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RESEARCH ARTICLE

Investigation of Some Physio-Chemical and Microbiological Quality of Fresh Meat Sold Online

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Online Sipariş Edilen Taze Etlerin Bazı Fiziko-Kimyasal ve Mikrobiyolojik Kalitelerinin İncelenmesi

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Öz

Amaç: Araştırma online satılan taze etlerin bazı kalite niteliklerini belirleyerek, online taze et satışına ve bu alanda alınması gereken önlemlere dikkat çekmek amacıyla yapıldı.

Gereç ve Yöntem: Numunelerin pH değerleri, dijital bir pH metre ile sıcaklık değerleri infrared sensörle çalışan batırma tipinde bir termometre kullanılarak tespit edildi. Numunelerin renk değerleri Chromameter renk ölçüm cihazı ile L*, a* ve b* renk değerleri ölçülerek belirlendi. Toplam canlı mikroorganizma sayısı Plate Count Agar besi yerinde, koliform bakteri sayısı Violet Red Bile Agar besi yerinde, *Staphylococcus* spp. sayısı Egg Yolk Tellurite Emulsion ilave edilmiş Baird Parker Agar besi yerinde klasik kültür teknikleri kullanılarak belirlendi.

Bulgular: Online ve müşteri olarak satın alınan parça et, kuşbaşı et ve kıymaların ortalama pH değerleri sırasıyla 5,62/5,62, 5,64/5,70 ve 5,81/5,84 olarak belirlendi. Online ve müşteri olarak satın alınan parça et, kuşbaşı et ve kıymaların ortalama sıcaklık değerleri sırasıyla 11.35/11.1°C, 11.26/11.7°C ve 12.07/12.7°C olarak belirlendi. Online ve müşteri olarak satın alınan parça et, kuşbaşı et ve kıyma numunelerinin toplam mezofilik aerobik bakteri sayısı sırasıyla 5,69/5,09, 6,34/5,68 ve 7,01/6,36 log10 kob/g olarak belirlendi. Soğuk zincir altında online olarak satın alınan ve soğuk zincir olmadan müşteri olarak satın alınan örneklerde sıcaklık değerleri ve mikrobiyolojik sonuçlar benzerlik göstermiştir.

Öneri: Elde edilen bulgular ışığında, mevzuatın yeniden düzenlenmesinde online etin sıcaklık değerleri, paketleme şekilleri ve sevkiyat koşullarının daha fazla öne çıkması gerektiği düşünülmektedir.

Anahtar kelimeler: Denetim, online et, soğuk zincir.

Abstract

Aim: The research was carried out to draw attention to online fresh meat sales and the precautions to be taken in this area by determining some quality characteristics of fresh meat sold online.

Materials and Methods: The pH values of the samples were determined using a digital pH meter and the temperature values were determined using an infrared sensor-operated immersion thermometer. The color values of the samples were determined by measuring the L*, a*, and b* color values with a Chromameter color measuring device. The total viable counts was determined in Plate Count Agar medium, coliform bacteria number in Violet Red Bile Agar medium, *Staphylococcus* spp. number in Baird Parker Agar medium supplemented with Egg Yolk Tellurite Emulsion using classical culture techniques.

Results: The mean pH values of pieced, cubed and minced meat purchased online and customers were respectively 5.62/5.62, 5.64/5.70, and 5.81/5.84. The average temperature values of pieced, cubed, and minced meat purchased online and customers were respectively 11.35/11.1°C, 11.26/11.7°C, and 12.07/12.7°C. Total viable counts of pieced, cubed, and minced meat purchased online and customers were respectively determined as 5.69/5.09, 6.34/5.68, and 7.01/6.36 log10 cfu/g. Temperature values and microbiological results determined in both meat samples purchased online with cold chain and customers without cold chain were similar.

Conclusion: In light of the results, it is thought that the temperature values, packaging forms, and shipping conditions of online meat should be more prominent in the reorganization of legislation.

Keywords: Legislation, online meat, cold chain.

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Introduction

Raw red meat can be contaminated during storage and distribution. In case of non-compliance with the storage temperature, it creates an ideal environment for the colonization of many pathogenic and saprophytic microorganisms (Liu et al 2019). Mainly saprophytic (e.g., *Pseudomonas, Acinetobacter, Aeromonas, Brochotrix thermopsphacta, Alteromonas putrafaciens, Lactobacillus spp.)* (Aidani et al 2014) and pathogenic microorganisms (e.g., *Salmonella* Enteritidis, *Salmonella* Typhimurium, *Listeria monocytogenes, Escherichia coli* O157:H7, *Staphylococcus aureus*) (Soyutemiz 2000, Aidani et al 2014) that can contaminate fresh meat create potential risks in terms of product quality and public health.

In recent years, consumers have been demanding fresh meat and meat products that are safe, high quality, delicious, and have a long shelf life. In line with consumer demands, various preservation methods are applied to preserve the quality characteristics of meat and extend its shelf life (Aymerich et al 2008). Besides these methods keeping the meat in the cold chain until it reaches the consumer is critical for meat hygiene, shelf life, and sensory properties (Ockerman and Basu 2004) and thus, cold storage is reported to be effective in reducing risks to food safety (James and James 2009) by eliminating and suppressing many pathogenic microorganisms that threaten public health.

Today, in addition to the changes in consumers' preferences and expectations, innovations in the food sector and their effects on the global market attract the attention of the scientific world. In parallel with all these developments, foodstuffs can be marketed directly to consumers within or between countries via the internet as it provides fast and easy shopping opportunities in recent years. It has been reported that online fresh food sales in China exceeded USD 20 billion in 2016 and the annual growth rate was over 70%, while laws and regulations on selling food online are relatively weak (Liu et al 2019). However, since fresh meat, which has an important place in human nutrition, undergoes various changes and deteriorations due to the activities of enzymes and contaminating microorganisms in its structure (Ertaş 1979), it must be preserved well until consumption. Liu et al (2019) analyzed the total volatile basic nitrogen, TVC, and coliform counts of 135 samples to investigate the quality of chilled pork collected from 45 online stores in China. Researchers reported that meat sold online in China poses potential hazards, temperature control, and package model are important in online meat quality to ensure meat safety. According to the Turkish Regulation on Food Hygiene (Anonymous 2011a), the vehicles and/or containers used in the transport of food should keep the transported food at suitable temperatures, enable monitoring of the determined temperatures, and the food business operator must protect and record the cold chain. According to Grunert (2006), the main issues that need to be addressed in the sale of fresh meat online, which is quite new to the consumer in terms of convenience, variety, and new experiences, are health concerns, quality, ethics, and the environment.

This study was conducted to determine some quality characteristics of fresh meat sold on the internet, which started a few years ago in Türkiye as well as all over the world, and to emphasize the need for regulation of online meat sales in food legislation.

Material and Methods

Material

In this research, pieced meat, cubed meat, and minced meat samples from four different companies that sell meat online and have market chains were ordered online. In addition, online purchase; pieced, cubed, and minced meat samples were obtained from these companies without applying the cold chain, in accordance with the routine conditions of the consumer's meat purchase. All samples were purchased at four different times. Physicochemical and microbiological analyzes were started immediately after the samples arrived at the laboratory.

Method

The pH values of the samples were determined using a digital pH meter (InoLab pH 720 model, WTW, GmbH, Germany) (Association of Official Analytical Chemist 1984). The temperature values were determined using an infrared sensor-operated penetration thermometer (testo 104 type). Color determination was carried out by measuring L*, a*, and b* color values with a Chromameter color measuring device (Chroma Meter CR-400, Konica Minolta) (Insausti et al 1999, Mancini and Hunt 2005). Total viable count (TVC) of samples in Plate Count Agar (PCA, Merck, 105463), coliform bacteria count in Violet Red Bile Agar (VRBA, Merck, 101406) (Harrigan and McCance 1976), *Staphylococcus* spp. count in Baird Parker Agar (BPA, Merck, 105406) with Egg Yolk Tellurite Emulsion (Merck, 103785) were detected after incubation at appropriate temperatures and times (Corry et al 2003, Halkman 2005, Tallent et al 2016).

Statistical analysis

Statistical analyzes of the values obtained as a result of the research were carried out by the variance analysis, the Duncan Test for the differences between the sources of variance, and the T-test to determine differences between online and customer-purchased meats using the SPSS package program (SPSS/PC version 21.00, SPSS Inc, Chicago, IL, USA) (Steel and Torrie 1981).

Results

The mean pH values of pieced, cubed, and minced meat purchased online were respectively 5.62, 5.64, and 5.81, and those purchased as customers were respectively 5.62, 5.70, and 5.84 (Figure 1A). Although the differences between the companies in terms of pH values of the meat purchased as a customer from four different companies were not significant ($p>0.05$), it was significant ($p<0.05$) in the pieced meat purchased online. According to the t-test results of the pH values of the samples obtained as online and customers, the differences in the pH values of the pieced meat and cubed meat were found to be significant ($p<0.05$).

The mean temperature values of pieced, cubed, and minced meat purchased online were respectively 11.35, 11.26, and 12.07°C, and the temperature values of those purchased as customers were respectively 11.1, ,11.7 and 12.7°C (Table 1). While the differences in the temperature values of pieced, cubed and minced meat purchased as customers from four different companies were not significant ($p>0.05$), the differences in the pieced and cubed meat purchased online were significant ($p<0.05$). The t-test results of the differences in temperature values of the samples purchased online and as customer were not significant ($p>0.05$).

Table 1. Temperature Values of Meat Purchased as Online and Customer

			Pieced meat				Cubed meat				Minced meat			
		n	Max	Min	Average(SD)	P	Max	Min	Average(SD)	P	Max	Min	Average(SD)	P
Online*	1	4	14,3	11,9	13,17±0,5^{a}		16,0	12,8	14,35±0,6^{c}		15,0	12,1	13,42±0,6	
	2	4	15,8	10,5	12,95±1,1^{a}		15,2	11,0	13,20±1,1^{bc}		16,8	9,2	13,27±1,6	
	3	4	9,7	6,1	7,92±0,7^{b}	0,00**	8,3	3,8	6,42±0,9^{a}	0,00**	11,5	9,4	10,37±0,5	0,20**
	4	4	14,1	9,4	11,37±1,0^{a}		12,8	9,1	11,07±0,9^{b}		14,8	9,4	11,22±1,2	
	T	16	15,8	6,1	11,35±0,6		16,0	3,80	11,26±0,8		16,8	9,2	12,07±0,6	
			Max	Min	Average(SD)	P	Max	Min	Average(SD)	P	Max	Min	Average(SD)	P
RCS*	1	4	17,0	9,40	12,2±1,6		14,0	8,70	11,6±1,2		18,7	12,7	14,9±1,3	
	2	4	12,90	6,80	9,72±1,2		14,9	9,00	11,5±1,3		16,1	4,10	10,3±3,2	
	3	4	16,10	6,10	10,2±2,0	0,4**	14,0	7,40	10,2±1,3	0,3**	14,2	11,0	12,9±0,6	0,4**
	4	4	13,50	11,50	10,5±2,0		15,2	11,4	13,5±0,8		15,1	10,7	12,6±0,9	
	T	16	17,0	6 ,10	11,1±0,7		15,2	7,40	11,7±0,6		18,7	4,10	12,7±0,9	
	Online	16			11,36±0,6	0,7*			11,26±0,8	0,2*			12,07±0,6	0,4*
	RCS	16			11,18±0,7				11,74±0,6				12,71±0,9	

RCS: Routine customer shopping, SD: Standart Deviation (+/-), *: t testi, **: Anova,

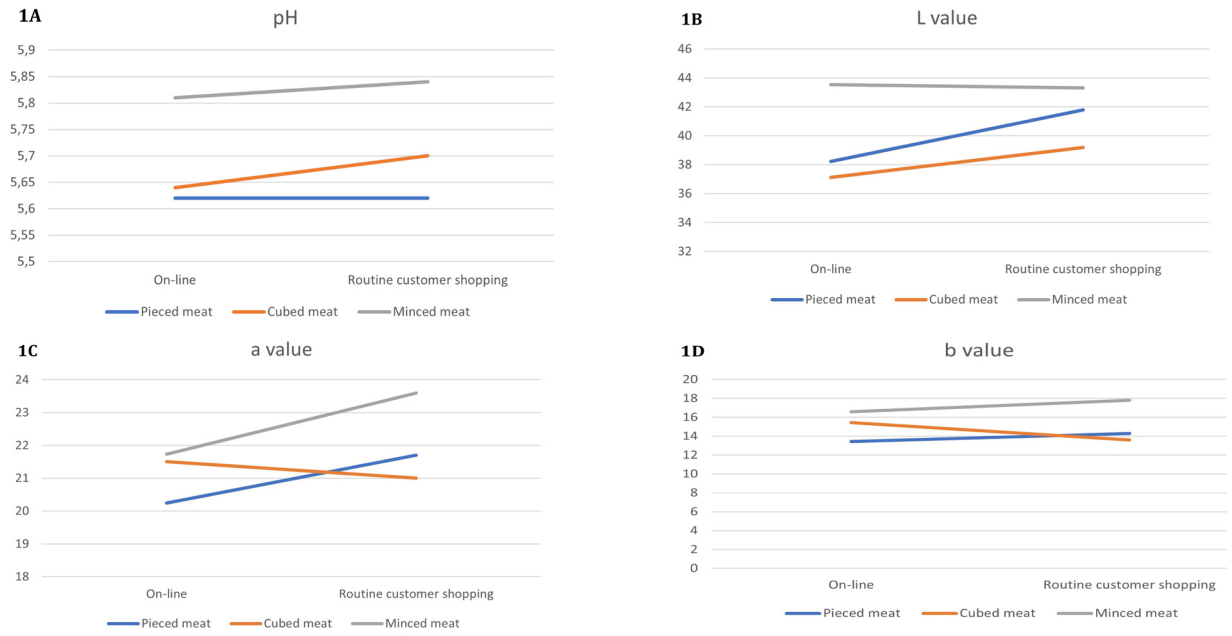

Figure 1. 1A: pH Values of Meat Purchased as Online and Customer, **1B:** L Values of Meat Purchased as Online and Customer, **1C:** a Values of Meat Purchased as Online and Customer, **1D:** b Values of Meat Purchased as Online and Customer.

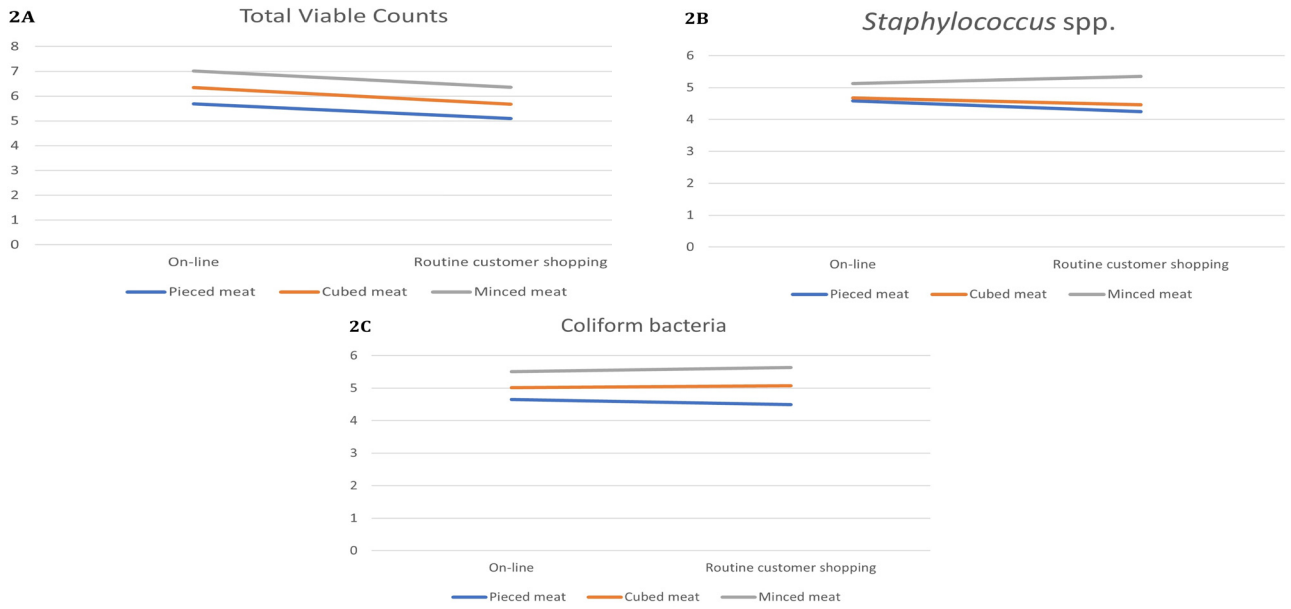

Figure 2. 2A: Total Viable Counts of Meat Purchased as Online and Customer, **2B:** Number of Staphylococcus spp. of Meat Purchased as Online and Customer, **2C:** Number of Coliform Bacteria of Meat Purchased as Online and Customer

The mean L values of pieced, cubed, and minced meats purchased online are 38.22, 37.13 and 43.55, a values are 20.25, 21.51, and 21.73, b values are 13.44, 15.44, and 16.59, respectively, and the mean L values of those purchased as a customer are 41.8, 39.2 and 43.3, a values are 21.7, 21.0 and 23.6, b values are 14.3, 13.6 and 17.8 (Figure 1B-D), respectively. Significant differences ($p<0.05$) were determined in the b values of minced meat samples purchased online and in the a values of pieced meat samples supplied as customers. The t-test results of the differences in the color values of the samples purchased online and as a customer were not found to be significant ($p>0.05$).

