human health due to its zoonotic feature besides poultry. TLR (Toll-like receptors), is one of the receptors involved in the natural immune system that acts as the first line of defence against many invading pathogens in the channel infection process. As a result of genome analysis, there are ten TLR (toll-like receptors) in chickens and five of them are determined to be ortholog with TLR2a, 2b, 3,4,5 and 7. Chicken TLR21 has been found in fish and reptiles, TLR15 reported phylogenetically specific to the TLR2 family and poultry species. It has been reported that while TLR2 is preserved in mammal and poultry species, the other TLR2 family member occurs spontaneously. Chicken TLR2 shows the same dimerization feature as TLR1a and 1b. TLR9 is not found in the chicken genome, while TLR21 is found in the mammalian genome. The aim of this review is to discuss the cellular role of TLRs that play a role in the antiviral response mechanism of chicken cells in the case of virus-borne infections.

**Key words:** Avian flu, TLR (Toll-like receptors), Infection, Antiviral response

### **INTRODUCTION**

Due to the high mortality rate in the herds of infected poultry, HPAI (Highly pathogenic avian influenza), which is also called chicken plague among the people, is one of the viruses that caused economic losses and decreased poultry populations in many countries. Due to its zoonotic feature, HPAI negatively affects human health as well as poultry. The HPAI epidemic, which has caused the loss of many people and millions of poultry in recent years, has caused major economic losses in countries where it has spread across the Asia-Europe continent.

In the United States, between 1983 and 1984, HPAI caused up to 90% deaths in poultry coops, and 17 million poultry were culled to control the disease during this period. During the epidemic of H7N1 type avian influenza in Italy between 1999-2001, 1 million poultry were been culled.

Similarly, between 2003 and 2004, H5N1 type virus outbreak including eight Asian countries (Japan, China, Indonesia, Laos, South Korea, Thailand, Cambodia and Vietnam) was

experienced same viruses and more than 100 million poultry were culled to take it under control. HPAI has been outbreak in Turkey between 2005-2006 and at the end of the year the number of birds culled across the country were reported to be more than 2 million (Acer and Besirbellioglu 2005; Arslan, M. O. (2006).

There are three different antigenic types of the rapidly spreading influenza virus, A, B and C. Influenza A, the most important and most common influenza virus, can cause infections in humans, birds, pigs, horses, marine mammals and many vertebrates. Influenza B causes infection humans only, Influenza C cause humans and pigs (Acar and Beşirbellioğlu 2005). Type A virus seen in poultry is caused by Neuroaminidase (NA) and Hemoglutenin (HA). HPAI could be transmitted via fecal and orally and have different variants (H5N1, H5N2, H1N1, H7N7, H7N1 H3N8 and H7N3) (Zhou et al., 1999; Van der Goot et al., 2005; Biswas et al., 2008; Stipkovits et al., 2012).

Studies have determined that HPAI virus is related to PB1, NA, M, NS genes. Especially in Asia, the NP gene was more common, and the NA gene isolated from infected chickens caused by the influenza subtype H5N1 virus was involved in the deletion of 19 amino acids. N1 protein has been reported to play a role in the removal of three glycolysis sites of this deletion. The deletions caused by the H5N1 virus; the NA gene affects the immune system by causing rapid amino acid changes when HPAI first enters the host. It has been reported that the highest number of HA genes in humans are associated with the flu and the risk of transmission to humans is higher (Zhou et al., 1999).

### **Chicken TLRs**

TLRs and PAMPs (pathogen-bound molecular patterns) expressed in various cell types (spleen, lung and brain), including macrophages of PPRs (pattern recognition receptors), leading to TLR understanding of poultry antiviral pathways and induction of interferons (IFNs) to understand the effect of HPAI, which has worrying consequences on chickens. Studies have been conducted to determine the role of the host in the formation of immune response (Ruan et al., 2015). Pattern recognition receptors, on the other hand, are involved in the recognition of pathogen-dependent molecular patterns (PAMPs) that play an important role in the innate immune system response (Zhou et al., 1999). PAMPs are molecules that extensively expressed and protected by microbial pathogens, and also recognized by TLR family members. TLR are a family of conserved trans-membrane protein family receptors that play a role in the recognition of preserved molecular motifs. In general, the structure of TLRs consists of the N-terminal and C-terminal domains domain, leucine-rich repeats (LRRs) and the highly conserved cytoplasmic Toll / IL-1 receptor (TIR) domain (Chen et al., 2013; Wu et al., 2018). Leucine-rich repeats (LRRs) support specific interactions between conserved membrane proteins (MAMP) and the TLR family. However, the TIR domeine, which is similar to human IL-1 cytoplasmic domein, is one of the most protected TLRs (Beutler and Rehli 2002). The interaction of TLR and MAMP enables initiation of the specific cascade signal, activation of transcription factors and expression of the gene responsible for innate immunity. Activation

of TLR, induction and activation of antimicrobial peptides (AMPs), pro-inflammatory cytokines/chemokines, interferons, congenital immune related enzymes and both MHC class I and class II co-stimulator molecules are up-regulated (Brownlie and Allan 2011).

To determine the host pathogen interaction, understanding the response of the pathogen to the immune system in chickens and the host's response to antiviral infection play a key role to identifying the virus. Thus, to understand the structure and role of TLRs have been identified in different types of receptors, including chickens, and 14 of this receptor family have been identified. Each TLR family member recognizes specific PAMPs, acting individually or in conjunction with another TLR (Wang et al 2016). Although there is not enough information about these receptors, it has been reported to be highly effective on interferons in inducing molecules infected with the HPAI virus (Brownlie and Allan 2011; Stewart et al., 2012; Lee et al., 2014).

TLR receptors consist of two families, cell surface and intracellular, depending on their location. TLR receptors on the cell surface include TLR1, TLR2, TLR4, TLR5, TLR6, TLR10 and TLR15 receptors, which mainly recognize microbial membrane components such as lipids, lipoproteins and proteins. Among these receptors, TLR3, TLR7, TLR8, TLR9, TLR11, TLR12 and TLR13 are localized in intracellular compartments (Zhou et al., 1999; Karpala et al., 2008; Ruan et al., 2015; Jo et al., 2019).

Chicken (Ch) TLR family, TLR1 (types 1 and 2), TLR2 (types 1 and 2), TLR3, 4, 5, 6, 7, 8, 9, 11, 12, 15 and 21. ChTLR1 (type 1 and type 2) and ChTLR2 (type 1 and type 2) play a role in the recognition of bacterial peptidoglycans (PGN), lipopolysaccharides (LPS) and lipid, which make up bacterial cell wall components (Brownlie and Allan 2011). Avian TLR (chTLR2, 4, 5 and 7) are orthologous to another mammalian including chTLR2a and chTLR2b (Chen et al., 2013).

Mammalian TLR1, 6 and 10 was determined to be expressed on cell surfaces. TLR2 complex dimerized with mammalian TLR1 is associated as a cofactor and enables the recognition of microbial triacyl lipoproteins. TLR2 dimerizes with TLR6 and plays a role in the recognition of viral infections and lipoteichoic acid of yeast diacyl lipopeptidase in Mycoplasma, Gram-positive bacteria. TLR2 play a role in recognizing

petidoglycans, glycolipids (Brownlie and Allan 2011). TLRs type 1 that rich in leucine trans membrane protein and there are amino acid differences between different chicken breeds. After infection with *Clostridium perfringens*, increased expression has been reported for several TLR receptor molecules in the TLR2 family, chTLR4, chTLR15, and chTLR21 in spleen and ileum (Lu et al., 2009; Brownlie and Allan 2011; Chen et al., 2013).

TLR4 play a role to recognition of bacterial LPS, the expression of chTLR4, was detected in wide tissue and cell types as mammals. TLR4 expression was also determined in various tissues of zebra finches and reported to be upregulated in peritoneal macrophages by LPS. TLR4 shows approximately 74% amino acid homology between zebra finch and chickens. It is located in a protected gene region. Mutations in chTLR4 in Leghorn chickens have been associated with *Salmonella enterica* infection of the level of TLR4 in chicks (Vinkler et al., 2009; Brownlie and Allan 2011).

TLR5 plays a role in the recognition of protein components of the bacterial flagellum. TLR5 in mammals recognizes the repeated structurally conserved monomer flagellin region in the bacterial flagella. ChTLR5 in chickens has approximately 50% amino acid homology with hTLR5 (human TLR), and its expression with hTLR5 has been reported to be similar in various tissue and cell types. Avian TLR5 genes are polymorphic, that associated with the resistance or susceptibility of birds to infectious diseases (Chen et al., 2013). In chickens, it is also thought that chTLR5 may play a role especially in chicks caused by bacteria (eg. *Salmonella enterica* serovar *Typhimurium*) (Brownlie and Allan 2011; Villanueva et al., 2011; Sławińska et al., 2013). Unlike other TLRs expressed in TLR, 3, 7, 8 and 9 nucleic acids found in mammals and are localized in the endosome. TLR3 have role to recognition of double-stranded RNA and artificial agonist poly (I: C). The chTLR7 and chTLR8 neutrophil activation has been reported to be important for recognition and inflammatory cytokine in the case of viral infection (Wang et al., 2008). Avian TLR3 and 7 have the ability to recognize the RNA virus, HPAI, while chTLR15 and chTLR21 are potential receptors that recognize both RNA viruses and bacteria (Chen et al., 2013). The chTLR3 also

recognize ssRNA through the complementary viral strand formed the transcription and replication of RNA viruses during infected host cells and play an important role in recognizing HPAI (Raven et al., 2017).

TLR7, recognition of single stranded RNA and synthetic antivirals (Imiquimod, Gardiquimod and R-848); TLR8 and TLR7 recognize similar synthetic antivirals and recognize TLR9, especially DNA rich in unmethylated CpG motifs. This TLR group is very important in supporting immune system against viral infections. In addition, TLR9 allows the recognition of bacteria that invade the cell. TLR3 and TLR7 were ortholog with the chicken and zebra finch genome. However, TLR9 is found ortholog in the genomes of other vertebrate species, while it is not functional in zebra finches. In general, both TLR7 and TLR9 are highly expressed in plasmacytoid dendritic cells (pDC) and are poorly expressed in mammals in conventional dendritic cells (cDC) and are thought to play a role in protecting pDC cells against viruses. HPAI virus is high potent inducer of TLR10 expression when compared seasonal influenza virus (Lee et al., 2014). In mammals, TLR3 is thought to play an important role in the induction of antiviral IFN during viral infections. After infection with the Marek disease virus, chTLR3 and chTLR7 are up-regulated in the lungs of chickens, and this has been associated with up-regulation of pro-inflammatory cytokines and macrophage infiltration (Abdul-Careem et al. 2009). TLR7 in chickens is expressed in many tissues (Hoshino and Kaisho 2008; Karpala et al. 2008; Brownlie and Allan 2011).

Karpala et al. (2008) performed intravenous virus infection in the leghorn chickens at 12th and 24th hours by using H5N1 virus to determine the role of type 1 IFN (IFN $\alpha$  and IFN $\beta$ ), which are vital in antiviral reactions, and euthanized RNA isolation at 12th and 24th hours. In addition, in order to determine the effect of the virus on chicken tissue cells, for each experiment, the level of spleen, lung and brain cells from 4-week-old chickens was investigated. Basal levels of TLR3 mRNA expression on the brain were found to be approximately 3 times lower than in the spleen and lung, it was reported that the inoculated chickens had lower IFN and TLR3 levels after 24 hours, this rate was 66 times higher in the brain and 25 times higher in the

lung. In case of viral infection, both TLR3 and IFN $\gamma$  mRNA expression were shown to increase the relationship between TLR3 expression and IFN $\gamma$ . After 2 hours of stimulation with (poli I: C), IFN $\alpha$  (136-fold) and IFN $\gamma$  (54-fold) have been reported to show changes in the mRNA level. ChTLR3 and ChTLR7 were thought to be involved in the recognition of viral double chain RNA, single chain RNA and artificial agonist poly (I: C) (Karpala et al., 2008).

TLR15 is unique to birds and some reptile species and has a tight compact asparagine structure. As TLR15, chTLR1La and chTLR1Lb are unique to birds (Chen et al., 2013).

Ku et al., (2014) reported that inducing innate immunity in different levels of various toll-like receptors (TLRs; TLR 1, 2, 3, 4, 5, 7, and 15) of the H7N9 or H9N2 viruses that cause HPAI in chickens. TLR15 expression was significantly upregulated in the lungs of chickens infected with both HPAI virus. (Ku et al., 2014). TLR15 also has an auto-activation mechanism in responding to *Salmonella enterica* infections and localized in the endoplasmic reticulum. Chicken TLR15 has been reported to be up-regulated in embryonic chicken fibroblasts after incubation with heat-killed *Salmonella*. The expression of chTLR15 against bacterial infection has been determined in both lymphoid and non-lymphoid tissues (Brownlie and Allan 2011).

chTLR21 in chickens is thought to exhibit wider species recognition than TLR9 found in mammals. chTLR21, has an immune response during viral infections as mammalian TLR9 (Chen et al., 2013).

