ISTANBUL UNIVERSITY CERRAHPASA

ISSN 2618-639X • EISSN 2619-905X

actaveteurasia.istanbulc.edu.tr

ACTA VETERINARIA EURASIA

Formerly Journal of the Faculty of Veterinary Medicine İstanbul University
Official Journal of İstanbul University-Cerrahpaşa Faculty of Veterinary Medicine

'19

VOLUME: 45 ISSUE: 2

ACTA VETERINARIA EURASIA

Formerly Journal of the Faculty of Veterinary Medicine İstanbul University

İstanbul Üniversitesi-Cerrahpaşa Veteriner Fakültesi adına sahibi / Owner on behalf of the İstanbul University-Cerrahpaşa Faculty of Veterinary Medicine: Güven Kaşıkçı • Sorumlu Yazı İşleri Müdürü / Responsible Manager: Serhat Alkan • Yayın türü / Publication Type: Yerel süreli / Local Periodical • Basım yeri / Printed at: Hamdioğulları İç ve Dış Ticaret A.Ş. Zübeyde Hanım Mah. Elif Sokak No.7/197 Altındağ, Ankara, Türkiye. Tel: +90 (542) 695 77 60 • Basım tarihi / Printing Date: Mayıs 2019 / May 2019

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Acta Veterinaria Eurasia (Acta Vet Eurasia) is an international, scientific, open access periodical published in accordance with independent, unbiased, and double-blinded peer-review principles. The journal is the official publication of İstanbul University-Cerrahpaşa Faculty of Veterinary Medicine and published three times a year (January, May and September). The publication language of the journal is English.

Acta Veterinaria Eurasia (Acta Vet Eurasia) aims to contribute to the literature by publishing manuscripts at the highest scientific level on all fields of veterinary medicine. The journal publishes original articles, reviews, case reports, short communications, and letters to the editor that are prepared in accordance with the ethical guidelines.

The scope of the journal covers all animal species including the topics related to basic and clinical veterinary sciences, livestock breeding and husbandry, veterinary genetics, animal nutrition and nutritional diseases, zooneses, veterinary medicinal products and public health, and food hygiene and technology.

The target audience of the journal includes specialists and professionals working and interested in all disciplines of veterinary medicine.

The editorial and publication processes of the journal are shaped in accordance with the guidelines of the International Committee of Medical Journal Editors (ICMJE), World Association of Medical Editors (WAME), Council of Science Editors (CSE), Committee on Publication Ethics (COPE), European Association of Science Editors (EASE), and National Information Standards Organization (NISO). The journal is in conformity with the Principles of Transparency and Best Practice in Scholarly Publishing (doaj.org/bestpractice).

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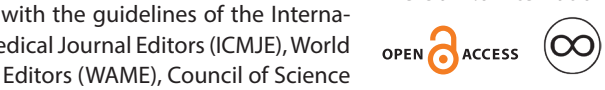

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ACTA VETERINARIA EURASIA
Formerly Journal of The Faculty of Veterinary Medicine İstanbul University

Contents

Original Article Acta Vet Eurasia 2019; 45: 37-41

Presence of the Parafollicular Cells in the Thyroid Gland of the One-Humped Camel

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Cite this article as: Ahmadpanahi, S.J., 2019. Presence of the Parafollicular Cells in the Thyroid Gland of the One-Humped Camel. Acta Vet Eurasia 45, 37-41.

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Abstract

The thyroid is an important endocrine gland that affects many organs of the body. A limited number of works have been done on the morphological and histological characteristics of this gland in the camel and controversial debates have been made on the presence of parafollicular cells. The aim of this study is to investigate the histological structure of the thyroid in the camel and determine the presence of parafollicular cells in this gland. This study was performed on 20 camels. The histological structure of the thyroid was studied using light microscope after preparing sections and staining it with Hematoxylin & Eosin, Verhoeff, and Toluidine blue. Thyroid gland has follicles of different sizes, follicular and parafollicular cells, and according to our results these cells are forming about 59.1% and 5% of the gland volume respectively. The large follicles are located in the peripheral part of the gland while the small follicles are seen in the central part of the gland. The central parts of the gland have a more extensive vascular bed than the peripheral parts. This study revealed that the thyroid gland in camel has parafollicular cells, but most of them are present in the central part of the gland.

Keywords: Camel, histology, parafollicular cell, thyroid

Introduction

Camel is a ruminant that is adapted to hot, dry, and inclement climates. Camel has many differences from animals that live in temperate climates. This animal can lose 25% of its body weight and they can concentrate their urine without illness. Camel's body temperature changes in a wide range. Stabilization of the internal situation of the camel's body depends on the endocrine system in hot weather and the thyroid gland influences many organs (Atoji et al., 1999). Thyroid gland contains both follicular cells that produce triiodothyronine (T3) and tetraiodothyronine (T4) hormones, and parafollicular cells, which produce calcitonin hormone. These hormones play an important role in the metabolism of animals (Abdel-Magied et al., 2000; Kausar and Shahid, 2006; Mason and Wilkinson, 1973).

Epithelial clusters that sprout from the caudal part of the fourth pharyngeal pouch are replaced by cells of the neural crest that are located around this pharyngeal pouch, making the ultimobranchial body. These cells incorporated into the thyroid gland and form parafollicular cells (Hyttel et al., 2010).