When the microbiological analysis findings of pieced, cubed, and minced meat purchased online were examined, the mean number of TVC were found to be 5.69, 6.34, and 7.01 log10 cfu/g respectively, number of *Staphylococcus* spp. 4.58, 4.67 and 5.12 log10 cfu/g, number of coliform bacteria 4.65, 5.01 and 5.51 log10 cfu/g. On the other hand, the mean number of TVC purchased as customers was found to be 5.09, 5.68, and 6.36 log10 cfu/g, respectively, and the number of *Staphylococcus* spp. was 4.24, 4.46, and 5.35 log10 cfu/g, and the number of coliform bacteria was 4.49, 5.07, and 5.64 log10 cfu/g (Figure 2A-C).

Significant differences in the numbers of TVC in cubed meat and *Staphylococcus* spp. in minced meat purchased online from four different companies were found ($p<0.05$). Significant differences ($p<0.05$) were determined in the number of TVC and coliform bacteria in pieced meat and *Staphylococcus* spp. in cubed meat purchased as a customers. According to the t-test results of the differences of meat samples purchased online and as a customer, *Staphylococcus* spp. in cubed meat and TVC in minced meat were found to be significant ($p>0.05$).

Discussion

Considering that cold chain management is important in terms of maintaining quality and safety in delivering fresh meat to the consumer due to its short shelf life, it was mainly aimed in this study to carry out to reveal the quality of pieced, cubed, and minced meat purchased online and to compare the quality of these piece of online meat to the same meat pieces obtained in accordance with the routine conditions of the consumer's meat purchase.

pH value is an important parameter that plays a role in the shelf life of meats. The high pH value of the meat is critical in the development and predominance of lactic acid bacteria, which play an important role in the deterioration of meat (Babji et al 2000). In this study, pH values of the meat samples supplied as online/customer were respectively determined as 5.62/5.62, 5.64/5.70 and 5.81/5.84, therefore the pH values were found to be appropriate. Similar to the findings of this study, Huidobro et al (2003) reported that the pH values of the meat decreased to 5.5 in the first 24 hours after slaughter, and there was no change in pH value during the next 5 days of cold storage. Unlike these research findings, Gök (2001) determined the pH value as 6.07 in minced meat measured before cold storage. In our study, the differences in the pH values of pieced meat purchased online from four different markets were found significant ($P<0.05$). Thus, significant differences between companies show that companies do not have certain standards in online sales procedures. On the other hand, considering the

statistical differences according to the results of the t-test in pH values of pieced meat and cubed meat procured online and as a customer, it has been concluded that if standards are established in online meat sales and controlled within the framework of the legislation, this will have significant advantages over the meats purchased as a customer.

Temperature seems to be the most influential factor in microbial growth (Liu et al 2019) and meat spoilage (Nychas et al 2008). Although some pathogenic or saprophytic microorganisms can grow at 0ºC or lower temperatures, risks to food safety can be significantly reduced by keeping fresh meat below 5ºC (James and James 2009). Cold storage of meat during the sale and transportation in the markets where it is delivered to the end consumer is critical in terms of quality and reliability (Nychas et al 2008). In the research, the average temperature values of pieced, cubed and minced meat purchased online/as customers were respectively determined as 11.35/11.1ºC, 11.26/11.7ºC, and 12.7/12.7ºC. Considering that the meat supplied online had been brought in the cold chain in transport containers, it was concluded that the temperature values detected in meat purchased online were high. Furthermore, the significant differences between companies in the temperature values of the cubed meat supplied online show that the companies do not have certain standards in online sales. However, the temperature values during the delivery of the meat are critical for the freshness, reliability, and overall quality of the meat. In this context, although it is possible to monitor and control the conditions affecting the whole chain with different methods, the complete implementation of the cold chain is still an important necessity for manufacturers, distributors, retailers, and consumers (Oliva and Revetria 2008, Nastasijević et al 2017).

The color of red meat is the first quality characteristic that the consumer sees as an indicator of freshness and health (Berruga et al 2005, Troy and Kerry 2010). Color can be adversely affected at all stages of the production chain including animal breed, diet, age, and slaughtering/marketing process (e.g., pre-slaughter treatments, stunning and bleeding, cooling variables, packaging, distribution, and marketing) (Insausti et al 1999). The increase in temperature during the cold storage and the retail sale of fresh meat accelerates the formation of metmyoglobin, causing a decrease in color stability and accelerating the formation of brown color (Rosenvold and Wiklund 2011). Similar to the findings obtained in the current research, Tolon et. al. (2000) determined the L value as 42.29 and the b value as 11.51 in fresh meat taken from the Musculus longissimus dorsi. Bozkurt et. al. (2009) determined the L value as 51.00, a value as 15.26, and b value as 5.88 of the Longissimus dorsi muscle. Alp (2008) determined the L value as 42.30, a value as 26.76, and b value as 9.28 of minced meat. In the research, significant differences ($P<0.05$) were determined in the b values of minced meat purchased online and in the a values of pieced meat supplied as customers. The fact that the significant differences between the companies in the b values of the minced meat supplied online, as in the same pH and temperature values, shows that the companies do not have certain standards in online sales procedures.

As a result of contamination by saprophytic bacteria in fresh meat, negative changes occur in sensory properties (e.g., color, flavor) (Aidani et al 2014). The most important factor in the control of meat spoilage is to protect from microbial contamination and to control the number of microorganisms that affect the safety and color of meat (Limbo et al 2010). A high TVC number can be considered as an indicator of low quality and/or reduced shelf life of the food (Aydemir Atasever and Atasever 2015). Furthermore, it has been found that the storage temperature during cooling and cold storage is associated with microbial growth of lactic acid bacteria, *Enterobacteriaceae* spp, *Clostridium perfingens*, and *Bacillus thermosphacta*, etc. (Liu et al 2019). According to the Turkish Food Codex Regulation on Microbiological Criteria (Anonymous 2011b), the number of aerobic colonies in minced meat is accepted as 5x106 cfu/cm2 in two of the five samples and 5x105 cfu/cm2 in three of them. The TVC count of meat is related to processing, storage conditions, and cooling methods (Liu et al 2019), and the initial TVC count is reported as 4 log10 cfu/g (Limbo et al 2010). In the study, the number of TVC in pieced, cubed, and minced meat purchased online/customer was respectively determined as 5.69/5.09, 6.34/5.68 ve 7.01/6.36 log10 cfu/g. Similar to the findings of this study, Aydemir Atasever and Atasever (2015) reported average TVC count as 7.33 log10 cfu/g in a total of 100 minced meat samples. Djordjević et al (2019) determined the TVC number of minced meat as 4.59 log10 cfu/g. Liu et. al. (2019) determined the number of TVC in the range of 3.84-6.86 log10 cfu/g. Unlike these research findings, Öztürk (2007) determined the TVC number of meat products between 3.49-4.18 log10 cfu/g.

One of the bacteria genus having foodborne pathogen strains frequently encountered in meat and meat products is *Staphylococcus* spp. (Erol 2007). *S. aureus* in these strains is the most important pathogenic strain associated with food poisoning. It is found mainly in the human nasopharynx and animal skin. The presence of *Staphylococcus* spp. in food indicates direct contamination of food by personnel (Gundogan et al 2005). In this research, the number of Staphylococcus spp. in pieced, cubed, and minced meat purchased online/as a customer were respectively determined as 4.58/4.24, 4.67/4.46 and 5.12/5.35 log10 cfu/g. Aydemir Atasever and Atasever (2015) determined the number of *S. aureus* at a maximum level of 4.51 $\log_{10}$ cfu/g and an average of 1.76 log cfu/g. The contamination ratio of *S. aureus* in fresh meat was reported by Gundogan et al (2005), as 60% of 90 beef, by Güven et al (1997) as

53.3% of 80 minced meat and by Sırıken (2004) as 21.4% of 70 minced meat. Öztürk (2007) reported the coagulase positive *Staphylococcus* number of the meat products as 3.42 log10 cfu/g. The number of *Staphylococcus* spp. in raw meat products was determined as minimum 2.0 log10 cfu/g (Selçuk ve Ağaoğlu 2019). It is believed that the low level of the agent detection in the studies given above may be due to the high TVC because *S. aureus* is a weak competitive feature and cannot grow well in cases where its initial number is not high in food. Its growth is easily suppressed by other microorganisms in mixed cultures (Erol 2007).

The number of coliform bacteria is another important indicator of meat quality. Some countries still use *Enterobacteriaceae* or fecal coliform as indicator, the others such as China, Norway, Israel, most of the EU countries use E. coli index to determine/predict coliform numbers in food (Liu et al 2019). In the research, the number of coliform bacteria in pieced, cubed and minced meat purchased online/as customer was respectively determined as 4.65/4.49, 5.01/4.46 and 5.51/5.35 log10 cfu/g. On the other hand, coliform bacteria number of minced meat samples was determined by Gök (2001) and Öztürk (2007), respectively as 2.87 log10 cfu/g. and 4.51 log10 cfu/g. Başkaya et. al. (2004) and Yapar (2006) found the coliform bacteria number of the minced meat as 1x104 log10 cfu/g and 21-43 EMS/g.

In the research, significant differences were found ($P<0.05$) in the number of TVC of the cubed meat and *Staphylococcus* spp. of minced meat purchased online and in the number of TVC and coliform bacteria of pieced meat and in the number of *Staphylococcus* spp. of cubed meat obtained as customers. These differences between companies show that companies do not have certain standards for online sales procedures. According to the t-test results of the samples obtained online and as customer, differences in the number of *Staphylococcus* spp. of cubed meat and TVC of minced meat samples were found significant ($P<0.05$). Considering the statistical differences in the samples purchased as online and customers, it has been concluded that if standards are established in online meat sales and controlled within the framework of the legislation, it will have significant advantages over meat purchased as a customer.

Conclusion

Due to the rapid changes in consumer's demand and the trends in food consumption of the future consumer, food manufacturers need innovation and studies on this subject as a way of survival in trade (Grunert 2006). Meat sales over the internet, which has emerged in recent years in parallel with the continuous improvement and innovation, has created a fast and easy shopping opportunity for consumers. This research was conducted to reveal the quality of the meat sold over the internet and to share the subject with all concerned, it was determined that the pH values of the samples were appropriate in the meat samples supplied online and as customers, and the temperature values were quite high. Statistical differences between companies in terms of pH, temperature, color and microorganism numbers in meats purchased online emphasize the necessity of companies' online sales procedures to have certain standards. When the samples are compared as online and customer, considering the statistical differences in pH values and microbiological findings according to the t-test results, it has been concluded that if standards are set in online meat sales and controlled within the framework of the legislation, it will have significant advantages over the meat purchased as a customer.

In the light of these findings, it is believed that online meat sales should be more prominent in the legislation, as potential food safety risk and poor quality may occur in meats. If the necessary care and control are not performed in online sales, which provide quick and easy shopping for the consumer, online meat will be a major public health problem. In the legislation, it is important to determine the values of the end point temperature when it reaches the consumer, and to define the packaging forms and shipping conditions.

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Conflict of Interest

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During this study, any pharmaceutical company which has a direct connection with the research subject, a company that provides and / or manufactures medical instruments, equipment and materials or any commercial company may have a negative impact on the decision to be made during the evaluation process of the study or no moral support.

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Motivation/Concept: AG, YA, AN; Design: YA, AG; Control/ Supervision: AG; Data Collection and/or Processing: TY, EG, AN; Analysis and / or Interpretation: EG, AN, TY.

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RESEARCH ARTICLE

Effects of Different Rearing Systems on Growth and Fattening Performance of American Bronze Turkeys

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Farklı yetiştirme sistemlerinin Amerikan Bronz hindilerinde büyüme ve besi performansına etkisi

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Öz

Amaç: Bu çalışma farklı yetiştirme koşullarında Amerikan bronz hindilerinin büyüme ve besi performanslarına etkisini araştırmak amacıyla yapılmıştır.

Gereç ve Yöntem: Çalışmada toplamda 123 hayvana ait veriler incelendi. Çıkım ağırlığı ölçülen palazlar ilk 10 hafta birlikte yetiştirildikten sonra deneme gruplarına ayrıldı. Birinci grup kesif yem ile ad-libitium beslenmiştir. Mera grupları yarı entansif ve ekstansif yetiştirme sistemdir. Yarı entansif grup, entansif grubun tüketmesi gereken kesif yemin %75 oranında ksıtlanmış beslemeye tabi tutuldu. Ekstansif yetiştirme sistemindeki hindi sürüsü ise entansif besleme grubundaki hindilerin tüketmesi gereken yemin %50 oranında kısıtlandırılmış oranında beslendiler. Mera grupları meradan 09:00-17:00 saatleri arasında faydalandı. Bu yetiştirme şartlarında 10-32 haftalık yaş döneminde yetiştirilerek iki haftada bir düzenli tartım ve ölçümler yapılarak, hindilere ait büyüme ve besi performansları incelendi.

Bulgular: Hindilerin 32 haftalık canlı ağırlıkları entansif, yarı entansif ve ekstansif sistemlerde sırasıyla dişilerde 4.60-4.70 ve 4.52 kg, erkeklerde 7.58-7.79 ve 6.71 kg olarak bulundu. Yetiştirme sisteminin etkisi erkeklerde 12-32. haftalarda anlamlı iken, dişilerde 14-28. haftalarda gruplar arasında fark ($p<0.05$) tespit edildi.

Öneri: İncelenen tüm parametreler değerlendirildiğinde, Amerikan bronz hindilerin mera koşullarına elverişli bir ırk olduğu söylenebilir. Çalışma gruplarının canlı ağırlık artışı ve yemden yararlanma değerleri esas alındığında, bu ırk için kaliteli meralar sağlandığında 32 haftadan daha uzun sürede de büyümesini devam ettirebileceği görüldü.

Anahtar kelimeler: Amerikan bronz, besi performansı, büyüme, yetiştirme sistemi, hindi.

Abstract

Aim: This study was carried out to investigate the effect of different rearing conditions on the growth and fattening performance of American bronze turkeys

Materials and Methods: In the study, data from a total of 123 American bronze turkeys were analyzed. The hatchlings, whose hatching weight was measured, were reared together for the first 10 weeks, and then divided into the experimental groups. The intensive rearing system group (control group) was fed only with concentrated ad libitum. Pasture groups are semi-intensive and extensive rearing systems. The semi-intensive group was subjected to 75% restricted feeding of the concentrate that the intensive group should consume. The turkey flock in the extensive rearing system was fed 50% of the feed that the turkeys in the intensive rearing system group should consume. Pasture groups benefited from the pasture between 09:00-17:00. The growth and fattening performances of the turkeys were examined by regular weighing and measurements every two weeks as they were being reared in these growing conditions between the ages of 10-32 weeks.

Results: The 32-week live weights of turkeys were found to be 4.60-4.70 and 4.52 kg in females and 7.58-7.79 and 6.71 kg in males, in intensive, semi-intensive, and extensive systems, respectively. The effect of the rearing system was significant in males at 12-32 weeks, moreover, a difference was determined among groups in females at 14-28 weeks.

Conclusion: As a result of, American bronze turkeys can be suitable breed for pasture conditions. Based on the live weight gain and feed efficiency values of the study groups, this breed can continue to grow for longer than 32 weeks when quality pastures are provided.

Keywords: American bronze, fattening performance, growth, rearing system, turkey.

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Introduction

Factors such as the rapid increase in the human population in recent years, as well as the worldwide COVID-19 pandemic and similar epidemics, have given rise to concerns about food supply and safety, especially in developing countries (Arslan et al 2020). Poultry meat production can be effective in reducing these concerns. Because, poultry have shorter generation intervals than other animal species (Mottet and Tempio 2017).

Considering its existence in the world and the usage areas of the meat obtained, it can be said that turkey is an important alternative to chicken (Arslan and Çetin 2022). The commercially grown turkeys are generally heavy turkeys, medium-heavy turkeys, and medium-weight turkeys. Males of this type of turkey can reach a live weight of 16.8-21 kg in 20 weeks, while females reach 9.3-11 kg at the same period (Murawska 2017). The production of turkey is advanced in terms of modernization and capacity in the poultry meat sector. However, feed costs, which are the most important expense, have a large impact on the development and sustainability of this sector (Arıkan et al 2022). Another problem affecting the profitability of the poultry businesses is the deformations that occur in chicken breasts and turkey meats due to the rapid growth of the animals (Nestor et al 1985, Petracci and Cavani 2012). This problem is less common in turkeys, as they take longer to complete their growth than broilers. Turkeys can also reach higher live weights than chickens. This feature makes them an alternative poultry species that can contribute to meeting the animal protein deficit in humans in the future (Jahan et al 2018).

In the intensive system, where heavy turkey breeds are preferred, the live weights of male turkeys with controlled environmental conditions can exceed 24 kg in approximately 200 days (Anonymous 2022a). However, in recent years, alternative rearing systems have been discussed, and the most frequently thought of is the free-range system. Reasons for this include consumer demand and the search for different tastes and flavors, and that the animals could roam freely (Aisyah et al 2018). In this regard, it has been reported that birds exhibit more natural behavior and are less exposed to stress compared to indoor rearing systems (Castellini et al 2002, Stadig et al 2016).

The choice of breed for the free-range system is very important (Devatkal et al 2019). It has been reported that generally slow growing genotypes should be used in free-range rearing systems (Ozbek et al 2020). Genotypes with slow growth rate have a better ability to utilize pasture and a greater resistance to tough climatic conditions than commercial hybrids (Castellini et al 2006, Garip et al 2017). Heavy turkeys with white feathers were preferred than bronze turkey. White turkeys can reach higher live weight and are more desirable for consumers. Bronze turkeys have also contributed to the development of other turkey breeds or lines with better fattening performance and feed efficiency (Arslan and Çetin 2022).

American bronze turkeys are one of the breeds most suitable for organic, ecological, and smallholder production because of good resistant to disease. They will continue to be useful for future production since provided high hatching rate by natural mating. Also, bronze turkeys have a better survival rate than other breeds. Depending on the care and feeding, bronze turkeys can reach slaughter maturity at around 28 weeks of age. Males have a live weight of 10-14 kg and females have a live weight of 5-8 kg. Since bronze turkeys are one of the medium-weight turkey breeds, various researchers (Ozer and Ozbey 2013, Arslan et al 2020, Miah et al 2020, Anonymous 2022b) have reported that they may be more suitable for semi-intensive rearing systems.