Similar to other TLRs, expression of chTLR21 was determined in both chicks and chickens' tissues and embryos, and was thought to protect against *Salmonella* infection. (He et al., 2007; Taghavi et al., 2008; Brownlie and Allan 2011). Chicken TLR21 plays a role in the recognition of microbial DNA and synthetic oligodeoxynucleotides (ODN), which are determined to be the ortholog of TLR21 in fish and amphibians. Chicken TLR21 is an unmethylated cytosine phosphate guanine dideoxy nucleotide motif. It has been reported that TLR21 is phylogenetically and evolutionarily related to the TLR11 family and has a close ortholog with the TLR13 family (Brownlie and Allan 2011).

## CONCLUSION

Viral diseases that pose serious threats for poultry and frequent changes of the virus are the main cause of immune deficiency. This cause epidemic viral disease outbreaks in poultry. Therefore, the development of genetic resistance to viruses offers an alternative control measure against viral infections. Therefore, TLRs, the basic sensor molecules in the innate immune system, are membrane-bound receptors on the cell surface or in endocytic compartments that can recognize a wide variety of pathogen-related molecular patterns can be used as a candidate molecular marker to select some disease tolerant individuals.

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## DIAGNOSIS OF CANINE PARVOVIRUS INFECTION BY POLYMERASE CHAIN REACTION AND EVALUATION OF HEMATOLOGICAL AND SEROLOGICAL CHANGES

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### **Abstract**

*Canine parvovirus (CPV) is the causative agent of canine viral hemorrhagic diarrhea, gastroenteritis, respiratory and myocardial disease that causes serious infections in canids and causes fatal systemic disorders especially in puppies. The causative agent of the Canine parvovirus (CPV) belongs to the Parvoviridae family, has small, non-enveloped single-stranded DNA. Canine parvovirus (CPV) infection is worldwide. Infection is a highly infectious and fatal viral disease characterized by hemorrhagic, extremely watery, and very smelly diarrhea, characterized by high mortality and morbidity, especially in puppies, usually with acute, fibrin, necrotic and hemorrhagic enteritis, and sometimes with myocarditis. There are two types of Canine parvovirus (CPV) isolated from dogs, canine parvovirus type-1 (CPV-1) and canine parvovirus type-2 (CPV-2). Type CPV-1, which is reported to cause mild gastrointestinal and respiratory infections in dogs, was first isolated in 1967. CPV-2 is an important virus in terms of its rapid spread among canids and causing infections that result in the death of many sick animals. This study aims to evaluate the effectiveness of serological, hematological, serum acute phase protein changes in dogs, as well as the diagnosis of canine Parvoviral infections by molecular viral DNA detection by Polymerase Chain Reaction.*

**Key words:** *Canine parvovirus, Infection, PCR, Hematology, C - reactive protein, Immunoglobulin*

### **INTRODUCTION**

Canine parvovirus (CPV) is a common cause of canine hemorrhagic diarrhea, which causes serious infections in dogs and causes fatal systemic disorders especially in puppies. The causative agent of the Canine parvovirus (CPV) is in the Parvoviridae family, has small, non-enveloped single-stranded DNA (Goddard and Leisewitz, 2010). Canine parvovirus (CPV) infection is found in many parts of the world. Infection is a highly infectious and fatal viral disease characterized by bloody and very poorly hooded diarrhea, characterized by high mortality and morbidity, especially in puppies, usually with acute, fibrin, necrotic and hemorrhagic enteritis, and sometimes with myocarditis. Canine parvovirus infection can also occur in puppies born from these vaccinated mothers because of the insufficient maternal antibodies from the vaccinated bitches or the formation of these antibodies or the protective antibody level cannot continue for a sufficient period (Prittie,

2004). Acute myocarditis in puppies (4-8 weeks) usually progresses with mild enteritis in adolescents (2-12 months) and in adults.

There are two types of Canine parvovirus (CPV) isolated from dogs, canine parvovirus type-1 (CPV-1) and canine parvovirus type-2 (CPV-2). Type CPV-1, which is reported to cause mild gastrointestinal and respiratory infections in dogs, was first isolated in 1967. Then, in 1978, the type that appeared in America and spread all over the world is CPV-2. The main pathogenic type CPV-2 soon becomes canine parvovirus type-2a (CPV-2a), canine parvovirus type-2b (CPV-2b) (Parrish et al., 2007; Binn et al., 1970) and some Western Europe. It has been transformed into antigenic forms called as canine parvovirus type-2c (CPV-2c) in Asia and South America countries with the same pathogenesis (Decaro et al., 2007; Perez et al., 2007). The CPV-2a, CPV-2b, and CPV-2c forms are broader in comparison to the CPV-2 form, and can cause the same disease that occurs naturally in cats

with panleukopenia infection (Greene, 2012). CPV-2 infection continues to be an important health problem for adults and puppies all over the world, although vaccines are used to be prevented disease (Panda et al., 2009).

It has been determined that it causes infection in dogs, cats, foxes, jackals, wolves and bears, and even in humans. Especially German shepherd dogs, Kangal dogs, known as Turkish shepherd dogs, are known to be used in rural environments such as search and rescue operations or protecting livestock. Since these dogs are likely to come into contact with infected animal feces in nature, they are also at higher risk of developing the disease. Parvovirus B19 infection or 5<sup>th</sup> disease as known by the public, which the bats are natural reservoirs, infects only humans and generally children (Yetkin et al., 2010).

Canine parvovirus (CPV) is a virus that is resistant to heat and pH changes and many disinfectants, maintains its longevity in the environment, is an important species-specific pathogen and causes disease in many mammals (Lamm and Rezabek, 2008; Uwe, 2006; Pollock and Coyne, 1993). The virus needs especially fast-dividing, DNA-synthesizing cells (enterocytes, bone marrow precursor cells, myocardiocytes) to reproduce. Since Canine parvovirus uses cell metabolism rather than cellular proteins to proliferate, it disrupts cell mitosis and causes cell death (Truyen, 1999, Nandi & Kumar, 2010).

Although CPV-2 infection is seen in dogs of all ages, genders and breeds, it is reported that puppies between the ages of 6 weeks and 6 months are particularly susceptible and cause fatal infections (Pollock & Goyne, 1993; Turgut, 2001; Prittie, 2004). In Canine parvovirus type 2, depression (lethargy and anorexia), fever, vomiting, and mucoid or severe hemorrhagic and very foul-smelling diarrhea with severe enteritis and diarrhea (Desario et al., 2005; Pollock and Goyne, 1993). The most effective way of transmission of CPV-2 in dogs is direct and indirect faecal-oral transmission. It has been reported that dogs that survive the disease after acute infection continue to spread the virus even weeks or months later (Nandi and Kumar 2010). So much so that an infection can occur in a new puppy that has been in contact with the uninfected leash, bait, water containers and beds for a dog that has died and died. Although

clinical findings may be sufficient in the diagnosis of the disease, it should be taken into consideration that different viral pathogens such as coronavirus, adenovirus, morbillivirus, and rotavirus can cause diarrhea in dogs. Clinical diagnosis should be supported by laboratory results. Generally, accurate and rapid diagnosis can be made from the samples taken from the feces of dogs or by direct examination of the intestinal contents of the deceased animals. It is reported that the examination of blood samples in the late stages of the infection is beneficial for the diagnosis since CPV-related viremia is long-term (Decaro and Buonavoglia, 2012). CPV-2 is an important virus in terms of its rapid spread among dogs and causing infections that result in the death of many sick animals.

In this study, it was aimed to diagnose the canine parvovirus infection by the Polymerase Chain Reaction and to investigate the hematological, serum C-Reactive Protein (CRP) and immunoglobulin changes in sick dogs.

#### **MATERIALS AND METHODS**

For the diagnosis of Canine parvovirus infection by polymerase chain reaction (RT-PCR and PCR), fecal samples is useful for viral DNA extraction. Blood samples need to be investigating to evaluate the hematological and serological changes. PCR is a sensitive test that consists of denaturation, annealing and extension steps. In the denaturation stage, the double-stranded template DNA molecule is denatured by applying 90-95 degrees of high heat. In this step, DNA becomes from single-stranded to single-stranded. In the second stage, annealing, primers are attached to a single-stranded DNA molecule at temperatures between 37-60 degrees. In the extension phase, the primers that are bound on DNA chains at 72 degrees are replicated (extension / elongation) by the DNA polymerase enzyme (Taq DNA polymerase). After these processes, millions of DNA particles can be reproduced from a single DNA sequence (Sevindik and Abacı 2013). Smaller and smaller amounts of CPV particles can be detected with the PCR test compared to conventional methods. Although PCR diagnoses are specific, vaccine virus and field viruses can be differentiated and false positive results due to vaccination can be prevented (Macintire and Smith-Carr, 1997; Nandi and Kumar, 2010). Complete blood count

and comprehensive hematological examinations and biochemical parameters are not sufficiently specific to identify the cause of infection and differential diagnosis in canine viral enteritis, however, they can provide clinically important information to create a list of differential diagnoses, evaluate the patient's response to treatment, and suggest prognosis. (Goddard et al., 2008; Kalli et al., 2010). Therefore, to investigate the clinical and laboratory findings together for the differential diagnosis of CPV enteritis and to characterize the clinical, molecular, hematological and biochemical findings together in dogs, various methods should be evaluated together. Veterinary specific auto hematology and biochemistry analyzers, ELISA tests and molecular tests should be combined together with better evaluation of sick dogs. The extensive and comprehensive molecular, hematological and biochemical analyses available to use in veterinary medicine for the definitive diagnosis of CPV infections. Evaluation of acute phase proteins based on the magnitude of increases and decreases in serum concentrations is also a very useful method at the diagnosis of CPV. Follow up the changes in serum concentrations can be an important indicator of the prognosis of the disease in dogs with canine parvoviral enteritis. It is also reported that acute phase proteins is a strong mortality marker in dogs with CPV (Denham et al., 2007; Kocatürk et al., 2010, Pinero et al, 2018).

## RESULTS AND DISCUSSION

CPV-2, which has been accepted as one of the most common enteric pathogens in dogs, causes serious and widespread infections all over the world. The emergence of new antigenic types and the resistance of the virus to environmental conditions are accepted as factors contributing to the preservation of the enzootic state of this disease (Decaro et al 2007; Nandi et al 2013). Despite all the treatments applied, CPV-2 infections, which have been determined to have high mortality and morbidity rates, cause local hemorrhagic gastroenteritis damage as well as causing a systemic infection is known (Prittie 2004).

It has been reported that canine parvovirus infection has a significant effect on all canids, and the rate of incidence is higher, especially in puppies younger than 6 months old especially

with the lacking or insufficient colostrum intakes (Aktaş et al 2011; Sakulwira et al 2003).

CPV-2, which has been characterized by severe hemorrhagic gastroenteritis findings especially in domestic and wild young dogs since its discovery, is a factor that has the ability to renew itself and evolve to more virulent and resistant subspecies. The canine parvo virus, which is thought to cause serious problems in pet livestock in the near future, even though there is no evidence of zoonosis. According to the data announced by the World Bank, which is classified as "emerging viruses", which are mostly composed of factors of zoonotic potentials; (calculated economical costs is around 80 billion dollars between the years 1997-2009). It is foreseen that it should be among the factors that can cause large losses in the economy (Johnson 2014).

Numerous studies in different countries of the world (Pereira et al 2000; Sakulwira et al 2003; Ntafis et al 2010; Decaro et al 2011; Ahmed et al 2012) have demonstrated how important and widespread CPV-2 infections are. Real-time PCR technique was used in the studies since it is thought to be more sensitive than the classical PCR method. However, features such as the endemic status of the infection in the country and region where the sampling was carried out, the age of the sampled animals (for example, almost all the animals that Cavalli et al sampled were 1-3 months old) should also be considered as factors to be considered, and such features should be considered as all the tests used in the research should not be forgotten in determining the reliability.

There are limited reserches and data about the curent situation of the CPV infection in Turkey till this date. The first report was based on the examination of CPV infected dogs in 1980 and the determination in 1981 based on macroscopic and microscopic findings in 16 puppies (Berkin et al 1981). The PCR evaluation of CPV infection in 2002 in Turkey was based on a CPV case with mild symptoms of parvovirus enteritis in the presence of a 6-month old dog. (Özkul et al, 2002). Dik and Şimşek were conducted a study on comparing the use and effectiveness of different methods as PCR, ELISA and on-side lateral immunochromatography in the diagnosis of the disease (2017). Finally Tekelioğlu et al., were conducted a study on the diagnosis and

effective treatment of the disease, including innovative antiviral treatment protocols (2019). Studies indicated acute phase proteins concentrations analysis is more effective than leukocyte count and procalcitonin concentration as an inflammatory biomarker in canine parvovirus enteritis. However, although serum acute phase protein concentrations is associated with clinical diagnostic and treatment responses outcome in puppies with canine parvovirus enteritis, but it has not been proven an efficient indicator when used alone. As a result, it is thought that using different diagnostic methods together with the diagnosis and effective treatment of CPV disease is important for the early and correct diagnosis of the disease and it will be effective in controlling the disease in line with the reports of the researchers.