Yagil et al. (1978) showed that the thyroid gland affects the animal's compatibility with inclement weather. Thyroid gland structure has been studied by both light and electron microscopy in different mammals (Fujita, 1975; Kurihara et al., 1990). Few histological and anatomical studies have been performed previously on the thyroid gland of camels. Also, the focus of previous histological studies of the camel thyroid gland was on the ultrastructure of the follicular cells (Abdel-Magied et al., 2000; Atoji et al., 1999; Kausar and Shahid, 2006). Although the results of these studies were similar with respect to the general structure of the camel thyroid gland, there has been a disagree-

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Received Date: 29 June 2018 • **Accepted Date:** 26 April 2019 • DOI: 10.26650/actavet.2019.18001

Available online at actaveteurasia.istanbulc.edu.tr

ment on the presence of the parafollicular cells. Moreover, the results of preliminary studies performed in this area differ with some results of recent studies. The present study was conducted with an emphasis on the presence or absence of parafollicular cells on the camel thyroid gland.

Materials and Methods

This study was conducted in spring on 20 (10 males and 10 females) 6-10 years old one humped camels, whose age was determined by their teeth (Hillson, 2005). Samples were collected from the Semnan industrial slaughterhouse, Semnan Province, Iran. The slaughterhouse authorities gave permission to use the animals in this study. It was concluded that the study is in compliance with the Principles of Scientific Research and Publication Ethics according to the examination that was made by Semnan University Veterinary Faculty Sub-Committee of Ethics. First, the anatomical characteristics of the thyroid glands were examined and their dimensions were measured. Then, for histological investigation, both lobes of the thyroid were removed, weighed, divided into pieces with a thickness of 5 mm, and fixed in 10% buffered formalin solution for 72 h. After routine histological processing, thyroid samples were embedded in paraffin and 6-µm sections were prepared. The sections were stained with Hematoxylin & Eosin and Verhoeff for light microscopic investigation. Hematoxylin & Eosin stained sections were used for histological inspection and stereological procedures, and Verhoeff stained sections were used for revealing connective tissue (Bancroft and Gamble, 2008; Culling and Allison, 1985). For toluidine blue staining some specimens were fixed in phosphate buffered glutaraldehyde (pH 7.4) and post fixed in osmium tetroxide (Sigma-Aldrich Co. LLC, Germany) in the same buffer at 4°C. Then they were dehydrated and embedded in epoxy resin (Epoxy Embedding Kit, Sigma-Aldrich Co. LLC, Germany) and semi-thin sections (1 µm thick) were prepared and were stained with toluidine blue (Bancroft and Gamble, 2008; Culling and Allison, 1985; Glauert and Lewis, 1998).

Toluidine blue is an acidophilic metachromatic dye that selectively stains acidic tissue components such as sulfates, carboxylates, and phosphate radicals. Toluidine blue can be used to stain connective tissue mucins, especially acid mucins, mast cell granules, endocrine cells and frozen sections (Sridharan and Shankar, 2012). Lead-Haematoxylin is well used for staining most endocrine cells of the pancreatic islets, thyroid, pituitary gland and adrenal medulla. The two methods of Toluidine blue and Lead-Hematoxylin have similar results in staining of the thyroid endocrine cells (Solcia et al., 1969). In our study, Toluidine blue staining method has been used for identifying and showing parafollicular cells in the thyroid gland.

The volume densities of parafollicular and follicular cells were determined by using the point- counting method of Weibel (1979). For cell volume density calculations, sections were taken from eight different parts of the thyroid glands of each animal. In each section, 50 different areas were evaluated under × 400 magnification. In each area follicular and parafollicular cell numbers touching to the points of counting graticule (100 point graticule) were recorded.

Statistical analysis

For statistical analysis, Statistical Package for the Social Sciences 2003 (SPSS, IBM Corp.; NY, USA) software was used and the differences between groups were determined using a Student's t-test. Overall, p-values less than 0.05 were regarded as statistically significant.

Results and Discussion

The thyroid gland of camel were consisting of two oval shaped lobes connected together by a distinct non-glandular isthmus across the ventral surface of the trachea at the level of the second and third rings. The right lobe of the thyroid gland was located on the dorsomedial aspect of the trachea at the level of the first to fifth rings and the left lobe was located at the level of the second to sixth rings on the medial aspect of the trachea. Weight and dimensions of the thyroid gland were slightly higher in female camels compared with the male ones, though the difference was not statistically significant (Table 1).

The thyroid glands were surrounded by a thin capsule of dense irregular connective tissue, containing a large number of thin collagen fibers (Figure 1). Some trabeculae were penetrating from the capsule into the gland and producing some irregular lobules (Figure 2). Each lobule was consisting of a number of follicles with different sizes and shapes. There were many fibroblasts, blood capillaries and thin collagen fibers between the follicles. Each follicle was surrounded by an epithelium, varying from simple cuboidal to low columnar, lying on a basement membrane. The secretion were stored in the follicles as a homogeneous eosinophilic colloid. In each follicle, the size of the cells were the same.