Free range breeding systems attract a lot of attention in the poultry industry for both consumers and farmer. This study was to perform the effect of different rearing systems on American Bronze turkeys' growth and fattening performance.

Material and Methods

Material

This study was conducted at the alternative poultry unit belongs to Prof. Dr. Hümeyra Özgen Research and Application Farm in Selcuk University. The grazing area provided to the turkeys was sheltered and fenced land. No additional fodder material was planted on the pasture; natural landform was used. Pasture compositions are given in Table 1.

Animal material

The animal material in the study consisted of 123 turkey poults purchased from a private farm with the necessary vaccinations (Newcastle, TRT). Turkeys were regularly monitored during the experiment.

Husbandry conditions

The temperature, light, humidity, and all biosecurity were checked the day before the poults brought to the rearing rooms. All poults were weighed on a 0.01 g digital scale and subsequently numbered using wing rings. During the experiment, plastic feeders and drinkers were used to meet the feed and water needs of the poults in the chick care and rearing rooms, which were 4x4 m in the alternative poultry unit.

Heater in the rearing room was adjusted into 36 oC on the first day. Then decreased by two degrees every week until the age of eight weeks, when the second feather change started. The heat was provided by electric heaters. Humidity conditions in the rearing room were regularly checked with a digital temperature and humidity measuring device. Poults were housed at eight and 10 weeks of age in open access, closed, and semi-open cages of 4x4 m, considering the weather, climatic, and other environmental conditions.

All animals were illuminated with 23 hours of light and one hour of darkness for the first eight weeks. While no additional lighting was applied to pasture groups (that is, the semi-intensive and extensive rearing system groups), lighting was provided to the intensive group with 23L:1D.

Experimental design

The turkey poults reared together for the first 10 weeks were randomly selected according to their live weights using the zigzag method (Inal 2005). They were divided into three groups. In the control group, the animals were fed ad libitum with concentrate under intensive conditions; in the second experimental group, the animals were fed with 75% of the amount of commercial feed consumed by the first group + pasture under semi-intensive conditions; in the third experimental group animals were fed with 50% of the amount of the commercial feed consumed by the first group + pasture under extensive conditions and were grown until the age of 32 weeks. In this study, animals benefited from pasture from 10 weeks of age to 32 weeks of age. This practice continued from the beginning of spring to the middle of autumn as a season. Literatures were consulted to determine the amount of concentrate to be given to the groups (ad libitum, 75%, 50%) and to give 75% and 50% of the feed consumed by the control group (ad libitum) the following week (NRC 1994). Pasture groups benefited from the pasture between 09:00-17:00.

Contents of feed

The feed used in the study was produced in a special feed factory by determining the energy and nutritional needs of the animals according to the NRC (1994). The purchased turkey feeds were packed in 50 kg bags and stored under appropriate storage conditions. During the experiment, the turkeys were fed with turkey starter feed in powder form at 0-4 weeks of age, granulated turkey grower feed at 5-8

Table 1. Nutrient content of the rations used in the experiment in dry matter

	Starter	Grower	Fattening-I	Fattening-II	Pasture composition
Crude Ash %	5.71	5.21	5.59	4.68	2.97
Crude Oil %	4.81	3.94	5.27	7.58	3.63
Crude Cellulose %	9.03	7.26	5.78	5.81	9.92
Crude Protein %	27.59	25.53	21.38	22.27	6.46
ME kcal/kg*	3004	3052	3127	3241	2920

*: Calculated by the formula (Karabulut and Canbolat, 2005).

Table 2. Live weights of turkey poults (g) for 0-8 weeks of age (initial period) according to sex ($\bar{x}\pm S\bar{x}$)

Age (week)	Male n=64	Female n=59	Total n=123	p
Hatched	47.73±0.48	47.28±0.55	47.50±0.37	-
1	79.58±1.30	74.22±1.04	76.79±0.86	**
2	129.36±2.44	114.52±2.58	121.63±1.90	***
3	231.71±5.17	201.98±4.95	216.24±3.81	***
4	368.21±8.39	315.69±7.85	340.66±6.18	***
5	513.58±11.96	438.86±10.42	474.70±8.56	***
6	665.34±15.00	561.72±12.19	611.42±10.64	***
7	895.30±0.02	746.40±0.02	817.80±0.01	***
8	1135.80±0.02	928.00±0.02	1027.60±0.02	***

Differences between values with different letters on the same line are significant. (-: p>0.05, **: p<0.01, ***: p<0.001)

Table 3. LWG, DFC, and FCR values of turkey poults in the initial period (0-8 week)

Age (week)	n	LWG (g)	DFC (g)	FCR
0-1	123	29.29	56.45	1.93
1-2	123	44.85	107.19	2.39
3-4	123	94.61	168.86	1.78
0-4	123	73.29	155.34	2.12
5-6	123	134.04	397.66	2.91
6-7	123	136.72	438.19	2.12
7-8	123	206.38	532.28	2.54
0-8	123	122.52	289.1	2.36

LWG: Live Weight Gain, DFC: Daily Feed Consumption, FCR: Feed Consumption Ration.

Table 4. Fattening performances of turkeys in different rearing systems (LWG, DLWG, DFC, FCR)

	Intensive				Semi-Intensive				Extensive			
Age/ week	LWG (g)	DLWG (g)	DFC (g)	FCR	LWG (g)	DLWG (g)	DFC (g)	FCR	LWG (g)	DLWG (g)	DFC (g)	FCR
11-12	314.72	22.48	110.02	4.89	231.59	16.54	81.77	4.94	116.86	8.35	52.55	6.3
13-14	427.14	30.51	118.51	3.88	366.19	26.16	100.62	3.85	208.5	14.89	69.05	4.64
15-16	448.7	32.05	135.04	4.21	431.2	30.8	120.75	3.92	522.5	37.32	88.1	2.36
17-18	261.94	18.71	172.62	9.22	526.68	37.62	145.16	3.86	332.8	23.77	101.7	4.28
19-20	689.92	49.29	195.14	3.96	426.0	30.43	147.54	4.85	376.2	26.87	101.57	3.78
21-22	469.0	33.5	221.02	6.6	617.0	44.07	150.0	3.4	563.0	40.21	100.0	2.49
23-24	462.98	33.07	185.63	5.61	292.0	20.86	168.64	8.09	304.0	21.71	98.4	4.53
25-26	308.98	22.07	199.74	9.05	694.0	49.57	169.92	3.43	783.0	55.93	111.7	1.20
27-28	440.02	31.43	196.92	6.26	135.0	9.64	159.52	16.54	222.0	15.86	118.34	7.46
29-30	141.96	10.14	164.07	16.18 .0	463.0	33.07	173.08	5.23	545.0	38.93	114.94	2.95
31-32	868.0	62.0	221.8	3.58	501.0	35.79	189.58	5.3	389.0	27.78	111.52	4.01
10-32	439.46	31.39	174.59	6.68	429.79	30.41	146.05	5.76	396.62	28.33	97.08	4.0

LWG: Live Weight Gain, DLWG: Daily Live Weight Gain, DFC: Daily Feed Consumption, FCR: Feed Consumption Ration.

weeks of age, pelletized turkey fattening feed (fattening-1) at 9-20 weeks and broiler feed (fattening-2) at 21-32 weeks until 32 week of age (Table 1). The feed consumed by the animals during the walk in the pasture is not included.

Method

Data collection

The animals were weighed individually once every week during the first 10 weeks and then every two weeks from 10-32 weeks of age throughout the study. The growth results were obtained with the data obtained from this weighing. The remaining amount of feed given to the animals in the experiment was measured at each weighing time with a digital scale with a precision of 0.01 g. All the data obtained were regularly recorded in Microsoft Excel throughout the study.

Non-repetitive group feeding was applied in the experimental groups. For this reason, feed consumption and feed efficiency values are given only as average values.

Feed conversion ratios were calculated daily by dividing the average weekly feed consumption of turkeys by the average live weight gain in the same week.

Statistical analysis

The SPSS 23.0 (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.) software was used to analyze the data. In evaluating the data, the independent t-test was used to compare groups by sex, while analysis of variance (ANOVA) was performed to determine the effect of the rearing system alone. Time-dependent analyses of different ages,rearing systems and

sex group were evaluated with the General Linear Model for Repeated Measure.

Results

The live weights of the male and female turkeys during the first eight weeks are given in Table 2.

In this study, no difference was found between the sex factor in terms of hatch weight, however the male poults had higher live weights than the females from one to eight weeks of age ($p<0.01$). Regardless of sex factor, the turkey poults reached about five times their hatch weight at three weeks of age, and about twenty times their hatch weight at eight weeks of age (Table 2).

The values calculated without including the effects of the sex and rearing system on the live weight gain (LWG), feed consumption (FC) and feed conversion ratios (FCR) of the turkey poults in the initial period (0-8 week) are given in Table 3.

As seen in Table 3, the amount of feed used increased as the birds aged. It was determined that the daily feed consumption (DFC) per animal in the initial period (0-4 weeks of age) was 155.34 g, while the FCR value was 2.12 (Table 3). Moreover, it was found that the DFC value per 0–8-week-old turkey poult was 289.10 g, while the FCR value was 2.36.

Table 5. Live weights of turkeys (kg) aged 10-18 weeks reared in different rearing conditions

Age (Week)	Rearing System	Male		Female	
		n	$\bar{x}\pm S\bar{x}$	n	$\bar{x}\pm S\bar{x}$
9-10	Intensive	17	1.43±0.05	16	1.10±0.05
	Semi-Intensive	19	1.44±0.03	27	1.19±0.02
	Extensive	23	1.39±0.05	21	1.20±0.05
	Total	59	1.42±0.02	64	1.17±0.02
	p		-		-
11-12	Intensive	17	1.79±0.06[a]	16	1.39±0.06
	Semi-Intensive	19	1.71±0.04[a]	27	1.41±0.03
	Extensive	23	1.50±0.05[b]	21	1.29±0.05
	Total	59	1.65±0.03	64	1.36±0.03
	p		***		-
13-14	Intensive	17	2.26±0.08[a]	16	1.76±0.06[a]
	Semi-Intensive	19	2.13±0.05[a]	27	1.74±0.03[a]
	Extensive	23	1.70±0.05[b]	21	1.49±0.06[b]
	Total	59	2.00±0.05	64	1.66±0.03
	p		***		***
15-16	Intensive	17	2.78±0.10[a]	16	2.12±0.07[a]
	Semi-Intensive	19	2.60±0.05[a]	27	2.13±0.04[a]
	Extensive	23	2.23±0.07[b]	21	1.94±0.07[b]
	Total	59	2.51±0.05	64	2.07±0.03
	p		***		*
17-18	Intensive	17	3.25±0.13[a]	16	2.47±0.07[a]
	Semi-Intensive	19	3.15±0.06[a]	27	2.58±0.04[a]
	Extensive	23	2.64±0.08[b]	21	2.24±0.07[b]
	Total	59	2.98±0.06	64	2.44±0.04
	p		***		***

Differences between values with different letters in the same column are significant (-:p>0.05, *:p <0.05, ***: p<0.001).

Table 6. Live weights of turkeys (kg) aged 19-32 weeks reared in different rearing conditions

Age (week)	Rearing System	Male		Female	
		n	x̄±Sx̄	n	x̄±Sx̄
19-20	Intensive	17	3.86±0.15^{a}	16	2.83±0.09^{ab}
	Semi-Intensive	19	3.72±0.07^{a}	27	3.00±0.05^{a}
	Extensive	23	3.11±0.08^{b}	21	2.62±0.08^{b}
	Total	59	3.52±0.07	64	2.84±0.05
	p		***		***
21-22	Intensive	17	4.48±0.19^{a}	16	3.25±0.11^{b}
	Semi-Intensive	19	4.49±0.09^{a}	27	3.57±0.07^{a}
	Extensive	23	3.68±0.10^{b}	21	3.09±0.09^{b}
	Total	59	4.17±0.09	64	3.33±0.06
	p		***		***
23-24	Intensive	17	5.12±0.22^{a}	16	3.56±0.09^{ab}
	Semi-Intensive	19	4.88±0.15^{a}	27	3.70±0.07^{a}
	Extensive	23	4.15±0.11^{b}	21	3.29±0.08^{b}
	Total	59	4.66±0.10	64	3.53±0.05
	p		***		***
25-26	Intensive	17	5.72±0.26^{ab}	16	3.81±0.08^{b}
	Semi-Intensive	19	5.99±0.22^{a}	27	4.25±0.08^{a}
	Extensive	23	5.21±0.14^{b}	21	3.95±0.10^{ab}
	Total	59	5.61±0.12	64	4.05±0.06
	p		*		***
27-28	Intensive	17	6.35±0.28^{a}	16	4.02±0.07^{b}
	Semi-Intensive	19	6.39±0.22^{a}	27	4.36±0.09^{a}
	Extensive	23	5.37±0.13^{b}	21	4.06±0.11^{ab}
	Total	57	5.99±0.14	64	4.18±0.06
	p		***		*
29-30	Intensive	17	6.63±0.31^{ab}	16	4.11±0.09
	Semi-Intensive	19	6.96±0.28^{a}	27	4.47±0.09
	Extensive	23	6.12±0.14^{b}	21	4.30±0.13
	Total	59	6.54±0.14	64	4.32±0.06
	p		*		-
31-32	Intensive	17	7.58±0.34^{ab}	16	4.60±0.11
	Semi-Intensive	19	7.79±0.33^{a}	27	4.70±0.11
	Extensive	23	6.71±0.19^{b}	21	4.52±0.13
	Total	59	7.31±0.17	64	4.61±0.07
	p		**		-

Differences between values with different letters in the same column are significant (-:p>0.05, *:p<0.05, **:p<0.01, ***:p<0.001).

The values for live weight gain (LWG), daily live weight gain (DLWG), DFC, and FCR of the turkeys at 10-32 weeks of age in the different rearing systems are given in Table 4.

Average live weight gain (LWG), daily live weight gain (DLWG), DFC, and FCR values were presented in Table 4. The LWG values of the intensive , semi-intensive and extensive system were determined as 439.46 , 429.79, 396.62 g; DLWG values 31.39, 30.41, 28.33 g; DFC values 174.59 , 146.05, 97.8 g per animals, respectively. In addition of these, FCR values of the groups in the intensive, semi-intensive and extensive systems were found to be 6.68, 5.76, and 4.0, in the same line. The live weights of the turkeys reared in intensive, semi-intensive, and extensive rearing systems at the age of 10-32 weeks are given in Tables 5 and 6.

Male turkeys were found to have a higher live weight ($p<0.001$) than female turkeys for all age periods examined in all experimental groups (Tables 5 and 6). The live weights of American bronze turkeys at 16 and 32 weeks of age in the intensive rearing system were 2.12-4.60 kg in females and 2.78-7.58 kg in males. The live weights of female and male turkeys at the age of 32 weeks in the semi-intensive rearing system were 4.70 and 7.79 kg respectively. In the extensive rearing system, it was found that the females and males had a live weight of 4.52 and 6.71 kg, respectively for the same weekly age.

Table 7. The interactions between Live Weight (kg), Age, Rearing Systems, and Sex in turkeys

Age (Week)	Live Weight (Mean ± SE)
12	1.51±0.02^{f}
16	2.30±0.03^{e}
20	3.19±0.04^{d}
24	4.12±0.05^{c}
28	5.09±0.07^{b}
32	5.97±0.09^{a}
Rearing System	
Intensive	3.83±0.08^{a}
Semi intensive	3.86±0.07^{a}
Extensive	3.39±0.07^{b}
Sex	
Male	4.31±0.06^{a}
Female	3.09±0.06^{b}
Interactions	
Age x Rearing System	*
Age x Sex	***
Rearing System x Sex	*
Age x Rearing System x Sex	-

Differences between values with different letters in the same column are significant (-:$p>0.05$, *:$p<0.05$, **:$p<0.01$, ***:$p<0.001$).

The effect of the rearing system on live weight was no significant at 10 and 12 weeks for female turkeys and at 10 weeks for male turkeys (Table 5).

As seen in Table 6, the rearing system affected the live weights at 19-32 weeks in females, except for the 30th and 32nd weeks ($p<0.01$). In male turkeys, it was determined that the rearing system had an effect at all these age periods ($p<0.05$). In addition, those female turkeys take advantage of pasture from the 26th week to slaughter showed a performance close to the intensively reared turkeys ($p<0.05$).

The interactions between live weight, age, rearing systems, and sex in turkeys are presented in Table 7.

As table 7, American bronze turkey effected by age, rearing systems, and sex factor. Moreover, Age x Rearing system ($p<0.05$), Age x Sex ($p<0.001$), and Rearing system x Sex ($p<0.05$) interactions were found to significantly.

Discussion

This study was to perform the effect of different rearing systems on American Bronze turkeys' growth and fattening performance. The growth of animals is a significant factor in poultry production for meat (Putra and Kırıkçı 2021). The most rapid development of muscle tissue in turkeys occurs during the initial period (Moore et al. 2005). The average live weights of turkeys for the beginning period are given in Table 2. It was determined that male and female turkeys with similar hatch weights had higher live weights in the first eight weeks of age ($p<0.05$). The values found in the present study for the beginning period of growth in turkeys are similar to the results of another research (Laudadio et al 2009, Rivera-Torres et al 2011, Mikulski et al 2012, Ozer and Ozbey 2013, Das et al 2018, Nwaodu et al 2018).

The LWG, DFC and FCR of the turkey poults in the initial period are presented in Table 3. In this study, the FCR in the first eight weeks of age was calculated as 2.36. This value is compatible with the values reported by Şengül et al (1999) in bronze turkeys. In commercial white turkeys, Laudadio et al (2009) found evidence of worse FCR between the ages of 31-44 and 45-58 days, varying between 2.06-2.38 and 2.16-2.26 respectively.