### CONCLUSIONS

The active ingredient of acute hemorrhagic enteritis and myocarditis, CPV-2 is one of the most important pathogenic viruses with high morbidity (100%) and frequent mortality rates of up to 10% in adult dogs and 91% in puppies. Over the years, the disease has become more complicated due to the appearance of a number of variants such as CPV-2a, CPV-2b and CPV-2c, and the participation of domestic and wild dogs. There are a number of different serological and molecular tests available for rapid, specific and accurate diagnosis of the disease. In addition, both live vaccines and inactivated vaccines are available to control the disease in animals. In addition, new generation vaccines, i.e. recombinant vaccines, peptide vaccine and DNA vaccine, are in different stages of the development and will allow better management of the disease in dogs. However, new generation vaccines are not licensed for use in field conditions yet. The presence of maternal antibodies often interacts with the live attenuated vaccine and active immunization, and although it follows the appropriate immunization regimen, there is always a sensitivity window. Finally, it should be applied to stray dogs and wild canids, not forgetting the new variants of CPV-2, with timely vaccinations in pet dogs, proper sanitation and disinfection practices for successful control of the disease.

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**DETECTION OF SOME PLANT-SPECIFIC RETROTRANSPOSONS IN GERZE CHICKEN BY IRAP-PCR  
METHOD**

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***Abstract***

*Retrotransposons are a class of mobile genetic elements, constituting part of genome in different ratios. In this study, we aimed to identify Nikita and Sukkula retrotransposons known as specific to barley (*Hordeum vulgare* L.) in chicken genome and also the relationships between these retrotransposons and mobile genetic elements found in chicken genome. For this purpose, genomic DNAs were isolated from one of the endemic breeds of Turkey, Gerze chicken also named as Hacı Kadi. Retrotransposon movements were investigated by using retrotransposon-based molecular marker, IRAP. Although polymorphism was not found in Sukkula movement, polymorphism ratios of Nikita retrotransposon were 0-60%. Moreover, in silico analyses also showed closely relationships among these two retrotransposons and other chicken mobile sequences. With the results of this study, poultry biology could be better understood, and a new perspective could provide to understand the evolutionary relationships between plants and animals.*

***Key words:*** Barley, IRAP-PCR, Sukkula, Nikita, Evolutionary relationships

## **LINEAR ASSESSMENT METHOD AND ITS IMPORTANCE IN DETERMINING THE DAIRY PROPERTIES IN GOATS**

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### **Abstract**

*In countries where goat breeding is developed, linear evaluation and regular yield control together with it contribute significantly to the correct determination of breeding animals. The fact that yield inspections outside of universities and research institutes in our country cannot be carried out regularly, especially in extensive and semi-intensive enterprises, brings out the external structure features in the selection of milk type goats. This can lead to some wrong decisions in herd management, especially in the selection and breeding of female breeds. As it is known, compliance with the direction of yield in animals, in a sense, helps to understand how it affects fertility and milk yield as well as having a solid composition. In other words, linear evaluation is critical to sustainable herd management. For this purpose, the purpose of the linear evaluation system in the selection of dairy goats gives dairy goats breeders more importance in the selection of breeders. The linear evaluation system includes 13 primary and one secondary features made by experts and used to determine the suitability for milk yield. Evaluation; Eight structural and functional areas that are evaluated as Excellent, Excellent, Good and Acceptable are examined. Only the linear property scores and the animal's latest score are part of the computerized linear assessment database used; other information is included to learn more about the animals evaluated to the herd owner. It will also assist in the development of the necessary database to determine the hereditary characteristics of the structural features and, consequently, the relationship between longevity and yield in dairy goats evaluated by the linear evaluation system. In this article; a general definition and importance of linear evaluation in dairy goats, information about primary and secondary features in the evaluation system, and finally information about general evaluation, strength, body capacity and breast system will be included.*

**Key words:** Dairy goat, Udder characteristics, Linear evaluation, Breeding selection

### **INTRODUCTION**

The selection of dairy goats in the past was based primarily on morphological traits, because no records were available for productive trait of the animals (Kızılay, 1983). In the past, morphological traits of animals played an important role in the identification of breeds with desirable characteristics. Productive life in dairy goats has been defined as total days in production (Castaneda-Bustos et al., 2017) or as stability (Valencia-Posadas et al., 2017). Milk production, reproduction, and health traits are among the factors that determine the longevity of an animal in a dairy herd (Solis-Gamit et al., 2019). Longevity values are determined by both voluntary and involuntary culling. Functional productive life, which is defined as the ability to

avoid involuntary culling (Mark, 2004), has been estimated by including first lactation milk, fat yield, protein yield, and final type score as covariates in statistical models for the genetic evaluation of longevity, to correct for the influence of voluntary culling (Valencia-Posadas et al., 2010). Nonetheless, the differences between the genetic parameters of productive life and functional productive life have been relatively small in goats (Castaneda-Bustos et al., 2014). In this case, the technicians usually recorded a large number of traits related to the morphology and to the type of the animal, with a significant increase in work and costs, but useless in breeding value prediction for important productive traits. Studies involving principal component analysis using morphological traits in



goats are scarce (Pesce et al., 2011). Thus, this study aimed to evaluate several variables recorded in different goat breeds by principal component analysis and select the most important ones accounting for total variation without loss of information.

The relation between longevity and type traits has been studied extensively in cattle, finding genetic correlations between type traits and longevity of  $-0.19$  to  $0.48$  (Scholtensa et al. 2018; Hermiz et al. 2004) and  $-0.34$  to  $0.74$  with stability (Manfredi et al., 2001), but this has not been studied in goats. In the case of type traits it is not enough to know the genetic correlations these may have with longevity, as in some studies (Torres-Vazquez et al., 2010) it has been observed that the relationship between longevity and type traits is not always linear, and a desirable optimum may be found at intermediate scores (McLaren et al., 2016). Their findings indicate that this type of study should consider the existence of nonlinear genetic relationships.

#### **DAIRY CHARACTERISTICS**

The term "linear" in a linear appraisal system refers to the fact that traits are rated on a linear scale that goes from one biological extreme for that trait to the other. The appraiser within the 50-point range that represents the biological range for the trait assigns scores for each primary and secondary trait (ADGA, 2012). Evaluation of linear traits is, except during training or in situations where verification is desired, based on observations by trained appraisers, rather than on actual measurements for each trait. As is true with dairy cattle linear systems, experienced appraisers achieve equal or greater accuracy and consistency while evaluation linear traits much more rapidly and less expensively using techniques based on observation compare to making actual measurements for every trait (Abu et al., 2013). The biggest problem associated with making actual measurements is the difficulty in trying to restrain an animal in a natural position long enough to make an accurate measurement, especially when the differences being measured are small. The following guidance is provided to help the herd owner understand the evaluation system for the all-primary traits, and one secondary trait that is included in the linear appraisal system (Ferreira et al., 2013). The appraiser evaluates these traits without regard

for age, management, or environment, or stage of lactation. The primary traits are traits that are believed to have economic importance and enough variation to provide a basis for selection when the data is summarized by the sire. The secondary trait has been included in order to gather research data for further evaluation of the trait's economic and genetic importance.

Three general criteria were used in the selection of the linear traits to be included in the linear appraisal system. The trait must have some economic value, either in terms of increased longevity, which reduces culling rate, or increased production.

The trait must be heritable (genetically controlled) enough so that progress or improvement can be made at an acceptable rate through the selection of sires. Traits that are not at least moderately heritable are more effectively handled through herd management practices and are not suitable for inclusion in a linear appraisal system (Berry et al., 2005). Generally, the heritability of 0.15 or higher is accepted as indicating at least moderate heritability of a trait. The heritability information that is available on dairy goats pertains mostly to production factors. Very little information exists on the heritability of structural traits in dairy goats. The heritability's used in the selection of traits for linear appraisal system were based on four years of dairy cattle linear data. Although the absolute heritability of traits is not known or expected to be the same for dairy goats, the relative indications of heritability of the various traits of interest should be similar (Janssens et al., 2004). One of the potential benefits of the linear appraisal system is the determination of the actual heritability of type traits for dairy goats when sufficient data has been gathered to perform the calculations. It must be possible to assign a value to the trait with acceptable repeatability among appraisers. This means that it must be possible to define the trait, all its components, and the associated evaluation criteria precisely enough that appraisers can evaluate the trait with acceptable repeatability.

#### **GENERAL APPEARANCE**

In evaluation, the general appearance of an animal, the appraiser considers the total structure of the goat, including the head, shoulder blades, back, loin, rump, legs, and feet (Koyuncu et al., 2016). Correctness in both size

and conformation is indicated by an attractive appearance that reveals vigour, a harmonious blending and correlation of parts, an impressive style and attractive carriage, and a graceful and powerful walk. Strength, upstandingness, vigor, stretch, sound feet and legs, a level topline, a wide and level rump, and smooth blending are necessary for an animal to be considered excellent or very good. Animals lacking these structural characteristics would be considered fair or poor, which animals intermediate in these characteristics, would be considered good plus or acceptable (Karadag et al., 2018).

### **Strength**

To determine a rating for dairy strength, the appraiser looks at the bone structure (including the neck, withers, ribs, and thighs), flanks, angularity, openness, degree of fleshing, animation, and skin and hair (Sadeghi et al., 2013).

A goat must have sharp lines, be angular and free from excess fleshing, have a strong but refined bone structure; a long and lean neck that blends smoothly into the shoulders' ribs spaced far apart to give openness to the body; incurving thighs are free from excess flesh to be rated as excellent or very good (Kouri et al., 2019). Animals that are intermediate for these characteristics are rated good plus or acceptable, while animals that have round, heavy bones and are coarse are rated fair or poor.

### **BODY CAPACITY**

The total volume of a goat concern to correct shape is considered in evaluating body capacity. A large, strong, vigorous animal generally can consume and utilize larger quantities of feed (Park and Haenlein, 2010). Goats that are wide and deep in proportion to their stature. It indicated by a deep, strongly supported barrel; ribs that are wide apart and well sprung and tend to increase in width and depth toward the rear barrel; a large heart girth resulting from long, well-sprung fore ribs; and a wide chest floor between the front legs and fullness at the point of elbow are rated excellent or very good. Goats that are not seriously deficient in body capacity, but lack some in the characteristics listed above, particularly if they are lacking in spring of rib, are rated good plus or acceptable (Yakubu et al., 2010). Goats that show definite deficiencies in body capacity, such as narrow, pinched heart

girth; overall frailness; short ribs, resulting in a shallow animal; or ribs that are close together, resulting in a short, cramped body, may be rated fair or poor.

### **MAMMARY SYSTEM**

In evaluating the mammary system, the appraiser considers capacity and shape, rear and fore udder attachments, texture, and teats. A capacious, strongly attached, well-carried udder of good quality, indicating heavy production and a long period of usefulness, is preferred (Onder et al., 2011). Udders with the following characteristics are rated excellent or very good; long, wide, strongly attached, and capacious udder that extends well forward, with a high, wide, rear udder attachment. Halves are evenly balanced and symmetrical; a fore udder that is carried well forward, is tightly attached, and blends smoothly into the body (Orman et al., 2011). A texture is soft, pliable, elastic, and free of scar tissue, so that the udder collapses well after milking; and teats that are uniform, of convenient length and size, cylindrical in shape, free from obstructions, set square and properly placed, and easy to milk. Goats with short, bulgy, or loose fore udders; low, narrow, loose, or pinched rear udders and udders that are tilted or pendulous are rated fair or poor. Udders intermediate in strength of attachment, balance, capacity, cleavage, and texture are rated good plus or acceptable (Upadhyay et al., 2014).

The traits suggested for disposal in this work have shown significant simple linear correlation with the others, in other words, they are redundant; on the other hand, the selected variables showed lower correlation with each other. In general, the correlations showed to be moderate; the variables "height rump" with "height at the withers" stood out for showing a highly positive correlation. Hence, the assessment of growth traits by height rump is already sufficient, and so, the indication of height at the withers could be discarded. Width rump and udder traits showed an average negative correlation, indicating that the smaller the limbs aperture is, the greater is the difficulty of the animal to contain in it the mammary system (Aktas et al., 2012). Variables body capacity and dairy type expressed a high and positive correlation with each other, and with variable final score, which represents an important result, because as the grade of the animal is increased,

its performance regarding its productive function improves. Udder shows an average positive correlation with dairy type and rear ligament. Okpeku et al. (2011) found high correlations for some traits in goats in southern Nigeria. The strong link between body capacity and the set that is part of dairy type is an indication that an animal that has a good score performance will therefore be satisfactory.