Table 1. Dimensions and weight of the right and left lobes of the thyroid gland in one-humped camel (mean ± standard deviation)

	Camel	Right lobe	Left lobe
Length (mm)	Male	5.7±0.4	4.6±0.9
	Female	5.9±0.3	5.2±0.7
	Mean	5.8±0.35	4.9±0.8
Width (mm)	Male	2.4±0.7	2.1±0.8
	Female	2.8±0.2	2.5±0.4
	Mean	2.6±0.45	2.3±0.6
Height (mm)	Male	0.9±0.65	0.9±0.46
	Female	0.9±0.25	0.9±0.73
	Mean	0.9±0.45	0.9±0.6
Weight (gr)	Male	52.71±0.83	50.67±0.21
	Female	54.2±0.34	51.94±0.17
	Mean	53.46±0.59	51.31±0.19

Depending on their size, follicles were divided into two groups. Large follicles were located in the peripheral area and the small follicles were placed in the central part of the gland (Figure 1). Sometimes, the epithelium of the large follicles were containing low cuboidal to squamous cells, while the epithelium of small follicles were varying from cuboidal to low columnar. The colloid in the large follicles was completely acidophilic and thick, but in small follicles, it was less acidophilic, thinner and uniform. The follicular cells, which constitute the largest cell population of the gland, were cuboidal and have a relatively basophilic cytoplasm. A large nucleus was present at the base of the cells (Figure 3). Small follicles were forming the active follicular population, which were having vacuoles in their colloid.

The parafollicular cells were found in very small numbers in the camel thyroid gland. These cells, which were often found individually or collectively between follicles, were stained lighter and were larger than follicular cells. Parafollicular cells were commonly found between the small follicles in camel thyroid gland. They were rarely present between the large follicles or between follicular cells (Figure 3). Parafollicular and follicular cell volume densities are given in Table 2.

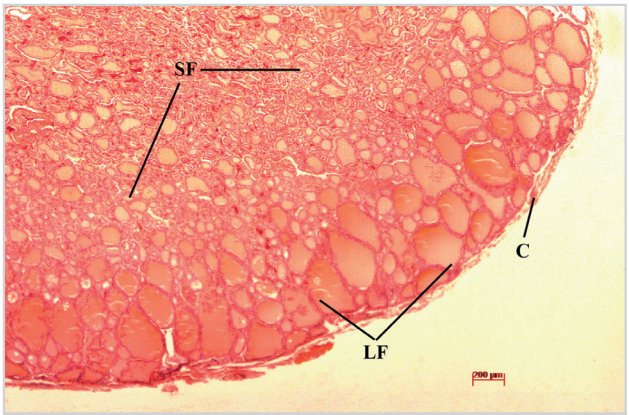

Figure 1. Thyroid gland of the camel. A thin collagenous capsule (C) surrounds the gland. The large follicles (LF) are located in the peripheral part of the gland and the small follicles (SF) in the central part of the gland. H&E.

Dimensions and weight of camel thyroid gland were not significantly different in the males and females. These results are similar to those of Kausar and Shahid (2006). The camel thyroid gland is located in a close contact with the trachea and on its lateral surface, as seen in other domestic mammals (Dyce et al., 1996; Nickel et al., 1979; Pousty and Adibmorady, 2003; Sisson et al., 1975). As Allen et al. (1998) have shown in their reports, the thyroid gland is surrounded by a capsule of collagenous connective tissue and penetration of trabeculae from the capsule into the gland divides it into various lobules. The lobules are made from follicles, which vary in size and shape. These features are also available in camels and are consistent with the findings of Kausar and Shahid (2006), Atoji et al. (1999), and Abdel-Magied et al. (2000). In contrary to the study of Kausar and Shahid (2006) reporting that the capsule of the camel's thyroid gland is thick, the results of this study show that the thyroid gland is surrounded by a thin capsule. Unlike other domestic mammals, in camel, the large (inactive) follicles are present in the peripheral part of the gland while the small (active) ones are located at the center of the gland (Abdel-Magied et al. 2000; Atoji et al. 1999; Pousty and Adibmorady, 2003). Loose connective tissue and vascular bed are

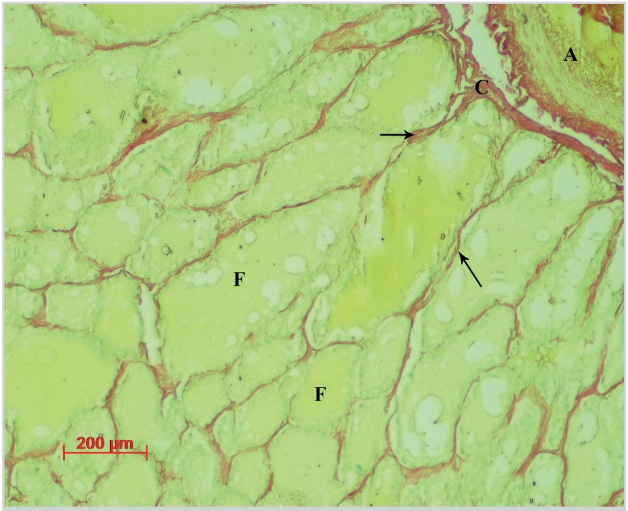

Figure 2. Thyroid gland of the camel. Penetration of trabeculae (arrow) from the capsule (C) into the gland. Artery: (A), Follicle: (F). Verhoeff.