That the different results obtained in the study may be due to differences in flock management (Scanes et al 1984). In a mixed flock of male and female turkeys from different turkey genotypes, Damaziak et al 2012 reported feed intake as 1.46 kg and 1.15 kg and FCR as 1.37 and 2.09 in primitive and commercial turkeys, respectively. Safiyu et al (2019) found the DFC and FCR values at 6-12 weeks of age in domestic turkey breeds to be 3.55 and 3.76 in white and black feather-colored turkeys, while they were 3.63 and 3.68 in open-access rearing systems and closed-access rearing systems, respectively. In the present study, it can be said that the DFC and FCR values that are worse than others reported may have been due to genotype, age, or care-feeding differences. Fattening performances of turkeys in different rearing systems are given in Table 4.

In this study, the FCR values at the age of 10-32 weeks were calculated as 6.68, 5.76 and 4.0in turkeys in the intensive, semi-intensive systems, and extensive system, respectively (Table 4). These values are worse than the values obtained by Mikulski et al (2012) from heavy whites aged 147 days and by Das et al (2018) from bronze turkeys, however better than the values reported by Karki (2005) for turkeys aged 0-28 weeks. While they were found to be consistent with the values reported by Sarıca et al (2009) for bronze turkeys reared in free-range systems, they were contrast with Bashir et al (2012) who found that intensive rearing is better than semi-intensive regarding FCR. In terms of DFC value, it is compatible with Karki's (2005) study, however the FCR value

was found to be lower than the FCR finding. This difference may be due to pasture productivity (Ozer and Ozbey 2013).

Laudadio et al (2009), reported the DLWG, FCR and DFC values of heavy whites at 4-16 weeks of age as 76-80 g, 3.77-4.26 and 222-245 g respectively. The fact that these values were not like the findings of the present study may be due to the difference in genotype (Miah et al 2020). Das et al (2018) reported the FC value under semi-intensive conditions in bronze turkeys as 24.29 g at 21 weeks of age. In this study, the DFC per animal was calculated as 44.07 g in turkeys aged 20-22 weeks reared in semi-intensive conditions. The difference between the two research findings in terms of DFC value may be due to pasture productivity and climatic conditions.

These differences may have arisen due to many factors such as genotype, age, pasture opportunities, ration content (Ozer and Ozbey 2013, Inci 2020, Miah et al 2020, Arslan et al 2022).

Inci (2020) has stated that pasture use improves feed efficiency ($p<0.05$). It was found to be 4.0 in this study. However, statistical significance could not be calculated at 10-32 weeks of age. In the group with 50% concentrate restriction, Sarıca et al (2009) and Ozer and Ozbey (2013) support the view that rangeland-based rearing systems have the potential to be more economical.

Efforts to improve the genetic capacity of animals, developments in nutrition and advances in poultry flock management can lead to rapid growth rates and high feed efficiency in poultry production for meat yield (Duclos et al 2007). The live weights of the turkeys reared in different conditions at the age of 10-32 weeks are given in Tables 5 and 6. The live weight values of turkeys during the rearing and fattening period have been examined by many researchers (Arslan 1999, Karki 2005, Laudadio et al 2009, Mikulski et al 2012, Ozer and Ozbey 2013, Das et al 2018, Nwaodu et al 2018, Inci 2020). In the present study, the live weight values for the females and males at 16 weeks of age were determined as 2.07 kg and 2.51 kg, respectively ($p<0.001$), and the effect of the rearing system on live weight was found to be significant ($p<0.05$). The fact that the values obtained were lower than the values in bronze turkeys reported by Arslan (1999) and Ozer and Ozbey (2013) for the relevant weekly age may be associated with the difference in care and feeding (Miah et al. 2020). The live weight values in this study were found higher than those reported by Arslan (1999) and Ozer and Ozbey (2013). However, this is not compatible with the finding of Arslan (1999), who stated that the rearing system affects live weight. The 20th week live weight values stated in Table 3.2.1.4 were found to be higher than the value obtained by Das et al (2018). However, the live weights of the 20-week-old females and males were lower than the value reported by Karki (2005), at 3.3 kg and 4.5 kg, respectively. This is compatible with males generally being heavier than females. This may be due to environmental and genetic factors (Arslan and Çetin 2022) or the difference in the abilities of the animals to use energy (Rivera-Torres et al 2011). In the present study, the live weights of 22-week-old turkeys in the intensive, semi-intensive and extensive systems were calculated as 4.48 kg, 4.49 kg and 3.68 kg in the males, and 3.25 kg, 3.57 kg, and 3.09 kg in the females respectively. When an overall assessment is made, the different results obtained in similar studies (Mikulski et al 2012, Inci 2020) may be due to differences in genotype, age, care and feeding, climate and pasture.

Conclusion

Fully exploiting the genetic potential of turkeys, as in other species, will only be possible with proper care and feeding. In the future, examining the fattening performances of American bronzes by providing free roaming opportunities in the fields with enriched quality pastures from the age of 16 weeks may create a more economical production model for this breed. In extensive and semi-intensive rearing systems based on pastures, an ecological and economical rearing model can be developed that can benefit from insect species such as grasshoppers and plants, especially acorns, by reducing feed consumption compared to intensive rearing system. As a result, breeders can choose to turkeys to avoid a possible future nutrient deficiency crisis for humanity and to meet protein needs or to seek a different taste. It can be said that turkeys as an alternative poultry species to chicken.

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Conflict of Interest

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Ethical Approval

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RESEARCH ARTICLE

Impact of deformed wing virus master variants (DWV-A, DWV-B, and DWV-C) in managed honey bee colonies of Türkiye

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Türkiye'de yer alan bal arısı kolonilerinde deforme kanat virüsü ana varyantlarının (DWV-A, DWV-B ve DWV-C) etkisi

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Öz

Amaç: Bu çalışmada, Türkiye'nin İç Anadolu ve Akdeniz Bölgelerinde yer alan bal arısı kovanlarında deforme kanat virüsü (DWV) ana varyantlarının belirlenmesi amaçlandı. Ayrıca arı kovanlarında sirküle olan DWV genotiplerinin bal arısı kovanlarında gözlenen klinik belirtilerle ilişkisi araştırıldı.

Gereç ve Yöntem: Bu çalışma için Aksaray, Isparta, Karaman, Konya ve Niğde illerinden 2019 yılı ilkbahar-yaz ve sonbahar sezonlarında aynı 25 kovandan yetişkin bal arıları toplandı. DWV'ye özgü nükleik asit ve DWV genotipleri, sırasıyla DWV gerçek zamanlı RT-PCR tahlili ve ABC tahlili ile tespit edildi.

Bulgular: DWV enfeksiyonu örnekleme yapılan her mevsimde tespit edildi. Örneklenen bir çok kolonide klinik bulgu görülmezken, bazı arılıklarda kanatlarda şekil bozukluğu, titreme, felç, karında şişlik, verim kaybı ve ölü arılar gözlemlendi. Erişkin bal arılarında DWV-A, DWV-B ve DWV-C yaygınlıkları sırasıyla %62, %82 ve %24 idi. Arı kovanlarında tespit edilen baskın genotip, DWV-B ana varyantıydı (%98). Ayrıca DWV-A ana varyantının virüs yükü, kışlama kayıpları görülen bal arısı kovanlarının tamamında yüksekti.

Öneri: Bu çalışmada, Türkiye'de sirküle olan DWV ana varyantlarının mevcut durumu ve bal arısı kolonileri üzerindeki etkileri ile ilgili veriler ilk kez rapor edildi. Böylece Türkiye arı kovanlarında yılın her mevsiminde değişen oranlarda verim kayıplarına neden olan DWV'nin dikkatle izlenmesi gerektiği önerilmektedir.

Anahtar kelimeler: Bal arısı, DWV ana varyantları, gerçek zamanlı RT-PCR, klinik belirtiler

Abstract

Aim: This study aimed to determine the deformed wing virus (DWV) master variants in managed honey bee hives in Central Anatolia and the Mediterranean Regions of Türkiye. Also, the relationship of DWV genotypes circulating in the apiaries with clinical signs observed in honey bee hives was investigated.

Materials and Methods: For this study, adult honey bees were collected from the same 25 hives in the spring-summer and autumn seasons of 2019 from the provinces of Aksaray, Isparta, Karaman, Konya and Nigde. DWV-specific nucleic acid and DWV genotypes were detected by DWV real-time RT-PCR assay and ABC assay, respectively.

Results: Deformed wing virus infection was detected in each sampling season. While many colonies were without any clinical signs, in some of the apiaries where samples were collected, wing deformity, trembling, paralysis, swelling in the abdomen, loss of productivity, and dead bees were observed. The prevalences of DWV-A, DWV-B, and DWV-C in adult honey bees were 62%, 82%, and 24%, respectively. The dominant genotype detected in bee hives was the DWV-B master variant (98%). Also, the virus load of the DWV-A master variant was high in all of the honey bee hives with wintering losses.

Conclusion: In this present study, data on the current status of DWV master variants circulating in Turkey and their impacts on honey bee colonies are reported for the first time. Thus, it is thought that DWV, which causes yield losses at varying rates in every season of the year in Turkish bee hives, should be carefully monitored.

Keywords: Clinical signs, DWV master variants, honey bee, real-time RT-PCR

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Introduction

The activities of managed honey bee (*Apis mellifera*) colonies are significant economically and ecologically (Venturini et al 2017, Lester 2021). Thanks to its rich floral biodiversity and more than 8 million honey bee hives, Turkey is among the most important countries for honey beekeeping (Doğanay 2017, TÜİK 2022). The critical decline of the honey bee population in recent years is worrying (Kevill et al 2019). Especially RNA viruses are among the causes of distress observed in honey bee hives (Kevill et al 2019, Brasesco et al 2021).

The deformed wing virus (DWV) is a member of the genus Iflavirus of the family Iflaviridae, belonging to the order Picornavirales. The single-stranded positive-sense RNA genome has a single open reading frame (ORF) (Valles et al 2017). Furthermore, DWV has three genotypes (Type A-B-C) that differ in their pathogenic effects and genetic diversity (Brettell et al 2017). DWV was previously reported as one of the severe honey bee viruses for hive health (ANSES 2011). Although subclinical DWV infections are common in hives (Mouret et al 2013), typical symptoms of disease caused by DWV are wing deformity, paralysis, abdominal swelling, discolouration, decreased lifespan, and death (Chen and Siede 2007, Ryabov et al 2016).

Having more than one strain or genotype of virus species is one of their well-defined characteristics (Piot and Smagghe 2022). DWV is one such virus that represents three genotypes or master variants (Martin et al 2012, Mordecai et al 2016, Martin and Brettell 2019). The DWV type-A master variant includes the original DWV genotype (Lanzi et al 2006) and the Kakugo virus (Fujiyuki et al 2004). Initially reported as Varroa destructor virus-1 (VDV-1) (Ongus et al 2004) in the taxonomy, the virus was assigned as the DWV type-B master variant (Mordecai et al 2016). Distinguished from the other two master variants, DWV type-C is the last master variant of the DWV species complex (Mordecai et al 2016). Infections of the DWV-A and DWV-B variants have been associated with overwintering colony death. Moreover, co-infection with DWV-B and Varroa destructor can be critical for a honey bee colony (Genersch et al 2010, Dainat et al 2012). It has also been reported that DWV-B may be more virulent than DWV-A (McMahon et al 2016, McMenamin and Flenniken 2018).

DWV is common in managed honey bee colonies globally (Cirkovic et al 2018, Hassanyar et al 2019, Avci et al 2022). Varroa mite is an effective vector for the transmission of DWV and mediates the worldwide spread of this virus (Berenyi et al 2006, Wilfert et al 2016). At the end of the summer, the prevalence of DWV increased with the mite infestation in the hives (De Miranda et al 2013), and it was reported that the adult honey bees with normal appearance were short-lived (Martin 2001). Thus, DWV infections can cause wintering losses or colony extinction in the spring (Ball 2001, Dainat et al 2012, Nazzi et al 2012).

Explaining the evolution of DWV and so the effect of its genotypic diversity on the honey bee is a biological process that may take many years (Martin and Brettell 2019). Some studies focused on exploring the selection or effects of DWV master variants to elucidate this dynamic process (Ryabov et al 2017, Brasesco et al 2021, De Souza et al 2021). Also, studies are concentrating on DWV prevalence and phylogenetic analysis in Turkey, and the prevalence rate of DWV has been reported to be between 1.8% and 84% (Kalayci et al 2020, Çağırgan and Yazici 2021, Avci et al 2022, Usta and Yıldırım 2022). However, there are no reports on the genotypic diversity of DWV yet. This research aimed to investigate and genotype the impacts of DWV on managed honey bee health to explain the influence of DWV variants at the colony level (representing apiaries) in the Central Anatolia and Mediterranean Regions of Turkey and to contribute to the dynamic nature of DWV variants.

Material and Methods

Material

Honey bee samples

The adult honey bees were collected from 25 hives at a 95% confidence interval and 5% expected prevalence, using the multistep sampling method in Aksaray, Isparta, Karaman, Konya, and Nigde provinces, where beekeeping is common. The samples of at least 30 adult honey bees were collected from the same colonies during the spring/summer and autumn months of 2019. Samples were kept on dry ice in sterile laboratory tubes during carry to the laboratory and stored at −80 °C until virus nucleic acid isolation. Clinical signs observed in the study colonies were recorded to understand the influence of DWV master variants at the colony level.

Method

Viral RNA isolation

Seven adult honey bees from each hive were placed in sterile centrifuge tubes and homogenized in 7 ml of PBS. These samples were centrifuged at 4000 rpm at 4°C for 10 min two times to obtain supernatants. Viral RNA was isolated from these supernatants according to the manufacturer's recommendations using an IndiSpin Pathogen Kit (Indical Bioscience, Germany).

DWV real-time RT-PCR assays

The DWV-specific RNA was specified with real-time RT-PCR using a QuantiNova Pathogen +IC Kit (Qiagen, Germany)

with a previously reported assay (Chantawannakul et al. 2006). The DWV real-time RT-PCR assay was carried out in a 20 µl reaction volume, containing 5 µL 4X Pathogen Master Mix, 5 µL template RNA,1 µL TaqMan probe (5 pmol/µL), 0.8 µL forward primer (10 pmol/µL), 0.8 µL reverse primer (10 pmol/µL), and 7.4 µL PCR-grade water. The assay conditions were as follows: reverse transcription at 50 °C for 10 min; reaction initial activation at 95 °C for 2 min followed by 40 cycles of denaturation at 95 °C for 5 sec, and primer annealing and extension at 60 °C for 30 sec.

Samples detected as DWV-positive were then analysed for DWV master variants with ABC assay (Kevill et al 2017), using a QuantiNova Pathogen +IC Kit. The reaction mixture and thermal cycling conditions for ABC real-time RT-PCR assay are shown in Table 1. Also, the primer sets used in the study are listed in Table 2.

Exogenous internal control assays

To consider the in-analysis reliability of the DWV real-time RT-PCR assay and ABC assay, the exogenous internal control assays were performed, in which both viral RNA isolation and RT-PCR efficiency were controlled. The exogenous internal control assay was based on visualising a commercially available synthetic RNA (200 bp) amplification from the QuantiNova Pathogen +IC Kit.

Results

Clinical signs

Deformed wing virus master variants infection was detected in each sampling season, and Varroa infestation was also observed in these hives. While many colonies were without any clinical signs, in some of the apiaries where samples were collected, wing deformity, trembling, paralysis, swelling in the abdomen, loss of productivity, and dead bees were observed. Additionally, no overt infections appeared in larvae and pupa samples, except for Varroa infestation, and the queen bees were also healthy in the colonies. The assays results of the study samples and the clinical signs observed in the apiaries are shown in Table 3.

Prevalence of DWV master variants

The result of DWV real-time RT-PCR and ABC assay revealed prevalence patterns and master variants of DWV in five provinces. The DWV real-time RT-PCR showed that the prevalence of DWV was 42/50 (84%) of the adult honey bees. Also, the prevalence rate of DWV-A, DWV-B, and DWV-C master variants was 62% (31/50), 82% (41/50) and 24% (12/50), respectively (Table 4).