#### **RELATIONSHIP BETWEEN CHARACTERISTICS**

The significant decrease of goats' body weight observed during the early lactation was expected, since after parturition the appetite of goats' decreases, so it becomes very difficult to satisfy their high-energy requirements of lactation. This weight decreasing was previously reported in Nigerian Red Sokoto goat (Djibrillou, Pandey, Gouro, Verhulst, 1998; Nwachukwu et al., 2012), local Burundian goat and ewe (Mbahyaga, Mandiki, Bister, Paquay, 1998) and Sudanese Nubian goat (Gubartalla, Abu Nikhaila and EL Khidir, 2002). The studied goats had a small udder with a floor well above the hocks level, which was not favorable to high milk production but reflected the adaptation of the mammary conformation of Bedouin goat to desert rangelands, with a predominance of shrubs increasing the risk of injury and contamination of deeper udders close to the ground. However, an udder floor at the same level of hocks or slightly above is more adapted for high-yielding goats maintaining under intensive management system, as reported in Saanen and Alpine goats (Mucha et al., 2015) and Fiurina goat (Cornale et al., 2014). The udder suspensory system was of a medium strength; the median ligament was well marked, the ratio between rear attachment width and udder depth was balanced but the fore attachment was loose. Assessment of teats characteristics did not show a form entirely favorable to milking. Usually, the teats inclination was vertical or cranial which was desirable, but divergent orientation was predominant, well-shaped teats with a medium size and cylindrical form were rare and supernumerary teats were frequent (Cedden et al., 2002). Udders with lower cistern depth tended to have thicker teats, and wide udders with deeper cisterns tended to have vertical teats rather than cranial ones. The significant correlation between the mean daily milk yield and each of goats' body weight withers height,

rump height, udder width and udder circumference emphasizes the importance of these traits both in predicting the milk yield in Bedouin goat and as selection markers for future improvement of its production (Federica et al., 2016). On the other hand, subjective linear udder appraisals were not significantly correlated with milk yield; thus, objective udder measurements were more appropriate for assessing milk production in this breed. Moreover, despite the ease of application of linear udder score method, it requires a trained technician, so breeders cannot adopt it as a routine method. The correlation of daily milk yield with udder width and circumference was in line with results of Amao, Osinowo, Onwuka, Abiola, and Dipeolu (2003) and Keskin, Kor, Karaca, and Mirtagioglu (2005) in Damascus, West African Dwarf and Akkeci goats and results of Emediato, Siqueira, Stradiotto, and Maestá. Fernandes (2008) and All udder morphological traits had positive and significant ( $P < 0.05$ ) correlation with doe's body weight at different intervals. Similar trend was also observed with the teat traits. From the results, it may be concluded that higher the body weight lower is the teat height from ground. This can be taken as judging criteria while selecting the animals. It is concluded that mammary system of this local goat is comparable with any other dual-purpose Indian goat breed. Milk production in this local goat was affected with udder parameters value. Similarly, it can be concluded that higher the litter size, higher the udder and teat parameters value (Solis-Ramirez et al., 2018).

#### **CONCLUSIONS**

Genetic improvement of type traits by selection is a viable option, as, in general, their heritability estimates allow for good response to selection. Final score, rump width, and fore udder attachment are highly correlated with productive life and functional productive life, as well udder depth in the case of functional productive life; thus, they may be used as selection criteria to indirectly increase productive life in dairy goats. Nonlinear relationships exist between many type traits and productive life. An indirect increase in longevity is possible by selecting goats with extreme scores for fore udder attachment and suspensory ligament. Dairyiness and udder depth have optimal intermediates for functional productive life; thus, the inclusion of these traits

in a genetic improvement program must consider this information in selection programs for increasing productive life in dairy goats.

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## GOOD RAM MANAGEMENT PRACTICES FOR LAMBING CONTROL

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### **Abstract**

*The idea is to share useful information and give the breeder a deeper knowledge of the potential of his flock. In the Mediterranean basin the latitude influences the reproductive cycle of the animals in such a way that they are cyclic at least 10 months a year with only two months of sexual rest (anoestrus). The resumption of reproductive activity is initiated by the male whose re-introduction into the flock, after an isolation period of at least 8 weeks, causes resumption of the ovarian cycle in adult sheep. Indeed in the fleece there are pheromones, chemical substances that stimulate the resumption of ovarian activity. This phenomenon is called the ram effect the physiological response of the sheep to a sudden re-introduction of the ram into the flock at the beginning of the breeding season. However the resumption of ovarian activity is also favored by several factors such: the number of males introduced, the age of the females, the physiological conditions of the flock and the time since last lambing (at least 4 months). Preparation of rams of proven fertility must start at least 50-60 days before mating, with particular attention to the general health of the animal, good nutrition plan and check of reproductive apparatus. If the farmer is not interested in knowing the paternity of newborn lambs he can take advantage of the male effect using rams without an apron; at least 4-6 males per 100 females is required to ensure a good conception rate. After 17-24 days from ram introduction the female will start to come on heat and be mated. If the farmer is interested on knowing the paternity of the new born lamb the ram effect need to be done with the help of ram with an apron and then the female conducted to the desired male, alternatively can be organized small breeding groups with one ram per 25 females. In a good mating system the breeder carefully plans the calendar of heats with a goal of lambing at the desired times. Lambing season can be established in advance via precise timing of the introduction of the males into the flock. To produce milk or meat all year around, it will be necessary to have at least three breeding groups with different birth dates. This program may be of particular interest to farms with irrigation systems.*

**Key words:** Sheep, Ram effect, Reproduction

## MILK SOMATIC CELL COUNT IN ANATOLIAN BUFFALOES

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### **Abstract**

*Somatic cell count (SCC) is commonly used as a reliable parameter to decide raw milk quality and detect any abnormality on udder health status of mammalian animals. The present study was aimed to reveal the SCC levels of Anatolian buffaloes reared in Turkey conditions. To estimate SCC means, ten scientific manuscripts published in national or international bases between 2008 and 2017 years were evaluated. The overall mean of SCC was calculated to be  $231 \times 10^3$  cells/ml and this level was found as suitable by Turkish Food Codex (TFC) declarations. Farm (26.66%) and stage of lactation (20.0%) had the highest effect on SCC as non-genetic factors. Finally, eliminating all factors affecting SCC is suggested to herd owners to prevent milk losses and boost quality of raw milk in Anatolian buffalo farms.*

**Key words:** Anatolian buffalo, Environmental factor, Milk production, Raw milk, Somatic cell count

### **INTRODUCTION**

Ecologic animal breeding has an elevated trend to achieve organic and healthy food products. Green lands, rivers, sufficient pasture and clear weather play important role on this system. Water buffalo farming is the principal model that comes to mind firstly. Today, approximately 200 million water buffaloes have been raised in different parts of the world. At this context, 185 thousand Anatolian buffaloes have been reared those especially exist as public in Turkey.

Anatolian buffalo is originated from river buffaloes those one of the important type of the buffaloes along with swamp buffaloes. Moreover, this breed is one of the sub-varieties of the Mediterranean buffaloes. The main farming purposes of the breed are beef and milk, and their products. Producing high quality and quantity raw milk is regarded by many dairy owners. Decision for milk quality is maintained by some laboratory tests and somatic cell counting is one of the reliable analyses methods those used for this purpose. Somatic cell count (SCC) is commonly used as a reliable parameter to decide raw milk quality and detect any abnormality on udder health status of mammalian animals. SCC of milk is a well known

indicator reflecting mammary health and milk quality.

For cattle milk,  $400 \times 10^3$  cells per ml is the highest limit for human consumption of the milk by EU directives. In Turkey, Turkish Food Codex (TFC) declared this limit as  $500 \times 10^3$  cells/ml. As seen, achieving high quality raw milk is based on its ingredients especially SCC amount. Revealing SCC of dairy animals in country base may reveal the general status of the quality level of the raw milk that used as consumption.

The present study was aimed to reveal the SCC levels of Anatolian buffaloes reared in Turkey conditions.

### **MATERIALS AND METHODS**

To estimate SCC means, ten scientific manuscripts published in national or international journals between 2008 and 2017 years were evaluated. While SCC levels of the conducted studies were noted, the non-genetic factors affecting SCC were assessed by frequency.

### **RESULTS AND DISCUSSION**

SCC levels of the papers published between 2008 and 2017 are presented in Table 1.



**Table 1.** Some study results on SCC in Turkey

Researcher	Location	SCC (cells/ml)
Özenç et al., 2008	Afyon	107000
Atasever et al., 2011	Samsun	829359
Şekerden, 2011	Hatay	90800
Şahin et al., 2012	Tokat	166000
Atasever & Erdem, 2014	Samsun	436978
Atasever et al., 2015	Samsun	148583
Erdem et al., 2015	Samsun	100332
Soysal et al., 2015	Istanbul	207000
Şahin et al., 2016	Tokat	134731
Şahin et al., 2017	Tokat	90701

As seen, many studies were conducted in the Middle Anatolian region.

The overall mean of SCC of those was calculated to be  $SCC = 231 \times 10^3$  cells/ml. In EU directives, the upper limit of bovine raw milk is  $400 \times 10^3$  cells/ml and there is no a schedule for the milk of buffaloes. Also, this limit is  $500 \times 10^3$  cells/ml by TFC. At this point, calculated mean was about half part of the declarations of both establishments. While the SCC means were evaluated by a threshold, 70% of the results were lower than  $200 \times 10^3$  cells/ml. From this view, SCC levels of Anatolian buffalo herds reared different parts of Turkey were found to be suitable limits.

**Table 2.** Non-genetic factors affecting SCC

Factors	Frequency (%)
Test day	6.66
Season of calving	6.66
Region	6.66
Milking method	6.66
Parity	13.33
Season	13.33
Stage of lactation	20.0
Farm	26.66

In this study, investigated papers were examined by the non-genetic factors affecting SCC of animals. According to frequency table (Table 2), stage of lactation (20.0%) and farm (26.66%) were the most effective environmental factors. Moreover, the percent of the region effect was estimated to be 6.66% here. When this rate is combined with the farm, the "enterprise (location) effect" had 33.32%. Thus, SCC had highly affected by enterprise difference in the evaluated papers.

## CONCLUSIONS

In this article, change of milk SCC in Anatolian buffaloes were discussed benefiting manuscripts published between 2008 and 2017 years. It was noted that SCC levels of the most studies could be assumed in the suitable thresholds. Also, the mean SCC was found to be half portion according to EU and TFC declarations for cattle milk. Enterprise and stage of lactation were determined as the main environmental factors for SCC.

Finally, it was suggested to herd owners that elimination of all non-genetic factors affecting SCC throughout the production period will enhance the quality of raw milk in Anatolian buffaloes.

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**STUDY OF THE INFLUENCE OF DIET ON BLOOD PARAMETERS AND THE COMPOSITION OF RAW  
CAMEL MILK RAISED IN THE SOUTH-EAST OF ALGERIA**

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***Abstract***

*The main objective of this study is the characterization of the biochemical profile of camels and the effect of rearing mode (diet) on different blood parameters and milk composition (lactose, calcium, fat and total protein). The study was conducted on "Sahraoui" camels aged between 4 and 8 years old and from two dairy farms (extensive and semi-extensive). In this study samples of raw camel milk are collected from both farms in the period from February to May. The blood samples were used to determine certain blood parameters (glucose, triglycerides, cholesterol, total proteins, urea and calcium) using spectrophotometric methods. The fat and calcium levels determined by complex metric and spectrophotometric methods showed a significant variation in the calcium content of the raw camel milk of the two farms studied ( $1425 \text{ mg / l} \pm 152.6$  against  $1177 \text{ mg / l} \pm 47.1$ ). As well as for the fat composition ( $27.5 \text{ g / l} \pm 3.32$ ) for camel milk from extensive rearing against ( $34 \text{ g / l} \pm 5.50$ ) for semi-extensive camel milk. Lactose and protein content did not show significant variation between the two farms. The determination of the blood parameters has shown that the effect of the breeding mode is highly significant for proteinemia with values ranging from  $71.52 \text{ g / l} \pm 12.54$  for camels from extensive rearing and  $47.10 \text{ g / l} \pm 15.82$  for those from the other breeding. On the other hand, the rest of the blood parameters showed no significant variation.*

***Key words:*** Camels, Rearing mode, Raw milk, Blood parameters, Biochemical characteristics

**STUDY OF INTESTINAL PARASITES AFFECTING DOE RABBIT REARED UNDER LOCAL ALGERIAN CONDITIONS**

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**Abstract**

*The purpose of this research work is to study the impact of rabbit breeding and animal type on the presence and frequency of intestinal parasites in doe rabbits and to examine the variability of these frequencies between individuals. Two farms were chosen, the first one is of the rational breeding (TIKOBAINÉ farm) and the second one is of the farmbreeding (TIZI-RACHÉD farm). Parasitological diagnosis, which consisted of coprological analyses, was carried out at the laboratory of the Mouloud Mammeri University of Tizi-Ouzou. It revealed and quantified the presence of helminth eggs and coccidia in the droppings and intestinal contents of the doe rabbit. A total of three species of parasites were identified, namely: *Passalurus ambiguus*, *Eimeria* sp and *Strongyloides* sp. Their frequencies vary according to the type of breeding; type of animals and according to the sampling. Fattening rabbits are reported to be the most infested compared to breeding doe rabbits. Finally, we can conclude that the *Oryctolagus cuniculus* doe rabbit is a real reservoir of many parasitic species.*