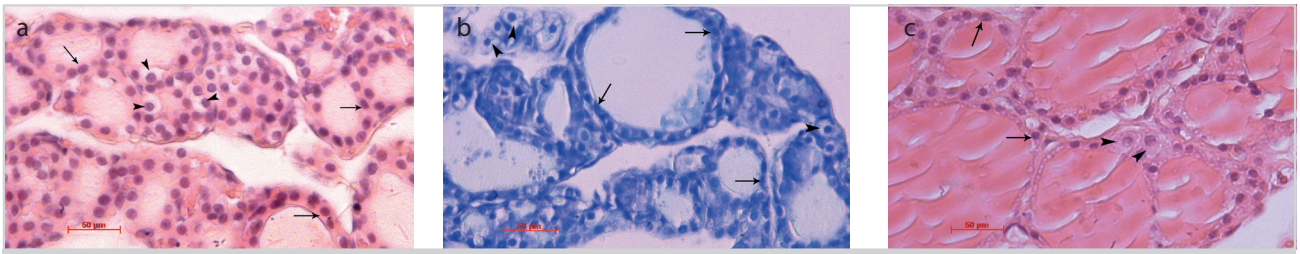

Figure 3. a-c. Thyroid gland of the camel. Hematoxylin & Eosin (a, c) and Toluidine blue (b); Follicular cells (arrows) that constitute the wall of the follicles. Parafollicular cells (arrowheads) with light cytoplasm are located between the follicles.

Table 2. Mean values (mean value ± standard deviation) of some stereological parameters of the thyroid gland in one-humped camel

Variable	Volume density
Follicular cell (%)	59.1±4.37
Parafollicular cell (%)	5.00±0.18
Colloid (%)	31.1±2.13
Stroma (%)	4.6±0.25

well developed in the central part of the gland, which is consistent with the activity of the central small follicles (Atoji et al., 1999; Kausar and Shahid, 2006; Pousty and Adibmorady, 2003). Camel is a ruminant, which is adapted to dry, very hot, and adverse climates (Atoji et al., 1999). In warm and dry areas, the internal condition of the camel's body is dependent on the endocrine system, and the thyroid affects many organs of the body (Banks, 1993). Reduction in thyroid function during dehydration in the summer helps to maintain water level by reducing pulmonary water loss and basal metabolism (Yagil et al., 1978). It seems that this specific arrangement of the follicles at the peripheral and central parts of the gland is effective in preparing the animal to cope with the hot and dry weather of the deserts. Although this condition has been observed in some rodents such as rats and guinea pigs (Pousty and Adibmorady, 2003), it has not been reported by researchers such as Abdel-Magied et al. (2000), Atoji et al. (1999) and Kausar and Shahid (2006) who studied camel's thyroid gland previously. Like other domestic mammals (Banks, 1993; Fujita, 1975; Mason and Wilkinson, 1973; Nickel et al., 1979), in the camel's thyroid gland, cuboid cells form the wall of the follicles and the inside of the follicles is full of colloid. These results are consistent with the findings of Abdel-Magied et al. (2000), Atoji et al. (1999) and Kausar and Shahid (2006).

Although there has been studies reporting that parafollicular cells are absent in camel's thyroid (Abdel-Magied et al., 2000; Atoji et al., 1999; Kausar and Shahid, 2006), this study showed that these cells are present in the camel's thyroid gland. The staining of these cells is paler than follicular cells. Also, they are brighter and larger than follicular cells and they are located between the follicles. These cells are significantly fewer than follicular cells and are more commonly seen between the small and active follicles of the central part of the gland, due to massive vascular bedding in this area. These cells are rarely present in the peripheral parts of the gland. The presence of ultimobranchial structure in the camel's thyroid gland has also been reported by Mubarak and Sayed (2005). In mammals such as horses, dogs, and cats, these cells are present as light and pale cells between the follicles and follicular cells and occupy a small number of thyroid cells (Banks, 1993; Manohar et al., 1995). In this study the presence of parafollicular cells in the thyroid gland of camel has been shown by routine histological methods, besides, further research is needed. They are located in the central part of the gland, between the small follicles. Like other domestic mammals, these cells form a small amount of the thyroid cells.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Semnan University.

Peer-review: Externally peer-reviewed.

Acknowledgements: We thank the officials of Semnan University to provide the necessary facilities for implementing this project. We would also like to thank Mr. Mohammad Mehdi Darvishi and Mr. Morteza Saberi for their assistance in this study.

Conflict of Interest: The author have no conflicts of interest to declare.

Financial Disclosure: The author declared that this study has received no financial support.

References

Abdel-Magied, E.M., Taha, A.A., Abdalla, A.B., 2000. Light and electron microscopic study of the thyroid gland of the camel *(Camelus dromedarius)*. Anatomia Histologia Embryologia 29, 331-336. [CrossRef]

Allen, A.I., Fretz, P.B., Card, C.E., Doige, C.E., 1998. The effects of partial thyroidectomy on the development of the equine fetus. Equine Veterinary Journal 30, 53-59. [CrossRef]

Atoji, Y., Yamamoto, Y., Suzuki, Y., Sayed, R., 1999. Ultrastructure of the thyroid gland of the one-humped camel *(Camelus dromedarius)*. Anatomia Histologia Embryologia 28, 23-26. [CrossRef]

Bancroft, J.D., Gamble, M., 2008. Theory and Practice of Histological Techniques. 6th ed. Philadelphia, Churchill Livingstone, pp. 295-307.