Table 1. ABC real-time RT-PCR assay reaction mixture and thermal cycling conditions

Reagent	Volume (µl)	Thermal cycling conditions	Temperature (°C)	Time	Cycle
4X Pathogen Master Mix	5	Reverse transcription	50	10 min	1
Primer fwd (10 pmol/µl)	0.8	PCR initial activation	95	2 min	1
Primer rev (10 pmol/µl)	0.8	Denaturation	95	5 sec	
EvaGreen dye	1	Annealing/ Extension	58.5* and 61.5**	30 sec	40
RNA template	5				
PCR-grade water	7.4				
Total volume	20				

* DWV-A and DWV-B primers annealing
** DWV-C primer annealing

Table 2. Primers used in this study

Virus	Primer	Primer sequence (5'-3')	Reference
DWV	DWV958F	CCTGGACAAGGTCTCGGTAGAA	Chantawannakul et al 2006
	DWV9711R	ATTCAGGACCCCACCCAAAT	
	DWV9627T	FAM-CATGCTCGAGGATTGGGTCGTCGT-TAMRA	
	DWVnew-F1	TACTAGTGCTGGTTTTCCTTT	
DWV Type A	DWVA-R1	CTCATTAACTGTGTCGTTGAT	Kevill et al 2017
DWV Type B	DWVB-R1	CTCATTAACTGAGTTGTTGTC	
DWV Type C	DWVC-R1	ATAAGTTGCGTGGTTGAC	

Table 3. Real-time RT-PCR assays results of the study samples and the clinical signs

Sample	Season	Clinical signs				C_T value of DWV[a]	C_T value of DKV-A[b]	C_T value of DKV-B[b]	C_T value of DKV-C[b]
		Varroa	Wing deformity	Death	Other signs				
Isparta-Bee-İ1	Spring/summer	+	-	+	-	22.92	21.40	29.60	No C_T
Isparta-Bee-S1*	Autumn	+	+	+++	+	6.95	11.99	29.78	26.62
Isparta-Bee-İ2	Spring/summer	+	-	-	-	30.34	30.77	29.53	No C_T
Isparta-Bee-S2	Autumn	++	-	+	+	15.54	30.35	16.80	No C_T
Isparta-Bee-İ3	Spring/summer	+	-	-	-	21.82	30.96	22.48	No C_T
Isparta-Bee-S3	Autumn	-	-	-	-	33.12	No C_T	33.84	No C_T
Isparta-Bee-İ4	Spring/summer	+	-	-	-	27.81	28.08	29.47	No C_T
Isparta-Bee-S4	Autumn	+	-	-	-	27.40	29.98	29.70	No C_T
Isparta-Bee-İ5	Spring/summer	+	-	-	-	29.19	28.75	28.90	No C_T
Isparta-Bee-S5	Autumn	+	-	-	+	22.27	32.77	23.99	No C_T
Konya-Bee-İ1	Spring/summer	+	+	-	+	23.83	No C_T	22.14	25.21
Konya-Bee-S1	Autumn	+	-	-	-	32.68	No C_T	33.57	No C_T
Konya-Bee-İ2	Spring/summer	+	-	-	-	27.88	No C_T	28.44	No C_T
Konya-Bee-S2	Autumn	+	-	-	-	29.57	No C_T	27.12	No C_T
Konya-Bee-İ3	Spring/summer	+	-	-	-	22.86	33.16	22.12	No C_T
Konya-Bee-S3	Autumn	-	-	-	-	32.29	No C_T	29.90	No C_T
Konya-Bee-İ4	Spring/summer	+	-	-	-	29.57	32.40	27.52	24.56
Konya-Bee-S4	Autumn	+	+	-	+	20.68	29.10	21.46	28.04
Konya-Bee-İ5	Spring/summer	+	+	++	+	14.30	15.00	28.77	27.15
Konya-Bee-S5*	Autumn	++	+	+++	+	12.39	12.13	26.47	23.49
Aksaray-Bee-İ1	Spring/summer	-	-	-	-	No C_T	No C_T	No C_T	No C_T
Aksaray-Bee-S1	Autumn	-	-	-	-	31.69	No C_T	32.00	No C_T
Aksaray-Bee-İ2	Spring/summer	-	-	-	-	33.73	No C_T	32.40	No C_T
Aksaray-Bee-S2	Autumn	-	-	-	-	33.42	No C_T	34.15	No C_T
Aksaray-Bee-İ3	Spring/summer	-	-	-	-	No C_T	No C_T	No C_T	No C_T
Aksaray-Bee-S3	Autumn	-	-	-	-	No C_T	No C_T	No C_T	No C_T
Aksaray-Bee-İ4	Spring/summer	-	-	-	-	30.70	No C_T	29.78	No C_T
Aksaray-Bee-S4	Autumn	-	-	-	-	No C_T	No C_T	No C_T	No C_T
Aksaray-Bee-İ5	Spring/summer	+	-	-	-	29.96	33.14	28.95	No C_T
Aksaray-Bee-S5	Autumn	+	-	-	-	19.52	30.80	20.16	No C_T
Karaman-Bee-İ1	Spring/summer	-	-	-	-	No C_T	No C_T	No C_T	No C_T
Karaman-Bee-S1	Autumn	++	+	-	-	24.94	29.45	24.70	No C_T
Karaman-Bee-İ2	Spring/summer	-	-	-	-	No C_T	No C_T	No C_T	No C_T
Karaman-Bee-S2	Autumn	+	-	-	-	24.63	28.08	24.56	No C_T
Karaman-Bee-İ3	Spring/summer	+	-	-	-	25.68	30.44	26.74	28.83
Karaman-Bee-S3*	Autumn	+++	+	+++	+	8.49	10.39	27.41	29.25
Karaman-Bee-İ4	Spring/summer	-	-	-	-	No C_T	No C_T	No C_T	No C_T
Karaman-Bee-S4	Autumn	-	-	-	-	No C_T	No C_T	No C_T	No C_T
Karaman-Bee-İ5	Spring/summer	++	-	-	-	22.65	29.07	24.00	No C_T
Karaman-Bee-S5	Autumn	++	-	-	+	23.08	29.80	22.40	No C_T
Nigde-Bee-İ1	Spring/summer	+	-	-	-	28.40	30.16	31.34	No C_T
Nigde-Bee-S1	Autumn	+	+	+	+	14.77	16.90	28.99	No C_T
Nigde-Bee-İ2	Spring/summer	-	-	-	-	15.73	21.62	22.41	24.26
Nigde-Bee-S2	Autumn	++	-	-	+	26.70	29.06	25.26	No C_T
Nigde-Bee-İ3	Spring/summer	+	-	-	-	26.92	29.05	27.10	No C_T
Nigde-Bee-S3	Autumn	++	-	+	+	12.90	29.79	14.08	No C_T
Nigde-Bee-İ4	Spring/summer	+	-	-	-	27.60	25.16	No C_T	20.36
Nigde-Bee-S4*	Autumn	+	+	+++	+	10.53	12.58	31.34	22.60
Nigde-Bee-İ5	Spring/summer	++	+	+	+	11.88	32.17	12.47	No C_T
Nigde-Bee-S5	Autumn	++	+	-	+	12.70	No C_T	13.00	26.18

*: Hive with wintering losses, a: Chantawannakul et al (2006), b: Kevill et al (2017), (+): Low, (++): Moderate, (+++): High, (-): Not observed, CT: Threshold Cycle, Other signs: paralysis, swelling in the abdomen, loss of productivity, wintering losses

Monitoring of exogenous internal control

The exogenous internal control assay allowed monitoring of the efficiency of the RNA isolation, and the reaction inhibitors and reverse transcription steps were monitored. Thus, the assays revealed an absence of PCR inhibitors or no trouble with the pathogen nucleic acid isolation or assay reaction. The Ct values of the exogenous internal control assay were in a range of 27 ± 3.

Discussion

The combined effect of biological and environmental potential stress factors is critical in reducing the honey bee population (Annoscia et al 2018, Brodschneider et al 2018, Bartlett et al 2021). Especially honey bee viruses can be determinant of hive distress, yield reduction and wintering losses (Dainat et al 2012, Martin et al 2012). However, it is difficult to identify potential causes of colony losses during the winter when hive inspection is infrequent (Kevill et al 2019).

Table 4. Season-based percentage prevalence of DWV master variants

Provinces	Year	Season	Number of hive	Prevalence of DWV	DWV-A	DWV-B	DWV-C
Aksaray	2019	Spring/summer	5	60% (6/10)	20% (1/5)	60% (3/5)	0% (0/5)
		Autumn	5		20% (1/5)	60% (3/5)	0% (0/5)
Karaman		Spring/summer	5	60% (6/10)	40% (2/5)	40% (2/5)	20% (1/5)
		Autumn	5		80% (4/5)	80% (4/5)	20% (1/5)
Konya		Spring/summer	5	100% (10/10)	60% (3/5)	100% (5/5)	60% (3/5)
		Autumn	5		40% (2/5)	100% (5/5)	40% (2/5)
Nigde		Spring/summer	5	100% (10/10)	100% (5/5)	100% (5/5)	40% (2/5)
		Autumn	5		80% (4/5)	80% (4/5)	40% (2/5)
Isparta		Spring/summer	5	100% (10/10)	100% (5/5)	100% (5/5)	20% (1/5)
		Autumn	5		80% (4/5)	100% (5/5)	0% (0/5)
Prevalence of spring/summer			25		64% (16/25)	80% (20/25)	28% (7/25)
Prevalence of autumn			25		60% (15/25)	84% (21/25)	20% (5/25)
Prevalence of DWV master variants			50		62% (31/50)	82% (41/50)	24% (12/50)

Turkey has a rich biodiversity and suitable climatic conditions for honey beekeeping (Muz and Muz 2018, Avci et al 2022). In recent years, managed honey bee viruses, a potential threat in apiaries of Turkey, have been investigated (Kalayci et al 2020, Çağırgan and Yazici 2021). However, data on the biology and epidemiology of honey bee viruses are limited. Beekeeping activities in the Mediterranean and Central Anatolia Regions, which this study focuses on, contribute to Turkey's ecology and economic dynamics.

DWV is a common honey bee virus in Europe and Turkey (Martin and Brettell 2019, Avci et al 2022). Generally, it has a low virulence compared to the infection pattern it creates in hives (Loope et al 2019). However, DWV can be devastating to bee hives after being transmitted between honey bees for several generations via Varroa infestation (Martin and Brettell 2019) and is one of the causes of overwintering loss in bee hives (Kevill et al 2019). Virus and mite infections observed together in honey bees can create a devastating and complex disease profile and cause new genotypes to circulate for a virus (Yang and Cox-Foster 2005, Kevill et al 2019). Varroa may also be the determinant in the selection of DWV genotypes (Martin et al 2012), and the dynamics of bee biology may play a role in the evolution of DWV master variants (Martin and Brettell 2019). Moreover, investigating the possible interaction of DWV master variants with other honey bee viruses may be critical for the evolution of DWV genotypes and elucidating their effects on bee health.

In the last decade, although studies have been reported on the epidemiology and infections caused by DWV master variants (McMahon et al 2016, Mordecai et al 2016, Tehel et al 2019), the effect of the genotypes of this virus on virulence or bee biology has not yet been comprehensively explained. However, it has been reported that the DWV-A genotype may be a more virulent variant compared to DWV-B. Moreover, the prevalence and viral load of the DWV-A variant were found to be higher than the other master variants in hives with overwintering losses (Kevill et al 2019). The DWV-B and DWV-C genotypes were generally detected at low viral load (Kevill et al 2019), and their effect was not devastating in managed honey bee hives (Mordecai et al 2016).

While the circulating DWV-C variant is infrequent among the honey bee (Mondet et al 2020), DWV-A and DWV-B are common master variants in managed hives (De Souza et al 2021). Initially, DWV-A was the major genotype (Ryabov ve ark 2017). However, in recent years it has been reported that the DWV-B variant is predominant and may be effective in the DWV genotypes selection (Kevill et al 2019).

DWV is one of the most common honey bee viruses detected in Turkey (Kalayci et al 2020). However, DWV genotypes have not yet been identified in managed honey bee hives. The DWV prevalence in the Central Anatolia and Mediterranean Regions was 84% (42/50) in adult honey bees. Hence, the prevalence of DWV determined in this study was consistent with the results of studies previously reported in Turkey (Avci et al 2022, Usta and Yıldırım 2022). Moreover, the prevalence of DWV in Aksaray, Konya, Karaman, Isparta, and Nigde was %60 (6/10), %100 (10/10), %60 (6/10), %100 (10/10), and %100 (10/10), respectively (Table 4). DWV infections usually peak in the autumn (Dainat et al 2012). However, the seasonal prevalence rates of DWV infections in Spring/Summer and Autumn were similar, with 80% (20/25) and 88% (22/25), respectively.

The prevalences of DWV-A, DWV-B, and DWV-C in hives were 62%, 82%, and 24%, respectively (Table 4). Also, the DWV master variants prevalences in the sampled provinces and seasons are shown in Table 4. In this study, DWV-B appeared to be the dominant genotype. The DWV-B genotype dominance (98%) detected in managed honey bee hives was consistent with the overall pattern reported by Kevill et al (2019) in bee hives in England and Wales.

Generally, the viral load of DWV is high in adult honey bees with deformed wing syndrome (Martin and Brettell 2019). However, the effect of DWV master variants on this syndrome is unclear (Brettell et al 2017, Tehel et al 2019).

Moreover, this syndrome can be an essential indicator of wintering losses in hives (Dainat and Neumann 2013). In this study, deformed wing syndrome was observed in colonies with wintering losses and some other honey bee hives.

Typically, the viral load of DWV is low in asymptomatic honey bees without Varroa infestation (Martin and Brettell 2019). Also, high Ct values were detected in DWV (ABC) real-time RT-PCR assays, indicating a low viral load, except in hives with overt infection in this study.

For this study, adult honey bees sampled had high Ct values (low viral load) in DWV real-time RT-PCR. Thus, the DWV load can be considered low for almost a year. However, the Ct value of DWV-A was notably lower (high viral load) in colonies with wintering loss (16%). Moreover, it was determined that the DWV-B genotype was the dominant variant (98%) at low viral load throughout the year in the surviving hives (84%) after the overwintering period. Previously, it has been reported that the incidence of wintering losses is low in honey bee hives with high DWV-B prevalence and dominance (McMahon et al 2016, Mordecai et al 2016, Natsopoulou et al 2017, Gisder et al 2009).

For a honey bee colony to be exposed to the devastating effects of DWV, the viral load of DWV-B must exceed the host tolerance threshold (Kevill et al 2019). Also, multiple infections of honey bees with DWV master variants can make the devastating effect on the hives obvious (Brasesco et al 2021). In this study, the more virulent DWV-A genotype was detected together with the DWV-B genotype in the bee hives with colony loss. Moreover, the fact that wintering losses were observed in colonies with high loads of DWV-A once again revealed the devastating effect of this variant on honey bees.

Conclusion

In conclusion, a report on the current status of DWV master variants circulating in Turkey and their impact on honey bee colonies is presented for the first time. In this study, it has been speculated that DWV, which causes varying levels of yield losses in every season of the year for the apiaries of Türkiye, should be carefully monitored. In particular, further studies in which whole genome sequences of DWV main variants and recombinations are determined to determine the role of the circulating DWV complex in colony losses will contribute to the elucidation of the ongoing evolutionary dynamism of DWV.

Conflict of Interest

The authors did not report any conflict of interest or financial support.

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RESEARCH ARTICLE

Geometric Morphometric Analysis of the Condylus Occipitalis and Foramen Magnum in Sheep and Goat

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Koyun ve Keçilerde Condylus occipitalis ve Foramen Magnum'un Geometrik Morfometrik Analizi

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Öz

Amaç: Yapılan çalışmada, foramen magnum ve condylus occipitalis' in türler arasındaki şekil analizi yapılarak dimorfik yapılarının ortaya konulması ve koyun-keçi arasındaki değişkenliklerinin belirlemesi amaçlandı.

Gereç ve Yöntem: Çalışmada toplam 81 (46 koyun, 35 keçi) kafatasından alınan veriler kullanıldı. Foramen magnum'un çerçeve şeklini belirlemek ve condylus occipitalis varyasyonunu anlamak için tip I (anatomik) ve tip III (semilandmark) landmarklardan faydalanıldı.

Bulgular: Buna göre toplam şekil varyasyonunun PC1, PC2 ve PC3'ün sırasıyla %30.76, 14.94 ve 14.07'sini açıkladığı, PC1'e göre şekil varyasyonundan birincil derecede condylus occipitalis'i, ikincil derecede ise foramen magnum'un sorumlu olduğu belirlendi. PC2'ye göre sağ condylus occipitalis şekil varyasyonunu büyük oranda açıkladığı, PC3'te ise sağ condylus occipitalis'in tüm, sol condylus occipitalis'in en lateral köşesi ile foramen magnum'un sol kenarı şekil varyasyonunun açıklanmasına neden olduğu belirlendi. Diskriminant fonksiyon analizi sonucunda Procrustes ve Mahalanobis distance sırasıyla 0.12293879 ($p<0.0001$) ve 67.7482 ($p<0.0044$) olarak tespit edildi.

Öneri: Sonuç olarak geometrik morfometri yöntemi, türler arası kafatası şeklindeki farklılıkları tespit etmek için kullanılabilir bir araç olduğu ve bu nedenle taksonomik, arkeolojik ve adli amaçlar için başarıyla kullanılabileceği düşünülmektedir.

Anahtar kelimeler: Craniometri, Geometrik morfometri, Şekil analizi, Temel bileşenler analizi

Abstract

Aim: The aim of this study was to reveal the dimorphic structures of the foramen magnum and condyle occipitalis through an interspecies shape analysis and to determine the variability between sheep and goats.

Materials and Methods: The study includes data from 81 skulls (46 sheep and 35 goat) for this aim. The foramen magnum frame shape and the condyle occipitalis variation were determined using type I (anatomical) and type III (semilandmarks).

Results: Accordingly, was determined 30.76, 14.94 and 14.07 of the total shape variation of PC1, PC2 and PC3, respectively. It was determined that condylus occipitalis was primarily responsible for the shape variation according to PC1, and foramen magnum was responsible for the secondarly. It was found to explain the shape variation of the right condyle occipitalis to a great extent compared with PC2, while in PC3, it caused the entire right condyle occipitalis to explain the shape variation of the extreme lateral corner of the left condyle occipitalis and the left edge of the foramen magnum. The discriminant function analysis determined the Procrustes and Mahalanobis distances to be 0.12293879 ($p < 0.0001$) and 67.7482 ($p < 0.0044$), respectively.

Conclusion: As a result, the geometric morphometry method is regarded to be a useful tool for detecting changes in skull shape between species and can thus be used successfully for taxonomic, archaeological, and forensic research.

Keywords: Craniometry, Geometric morphometry, Principal component analysis, Shape analysis.

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Introduction

In classifying species that are taxonomically close to each other, differences in the skeletal system are used as a reference. The intraspecific and interspecific data revealed by using these differences are of great importance not only to the science of taxonomy, but also to the archeological and forensic sciences (Tecirlioğlu 1983). In the skeletal system, the bones that are the most commonly used to distinguish sex, species, and race are the skull and pelvis (Scheuer 2002, Bärmann et al 2013). Species identification based on skull morphology is very difficult because it shows high intraspecific diversity (Bärmann et al 2013). Classical morphometry alone is also usually insufficient for differentiation. For this reason, geometric morphometry has been increasingly preferred in recent years (Bernal 2007, Aytek 2017, Demircioğlu et al 2021).