**Key words:** *Intestinal parasites, Coprology, *Oryctolagus cuniculus*, Rational breeding*

## STUDY OF STRUCTURAL CHANGES OF THE MAMMARY GLAND IN THE DOE RABBIT DURING PREGNANCY STAGE

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### **Abstract**

*The objective of our study is to determine mammary gland changes in doe rabbits during pregnancy, in comparison with empty rabbits (controls). A Histomorphometric study was carried out in rabbits of synthetic SS strain, issued from a cross between the local population and the French strain INRA2666, selected for its prolificity; however, productivity at weaning was very low, especially in the summer period. These low productivity levels are linked to high mortality during the lactation phase. The latter is carried out by the mammary gland and its development, which conditions the survival of the bunnies during this phase. Six 4-month-old female doe rabbits were divided into two batches; one batch of three empty control rabbits and one batch of three pregnant doe rabbits at 24 days of age. Females were weighed and sacrificed through decapitation. Mammary tissue was collected, weighted and fixed with 10% formol. The tissue is then prepared for the histological study with standard topographic coloration with Haematoxylin-Eosin and for the morphometric study, performed using AxioVision software and targeting the measurement of the different structures of the mammary tissue. The preliminary results obtained show that in the pregnant doe rabbit compared to the empty doe rabbit; the difference is highly significant ( $P < 0.0001$ ) for the diameter and surface area of the acini, the surface area of the acini lumen and the nucleocytoplasmic ratio, a highly significative difference ( $p < 0.01$ ) for the surface area of the acini epithelium and a non-significant difference ( $p > 0.05$ ) for the cell height. Our results confirmed that the mammary gland undergoes structural modifications allowing the female to carry out the lactation activity on which her litter's survival depends.*

**Key words:** Mammary gland, Doe rabbit, Synthetic strain, Histomorphometry

**STUDY OF HISTOMORPHOMETRICAL CHANGES IN THE OVARY AND THE UTERINE STRUCTURES OF SYNTHETIC STRAIN DOE RABBITS IN RELATION TO PREGNANCY STAGE**

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**Abstract**

*The aim of our study was to study the histological and morphometric changes of ovarian and uterine structures in does rabbits according to their physiological state (empty or pregnant) in order to characterize their reproductive performance (fertility, mortality). A total of 6 rabbits of the synthetic strain (SS) from the crossing of the local population (L) and the INRA2666/France strain, 4 months old and coming from the rabbit breeding centre of Tizirt (Tizi-Ouzou) were divided into 2 lots in function of the physiological activity of the rabbits: empty rabbits and rabbits on the 24th day of gestation, the latter were artificially inseminated (IA). The females were weighed and then sacrificed by decapitation. The ovaries and uterine horns were removed, weighed and fixed with 10% formaldehyde. They were then treated for histological study with standard topographic staining with Haematoxylin-Eosin. A morphometric study was carried out using the AxioVision software, targeting the measurement of the different structures of the organs removed (ovarian follicles, myometrium, endometrium and endometrial glands). Results of the histomorphometric study, measurements of follicular and oocyte components reveal some differences between the two lots. Some microscopic parameters of the uterus reveal very significant differences ( $P < 0.01$ ), an increase in myometrial size and endometrial gland diameter in pregnant doe rabbits. There was an increase in epithelial proliferation and shape of crypts, an increase in luminal folding complexity, an increase in the glandular abundance of the uterine endometrium and a richly uterine vascularized lace. Some microscopic parameters of the endometrial glands, such as epithelial surface area, gland surface area and luminal surface area showed a very highly significant ( $P < 0.0001$ ), increase in pregnant doe. From the cellular point of view, the nucleocytoplasmic ratio of endometrial gland epithelial cells increased significantly in empty doe rabbits. Our results show that female fertility, fetal maintenance and litter survival depend on the proper functioning of the ovarian and uterine structures.*

**Key words:** Synthetic strain, Ovary, Uterus, Histomorphometry

**STUDY OF CHANGES IN HISTOMORPHOMETRY OF THE EPIDIDYMAL STRUCTURES OF RABBIT  
BUCKS IN RELATION WITH THEIR AGE**

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**Abstract**

*Rabbit farming in Algeria is arousing great interest by the private and state sectors. Our work is part of the project of knowledge of the physiological specificities of local populations and its objective is the characterization of the postnatal development of the epididymis of rabbits of the synthetic strain resulting from the crossing of the local population and the strain INRA2666 / France, exploited in Kabylia. The animals used from the Tigzirt rabbit breeding center (Tizi-Ouzou) are divided into 3 batches of: one month, three months and six months. After weighing the body weight, testis and epididymis, the organs are fixed with 10% formalin and then treated for a histo-morphometric study. The morphometric parameters of the epididymal ducts were evaluated using the AxioVision-4.8 software. The preliminary results obtained show a progressive increase in body weight and the weight of the organs aroused during development. Histological study reveals a cubic type epithelium at the age of one month, formed of main cells and becomes prismatic and pseudostratified at 3 months, where it is enriched in basal cells, with the appearance of stereocilia, at the level of the cells main, testifying to the development of the epididymal epithelium, but the lumen devoid of spermatozoa. At six months of age, the epididymal lumen contains a large amount of sperm, indicating the significant activity of spermatogenesis. Morphometric measurements of the epididymis show a significant increase for all the parameters measured (diameter, area, lumen of the epididymis) in relation with their age (1, 3, 6 months). In addition, the height of the main cells of the epididymis increases significantly from postnatal age of one month to 3 months, this dimension maintained at 6 months postnatal age. All the histological and morphometric results obtained in the synthetic strain are comparable to the results reported in rabbits from the white population also, exploited in farms in Algeria.*

**Key words:** *Epididymis, Synthetic strain, Histology, Morphometry*

**BIOCHEMICAL PARAMETERS OF THE KYRGYZ YAK POPULATION KEPT ON HIGH ALTITUDE  
PASTURE IN ISYK-KUL, KYRGYZSTAN**

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**Abstract**

*The semi-wild yak (*B. grunniens*) remains a poorly studied species among other farm animals, since its breeding is limited mainly to high-mountain hard-to-reach territories of the Asian continent. With minimal labor costs and funds for their maintenance, yaks produce varied, high-quality products. The Kyrgyz yak population has significant differences from other populations in their biological and economic characteristics. This work was performed in the biochemistry laboratory of Biology Department of the Faculty of Science at Kyrgyz-Turkish Manas University. The experimental yaks were kept in the Ak-Shiyrak village (3200 m above sea level), Jeti-Oguz area of the Issyk-Kul region. The studies were carried out simultaneously on 20 clinically healthy yaks both sexes at the age of 2-3 years. Blood for examination was taken from the jugular vein before morning grazing. The following biochemical parameters of serum were evaluated by a PERFECT MINDRAY 400 automated biochemical analyzer: the activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), the levels of total bilirubin, direct and indirect bilirubin, total protein, albumin and globulin. The experiment results were statistically processed using biometric analysis methods taking into account the Student's t-test. The aim of this work was to study the activity of tissue enzymes, bilirubin and protein fractions in the blood serum of the Kyrgyz yak population of the high altitude Ak-Shiyrak zone. In the conditions of the high-mountain Ak-Shiyrak pasture (3200 m above sea level) in the Issyk-Kul region the concentrations of AST and ALT enzymes in the blood serum of yaks (*B. grunniens*) were  $122.25 \pm 4.02$  U/L and  $43.9 \pm 1.12$  U/L respectively. When determining the level of total protein and its fractions in experimental animals, the following picture was revealed: the total protein in the blood serum of the yaks was  $6.70 \pm 0.08$  g/dL, the albumin concentration was  $3.96 \pm 0.06$  g/dL. Statistical analysis of the globulin level in the blood serum of yaks showed  $2.72 \pm 0.10$  g/dL. In the chain of biochemical reactions, indirect bilirubin forms first, which in the blood serum of animals was  $0.46 \pm 0.02$  mg/dL. Under the influence of an enzyme indirect bilirubin transforms into direct bilirubin in the liver structure, namely in hepatocytes, and its level was equal to  $0.23 \pm 0.03$  mg/dL. The total bilirubin in the Kyrgyz yak population serum was  $0.68 \pm 0.03$  mg/dL. It has been established that the high-altitude condition is the reason for the formation of adaptive qualities in yaks and indicates the presence of a high level of metabolic processes, which is manifested by an increased content of tissue enzymes, total bilirubin and its fractions within the physiological norm compared to published data.*

**Key words:** Biochemical parameters, The Kyrgyz yak population, High altitude pasture zone



**EFFECT OF ARUM KOROLKOWII REGEL TUBER TINCTURE ON THE HEMATOLOGICAL AND SERUM BIOCHEMICAL PARAMETERS, MORPHOLOGY OF THE TESTIS AND LIVER IN THE MALE GUINEA PIGS (CAVIA PORCELLUS LINNAEUS, 1758)**

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**Abstract**

*One of the medicinal plants often used in folk medicine of Central Asia since ancient times, which has not lost its relevance even nowadays, is Arum korolkowii Regel. The vernacular name of this herb is kuchala. The plant is very poisonous and in folk medicine, small doses of tuber tincture are used as medicinal raw material to increase human potency. This scientific and practical work was carried out using modern ethological, hematological, biochemical, statistical and morphological methods. The aim of the experiment was to study the effect of 10% tincture of Arum korolkowii Regel tuber in 70% ethanol on the hematological, biochemical, and ethological parameters and on the morphology of individual organs (blood cells, liver, testis) of guinea pigs (Cavia porcellus Linnaeus, 1758). For the study, 22 Abyssinian guinea pigs (Cavia porcellus Linnaeus, 1758), which were kept under recommended conditions in the laboratory where the experimental work was organized, were taken. During the study, experimental animals were orally given 0.15 µl of ethyl alcohol tinctures, and the control group - 0.15 µl of water for 30 days. Also characteristics of 10% tincture from dried and ground Arum korolkowii Regel root tuber in 70% ethanol were obtained. According to the results of ethological observations relative activity and increased appetite of experimental animals were noted. Individual hematological parameters of guinea pigs significantly differed between the control and experimental groups of animals. Thus, the number of neutrophils in experimental guinea pigs was significantly lower than in control animals ( $P < 0.001$ ). The number of lymphocytes, on the contrary, was significantly higher in experimental individuals ( $P < 0.001$ ). The percentage of hematocrit and hemoglobin was higher in experimental male guinea pigs than in control individuals. Biochemical analysis of the blood serum of experimental animals showed a statistically significant increase in the concentrations of testosterone and hemoglobin, as well as reduced concentrations of ALT and AST (all  $P < 0.001$ ). However, the methods of anatomical and histological studies did not reveal significant changes in the liver, heart and testis of animals.*

**Key words:** Arum korolkowii Regel tuber, 10% ethyl alcohol tincture, Serum biochemistry, Testosterone, Guinea pig

**FINANCIAL BENEFITS ANALYSIS OF BROILER CHICKEN FARM OPERATORS IN DELTA STATE  
NIGERIA**

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***Abstract***

*This study examined financial benefits of broiler chicken farm operators in Delta State, Nigeria. Data were collected from 168 randomly selected broiler farm operators with the aid of structured questionnaire and analyzed using descriptive statistics, cost and return analysis and regression analysis. The result showed that 58.3% of the farmers were between age brackets of 31-40 years with 82% of them male and 76.2% were married with 67.9% having household size of 6-10 persons. About 96.4% of the broiler chicken farmers were literate with 66.7% of them having between 11-15 years of experience. The average net profit, gross margin and average net profit per bird as well as return per naira invested were N 928720, N1034606, N 1254.82 and N 2.89 respectively. This implies that broiler production is profitable. Poultry droppings/litters were also essential by-products that generate enough income to the broiler chicken farmers. The result of the regression model showed that cost of chicks, cost of feed, cost drugs and medication cost positively influenced profit while labour cost, water supply cost, transportation cost and mortality rate had inverse relationship with profit. High cost of feeds, bird mortality and inadequate capital were the major constraints associated with broiler chicken production. It is recommended that if these aforementioned constraints are tackled will help boost broiler chicken production in the study area.*