Banks, W.J., 1993. Applied Veterinary Histology. Philadelphia, Mosby, pp. 414-424.

Culling, C.F., Allison, T.R., 1985. Cellular Pathology Technique. 4th ed. London, Butterworths, pp. 113- 479. [CrossRef]

Dyce, K.M., Sack, W.O., Wensing, C.J.G., 1996. Textbook of Veterinary Anatomy. Philadelphia, W.B. Saunders Company, pp. 211-649.

Fujita, H., 1975. Fine structure of the thyroid gland. International Review of Cytology. 40, 197-274. [CrossRef]

Glauert, A.M., Lewis, P.R., 1998. Biological Specimen Preparation for Transmission Electron Microscopy. Vol. 17, Portland press, London. [CrossRef]

Hillson, S., 2005. Teeth. Cambridge University Press, New York, pp. 223-245. [CrossRef]

Hyttel, P., Sinowatz F., Vejlested, M., 2010. Essentials of Domestic Animal Embryology. Philadelphia, W.B. Saunders Elsevier, pp. 230.

Kausar, R., Shahid, R.U., 2006. Gross and microscopic anatomy of thyroid gland of one humped camel *(Camelus Dromedarius)*. Pakistan Veterinary Journal 26, 88-90.

Kurihara, H., Uchida, K., Fujita, H., 1990. Distribution of microtubules and microfilaments in the thyroid follicular cells of normal TSH-treated and hypophysectomized rats. The Histochemical Journal 93, 335-345. [CrossRef]

Manohar, M., Goetz, T.E., Saupe, B., Hutchens, E., Coney, E., 1995. Thyroid, renal and splanchnic circulation in horses at rest and during short-term exercise. American Journal of Veterinary Research 56, 1356-1361.

Mason, R., Wilkinson, J.S., 1973. The thyroid gland. A review. Australian Veterinary Journal 49, 44-49. [CrossRef]

Mubarak, W., Sayed, R., 2005. Ultramicroscopical study on thyrocalcitonin cells in the camel *(camelus dromdarius)*. Anatomia Histologia Embryologia 34, 35. [CrossRef]

Nickel, R., Schummer, A., Seiferle, E., 1979. The Viscera of the Domestic Mammals. Berlin, Verlag Paul Parey, pp. 64-235. [CrossRef]

Pousty, I., Adibmorady, M., 2003. Comparative Histology and Histotechnique. University of Tehran Press, Tehran, pp. 253-254.

Sisson, S., Grossman, J.D., Getty, R., 1975. The Anatomy of the Domestic Animals, Volume 1, Philadelphia, W.B. Saunders Company, pp. 150-154.

Solcia, E., Capella, C., Vassallo, G., 1969. Lead-Haematoxylin as a Stain for Endocrine Cells, Significance of Staining and Comparison with other Selective Methods. Histochemistry and Cell Biology 20, 116-126. [CrossRef]

SPSS Inc. Released 2003. Released 2003. SPSS for Windows, Version 12.0. Chicago, SPSS Inc.

Sridharan, G., Shankar, A.A., 2012. Toluidine blue: A review of its chemistry and clinical utility. Journal of Oral and Maxillofacial Pathology 16, 251-255. [CrossRef]

Weibel, E.R., 1979. Stereological methods. Practical methods for biological morphometry. Vol. 1. Academic Press, London, pp. 101-108.

Yagil, R., Etzion, Z., Ganani, J., 1978. Camel thyroid metabolism: Effect of season and dehydration. Journal of Applied Physiology 45, 540-544. [CrossRef]

ISTANBUL UNIVERSITY CERRAHPASA

The Investigation of the Presence and Antimicrobial Profiles of *Arcobacter* Species in Sheep Carcasses and Feces

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Cite this article as: Çelik, C., İkiz, S., 2019. The Investigation of the Presence and Antimicrobial Profiles of *Arcobacter* Species in Sheep Carcasses and Feces. Acta Vet Eurasia 45, 42-49.

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Abstract

This study was designed to investigate the presence and the prevalence of *Arcobacter butzleri, Arcobacter cryaerophilus,* and *Arcobacter skirrowii* in sheep carcass swabs and feces from sheep with and without diarrhea raised in Istanbul. Also it was aimed to determine their resistance profiles against some antimicrobials. For this purpose, 50 fecal samples from sheep without diarrhea, 50 fecal samples from sheep with diarrhea, and 50 carcass swab samples from sheep were the material of this study. *Arcobacter* spp. were isolated from 49 (32.6%) of total 150 samples. 34 samples (68%) of 50 fecal samples from sheep with diarrhea, 5 samples (10%) from 50 fecal samples of sheep without diarrhea and 10 samples (20%) of 50 sheep carcass swabs were found to be positive according to isolation results. According to multiplex Polymerase Chain Reaction (mPCR) results, 31 of 49 were identified as *A. skirrowii* (63.3%), 9 of 49 were *A. butzleri* (18.3%), while 9 of 49 were *A. cryaerophilus* (18.3%). As a conclusion, *Arcobacter* species should be taken into consideration especially in sheep with diarrhea. Additionally, it should be considered that arcobacters have started to gain resistance against fluoroquinolones.