Geometric morphometry is a method that determines the shape differences of objects based on landmark coordinates (LM) and indicates the degree of shape change (Viscosi and Cardini 2011, Zelditch et al 2012). By analysing the orientation of the coordinates of LMs identified on the Cartesian coordinate plane, the intra-group and inter-group differences and similarities of the revealed structure are revealed. LMs are identified as points common to all samples and located in the same positions (Slice 2007, Bigoni et al 2010). They are divided into three types based on their anatomical location. Type I LMs are the group that is the most suitable for geometric morphometry and easiest to replicate. They are points with positions and definitions that are clear and easy to identify. Type I LMs are the group best suited for geometric morphometry and are the easiest to replicate. They are points whose positions and definitions are clear and easy to identify. Type II LMs are points positioned at the most extreme or distinct parts of anatomical structures (e.g., columns and appendages). Type III LMs (semi-landmarks) are points placed on the base of other LMs (Aytek 2017). The method of geometric morphometry, which is applied in many fields, has been intensively studied for some time, especially in connection with sheep and goat breeds, which show a high intraspecific polymorphism (Parés Casanova 2014, Parés Casanova and Bravi 2014, Demircioğlu et al 2021, Gündemir et al 2023, Yaprak et al 2023).

The occipital bone is one of the bones of the neurocranium that shape the caudal part of the cranium. It consists of the basilar part, the two lateral parts and the squamous part. At the junction of these three parts, there is the foramen magnum (FM) that constitutes the transition between the cavum cranii and canalis vertebralis. The occipital condyles is articulated with the atlas, which is found in the lateral partes region of the occipital bone. It also marks the lateral boundaries of FM (Bahadır and Yıldız 2008, Demiraslan and Dayan 2021). The size and shape of FM and the occipital condyles show dimorphism based on sex and breed and provide information about cranio-vertebral biomechanics (Murshed et al 2003, Naderi et al 2005, El-Barrany et al 2016).

The aim of this study was to perform a morphological analysis of FM and the occipital condyle between species, to visualise dimorphic structures, and to identify variations between two species.

Material and Methods

Material

Research Samples

Data obtained from the crania of a total of 81 animals (46 sheep and 35 goats) were used. The materials were samples that were being used for educational purposes at the laboratories of the Anatomy Departments of the Veterinary Medicine Faculties at Harran University, Burdur University, and Bingol University. Therefore, no animals needed to be euthanized for the study. There was no pathology in the samples. In addition to this issue, it was ensured that the laboratory records of the included samples did not have conditions (e.g., orthopedic or neurological conditions) that could affect the results. Based on dental examinations, all samples came from adult animals. While the analyses in this study were carried out only based on the species factor, the breed and sex information of the samples is presented in Table 1.

Table 1. Distributions of the samples according to breed and sex, n

Material	Sheep		Goat	
Sex	Akkaraman	Morkaraman	Kil	Honamlı
Female	10	14	10	9
Male	10	12	9	7

Methods

Photography and digitization

For 2D analyses, the samples were photographed (Canon 650D) from a 30 cm distance with a focus on the center of FM. Care was taken to ensure that the transverse axis of FM and the lens of the camera were in parallel with each other. The photographs were saved on a computer as JPG files. Type I (anatomical) and type III (semi-landmark) LMs were utilized to determine the outline of the shape of FM and understand the variations of the occipital condyles. For this, first of all, tps file was created in the tpsUtil (version 1.79) program (Rohlf 2019). On this file, using the tpsDIG2 (version 2.31) (Rohlf 2018) program, 40 LMs in total (9 type I, 31 type III) were marked (Figure 1). In this process, the x and y Cartesian

coordinates of the LMs, which are the most fundamental requirements for measuring morphological variations, were identified. LM1 represented the dorsal median point of FM (superimposed with LM32 in the figures), LM33 represented the dorsomedial corner of the left occipital condyle, LM34 represented the lateralmost corner of the left occipital condyle, LM35 represented the ventromedial corner of the left occipital condyle, and LM36 represented the medial junction point of the dorsal and ventral articular surface parts of the left occipital condyle. LM37, LM38, LM39, and LM40 respectively corresponded to the LMs on the right side contralateral to the ones on the left.

To determine morphological differences, a generalized Procrustes analysis (GPA) of the coordinate values of the LMs that were marked in the study was carried out. This way, by eliminating differences in the photographs such as those in size, position, and direction, (Aytek 2017) Procrustes coordinates were obtained. Using these new values, to reduce dimensionality and demonstrate the variations in the principal components, a principal component analysis (PCA) was conducted (Zelditch et al 2012, Villalobos-Leiva and Benítez 2020).

The LMs around which morphological differences were gathered, the presence of an allometric effect (multivariate regression on Procrustes coordinates), and the clustering characteristics of the samples (Discriminant Function Analysis-DFA) were analyzed. All these analyses were performed using the MorphoJ program (Klingenberg 2011).

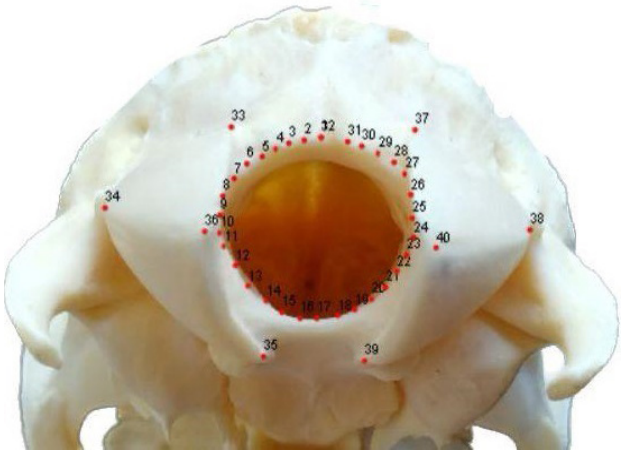

Figure 1. Landmarks

Results

In this study, a small allometric effect (2.6%) of the centroid side on the data was identified. Despite this, the allometric effect was significant in the 10000-round permutation test (p=0.0283). Based on the results of the regression analysis conducted to determine the effects of the allometry on the

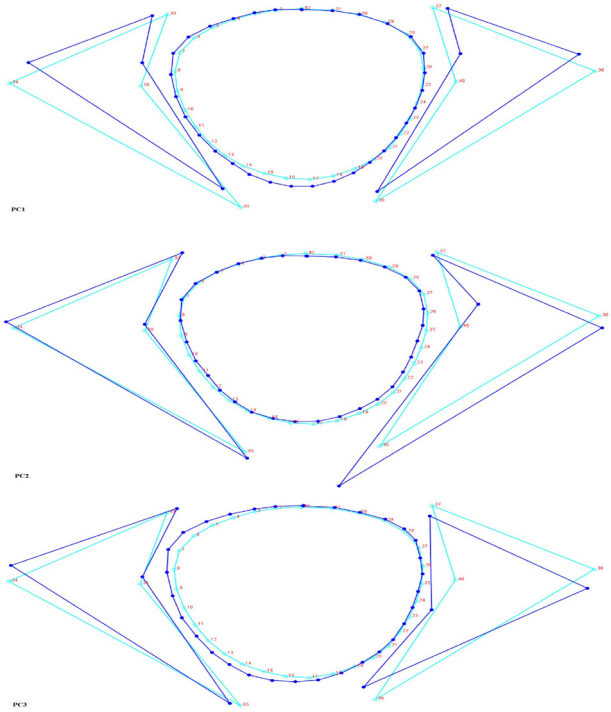

Figure 2. Wireframe morphological change plots according to PC1 (30.76%), PC2 (14.94%), and PC3 (14.07%)

principal components, 9.64% of the morphology according to PC1 (p=0.005) and 0.40% of it according to

PC2 (p=0.569) was estimable by dimension. Accordingly, it was seen that in the comparisons of the individuals based on the species factor, morphological variations were dimension-independent.

In the PCA, 76 PCs were calculated. It was determined that PC1, PC2, and PC3 explained the total variance in morphology by 30.76%, 14.94%, and 14.07%, respectively. According to PC1, the occipital condyle was the primary factor for the variation in morphology, whereas the upper-left corner and ventromedial side of FM were the secondary factors (Figure 2). According to PC2, the right occipital condyle explained the variation in morphology to a large extent (Figure 2). In the case of PC3, the variation in morphology was explained by the entire right occipital condyle, the lateralmost corner of the left occipital condyle, and the left side of FM (Figure 2).

The scatterplot of the individuals that was obtained as a result of the PCA is presented in Figure 3. According to this scatterplot, the individuals were noticeably distinguished from each other. The results of the DFA that was performed to observe the relationship between the groups more clearly showed that the Procrustes and Mahalanobis distances were consecutively 0.12293879 (p<0.0001) and 67.74 (p<0.0044). According to the cross-validation results, the goats were grouped with 83% accuracy (29:6), while the sheep were grouped with 82% accuracy (37:9) (Figure 4). The results

on the morphological variations between the groups were compatible with the PCA results. The DFA results (Figure 4) revealed larger occipital condyles and more basal localization of the ventral parts of these condyles in reference to FM in the goats compared to the sheep. The goats also had a more dorsal placement of the ventral articular part of the occipital condyle in reference to FM. In the sheep, FM was broader along the ventromedial and left lateral lines in comparison to the goats. The mean morphologies of the regions that were analyzed in the sheep and goats are shown in Figure 5. Based on these results, the ventromedial edge of FM in the sheep was more conical compared to that in the goats. The FM of the goats had an elliptical appearance.

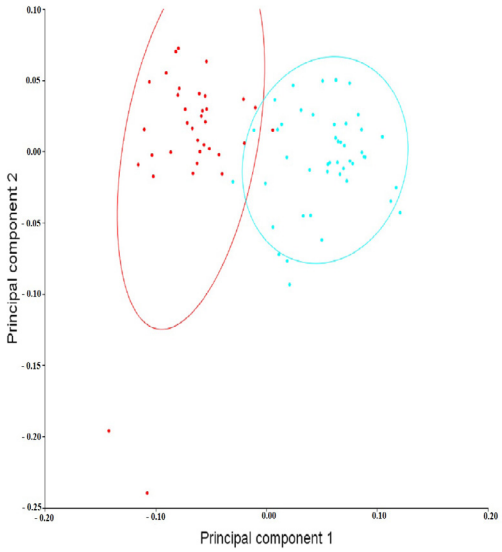

Figure 3. Scatterplot of goats and sheep according to PCA (G: Goat: Red, S: Sheep: Blue)

Discussion

In areas where visual morphology can fall short in terms of interspecies classification, morphometry, which reveals the variety and differences of morphologies with metrics, is utilized (Rohlf and Marcus 1993). Classical morphometry alone is also inadequate in terms of the comprehensive analysis of the shapes of structures (Zeder 2005). Although there are different studies in which the cranial morphologies of sheep and goat breeds have been investigated from dorsal, ventral, and lateral directions (Parés Casanova 2014, Parés Casanova and Bravi 2014, Demircioğlu et al 2021, Parés-Casanova and Domènech-Domènech 2021, Yaprak et al 2022, Yaprak et al 2023), morphological analyses carried out from the caudal aspect of the cranium are highly limited, and this dearth in the literature constitutes the most significant limitation of this study.

The size and shape allometries of the cranium provide important clues in the revelation of evolutionary and developmental changes (Parés Casanova and Sabaté

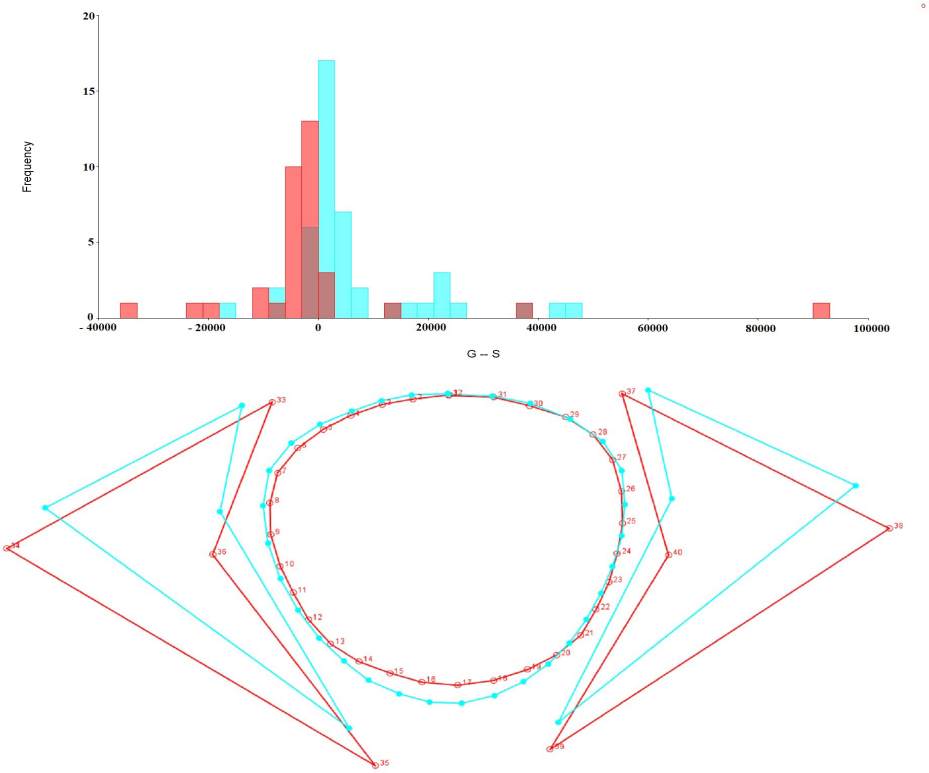

Figure 4. Cross-validation and intergroup morphological variation plots according to DFA (G: Goat: Red, S: Sheep: Blue)

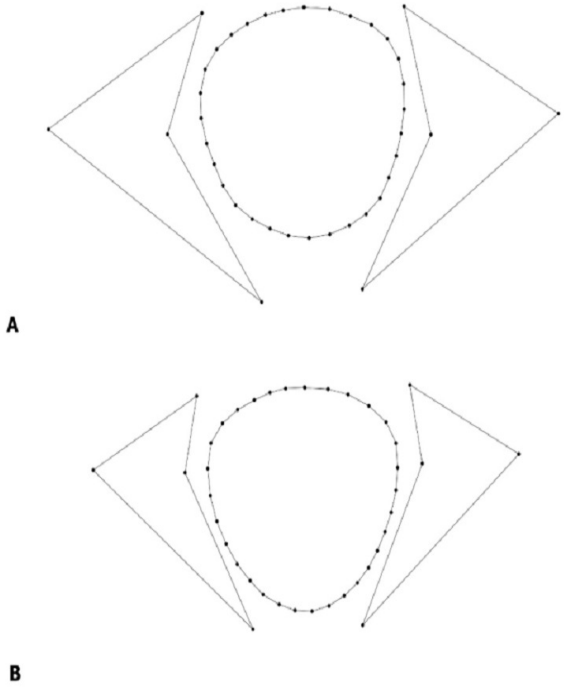

Figure 5. Mean morphologies of regions that were analyzed in sheep and goats (G: Goat, S: Sheep)

2013). It was reported that the Awassi and Hamdani sheep breeds, which have similar conditions in the geographical areas where they are bred, had morphologically different crania, and these intergroup morphological differences are significant (Demircioğlu et al 2022). In another study (Demircioğlu et al 2021) noticeable sexual dimorphism was found from the lateral to the dorsal in the crania of Awassi sheep. In their morphological analyses of the os sphenoidale of domestic sheep and goats, Parés-Casanova and Domènech-Domènech (2021) showed that the two species had morphological differences, and they stated that the first three components in their PCA (PC1, PC2, and PC3) explained these differences at a rate of 71.456%. In this study, the rate of the total variance in morphological differences explained by the first three components in the PCA (PC1, PC2, and PC3) was found as 59.776%. Therefore, it is seen that in the cranial morphology analyses of sheep and goats, the sphenoidal bone shows more allometric variation compared to the occipital condyle and FM.

The occipital bone is the most mobile part of the vertebral column by which the head and neck movements in the craniocervical junction (CCJ) constituted by the atlas and the axis are performed. The rotation, extension, and flexion movements of the cranium are associated with the harmony of the bones constituting this compound structure with each other (White III and Panjabi 1978, Bellabarba et al 2006). Goats usually graze at rockfaces and highlands, while sheep graze in tablelands and foothills. The chins of sheep stay close to the ground during grazing, and they are suitable for grazing close to the soil. On the other hand, when they can stand on their hind limbs, goats can feed on sprouts, buds, and leaves that are found on trees in higher areas (Shackleton and Shank 1984, Altın 2005, Garip 2013). According to the DFA results of our study, it was seen that compared to the sheep, the goats had larger occipital condyles, the ventral ends of their occipital condyles were localized in a more basal direction in reference to FM, and their ventral articular parts were more dorsally positioned in reference to FM. The sheep, on the other hand, had a broader FM along the ventromedial and left lateral lines in comparison to the goats. The mean morphologies of the regions that were analyzed in the goats and sheep revealed that the ventromedial side of the FM of the sheep was more conical compared to that in the goats, and the FM of the goats had a more elliptic appearance. It is believed that these data demonstrated in our study resulted from changes in the biomechanics of CCJ originating from differences in grazing behaviors. Furthermore, in our study, it was found that according to PC1, the occipital condyle was the primary factor for the variation in morphology, whereas the upper-left corner and ventromedial side of FM were the secondary factors. According to PC2, the right occipital condyle explained the variation in morphology to a large extent. Based on PC3, the variation in morphology was explained by the entire right occipital condyle, the lateralmost corner of the left occipital condyle, and the left side of FM. These asymmetries suggested that there may be a dominance on one side of the body originating from the development of the associated parts of the brain.