***Key words:*** Broiler chicken, Production, Profit, Farmers, Gross margin analysis

## **SOCIOECONOMIC IMPACT OF HIGH PATHOGENIC AVIAN INFLUENZA ON POULTRY FARMERS IN RIVERS STATE**

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### **Abstract**

*The socio-economic impact of the 2015 HPAI outbreak on poultry farmers in Rivers State was evaluated. 44 farmers (farms) across three LGAs viz. Obio/Akpor (81.8%), Ikwerre (11.4%) and PHC (6.8%) were affected, 23 (52.3%) of them being women and 21 (47.7%) men. 19/44 (43%) of the farmers suffered heartbreak and never went back to poultry farming. 90.9% of the poultry population affected were chicken while 9.1% were turkeys. 87,485 layers were culled and ₦47,808,250.00 compensation paid at ₦ 546 per bird instead of ₦ 104,982,000 at the prevailing market price of ₦1200 per spent layer at the time. 8,478 broilers were culled and compensation of ₦ 3,190,950.00 paid at ₦ 376 per bird instead of ₦ 12,717,000 at average price of ₦ 1500 per average sized broiler at the time. 65 cockerels were culled and ₦ 47,625.00 was paid as compensation at ₦ 733 per bird as instead of ₦ 97,500 at ₦ 1500 per mature cockerel at the time. 670 turkeys were culled and ₦ 467,500 was paid as compensation at ₦ 698 per bird instead of ₦ 6,700,000 at ₦ 10,000 per average sized turkey at the time. Egg destroyed were 27, 900 and ₦ 209,250.00 was paid at ₦ 7.5 per egg instead of ₦ 697,500 at ₦ 25 per egg wholesale price at the time. It took 2 years, from 2015 to 2017 before compensation was paid. The number of dead layers before report was made was 23,565, broilers were 1,480, and turkeys were 450. These numbers were not compensated. Total compensation paid by FGN to the farmers was ₦ 52, 723,575.00 instead of ₦ 125,194,000 (the prevailing market price at the time). In monetary terms, the farmers lost ₦ 73,470,425 and ₦ 9,547,800 from the dead layers, broilers and turkeys which were not compensated for plus the uncalculated amount spent on drugs. In spite of the Federal Government bearing the monetary cost in terms of payment of compensation to farmers, the private sector (farmers) suffered more economic losses.*

**Key words:** HPAI, Socio-economic, Impact, Poultry, Farmers, Compensation, Heartbreak

## SILKWORM MODEL IN PHARMACOLOGICAL AND TOXICOLOGICAL STUDIES

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### **Abstract**

*For long and healthy life, new drugs, medical services, and care must be developed against diseases. Laboratory animals are used in pharmacology and toxicology studies. The use of laboratory animals causes serious problems such as animal welfare and cost. Cell culture, which constitutes the first step of drug development, is used to evaluate the safety and effectiveness of drugs; however, it can cause failure due to unpredictable liver toxicity and bioavailability problems. Invertebrates are used to determine the desired effect in the early stages of drug development. Silkworm, an invertebrate, is one of the best models to represent genetic, biochemical studies due to its complex metabolism, large body, the abundance of mutants. Silkworms have been used in many studies on pathogenic microorganisms in the world. In this review, the information will be given on the use of silkworms as a pharmacological and toxicological animal model.*

**Keywords:** *Silkworm, Pharmacology, Toxicology, Experimental Animal*

### **INTRODUCTION**

Silkworm *Bombyx mori* has been tamed to produce silk for nearly 5000 years. Due to its complex metabolism, large body, the abundance of mutants, silkworm in the *Lepidoptera* team is one of the best models to represent genetic and biochemical studies (Nagaraju; Goldsmith, 2002; Goldsmith et al., 2005). Silkworm's ' egg, larvae, pupae, butterfly forms, and their products' are used as animal drug sources. Amino acids, adipokinetic hormone, beta N-acetylglucosaminidase, sex pheromone bombykol, chymotrypsin inhibitors, etc., found in the form of larvae. The substances are used in the treatment of trigeminal neuralgia, vocal nodules and polyps, facial paralysis and pain. Substances such as vitamins B1, B2 and E, diapause hormone, proteins and amino acids in the form of pupa fall into the structure of antibacterial and antihistamine drug preparations.

The male butterfly form is used for sterility treatment. Extract of silkworm feces contains pectin, chlorophyll, carotene, phytol, and triacontanol, solanesol, etc. and is used in various diseases such as leukocytopenia, acute

pancreatitis, hepatitis, chronic nephritis, stomach ailments; In addition, these substances have the effect of reducing cholesterol and blood sugar. Since silkworm products contain stearic, palmitic, linoleic acids, they are used in pharmaceutical preparations and as food additives (Singh and Jayasomu, 2002). For people who want to live a long and healthy life, new drugs, medical services and care must be developed against diseases. Laboratory animals (mice, rats, rabbits) are used in medicine, oncology, toxicology, mycology, tissue and organ culture, immunology and reproductive studies (Sarıözkan, 2005). In order to evaluate the results correctly in the pharmaceutical and food production stages, the sacrifice of many animals such as mice and rats; it causes serious problems such as animal welfare and cost.

In the text of Russell and Burch's Principles of Human Experiment Technique in 1959, it was stated that there should be alternative methods of reduction, replacement and refinement in laboratory animals with the 3R rule (Russell; Burch, 1959). Sensitivity to animal welfare in the world has been affecting the drug development sector negatively in recent years (Sekimizu and

Hamamoto, 2016). Cell culture, which constitutes the first step of drug development steps, is an expensive method used to evaluate the safety and effectiveness of therapeutic drugs before preclinical animal studies. (Breslin, 2013; Mazzoleni, 2009). The system used to assess drug intake and metabolism, hepatotoxicity causes failure due to unpredictable liver toxicity and bioavailability problems. Many of the substances studied from in vitro cultured cell systems have no therapeutic effect since pharmacodynamics cannot be determined in the target animal (Sivaraman et al., 2005; Hopkins, 2008; Sekimizu; Hamamoto, 2016). Mammalian models are used in pre-clinical animal studies from drug development stages. Collecting information by working on a large number of mammals in preclinical studies causes financial and ethical problems (EU, 1986; Orlans et al., 1998). Invertebrate animals can be used in the early stages of drug development to solve these problems. Nematodes used as invertebrates (*Caenorhabditis elegans*), fruit flies (*Drosophila melanogaster*) honeycomb moth (*Galleria mellonella*) are very small in the evaluation of therapeutic effects, causing problems in the evaluation of the results due to the injection area and the sample volume to be taken (Needham et.al,2004; Breger,2007; Mylonakis,2005).

Silk beetles, one of the invertebrate models, have been used as models for infection in human pathogenic bacteria (Hamamoto et al., 2004; Kaito et al., 2002). Silkworm has been used as a model for a long time due to the ease of growing in the laboratory, small growing area, body size and contribution to the economy. (Fujii et al., 1998). With the silkworm infection model, the pharmacology and toxicology of the compounds investigated in the studies are also evaluated (Hamamoto et al., 2009). A large number of silkworm can be produced with artificial food at any time of the year at low cost. It has been determined that some antibiotics of ED50 values are compatible with mammalian animal models in silkworm infected with human pathogen infectious agents (Hamamoto et al., 2004; Hamamoto; Sekimizu, 2005; Kaito et al.,2002).In silkworm diseases, Flacherie (*Escherichia coli*, *Streptococcus*, *Bacillus*, *Proteus sp* and *Staphylococcus*) Muscardine (*Beauveriana bassiana*, *Aspergillus flavus*, *A. oryzae*, *A. tanei*, *Paecilomyces farinosus*, *Sporosporella uvella*,

*Metarhizium anisophia*), *Nosema spp.*, nuclear polyhedrosis virus, cytoplasmic polyhedrosis virus and densonucleosis virus infect silkworm (Kumar et al.,2009; Fujiwara 1980; Watanabe,2002). *Microsporidia* are commonly found in nature that infects all vertebral and non-vertebral hosts. It also infects silkworm. It causes diarrhea in humans (*Encephalitozoon species*) (Oğuz kaya et al., 2008). In the laboratory, rabbits, mice and rats can accommodate many viral, parasitic, fungal and bacterial agents. Researchers should be aware of the effects of these agents on studies.

The reliability of a scientific study also requires laboratory animals to be free from viral, bacterial and fungal diseases (Müftüoğlu; Albayrak, 2019). In mice rats and rabbits, Mousepox, Lymphocytic Choriomeningitis Virus, Minute Virus, Adenovirus, Mouse Cytomegalovirus, Mouse Hepatitisvirus, Rotavirus, Reovirus Type 3, Sendai Virus, Encephalomyelitis virus, Kilman Rat Virus, Toolan H-1 Virus, Rat Coronavirus, Hemorrhagic Syndrome Virus, *Klebsiella pneumoniae*, *Mycoplasma pulmonis*, *Streptococcus pneumoniae*, *Helicobacter spp*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bordetella bronchiseptica*, *Clostridium spiroforme*, *Staphylococcus aureus Pneumonia Stiedae*, *Intestinal coccidiosis*, *Sarcoptes scabiei* agents cause disease. Such animals may cause lots of diseases such as Leptospirosis, Listeriosis, Pseudotuberculosis, Salmonellosis, Toxoplasmosis, Rat Bite Fever, Tularemia, Tuberculosis, Tyzzer Disease, Hantavirus Infection, Lymphocytic Choriomeningitis Pasteurellosis, Triposomiasis, Dermatophytosis via direct contact, respiration, fecal-oral, bite wounds and indirect ways (Baker, 1998; Gül et al.,2013).

Silkworms have cytochrome P450s and conjugating enzymes (Hamamoto et al., 2005; Li et al., 2005). Drug injection is provided in silk beetles through midgut and hemolymph. Many pathogens have been studied in the world with silkworm. With the silkworm infection model, the therapeutic effectiveness of antiviral agents used in viral diseases of humans has been tested in silkworm compatibility. In the study with Baculovirus, the amount of IC50 of some antiviral drugs required to inhibit the ED50 and the virus was determined. It was stated that the amount of

antiviral agents used at the end of the study is consistent with the amount used in humans (Kool et al., 1995; Szewczyk et al., 2006; Orihara et al., 2008). *Cryptococcus neoformans*, a pathogenic fungus in humans, is caused by cryptococcosis. As an invertebrate animal, *Bombyx mori*, nematodes (*Caenorhabditis elegans*), fruit flies (*Drosophila melanogaster*), honeycomb moth (*Galleria mellonella*), pathogen in humans in *Mus musculus* animals *Cryptococcus neoformans* comparison was made as a result, silkworm's body size, human body temperature 37 C° survival. It has been stated that injection can be used instead of mice and rats in order to create *Cryptococcus neoformans* infection due to the injection of two ways, namely hemolymph and appropriate sample taking (Ishii et al., 2016). The therapeutic effects of antifungal drugs, amphotericin B, flucytosine, fluconazole, and ketoconazole were determined in animals that had *C. neoformans* infection in silkworm.

As a result, it was emphasized that to evaluate the therapeutic effects (ED50) of antifungal drugs, each drug can be studied in vivo toxicity and pharmacokinetics, and results matching mammalian infection models were obtained. (Matsumoto et al., 2012). In silkworm, hyperglycemia can be created with foods containing high glucose. Extract of *Rehmanniae Radix*, a hypoglycemic effect was shown in the study conducted with the hyperglycemic silkworm model. As a result, it has been stated that silkworm can be used in herbal studies (Matsumoto; Sekimizu, 2016). Silkworm larvae are sensitive to some human pathogens (*Pseudomonas aeruginosa*, *Candida albicans*, *Staphylococcus aureus*, *Vibrio cholerae*) and even cause their death. The application of antimicrobial or antifungal agents in the treatment of these diseases prevents the silkworm from killing these pathogens (Kaito et al., 2002; Hamamoto et al., 2004). Silk beetles are used to investigate the treatment of diseases, but cannot fulfill basic obligations of pathological study. (Guo-Ping; Xi-Jie; 2011).

## CONCLUSIONS

As a result, we think that silkworm can be used as an experimental animal model in pharmacological and toxicological studies. More studies are needed in this direction.