Keywords: *Arcobacter butzleri, Arcobacter cryaerophilus, Arcobacter skirrowii,* antibiotic sensitivity test, mPCR, sheep

Introduction

The importance of arcobacters that are described as foodborne and zoonotic entero-pathogens has increased in recent years. *Arcobacter* species (spp.) are considered emerging food-borne entero-pathogens (Abay et al., 2012; Atabay and Corry, 1998). Although it varies among species, these bacteria can be found in animals showing signs of gastroenteritis, abortion and mastitis whereas it can also be seen in healthy animals which do not have these symptoms (On et al., 2002, Vandamme et al., 1992b). Especially *Arcobacter butzleri (A. butzleri)* is the most known species that they can cause food-borne gastroenteritis and septicemia in human beings (Lau et al., 2002; Vandamme et al., 1992b).

Lately, *Arcobacter* spp. has been frequently isolated from animal source foods (Fernandez et al., 2015; Sekhar et al., 2017). Although they have shown significant phenotypic heterogeneity in terms of their biochemical and physiological characteristics, the genus *Arcobacter* is classified together with *Campylobacter* genus under the *Campylobacteriaceae* family (Vandamme and De Ley, 1991). Their ability to grow at temperatures below 30°C and aerotolerance characters are the features that seperate *Arcobacter species* from *Campylobacter* species. Therefore, they have been defined as "aerotolerant campylobacters" (Neill et al., 1978).

Arcobacters have been identified as a potential zoonotic agent of food and water origin. The discovery of new species has enlarged the genus. Recently, the whole *Arcobacter* genus has been defined to contain 25 species (Ramees et al., 2017). In the genus, *A. butzleri*, *A. cryaerophilus* and *A. skirrowii* can more probably cause human diseases (Assanta et al., 2002). Among several *Arcobacter* species, *A. butzleri*, *A. cryaerophilus*, and *A. skirrowii* are reported to have veterinary and public health sig-

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Received Date: 25 July 2018 • **Accepted Date:** 05 March 2019 • DOI: 10.26650/actavet.2019.18007

Available online at actaveteurasia.istanbulc.edu.tr

nificance. *Arcobacter* spp. have been included among microorganisms that pose a high risk for human health and *A. butzleri* can be isolated from many human cases, it has been accepted as the most significant species in this genus by International Food Microbiology Specification Committee (Collado and Figueras, 2011).

Although there are many medium and different procedures for the identification of *Arcobacter* spp., no standard reference methods have been suggested. Atabay and Corry (1998) used an arcobacter broth with the addition of cefoperazone, amphotericin, and teicoplanin (CAT) supplements in their research, and more recently, Houf et al. (2001) developed an *Arcobacter*-specific isolation method with the use of an arcobacter medium. This medium consisted of five antibiotics, including cefoperazone, trimethoprim, amphotericin, novobiocin, and 5-fluorouracil.

Modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA) with the addition of a CAT supplement was used by Kemp et al. (2005). Columbia agar containing 5% (vol/vol) of defibrinated horse blood was also used by Merga et al. (2011).

The biochemical properties of *Arcobacter* spp. reported by Vandamme et al. (1992a) are that *A. butzleri, A. cryaerophilus, and A. skirrowii* strains have oxidase and catalase activities and can not produce hydrogen sulfide on Triple Sugar Iron agar.

Methods used for direct detection and/or typing of the species in the genus include PCR, multiplex PCR (mPCR), real time PCR (RT-PCR), fluorescence *in situ* hybridisation (FISH), denaturing gradient gel electrophoresis PCR (DGGE-PCR), restriction fragment length polymorphism (RFLP) and matrix assisted laser desorption ionization mass spectrometry (MALDITOF MS) Enterobacterial repetitive intergenic consensus (ERIC) PCR was set up and optimized for the characterization of *A. butzleri, A. cryaerophilus, and A. skirrowii* strains (Houf et al., 2002).

Recently, mPCR has been developed as a very useful method for quick identification of *Arcobacter* species. It has been reported that there is no risk of false positive results based on the contamination with other bacteria from *Campylobacteriaceae* family. It can reduce the time required for the identification of *Arcobacter* spp. and eliminate the likelihood of false-positive results because of other *Campylobacteriaceae* family members (Neubauer and Hess, 2006; Snelling et al., 2006).

Fluroquinolones and tetracycline have been suggested for the treatment of infections caused by *A. butzleri* in humans and animals (Son et al., 2007; Vandenberg et al., 2006). It is stated that the resistance to erythromycin and the susceptibility to ciprofloxacin may have human health effects, as the two antimicrobials are generally used as first-line drugs to treat the bacterial infections due to bacteria in the *Campylobacteriaceae* family (Rahimi, 2014).

Abay et al. (2012) argued as a conclusion of their study that gentamycin, streptomycin, and tetracycline would be suitable antibiotics for the treatment or control of the disease caused by *Arcobacter* spp. in veterinary and human medicine. Aski et al. (2016) reported that all *Arcobacter* isolates were resistant to rifampicin, vancomycin, ceftriaxone, trimethoprim, and cephalothin, and furthermore, the isolates showed high susceptibility to tetracycline, oxytetracycline, erythromycin, ciprofloxacin, kanamycin, amikacin, gentamicin, and enrofloxacin.