Factors such as nutrition, breeding style, and climate conditions can result in some variations, even among individuals of the same breed. This is why various metric measurements are needed to identify not only interspecies but also intraspecies dimorphisms.

Conclusion

In conclusion, it is believed that with this study, data that will contribute to several different disciplines are provided by presenting interspecies similarities and differences by conducting the morphological analyses of the foramen magnum and the occipital condyle, which participate in the formation of the caudal part of the cranium in sheep and goat breeds, which have existed in the history of humanity for millennia.

Conflict of Interest

The authors declare no conflict of interest.

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RESEARCH ARTICLE

The enigmatical manipulators in the capsule synthesis of *Pasteurella multocida*: Iron acquisition proteins

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Pasteurella multocida'nın kapsül sentezindeki esrarengiz manipülatörler: Demir alım proteinleri

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Öz

Amaç: *Pasteurella multocida*'daki spontan kapsül kaybı veya kapsül değişiklikleri, tekrarlanan laboratuvar geçişlerinden, pozitif veya negatif düzenleyici genlerden veya bilinmeyen bir genden kaynaklanabilir. Bu çalışmada, tipik olmayan ve tipik *P. multocida* suşlarının fenotipik, genotipik ve biyotipik özelliklerinin karşılaştırılması, kapsül sentezindeki baskın genlerin belirlenmesi amaçlanmıştır.

Gereç ve Yöntem: Bu çalışmada kapsül tipi belirlenen 56 suş ve kapsül tipi belirlenemeyen otuz altı suş kullanıldı. İzolatlarda baskın genlerin (serogrup, serotip, toksin, adezin, demir alımı ve koruyucu) varlığına dayalı olarak çoklu doğrusal regresyon analizi kullanıldı.

Bulgular: Bu suşların kültür yöntemleri ile koloni morfolojileri değerlendirildiğinde, tipik suşlarda (%87,5) mukoid koloni oluşumu, tipik olmayan suşların aksine (%27,7) yaygın olarak saptanmıştır. Tipik suşlarda en yüksek ptfA, ompA ve tadD gen yüzdeleri sırasıyla %78,57, %75 ve %69,64 idi. Tipik olmayan suşlarda en yüksek ompA, ptfA ve tadD gen oranları sırasıyla %61,1, %52,78 ve %52,78 idi. Çoklu lineer regresyon analizi sonuçlarına göre, hgbA ve hgbB genlerinin birlikteliği tipik olmayan suşlarda kapsül sentezinin artmasına neden olmuştur. Bu suşlarda ompA geninin varlığı, ikinci olarak bir indüksiyondu. Diğer genler, tipik olmayan suşlarda kapsül sentezinde etkili değildi.

Öneri: Tipik olmayan *P. multocida* suşlarının oluşumundaki en önemli etkinin HgbA ve HgbB genlerinin yeterli olmaması ile ilgili olduğu belirlendi. P. multocida'nın demir kısıtlamalı koşullar altında yoğun bir şekilde kapsüllenmemiş olabileceği düşünüldü. Sonuç olarak, *P. multocida*, demir alma proteinlerine bağlı olarak kapsülünü değiştirebilir veya kapsülünü kaybedebilir.

Anahtar kelimeler: Çoklu linear regresyon analizi, *Pasteurella multocida,* pnömoni, virülans ilişkili genler

Abstract

Aim: Spontaneous capsular loss or capsular changes in *Pasteurella multocida* can result from repeated laboratory passages, positive or negative regulatory genes, or an unknown gene. This study, it was aimed to compare the properties of phenotypic, genotypic, and biotypic of each non-typical, and typical *Pasteurella multocida* strain, to determine the dominant genes on capsule synthesis.

Materials and Methods: Fifty-six strains, which capsular type was determined, and thirty -six, which capsular type was not determined, were used in this study. Multiple linear regression analysis was used based on the presence of dominant genes (serogroup, serotype, toxin, adhesin, iron acquisition, and protectin) in the isolates.

Results: When colony morphologies of strains were evaluated of these strains by culture methods, mucoid colony formation was commonly detected in typical strains (87.5%), in contrast to non-typical strains (27.7%). In typical strains, the highest percentages of ptfA, ompA, and tadD genes were 78.57%, 75%, and 69.64%, respectively. In non-typical strains, the highest rates of ompA, ptfA, and tadD genes were 61.1%, 52.78%, and 52.78%, respectively. According to multiple linear regression analysis results, the together hgbA with hgbB genes caused the increase of capsule synthesis in these strains. The presence of the ompA gene in these strains was secondly a induction on these strains. Other genes were not effective in capsule synthesis in these strains.

Conclusion: It was determined that the most significant effect in the forming of non-typical *P. multocida* strains was related to not enough HgbA and HgbB genes. It was supposed that *P. multocida* may not be heavily encapsulated under iron-restricted conditions. Consequently, *P. multocida* may change its capsule or lose its capsule related to iron acquisition proteins.

Keywords: *Pasteurella multocida*, pneumonia, multiple linear regression analysis, virulence-associated genes

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Introduction

Healthy cattle have pathobionts in their nasal cavities, which are commonly *Pasteurellaceae family*: *Pasteurella multocida, Histophilus somni,* and *Mannheimia haemolytica* (Capitini et al 2002). *Pasteurella multocida* is a heterogeneous species that cause various infection such as avian fowl cholera, snuffles in rabbits, enzootic pneumonia, and bovine hemorrhagic septicemia in a wide range of animal species (Weber et al 1984). *P. multocida* strains are classified into serogroups based on five capsule antigens (A, B, D, E, and F) and typed primarily on lipopolysaccharide antigens into 16 serotype (1–16) (Carter 1952, Heddleston et al 1972). Also there are the various virulence proteins (adhesins, hyaluronidase, outer membrane and porin proteins, iron acquisition proteins, sialidases, and toxins) (Boyce and Adler, 2000). These factors play important role in the efficiency of vaccination (Ujvari et al 2019). To survive bacteria, iron is an essential element, and it is believed that iron acquisition proteins play a role in the disease process. Through hemin, hemoglobin binding protein (hgb) A and hgbB help for the growth of bacteria (Rimler 2001). Outer membrane protein (omp) A, which is the immunogenic and protected structure of the outer membrane, plays of role in epidemics (Luo et al 1997). OmpA has been used to assess the pathogen's interaction with the host, as well as the association of this construct with infection and its diversity within different species (Lin et al 2002). Since OmpA plays an important role between the host and the pathogen, has high immunogenicity, and has no similarity with other structural proteins of the bacteria, it must be among the components of the vaccine for the vaccine to be effective (Marandi and Mittal 1997).

Especially, particularly serotype A:3 or A:1, can give rise to critical respiratory diseases in cattle and has largely caused epidemics in beef calves (Ewers et al 2006). It is known that acapsular strains (non-typical) of *P. multocida* are less virulent than capsular strains (typical) (Oh et al. 2019). But it has been presented that the isolation rates of non-typical *P. multocida* strains may be different from ~ 0.5% to 10.6% in farm animals with respiratory system infections in the different regions (Harper et al 2006, Riley et al 2020, Shayegh et al. 2008). Interestingly, we detected non-typical *P. multocida* strains with 39.13% from sheep, goats, and calves with respiratory tract infections (Sakmanoglu et al. 2021). Protective immunity was obtained in chickens vaccinated with high doses of acapsular mutant (Chung et al 2003). Because of capsular type variety, a wide range of hosts, and especially acquired immunity of serotype-specific, there are enormous difficulties in the protection with vaccines from this infection (Harper et al 2006).

Therefore, several commercial vaccines do not provide protection from this convention as they do not have the desired level of efficacy (Chung et al 2003).

In this study, it was aimed to compare the fenotypic, genotypic and biotypic properties of each one of non-typical *P. multocida* with typical *P. multocida*, to determine of the dominant genes on capsule sythesis.

Material and Methods

Bacterial strains and culture

A fifty-six strains, which capsular type were determined, and thirty -six, which capsular type were not determined, were used in this study. Also, *P. multocida* type strains (ATCC 12945, NCTC10323, ATCC 12948, and ATCC 43020) were used as positive controls. At least one of the clinical symptoms of respiratory infection as fever, nasal discharge, and cough were seen in all the animals. *P. multocida* was isolated from a blood agar base supplemented with 5% sheep blood, and incubated in a 7% CO2 atmosphere for 24 h at 37°C in. Colony formation results of these strains were investigated as stated in this study (Sakmanoglu et al 2021).

Determination of virulence factors by PCR

This section was carried out in our study previously (Sakmanoğlu et al 2021). Briefly, the Wizard® Genomic DNA Purification Kit (Promega, USA) was used to obtain all DNA extracts from the isolates. The serogroup (capA, capB, capD, capE, capF) (Townsend et al 1998, Townsend et al. 2001, Sakmanoglu et al 2021), and serotype (L1-8, L3A-L6A) (Harper et al 2015) of isolates were determined. Also, iron acquisition (tbpA, hgbA, hgbB, tonB), protectin (ompA, ompH, omp87, plpB), toxin (toxA), and adhesion (ptfA, pfhA, tadD) genes of isolates were determined as described previously protocol (Ewers et al 2006, Sakmanoglu et al 2021).

Statistical analysis

The obtained results were evaluted by multiple linear regression analysis (IBM SPSS Statistic 21 Program) were used to compare the risk values of related genes in the both typical and non-typical strains. These values were p value, spesivite, sensivite, odds ratio, and confidence interval.

Results

When colony morphologies of strains were evaluated of these strains by culture methods, mucoid colony formation was commonly detected in typical strains (87.5%), in contrast to non-typical strains (27.7%). The A capsular type was the most common serogroup in typical (85.71%). The L3A was the most common serotype in typical (69.64%) and non-typical (47.22%) strains. All strains possessed at least one gene from adhesins (tadD, ptfA), toxin (toxA), protectins (plpB, ompH, ompA), iron acquisition (tonB, exbB, hgbA, exbD) in contrast to pfhA with Oma87. In typical strains,

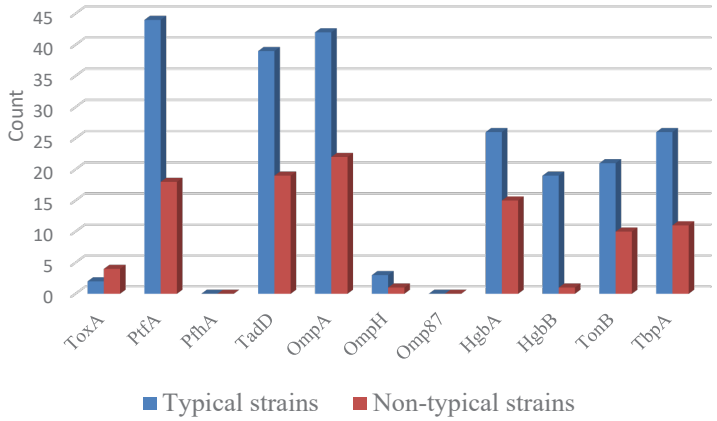

Figure 1. Graphic of virulence-associated genes in both typical and atypical Pasteurella multocida strains

Table 1. Percentages of virulence-associated genes in typical and non-typical Pasteurella multocida strains

Strain types→		Typical strains		Non-typical strains	
Virulence-associated genes↓		Count	%	Count	%
Toxin	ToxA	2	3.57	4	11.11
Adhesins	PtfA	44	78.57	18	52.78
	PfhA	0	0	0	0
	TadD	39	69.64	19	52.78
Protectins	OmpA	42	75	22	61.11
	OmpH	3	5.35	1	2.77
	Omp87	0	0	0	0
Iron acquisition	HgbA	26	46.42	15	41.66
	HgbB	19	33.92	1	2.77
	TonB	21	37.5	10	27.77
	TbpA	26	46.42	11	30.55

the highest percentages of ptfA, ompA, and tadD genes were 78.57%, 75%, and 69.64%, respectively. In non-typical strains, the highest rates of ompA, ptfA, and tadD genes were 61.1%, 52.78%, and 52.78%, respectively (Sakmanoglu et al. 2021) (Table 1, Figure 1). Because, it was found that spesivite, sensivite, and p values of hgbA with hgbB genes were detected 78%, 58%, value <0.05, respectively. Odds ratios of hgbA with 2.933, and hgbB with 32.154 were highest values in the genes. Also, whereas confidence interval up values of hgbA and hgbB were 7.471, and 269.102, respectively, confidence interval low values of hgbA and hgbB were 1.152, and 3.842, respectively. According to regression analysis results, the hgbA with hgbB genes were the highest risk on capsule synthesis in these strains. Presence of ompA gene in these strains were secondly as a possible risk on these strains. Other genes were not effective on capsule synthesis in these strains.

Discussion

Capsule (A, D, and F) structure, composed of chondroitin, hyaluronic acid (HA), and heparin is known, better than structures of serogroup E and B capsules, which have a more complex structure (Cifonelli et al 1970, DeAngelis et al 2002). Capsular type A of *P. multocida* causes respiratory disease in cattle (Ewers et al 2006). Mucoid colony of *P. multocida* strain is observed in lung samples of cattle, rabbits, and pigs although non-mucoid colonies are isolated from poultry (Harper et al 2006, Gluecks et al 2017).

Previous to our study, we detected that the rate of non-typical strains was interestingly more than that reported so far, at the same time we isolated from farm animals with respiratory disease. Also, mucoid colony formation was commonly detected in typical strains (87.5%), in contrast to non-typical strains (27.7%) (Sakmanoglu et al 2021). This variation has been ignored until now. Because of various determinants, spontaneous capsule loss has been seen in *P. multocida* (Steen et al 2010, Smallman et al 2022).

Capsule spontaneous loss in *P. multocida* can originate from the repeated passage of one then more (30 sub-cultures) (Muniandy et al 1992, Steen et al 2010). According to sequence analysis results, these acapsular variants are caused by two nucleotide changes in the cap locus, but these changes were not explained in how being on effective the acapsular phenotype formation (Watt et al 2003). Factor for inversion stimulation (fis) (Steen et al 2010) with hfq (Smallman et al 2022) genes encode known positive regulators of *P. multocida* capsule. There need for information about the cellular signals, which control regulatory mechanisms and capsule production in *P. multocida*. It was reported that the regulator fis both controls the expression of capsule biosynthesis genes and regulates known and putative

virulence factors in *P. multocida* (Dorman et al 2018). Also, fis proteins are synthesized at the highest level in the active growth phase of bacterial cells contrary to the stationary phase (Steen et al 2010). Iron plays a critical role in metabolic electron transport chains for most organisms. Transferrin and lactoferrin in body fluids in avian and mammalian hosts can affect the concentration of free iron normally present and the growth of bacteria in vivo impress with negative because of less iron amount (Bullen 1981). Therefore, to survive negative conditions, pathogens must possess an effective response to protect from the limited iron conditions encountered upon entry into a host (Veken et al 1996). It is reported that iron acquisition proteins play a role in the disease process, because hemoglobin binding protein (hgb) A and hgbB help for the growth of bacteria (Rimler 2001). The hgbA and HgbB proteins are used to obtain iron directly from the haem component. The prevalence of hgbB gene in strains alters relative to the host origin and the animal disease status, while hgbA gene is more regularly among isolates. TbpA, an epidemiological marker among cattle, plays an essential in the obtaining of iron from transferrin by transferrin-binding protein role (Paustian et al 2001). Iron-restricted conditions with iron deprivation effects markedly in decreasing the capsular amount of *P multocida*. These chelators affecting capsule structure are inhibited by the addition of iron neutralized (Jacques et al 1994).

Conclusion

In conclusion, it was determined that the most significant effect on the capsule synthesis of *P. multocida* was related to HgbA and HgbB genes. *P. multocida* may not be heavily encapsulated under iron-restricted conditions. Additionally, *P. multocida* may change its capsule or lose its capsule related to iron acquisition proteins.

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Conflict of Interest

The authors did not report any conflict of interest or financial support.

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Author Contributions

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Ethical Approval

This research has been approved (grant number: 2020-69, Date: 20.08.2020) by the Ethics Committee of the Faculty of Veterinary Medicine at the University of Selcuk in Konya, Turkey.

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RESEARCH ARTICLE

Lungworm infections in small ruminants in Uşak province

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Uşak yöresinde küçük ruminantlarda akciğer kılkurdu enfeksiyonları

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Öz

Amaç: Bu çalışma, Uşak Yöresinde küçük ruminantlarda akciğer kıl kurdu enfeksiyonlarının yaygınlığını belirlemek amacıyla yapılmıştır.

Gereç ve Yöntem: Çalışma, Uşak bölgesinde 5 farklı yerleşim yerinden toplanan 250 koyun ve 250 keçinin dışkı örneklerinin Baermann-Wetzel yöntemi kullanılarak test edilmesiyle gerçekleştirildi.

Bulgular: Akciğer kıl kurdu enfeksiyonlarının yaygınlığı koyunlarda %9,6 iken keçilerde ise %34,4 olarak belirlendi. Tüm koyun ve keçilerde enfeksiyon yaygınlığı %22 olarak tespit edildi. Koyunlarda akciğer kıl kurdu enfeksiyonlarının yaygınlığı dişilerde %9,8 olarak belirlenirken, erkeklerde enfeksiyona rastlanmadı. Keçilerde enfeksiyonun yaygınlığı, dişilerde %33,3 ve erkeklerde %53,8 olarak tespit edildi. Koyunlarda akciğer kıl kurdu türlerinden *Muellerius capillaris*'in %5,6, *Dictyocaulus filaria*'nın %2, *Protostrongylus* sp.'nin %1,2 ve Cystocaulus ocreatus'un %0,8 oranlarında yaygınlık gösterdiği tespit edildi. Keçilerde ise *M. capillaris*'in prevalansı %34,4 ve *C. ocreatus*'un %0,4 olduğu saptandı. Üç koyunda iki akciğer kurdu türünün neden olduğu miks enfeksiyonlar tespit edildi. Ancak sadece bir keçide miks enfeksiyon saptandı. Koyunlarda akciğer kurdu enfeksiyonu prevalansı ırklara göre karşılaştırıldığında beş koyun ırkından ikisinde enfeksiyon saptanmıştır. Akciğer kurdu enfeksiyon prevalansı Eşme ırkı koyunlarda %14,1 ve Kıvırcık ırkı koyunlarda ise %9.1 olarak bulundu.