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**OCCURRENCE, DETECTION, PRECAUTIONARY MEASURES OF AFRICAN SWINE FEVER VIRUS  
OUTBREAK IN BUEA, SOUTH WEST REGION OF CAMEROON**

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**Abstract**

*African swine fever (ASF) is a highly contagious, haemorrhagic and fatal disease of pigs, caused by a virus known as African swine fever virus (ASFV). The ASFV can last for long periods in contaminated environments or cured pork products, which can be a source of infection or introduction of the disease to distant areas. The disease may occur in acute, sub-acute or chronic forms. Mortality is usually close to 100% and pigs of all ages are affected. Pigs are infected as a result of contact with infected wild or domestic pigs and by transmission from infected soft ticks of the Ornithodoros genus. Occurrence and study of ASF in Buea The disease began in Buea, Fako Division of the South West Region in May 2016 but it was not immediately diagnosed as ASF. This is due to the fact that the disease started by attacking one or two pigs and when the animals were treated with Pencilline/Streptomycine combination of antibiotics; dexamethasone, which is an anti-inflammatory drug and multivitamins, the disease subsided. But two weeks later, by early June the disease re-occurred with pigs and piglets presenting with reddened ears and areas of the legs. This time it responded to no treatment. It was at this point that concerned farmers started reporting the disease outbreak to Farming and Animal Husbandry Technical Team, who in turn collected samples from pigs and sent to the National Veterinary Laboratory in Yaoundé the nation's capital city. Collection, analysis of samples a total of 44 blood samples were collected from twelve heavily infected farms in Buea, Fako Division of the South West Region and sent for analysis. Samples for analysis were also collected from the piggery unit of Farming and Animal Husbandry Project (FAHP -Cameroon). The collection points were the lymph nodes, spleen and serum of all infected pigs. The blood samples were treated with EDTA. The samples were analyzed and confirm positive for the disease. FAHP staffs were then sent out immediately to locate the pig farms in the whole region and collect more statistics including the total number of infected pigs, mortality and survival if any. Precautionary Methods or Control of ASF •There should be stricter control over the import of meat and meat products primarily to guard against re-introduction of animal diseases. •Practicing good bio-security at all times can help reduce the risk of many diseases like ASF spreading. •Farmers should be properly educated to be able to recognize disease symptoms early so as to act promptly and avoid the serious socio-economic consequences that could follow. Conclusion Despite the present knowledge on ASF, the disease continues to re-occur in Cameroon due to the general fact that farmers only reluctantly heed to or simply shun instructions to confine their animals and disinfect their piggeries as well as report suspected cases. It is possible that the ASF outbreak that occurred in the Buea, Fako Division, South West Region of Cameroon was introduced through contaminated meat or meat products. The success of the prevention measures depends on strengthening national epidemiological capabilities to render support.*

**Key words:** African swine fever, Buea, South West region, Farmers



### **THREE - DIMENSIONAL RECONSTRUCTIVE ANATOMY OF THE OS HYOIDEUM IN VAN CATS**

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#### ***Abstract***

Aim of the Study: Os hyoideum (apparatus hyoideus) are situated between the ramus mandibulae at the base of the tongue and act as a suspensory mechanism for the tongue and larynx. This study was carried out to perform three-dimensional reconstruction of the os hyoideum using computed tomography images in Van Cats and to determine their anatomical features. Material and Methods: A total of 16 adult Van cats were used in the study. The cats that were anesthetized with the ketamine-xylazine combination were scanned with a CT (Computed Tomography) device and their images were obtained. From these images, three-dimensional reconstructions of the os hyoideum were performed through the MIMICS 20.1 (The Materialise Group, Leuven, Belgium) program. Later, these bones were examined for their anatomical features. Results: It was observed that os hyoideum consists of the six segments in Van cats, including the basihyoideum, thyrohyoideum, ceratohyoideum, epihyoideum, stylohyoideum, and tympanohyoideum. The basihyoideum was a short transversal bone, and there was no processus lingualis on its cranial border. The rostral end of the thyrohyoideum fused with the basihyoideum, while the caudal end was attached to the cornu rostrale of cartilago thyroidea. The ceratohyoideum was a pair bone that extended caudoventrally between the epihyoideum and basihyoideum. The epihyoideum and stylohyoideum bone pairs were located cranioventrally. Finally, the tympanohyoideum was a cartilage pair of bones jointed with the processus mastoideus of the pars petrosa part of the temporal bone and was located in the caudoventral of the meatus acusticus externus. Conclusion: As a result, it was observed that the anatomical structures of the os hyoideum in Van cats were similar to domestic cats.

***Key words:*** Anatomy, Computed tomography, Os hyoideum, Three-dimensional modeling, Van cat.

**ASSESSMENT OF COMMERCIAL SMALLHOLDER EGG PRODUCTION SYSTEMS IN GREATER PORT  
HARCOURT CITY, NIGERIA**

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**Abstract**

*Commercial smallholder egg production systems in Greater Port Harcourt City were assessed for problems and opportunities to intervene. Desk study, survey of 94 farmers using semi-structured questionnaires and focus group discussion with eight farmers were carried out. Commercial smallholder farmers with maximum of 2,500 layers were purposively sampled. Subjects were selected using snowballing sampling technique. Three local government areas (Obio-Akpor, Oyiibo and Etche) of the eight in Greater Port Harcourt City were surveyed. Quantitative data was analyzed using descriptive statistics in Statistical Package for Social Sciences while matrices were used to analyze focus group discussion data. Results show low participation of youths in commercial smallholder egg production. All farmers attained some level of education. Majority (50%) had Bachelor's degrees, hence, potential for innovation adoption. Farmers were motivated by self-employment (68%) and extra income (32%) to produce eggs. Average flock size (1100) was low and inadequate to meet egg demand. Farms were mainly (91%) self-financed, thus confirming weak support for farmers by banks and government. No farm activity was automated except watering (21%). Though all farms use some form of electricity, most (70%) depended on electricity generators, which increased production costs. Majority (94%) of farmers use commercial compound feed. Most (59%) bought their feed through middlemen while others (41%) buy direct from feed manufacturing companies to gain 15% margin. Similarly, 62% bought day-old-chicks through day-old-chicks distributors while 38% procure direct from hatcheries to gain 21% margin. To improve profits, farmers should form egg producers' cooperatives to enhance bulk input purchases, and reduce costs.*

**Key words:** Motivation, Farming Systems, Feed

**DETERMINATION OF SELECTION CRITERIA FOR EGG WEIGHT WITH PATH ANALYSIS IN  
POTCHEFSTROOM KOEKOEK LAYER BREEDING**

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**Abstract**

*Chicken eggs are the cheapest source of animal protein sustaining human population nutritional requirements. The study was conducted to determine the relationship between egg weight (EW) and egg quality traits such as egg length (EL), egg width (EWD), yolk weight (YW), albumen weight (AW), shell weight (SW), shape index (SI), shell ratio (SR), albumen ratio (AR) and yolk ratio (YR), and investigate direct and indirect effects of egg quality traits on EW. A total of 2000 eggs of Potchefstroom Koekoek layer breed were used. Pearson correlation was used to determine the relationship among measured traits and path analysis was used to examine direct and indirect effects of egg quality traits on EW. Correlation results revealed that EW had a positive highly significant correlation with AW ( $r = 0.91^{**}$ ), EWD ( $r = 0.84^{**}$ ), EL ( $r = 0.84^{**}$ ) and SW ( $r = 0.80^{**}$ ). Path analysis indicated that SW (2.14) had a highest direct effect while EL had a higher indirect effect on EW via SW. It is concluded that correlation findings suggest that improvement of AW, EWD, EL and SW might result to increase in egg weight of Potchefstroom Koekoek layer breed. Path analysis results suggest that SW and EL might be used as a selection criterion during breeding to increase egg weight of Potchefstroom Koekoek layer breed. The results of the current study might be used by chicken layer farmers to estimate egg weight using egg quality traits.*

**Key words:** Path analysis, Egg weight, Koekoek layer

## EFFECT OF BREED ON BODY WEIGHT AND MORPHOLOGICAL TRAITS OF TWO LAYER CHICKENS

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### **Abstract**

*Body weight is a trait of economic importance in livestock breeding. The study was conducted to examine the effect of layer breed namely, Hy-Line silver brown and Potchefstroom koekoek on body weight (BW) and morphological traits such as wing length (WL), keel length (KL), shank circumference (SC), chest circumference (CC) and beak length (BL). A total of 200 layers with 100 for each breed were randomly selected at the age of 24 weeks for data collection and measurements were taken once per bird. The study was conducted at the University of Limpopo experimental farm, South Africa. Pearson's correlation were used to determine relationship among measured traits and analysis of variance were used to examine effect of breed. Results showed that among morphological traits, the highest correlation was recognized between BW and WL ( $r = 0.76^{**}$ ;  $P < 0.01$ ) in Potchefstroom koekoek breed, and between CC and BL ( $0.75^{**}$ ;  $P < 0.01$ ) in Hy-Line silver brown breed. Results indicated that there were a statistical significant differences ( $P < 0.05$ ) in BW and morphological traits between breeds. Potchefstroom koekoek breed ( $1.64 \pm 0.02$ ) was heavier than Hy-Line silver brown breed ( $0.82 \pm 0.05$ ). Potchefstroom koekoek had longer wing length ( $18.79 \pm 1.00$ ) and chest circumference ( $4.69 \pm 0.01$ ) than Hy-Line silver brown while Hy-Line silver brown had longer keel length ( $8 \pm 0.31$ ), shank circumference ( $2.85 \pm 0.50$ ) and beak length ( $31.05 \pm 90$ ) than Potchefstroom koekoek. Findings of the study suggest that Potchefstroom koekoek breed is a weightier indigenous layer breed but keel length, shank circumference and beak length might require improvement. It also suggests that improvement of WL might improve BW of Potchefstroom koekoek breed while improvement of CC might improve BL of Hy-Line silver brown breed.*

**Key words:** *Body weight, Beak length, Correlation, Potchefstroom koekoek, Hy-Line silver brown*

**EFFECT OF HATCHABILITY AND SEX ON GROWTH PARAMETERS IN A CROSSBREED  
POPULATION OF ARIAN AND URMIA NATIVE**

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**Abstract**

*There is a good correlation between growth parameters and production of the birds in the field and it is important to manage the herd production. In this study 2nd generation of a crossbreed population of Arian and Urmia native chicks constructed and growth data at birth to 12 weeks of age collected on 312 birds. The information of all birds categorized in 5 groups of 5 hatchability in 2 sex. The Gompertz model was determined as the best model of growth parameters prediction in previous study, therefore the growth parameters of each bird estimated by this model. The results showed that sex and hatchability have different effect on growth parameters of the model. In all groups, initial weight, final weight and growth rate were high in males as it could have been indicated. Values of maturity rate in males were equal or higher than females. Weight and age at inflection point in all groups were higher in males. The data indicated that the growth trend was different between males and females. Initial weights of the birds of group 1 was higher than other groups, in opposite, initial weight of the birds of group 4 was lower than other groups. Growth rate of male birds of the group 1 and females of the group 3 were more than other groups. Subsequently growth at inflection point of males of the group 1 in contrast to other males, and in females of the group 3 in contrast to other females displayed lower values. In order to confirm the signification of these results (sex and hatchability effect on Gompertz and growth parameters) GLM was carried out in SAS software. The final results showed that sex and hatchability have significant effect on growth parameters.*

**Key words:** Gompertz model, Growth parameter, Chick, Hatchability, Sex

**WEIGHTED AND UN-WEIGHTED ESTIMATION OF ECONOMIC TRAIT' HERITABILITY IN NATIVE  
IRANIAN CHICKENS BY META-ANALYSIS METHOD**

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**Abstract**

*Meta-analysis is actually an analysis of analyzes that combines previous information for giving them a weighted coefficient to be compared and re-evaluates the results providing an opportunity to make an explicit judgment that inevitably influences the conclusions of any summary of research to increase the power of the test and to achieve a unit and more meticulous result. In Iran, there are 6 distinct native chicken populations that they belong to different geographical region named Fars, Isfahan, Mashhad, Mazandaran, West Azerbaijan, and Yazd, which are valuable genetic resources and also they are the main source of the livelihoods for rural families. So that a meta-analysis of 20 Iranian published papers on the native chicken was conducted to calculate weighted and un-weighted average of direct heritability of growth and production traits including day-old weight, 8, 12 weeks weight, weight and age in sexual maturity, egg number and average egg weight by Meta-analysis method. The results of this meta-analysis showed that the average heritability of the mentioned economic traits was calculated separately in Iranian native chickens. On average, the total heritability for day-old, 8, 12 weeks weight and weight in sexual maturity, egg number, average egg weight, age in sexual maturity was 0.425, 0.232, 0.438, 0.315, 0.21, 0.242 and 0.41 respectively. 8 weeks, 12 weeks body weights and the number of eggs in the Fars population showed the highest heritability, which were 0.36, 0.52 and 0.31, respectively. The heritability of sexual maturity weight in Yazd population chicken was 0.58 and the highest. The lowest heritability of sexual maturity weight belongs to the population of Azerbaijan chickens ( $h^2= 0.22$ ) which has the highest average weight and the gain trend until the sexual maturity age. Also, the lowest heritability of 12 weekly weight was observed in Isfahan native chickens. The present finding illustrated that weighted average heritabilities are more reliable because weighted heritability for some traits is more conservative than average of un-weighted estimated heritabilities. The data extracted and summarized from the published articles provide a more obvious picture of the specific characteristics of the Iranian indigenous chicken populations, revealing their differences, and paves the process of decision making and the formulation of an efficient and sustainable breeding or conservation programs.*

**Key words:** Growth, Production traits, Iranian native chicken, Meta-analysis, Weighted, Unweighted

**PERFORMANCE OF OWNER-MANAGED AND EMPLOYEE-MANAGED SMALL-SCALE POULTRY  
FARMS IN NIGERIA**

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***Abstract***

*This study was conducted to establish the linkage between farm manager's identity and performance of poultry farms in Lagos State, Nigeria. Primary data on poultry farm features were elicited with well-structured questionnaire from randomly selected 130 poultry firms. Descriptive statistics, regression model and enterprise budget were applied for data analysis. From the result, it is seen that 78.94% of the poultry farms are owner-managed while the other 21.05% are employee-managed. The result revealed that poultry farms are mainly owner managed and had survived for past 4 years in operations. Result shows a return on investment 53% and 61% for employee-managed and owner-managed poultry farms, respectively. The finding shows that the extent to which farm manager's identity affects financial performance of the poultry firm is 72%. The result shows that there is a positive and significant relationship between farm manager's identity and the financial performance of poultry farms. Farm manager's identity ( $P < 0.05$ ) is a predictor of the financial performance of poultry farms in Nigeria. We concluded that owner-managed poultry farms performed better than employee-managed farms. We recommended that small scale poultry farm owners should personally manage their farms, provided they have the rudimentary knowledge of farm management and the technical know-how of poultry production.*