This study aimed to investigate the presence and the prevalence of *A. butzleri, A. cryaerophilus,* and *A. skirrowii* in sheep carcass swabs and feces of sheep with and without diarrhea. It also aimed to determine their resistance profiles against some antimicrobials. In addition, the possible effects of season, gender, sample type, and age on the prevalence of arcobacters were examined.

Materials and Methods

This study was approved by Ethic Committee of the İstanbul University Faculty of Veterinary Medicine (Approval number: 2013/74).

Samples

Fifty fecal samples from healthy sheep, 50 fecal samples from sheep with diarrhea, and 50 swab samples from sheep carcasses were taken from different farms and slaughterhouses located in İstanbul, Turkey. All the samples were gathered in the same year and only one sample was received from each sheep. 150 samples were taken from 73 male and 77 female animals. The details of the samples and their collections were given in Table 1. The fecal samples were collected from rectum and stored in sterile plastic containers and the carcass swab samples were taken by using sterile cotton swabs. The swab samples were taken from the area from neck to the hips (50 cm^2) according to the EU Regulation 2073/2005. All the samples were analyzed in the microbiology laboratory in 3 hours after they were collected from the sheep.

Reference strains

The positive control strains belong to *A. butzleri*, *A. cryaerophilus* and *A. skirrowii* were taken from Victor Segalen Bordeaux II University Hospital's Bacteriological Laboratory.

Culture

Two grams of fecal materials were transferred into the test tubes including 9 ml of arcobacter broth (Oxoid, Basingstoke, UK), and each swab sample was transferred into the test tubes including 2 ml of arcobacter broth with the addition of 5-fluorouracil (100 mg/1000 mL) (Merck, Darmstadt, Germany), amphotericin B (10 mg/1000 mL) (Bioshop, Ontario, Canada), cefoperazone (16 mg/1000 mL) (Sigma-Aldrich, Saint Louis, USA), novobiocin (32 mg/1000 mL) (Sigma-Aldrich, Saint Louis, USA), and trimethoprim (64 mg/1000 mL) (Sigma-Aldrich, Saint Louis, USA) (Houf et al., 2001) as an enrichment broth, and were incubated aerobically

Table 1. The information regarding the samples collected in one year

Sample type (n:number)	Farm code	Sample number	The month of sample collection
Fecal samples from sheep without diarrhea (n=50)	FARM A	20	MAY
	FARM B	20	JUNE, JULY
	FARM C	10	AUGUST
Fecal samples from sheep with diarrhea (n=50)	FARM A	3	MAY
	FARM D	6	SEPTEMBER
	FARM E	10	OCTOBER
	FARM F	4	NOVEMBER
	FARM G	14	NOVEMBER
	FARM H	13	DECEMBER
Carcass swab samples (n=50)	SLAUGHTERHAUSE A	10	JULY
	SLAUGHTERHAUSE B	15	JULY
	SLAUGHTERHAUSE C	15	AUGUST
	SLAUGHTERHAUSE D	10	OCTOBER

Table 2. Primer sequences used in this study (Houf et al., 2000)

	Primers	Sequence (5' to 3')
A.butzleri	BUTZ	CCT GGA CTT GAC ATA GTA AGA ATGA
16Ss r DNA	ARCO	CGT ATT CAC CGT AGC ATA GC
A.skirrowii	SKIR	GGC GAT TTA CTG GAA CAC A
A.cryaerophilus	CRY1	TGC TGG AGC GGA TAG AAG TA
23S rDNA	CRY2	AAC AAC CTA CGT CCT TCG AC

at 30°C for 24 h. At the same time, motility tests were performed with each sample taken from arcobacter broth after 24 h as soon as the pre-enrichment stage was completed. Spiral or corkscrew motility types were observed. After the pre-enrichment phase, 15 µl was collected from broth culture and was inoculated onto modified charcoal-cefoperazone-deoxycholate agar (Scharlab, Barcelona, Spain) as a selective medium which consisted of CAT supplement (Oxoid, Basingstoke, UK).

It was incubated for 48 h at 30°C under aerobic conditions. At the end of incubation, Gram staining was performed on gray-white, small, round, and S-type colonies. Gram negative colonies were transferred to Columbia agar (Biolife, Milan, Italy). The Gram staining properties of gray-white, small, round, and S-type colonies were reexamined after 24-48 h (Merga et al., 2011). The biochemical features of gram negative bacteria showed catalase and oxidase activities, but no production of hydrogen sulfide was detected from any of the strains. Additionally all reference strains were isolated with the same method.

Multiplex polymerase chain reaction (mPCR)

The isolates were then identified to species level by multiplex PCR (Houf et al., 2000). A boiling method was used to extract DNA from the *Arcobacter* isolates.

Selected primer sequences shown in Table 2 were amplified according to the method by Houf et al. (2000) through PCR, using a reaction mixture containing 4 µl 10X PCR buffer+$MgCl_2$, 0.8 µl 10mM dNTPs, 2 µl (20 pmol/mL) of each of the four primers (BUTZ, ARCO, CRY1, CRY2) and 1 µl (20 pmol/mL) of SKIR primer, 0.3 µl of Taq DNA polymerase, 15.9 µl DNase-free water, and 10 µl bacterial DNA for each sample. PCR amplification kit (Qiagen, Germantown, USA) was used in the study. The reactions were performed in a thermal cycler (Biometra UNO-Thermoblock, Dublin, Ireland) with the following amplification conditions: a denaturation step for 3 min at 94°C; 37 amplification cycles: denaturation for 2 min at 94°C, annealing for 45 sec at 61°C, and for 30 sec. extension at 72°C; the final extension step was 7 min at 72°C.