Öneri: Küçükbaş hayvan yetiştiriciliğinde akciğer kurdu enfeksiyonlarının yaygınlığı ve risk durumu ortaya konmuştur. Ayrıca, koyun ve keçilerin birlikte yetiştirilmesi ve farklı yaş gruplarından hayvanların birlikte otlatılması akciğer kurdu enfeksiyonları açısından risk oluşturabileceği tespit edilmiştir.

Anahtar kelimeler: Akciğer kıl kurdu, koyun, keçi, uşak yöresi

Abstract

Aim: This study was carried out to determine the prevalence of lungworm infections in small ruminants in the Uşak province.

Materials and Methods: The study was carried out by testing the stool samples of 250 sheep and 250 goats collected from 5 different localities in the Uşak province using the Baermann-Wetzel method.

Results: While the prevalence of lungworm infections was 9.6% in sheep, it was 34.4% in goats. The prevalence of infection was found to be 22% in all sheep and goats. While the prevalence of lungworm infections in sheep was determined to be 9.8% in females, no infection was found in males. The prevalence of infection in goats was determined as 33.3% in females and 53.8% in males. The prevalence of *Muellerius capillaris* was 5.6%, *Dictyocaulus filaria* 2%, *Protostrongylus* sp. 1.2% and *Cystocaulus ocreatus* 0.8% in sheep. In goats, the prevalence of *M. capillaris* was 34.4% and *C. ocreatus* was 0.4%. Mix infections caused by two lungworm species were detected in three sheep. However, mix infection was detected in only one goat. When the prevalence of lungworm infection in sheep was compared according to breeds, infection was detected in two of the five sheep breeds. The prevalence of lungworm infection was 14.1% in Eşme breed sheep and 9.1% in Kıvırcık breed sheep.

Conclusion: The prevalence and risk status of lungworm infections in small ruminant breeding have been demonstrated. In addition, it has been determined that raising sheep and goats together and grazing animals from different age groups together may pose a risk in terms of lungworm infections.

Keywords: Lungworm, sheep, goat, uşak province

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Introduction

Lungworm infections in small ruminants (sheep-goats) are chronic and long-term infections characterized clinically by respiratory problems, pathologically by bronchitis and bronchopneumonia. Lungworms of small ruminants are nematode parasites found in the *Trichostrongylidea* and Metastrongylidea superfamilies of the order *Strongylida*. Trichostrongylid lungworms have a monoxene life cycle and are located in the trachea and bronchial branches in their hosts. Although they are more common in temperate climatic conditions, they have a cosmopolitan distribution. Metastrongylid lungworms have a heteroxenous life cycle and are localized in the lung parenchyma, bronchioles and alveoli. Various gastropods (land snails) serve as intermediate hosts to the metastrongylid lungworms (Urquhart et al 1996, Schnieder 2005, Eckert et al 2008).

Small ruminant breeding is a large component of the agricultural economy in developing countries and constitutes a significant part of the livelihoods of the rural population. The presence of infections, the species causing the infection, the prevalence of these species and the risks they cause should be revealed in order to determine the control strategies and to prepare the control programs against the lungworms that threaten animal health and cause significant yield losses in small ruminant breeding (Hansen and Perry 1994).

In previous studies in Turkey, the prevalence of lungworm infections in sheep varies between 10.86-62.5% according to stool examination (Doğanay et al 1989, Celep et al 1995, Dik et al 1995, Umur and Arslan 1998, Değer et al 2000, Yıldız and Aydenizöz 2001, Yıldız 2006, Bağcı and Bıyıkoğlu 2003, Yıldırım and İça 2005, Sevimli et al 2006, Gül and Kılınç 2016, Can et al 2018) and 19.85-86.18% according to necropsy examinations (Güralp 1952, Gargılı 1995, Taşan et al 1997, Umur and Arslan 1998, Değer et al 2000, Yıldız and Aydenizöz 2001, Yıldız 2006, Bağcı and Bıyıkoğlu 2003, Can et al 2018). According to previous studies, the prevalence of lungworm infections in goats varies between 4.27% and 36% (Şenlik et al 2001, Yaman et al 2006, Gül and Kılınç 2016, Sevimli et al 2018).

This research was carried out to determine the prevalence of lungworm infections and the species responsible for the infection in sheep and goats raised in Uşak province.

Material and Methods

Material Sampling

Each stool samples of 5 grams were collected rectally from 50 sheep and 50 goats from 5 localities (Alahabalı, Alıçlı, Davutlar, Delibaşlı and Konak villages) in the Uşak province. Stool samples were collected from 245 female and 5 male sheep and 237 female and 13 male goats. A total of 500 samples were brought to the laboratory in labeled stool sample containers. Information on stool samples (locality, farm, species, sex, breed and age) was recorded for use in data analysis.

Method

Stool samples were tested with the Baermann-Wetzel method in the parasitology laboratory (Yıldız 2006, Yıldırım and İça 2005). First-stage larvae collected from stool samples were identified by Nikon Elipse 80İ-DS-5-L1 light microscopy and photographed.

Statistical analysis

The data obtained in the research were analyzed with the SPSS for Windows package program. The variables in the study were described with frequency and percentage distributions, and the relationships between categorical variables were determined by the Chi-Square test. Statistical significance level was determined as 0.05.

Results

In this study, the prevalence of lungworm infections was determined as 9.6% in sheep, 34.4% in goats and 22% in all small ruminants (Table 1). The difference between sheep and goats in terms of the prevalence of lungworm infections was statistically significant ($p<0.05$). While the prevalence of lungworm infections in sheep was 9.8% in females, no infection was found in males. The prevalence of infection in goats was 33.3% in females and 53.8% in males. Considering all small ruminants, the prevalence of infection was 21.4% in female animals and 38.9% in males (Table 1). The difference between the sexes in terms of the prevalence of lungworm infections in sheep, goats and all ruminants were not statistically significant $p>0.05$).

The prevalence of lungworm infections in sheep by age groups was determined as 16.7% in the 1-3 age range, 11.1% in the 4-7 age range, and 3.0% in the 8 years and older age group (Table 2). When the prevalence of lungworm infections in sheep was compared according to age, the difference was not statistically significant ($p>0.05$). The prevalence of lungworm infections in goats according to age groups was 33.3% in the 1-3 age range, 16.2% in the 4-7 age group, and 53.5% in the 8 years old and older age group (Table 2). The difference between age groups was significant in goats ($p<0.05$). When all sheep and goats are evaluated together, the prevalence of infection was 29.8% in the 1-3 age range, 12.7% in the 4-7 age range, and 29% in the 8 years and older age group (Table 2). The difference between age groups was statistically significant in all small ruminants ($p<0.05$).

Table 1. Distribution of lungworm infections in small ruminants by host and gender

Host	Gender (n)	Infection Rate by Gender (%)	Infection Rate by Host (%)	χ^2	P
Sheep	♀ (245)	24 (9.8)	24(9.6)	0.542	0.601*
	♂ (5)	-			
Goat	♀ (237)	79 (33.3)	86(34.4)	0.143	0.114*
	♂ (13)	7 (53.8)			
Total	♀ (482)	103 (21.4)	110(22)	0.087	0.076*
	♂ (18)	7 (38.9)			

(*P>0.05)

Table 2. Distribution of lungworm infections in small ruminants by age groups

Host	Age Groups			χ^2	P
	1-3	4-7	8≥		
Sheep	16.7%	11.1%	3%	5.507	0.064
Goat	33.3%	16.2%	53.5%	21.566	0.000*
Total	29.8%	12.7%	29%	20.119	0.000*

(*P<0.05)

Table 3. Comparison of prevalence of lungworm species in sheep and goats

Lungworm species	Lungworm infections Sheep(%)	Goat(%)	χ^2	P
D. filaria	5 (2)	-	39.417	0.000*
M. capillaris	14 (5.6)	86 (34.4)		
C. ocreatus	2 (0.8)	1 (0.4)		
Protostrongylus sp.	3(1.2)	-		
M. capillaris + C. ocreatus	3 (1.2)	1 (0.4)		
M. capillaris + Protostrongylus sp.	1 (0.4)	-		

(*P<0.05)

It was determined that the prevalence of *M. capillaris* was 5.6% (Figure 1a), *D. filaria* 2% (Figure1b), *Protostrongylus sp.* 1.2% (Figure 1c) and *C. ocreatus* 0.8% (Figure 1d) in sheep (Table 3). While the prevalence of *M. capillaris* and *C. ocreatus* in goats was 34.4% and 0.4% respectively, *Protostrongylus* sp. and *D. filaria* were not determined (Table 3). When all small ruminants were evaluated, it was determined that *M. capillaris* had a prevalence of 20%, *D. filaria* 1%, *Protostrongylus* sp. 0.6% and *C. ocreatus* 0.6% (Table 3). In both sheep and goats, the predominant species was *M. capillaris*. When the prevalence of lungworm species in sheep and goats was compared, the difference was statistically significant (p<0.05). Mixed infections were detected in three sheep. *M. capillaris* and *C. ocreatus* were observed together in two of them, and *M. capillaris* and *Protostrongylus* sp. were observed together in one. Mixed infection with *M. capillaris* and *C. ocreatus* was detected in only one of the goats. (Table 3). When the prevalence of lungworm infections in sheep was compared according to breed, a significant difference was found (p<0.05). The prevalence of lungworm infections was 14.1% in Eşme breed sheep and 9.1% in Kıvırcık breed sheep (Table 4).

Discussion

In this study, the prevalence of lungworm infections was 9.6% in Uşak sheep, according to stool examination. Although this result is lower than the results of other studies conducted in various regions of Turkey, it is similar to the results (10.6% and 14%) of two studies conducted in Kırıkkale (Yıldız and Aydenizöz 2001, Yıldız 2006). It is thought that these differences may be due to the climatic characteristics and breeding methods of the regions. According to previous studies in Turkey, the prevalence of lungworm infection in goats varies between 4.27% and 36% (Şenlik et al 2001, Yaman et al 2006, Gül and Kılınç 2016, Sevimli et al 2018). In this study, the prevalence was 34.4%. While this result was found to be higher than the results of the studies conducted in Hatay (Yaman et al 2006) and Afyonkarahisar (Sevimli et al 2018), it was found to be similar to the results of the studies conducted in the Southern Marmara Region (Şenlik et al 2001) and Bingöl (Gül and Kılınç 2016). In this study, the difference was statistically significant between sheep (9.6%) and goats (34.4%) in terms of lungworm infection prevalence (p<0.05).

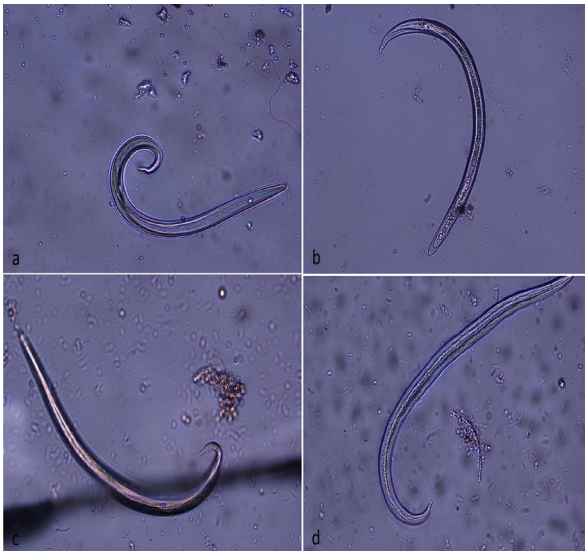

Figure 1. a) *Muellerius capillaris* L1(X40), b) *Dictyocaulus filaria* L1(X40) c) *Protostrongylus* sp. L1(X40), d) *Cystocaulus ocreatus* L1(X40)

When the prevalence of lungworm infections was examined by gender, the prevalence of infection was found to be higher in female sheep in some studies (Gargılı 1995, Sevimli et al 2006), while it was higher in males in one study (Yıldız and Aydenizöz 2001). The prevalence of lungworm infections in this study was 9.8% in female sheep and no infection was found in male sheep. The prevalence was found 33.3% in female goats, while 53.8% in male goats. The relatively high rate of infection in male goats can be explained by the low number of male goats in the herds and the fact that they are breeding animals at an advanced age. Considering all small ruminants, the prevalence of infection was found to be 21.4% in female animals and 38.9% in males. In this study, the difference between the sexes in sheep, goats and all small ruminants was not statistically significant (p>0.05).

When the prevalence of lungworm infections is examined by age groups, it is seen that the prevalence of infection increases with age. In a study conducted in sheep (Yıldırım and İça 2005), the prevalence of lungworm infections was reported as 53.3% in the ≥6 age group, 41.3% in the 3-5 age group, and 9.4% in the ≤2 age group. In this study, it was determined that the prevalence of lungworm infections in sheep was 16.7% in the 1-3 age range, 11.1% in the 4-7 age range, and 3.0% in the 8 years and older age group. The difference was not statistically significant in the prevalence of infection between age groups (p>0.05). The prevalence of lungworm infections in goats was 33.3% in the 1-3 age group, 16.2% in the 4-7 age group, and 53.5% in the 8 years and older age group. When the prevalence of lungworm infections in goats was compared according to age, the difference was statistically significant (p<0.05). When all sheep and goats were evaluated together, the prevalence of lungworm infection was found to be 29.8% in the 1-3 age range, 12.7% in the 4-7 age range, and 29% in the 8 years and older age group. When the age groups were examined in all small ruminants, the difference was statistically significant (p<0.05). Although it is expected that the infection rates will be higher in animals that go out to the pasture for the first time and in older animals with reduced immunity, *M. capillaris* is less responsive to treatment compared to other lungworm species.

Table 4. Comparison of the breed-specific prevalence of lungworm infections in sheep

Breed	Number of examined sheep	Lungworm infections Negative (%)	Positive (%)	χ^2	P
Pırlak	36	36 (100)	-		
Kıvırcık	22	20 (90.9)	2 (9.1)		
Eşme	156	134 (85.9)	22 (14.1)	11.297	0.023*
Sakız Half-breed	25	25 (100)	-		
Pirit	11	11 (100)	-		
Total	250	226 (90.4)	24(9.6)		

(*P<0.05)

C. ocreatus is the most common species of lungworm observed in sheep in most of the studies conducted in different regions of Turkey (Güralp 1952, Doğanay et al 1989, Taşan et al 1997, Dik et al 1995, Değer et al 2000, Bağcı and Bıyıkoğlu 2003, Yıldız 2006, Can et al 2018). There are fewer studies reporting that *D. filaria* is the most common species in sheep (Celep et al 1995, Gargılı 1995, Umur and Arslan 1998, Yıldız and Aydenizöz 2001, Gül and Kılınç 2016). Only one study reported *Protostrongylus* sp. as the most common lungworm species in sheep (Yıldırım and İça 2005). In this study, *M. capillaris*, *D. filaria* and *Protostrongylus* sp. were detected at a rate of 5.6%, 2% and 1.2%, respectively. This can be explained by the fact that sheep are breeding together with goats and *M. capillaris* is the predominant lungworm species in goats. Although mix lungworm infections are common in sheep, mix infections with two species are more common than mix infections with three species (Umur and Arslan 1998, Değer et al 2000, Bağcı and Bıyıkoğlu 2003, Yıldırım and İça 2005, Yıldız 2006, Gül and Kılınç 2016, Can et al 2018). Mix infections with two species were detected in only three of the infected sheep in the region where this study was conducted. This result is quite low compared to the results determined in other studies.

In two studies (Cantoray et al 1992, Şenlik et al 2001) on goats in Turkey, C. ocreatus, in the other two (Gül and Kılınç 2016, Sevimli et al 2018) *M. capillaris* and in one study (Yaman et al 2006) *D. filaria* were reported to be the most common lungworm species. In this study, *M. capillaris* was found in 34.4% and *C. ocreatus* in 0.4% in goats. In all small ruminants, *M. capillaris* was found to be 20%, *D. filaria* 1%, *Protostrongylus* sp. 0.6% and *C. ocreatus* 0.6%. *M. capillaris* was the predominant species in both sheep and goats. In a study conducted in Bingöl, the rate of mixed lungworm infection in goats was reported as 3% (Gül and Kılınç 2016). In this study, mixed infection was detected in only one goat (0.4%).

This study was carried out on 5 different breeds of sheep, and lung pinworm infection was detected only in Eşme and Kıvırcık breed sheep. There has not been a study comparing different sheep breeds in terms of lungworm infections in Turkey before.

Conclusion

As a result, it has been revealed that lungworm infections seen in small ruminants in the Uşak province are very important in terms of livestock and farm economy. Parasitic infections are an important threat in sheep and goat farming, which is an important part of rural development, and timely diagnosis and treatment of these infections and prevention and control measures are also important. Raising sheep and goats together and grazing animals from different age groups together may pose a risk in terms of lungworm infections. It is noteworthy that *M. capillaris* infections, which are more difficult to treat than infections caused by other species, dominate. In this study, attention was drawn to lungworm infections in sheep and goats in Uşak province.

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Conflict of Interest

The authors did not report any conflict of interest.

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Author Contributions

Motivation/Concept: MK; Design: MK; Control/Supervision: MK; Data Collection and/orProcessing: EE; Analysis and /orInterpretation: MK, EE; LiteratureReview: MK, EE; WritingtheArticle: MK; Critical Review: MK, EE.

Ethical Approval

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