***Key words:*** Farm manager's identity, Owner-managed, Financial performance, Poultry farm

**COST AND RETURN ANALYSIS OF BIOSECURITY MANAGEMENT IN POULTRY FARMS IN RIVERS  
STATE, NIGERIA**

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**Abstract**

*Cost of preventing disease incursion and containment in poultry management is gaining attention in scientific and public debates. Biosecurity involves the use of relevant resources to secure poultry-based food production system (products, producers and consumers health) from diseases. This study investigates whether biosecurity resources are efficiently utilized to enhance maximum profit in poultry-based food production. Primary data used for the study were obtained from 120 sampled poultry farms. Multi-stage procedure was adopted in the selection of the sampled farms. Structured questionnaire was the data collection instrument used for the investigation. Descriptive and inferential statistical tools were employed in data analysis. Also cost efficiency model and profit functions were used to analyze data. The result shows that bio-secured poultry farms had a mean profit of N 150,230 higher than N 92,590 earned by bio-insecured farms. Profitability of biosecured poultry farms significantly and positively correlated (2.02)\*\* with bio-security management index ( $p < 0.05$ ). The major constraints to bio-security management of poultry farms were high cost of effective disinfectant/vaccines and unwillingness of farmers to adopt effective biosecurity management practices. The researcher has sufficient reasons to conclude that biosecurity resources were efficiently utilized, hence the relative high profit that was realized. However, the biosecurity management index gap of 37.5% observed in the study suggests that there is still room for improvement.*

**Key words:** Biosecurity management, Resource use efficiency, Poultry-based food, Producers



## GENETIC DIVERSITY OF PROLACTIN GENE IN FULANI AND YORUBA ECOTYPE NIGERIA CHICKEN

Osaoyuwu OH<sup>1</sup>, Olanhanmi OE<sup>1</sup>, *Fijabi OE<sup>1\*</sup>*, Akinyemi MO<sup>1</sup>, Fatai RB<sup>1</sup>

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### **Abstract**

Yoruba and Fulani ecotype chickens are native to Nigeria. The prolactin (PRL) gene is connected with egg production traits in birds. This study was carried out to investigate polymorphism at the PRL gene locus in order to gain insight into the population structure and determine the genetic relationship between the Yoruba and Fulani ecotype chickens using PCR-RFLP marker. Blood of 50 birds from each ecotype was randomly sampled for DNA assay through jugular venipuncture and preserved using the FTA card. PRL gene amplification was done at 24 indel (insertion-deletion) on the nucleotide point of 358 with 3% gel through electrophoresis the bands were read. Allele frequency, observed heterozygosity, genetic distance, F-statistics, and test of Hardy-Weinberg Equilibrium (HWE) was used to characterize the population through Popgene 1.31 version software. The birds were raised in an intensive system and fed ad libitum with commercial feed. A total of 182 alleles were observed at PRL gene locus with alleles A and B having frequencies 0.50 respectively and each controlling three genotypes AA, AB, and BB with genotype frequencies 0.32, 0.36, and 0.32 respectively. 0.40, 0.33, and 0.36 were the heterozygote frequencies observed for Fulani ecotype, Yoruba ecotypes, and the overall population respectively. Genetic distance obtained between the ecotypes was 0.0010. The observed FIS for Fulani and Yoruba ecotypes was 0.21 and 0.25 while that of the overall population is 0.27. The chi-square (2.29) value revealed that Fulani ecotype chicken did not significantly ( $P > 0.05$ ) deviate from HWE but Yoruba ecotype and the overall population with values of 5.58 and 7.15 respectively showed significant ( $P < 0.05$ ) deviation from HWE. The phylogenetic relationship shows that both ecotypes originated from the same root for the PRL gene. The results of this investigation detected polymorphic sites with moderate genetic differentiation between the ecotypes of Nigeria native fowls.

**Key Word:** Prolactin, Polymorphism, PCR-RFLP, Nigeria Chicken, Ecotypes

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## FEATURES OF DAYLIGHT AT THE BIRTH TIME OF NORDUZ SHEEPS

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### **Abstract**

*Sheep are animals that highly affected by daylight with a panoramic viewing angle of up to 330-360 degrees. It is known that the intensity and length of daylight are also effective in hormonal cycle and birth of these animals. In this study, the relationship between the birth times of sheep and the current daylight data (Blue hour, Golden Hour, Sunrise/Sunset, Moonrise/Moonset) has been discussed in detail. For this purpose, 67 pregnant Norduz sheeps, 3-4 years old, raised in the village of Bardakçı, Tusba, Van (coordinates: 38° 34' 14.7864" N, 43° 15' 46.8576" D), used as live material. Data on the births (date of birth, date of birth, type of birth and gender of lamb) of sheeps between February 8 and March 21, 2018 were used in the analyzes.*

**Key words:** Norduz, Blue hour, Golden hour, Sheep, Twilight

**EFFECTS OF DIAKUR PLUS ADDED IN WHOLE MILK ON CALF DIARRHEA AND GROWTH PERFORMANCE**

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**Abstract**

*The aim of this study is to investigate the effect of Diakur © Plus (electrolytes, glucose and inactive yeast (MOS)), which is used to prevent fluid loss formed after diarrhea cases, on diarrhea and average daily gain by using on calves without diarrhea. Healthy calves taken from the third day of the trial are divided into two groups as experimental (DPG) and control (CG) groups, with 20 calves in each group. The trial was continued until the first two weeks when diarrhea cases were most intense and was terminated on the thirteenth day. Calves were given 5 litres of whole milk twice a day in total. While the control group was given only whole milk, the experimental group was given 1 packet (100 g) of Diakur© Plus once a day with whole milk. Cases of diarrhea between the two groups, the number of days with diarrhea and average daily gain were statistically similar ( $p>0.05$ ). Faecal score was higher in the group given Diakur© Plus ( $p<0.05$ ). Although there was no statistically significant difference, it was observed that diarrhea incidence was lower (30%), average daily gain was higher (65%), and the number of days with diarrhea was less in the experimental group. According to these results, 100 g Diakur© Plus taken daily with whole milk has some positive effects on diarrhea cases and performance.*

**Key words:** Diakur plus, Diarrhea, Performance

**GENETIC RESISTANCE TO NEWCASTLE DISEASE IN YORUBA SMOOTH FEATHERED AND YORUBA FRIZZLED FEATHERED NIGERIAN INDIGENOUS CHICKEN**

**PO AKINOLUWA<sup>1\*</sup>, AE SALAKO<sup>1</sup>, TO OLONIJOLU<sup>1</sup>**

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***Abstract***

*An experiment was conducted to assess and compare the genetic resistance to Newcastle disease in Yoruba Smooth Feathered (YSF) and Yoruba Frizzled Feathered (YFF) Nigerian Indigenous Chicken. A total of forty (40) points of lay chickens were used for this study, comprising of twenty (20) chickens each. Assessment and comparison of genetic resistance were done by the evaluation of hemagglutination Inhibition (HI) TITRE, clinical signs and mortality following physical contact with Newcastle disease infected chickens. The experimental chickens developed clinical signs of Newcastle disease from day 9 after infection. Mortality commenced on day 10 after infection. 14 chickens from Yoruba Smooth Feathered, 8 chickens from Yoruba Frizzled Feathered. HI titre was determined on days 0, 21 and 28 post infection. On day 0, the HI titre of the 2 genotypes were below 3log<sub>2</sub>. On day 21, there were significant differences within the each HI titre ( $P < 0.05$ ) in which Yoruba Frizzled Feathered had the highest mean HI titre of 6.25. The experiment showed that Yoruba Frizzled Feathered is more resistance to Newcastle disease. However there may be need to further compare Yoruba Frizzled Feathered to other variety within the Yoruba Ecotype.*

***Key words:*** Nigerian indigenous chicken , Hemagglutination inhibition, Newcastle Disease

**IMMUNE RESPONSES OF TWO GENOTYPES OF YORUBA ECOTYPE NIGERIAN INDIGENOUS  
CHICKEN TO NEWCASTLE DISEASE**

**PO AKINOLUWA<sup>1\*</sup>, AE SALAKO<sup>1</sup>, TO OLONIJOLU<sup>1</sup>**

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**Abstract**

*This study assessed the effects of Newcastle disease on the Immune responses of Yoruba Ecotype Nigerian Indigenous Chicken. A total of forty (40) indigenous chickens made up of twenty (20) each of Yoruba Frizzled feathered and Yoruba Naked Neck were infected with New Castle Disease through physical contact. Already Newcastle infected chickens were introduced into their different compartments. Clinical signs, mortality and hemagglutination Inhibition (HI) Titre evaluation were used to assess the immune responses. The experimental chickens developed clinical signs of Newcastle disease from day 10. Mortality were recorded from day 11 after infection, with 35% in Frizzled feathered and 30% in Naked Neck subsequently the chickens were bled on day 0, 11 and 28 post infection for HI titre determination. On day 0, HI Titre of the two Genotypes were below 3log<sub>2</sub>, on day 21 there was significant differences within each HI Titre ( $P < 0.05$ ) in which Yoruba Naked Neck had the highest mean HI Titre of 8.0 and Yoruba Frizzled feathered 6.5. There was decrease in the mean HI titre in the two on day 28 with Yoruba Naked Neck having the least reduction. It could therefore be concluded that, the naked neck was more resistant to Newcastle disease than the frizzled feathered. It is therefore recommended that Naked Neck should be selected and crossed with other Genotypes within the Yoruba Ecotype.*

**Key words:** Genotype, Indigenous chicken, Newcastle disease, Hemagglutination inhibition

**EFFECTIVENESS AND SIDE EFFECTS OF TREATMENT METHODS AGAINST VARROA DESTRUCTOR  
(VARROASIS) PARASITE OF THE HONEY BEE**

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**Abstract**

*The Varroa destructor mite is responsible for the pathology called: varroasis, which has become the main disease that causes weakening and loss of colonies. Globally prevalent, it is responsible for numerous damages at the individual and colony level, due to the association of the parasite with several bee viruses. In Algeria, several kinds of methods and products are used by our beekeepers, the study of the effectiveness of these products and their side effects are the main objectives of this study. The trials were carried out on 15 apiaries belonging to a dozen beekeepers located in the central region of Algeria (Blida and Boumerdes). Natural chemicals were tested during these experiments. Our study on the effectiveness of chemical and biological treatments, available on the market, showed the decrease in the effectiveness of chemical molecules between other fluvalinate and flumethrin. This confirms the strong resistance of the mite to these products in Algeria. The field study revealed the importance of organic thymol products (Apilife Var®, Oxuvar® and Apibioxal®) in alternative control; a more interesting efficacy has been found with these products but with a negative influence on bee colonies (desertion, queen mortality, lay stop). The application of a few plants and essential oils in the colonies produced very variable results, the majority of them having average effectiveness with a disturbance in the behavior of the colonies. The use of oxalic acid, on the other hand, recorded the most effective results in the absence of brood (more than 90%). Other efficacy tests in the laboratory are necessary to be able to recommend to beekeepers the use of natural substances in the fight against this bee pathology. The use of a combination of chemical, natural and biotechnical control is the best solution in order to effectively combat varroasis while observing the side effects on the colonies.*

**Key words:** Honey bee, Varroa, Methods



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**Nationality of Presenters**

<b>Country</b>	<b>n</b>	<b>%</b>
Algeria	6	6,59
Cameroon	1	1,1
Hungary	2	2,2
India	1	1,1
Indonesia	1	1,1
Iran	5	5,49
Italy	1	1,1
Kazakhstan	1	1,1
Kyrgyzstan	3	3,3
Malaysia	1	1,1
Nigeria	11	12,09
Pakistan	3	3,3
Poland	2	2,2
South Africa	5	5,49
Turkey	44	48,35
United Kingdom	2	2,2
USA	2	2,2
<b>TOTAL</b>	<b>91</b>	<b>100</b>

**Nationality of Authors**

<b>Country</b>	<b>n</b>	<b>%</b>
Algeria	16	8,08
Belarus	1	0,51
Cameroon	2	1,01
Ghana	1	0,51
Hungary	2	1,01
India	6	3,03
Indonesia	3	1,52
Iran	12	6,06
Italy	1	0,51
Kazakhstan	1	0,51
Kyrgyzstan	8	4,04
Malaysia	1	0,51
Mozambique	2	1,01
Nigeria	19	9,6
Pakistan	8	4,04
Poland	3	1,52
South Africa	14	7,07
Turkey	93	46,97
United Kingdom	3	1,52
USA	2	1,01
<b>TOTAL</b>	<b>198</b>	<b>100</b>