The electrophoresis (Biometra, Dublin, Ireland) was performed approximately at 125V for 45 min. The bands were visualized on an UV illuminator. The resulting products sizes were 257 bp for *A. cryaerophilus*, 401 bp for *A. butzleri,* and 641 bp for *A. skirrowii*. Furthermore, all reference strains were identified with the same technique.

Antibiotic sensitivity test

The antibiotic resistance profiles of *Arcobacter* strains were determined by using disc diffusion technique. Blood agar that comprised 5% (v/v) of defibrinated horse blood in blood agar base no. 2 was used for this purpose. Sterile cotton-tipped swab was used for spreading on the plate. Later on, each antibiotic disc was placed onto the agar and the plates were kept at 4°C for about 15 min. The plates incubated in a micro-aerobic atmosphere at 30°C for 48 h and the diameters of the inhibition zones were measured with calipers. The diameters of the zones occurred around the discs were evaluated according to the criteria defined for materials for *Campylobacter* spp. by the National Committee for Clinical Laboratory Standards and also

according to the criteria specified for *Enterobacteriaceae* by the European Committee on Antimicrobial Susceptibility Testing (2015), since no standardized source was described yet to make comparison.

Statistical analysis

In order to determine the statistical significance of the findings, the results were evaluated with "Chi-square (x^2) test" by SPSS 13.0 programme (SPSS Inc.; IL, Chicago, USA) by comparing the positive rates of *A. butzleri, A. cryaerophilus,* and *A. skirrowii* under sex, age, season, and sample subgroups.

Results

Culture and isolation studies

A total of 150 samples were collected from 50 sheep with diarrhea, 50 healthy sheep, and 50 sheep carcasses from the slaughterhouses and farms in and around Istanbul province. Fourty-nine (32.6%) of 150 samples were detected as *Arcobacter* spp.

Thirty-four (68%) of 50 fecal samples from sheep with diarrhea, 5 (10%) of 50 fecal samples from sheep without diarrhea and 10 (20%) of 50 sheep carcass swab samples were found to be positive according to isolation results.

Multiplex Polymerase Chain Reaction (mPCR)

Presumptive colonies were chosen according to their macroscobic and microscobic features. Only one suspicious colony from each culture were applied for PCR identification. A total of 49 culture positive samples were tested by mPCR and all 49 (100%) samples were positive in terms of *Arcobacter* species. As a result of the agarose gel electrophoresis of the mPCR products, 257 bp for *A. cryaerophilus*, 401 bp for *A. butzleri,* and 641 bp bands for *A. skirrowii* were detected. According to mPCR, 31 of 49 samples were identified as *A. skirrowii* (63.2%), 9 of 49 as *A. butzleri* (18.3%), while 9 of 49 as *A. cryaerophilus* (18.3%) (Figures 1, 2).

Antibiotic sensitivity testing

All strains identified as *A. butzleri* were susceptible to methicillin (30 µg) (Bioanalyse, Ankara, Turkey) and gentamycin (10 µg)

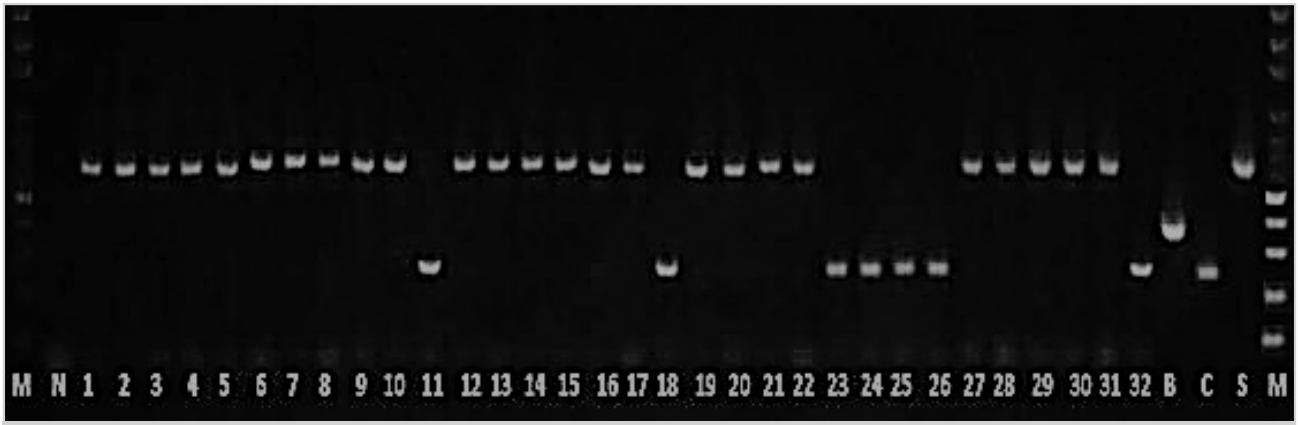

Figure 1. M: Marker, N: Negative Control, B: *A.butzleri* Positive Control, C: *A.cryaerophilus* Positive Control, S: *A.skirrowii* Positive Control, 1-32: Samples

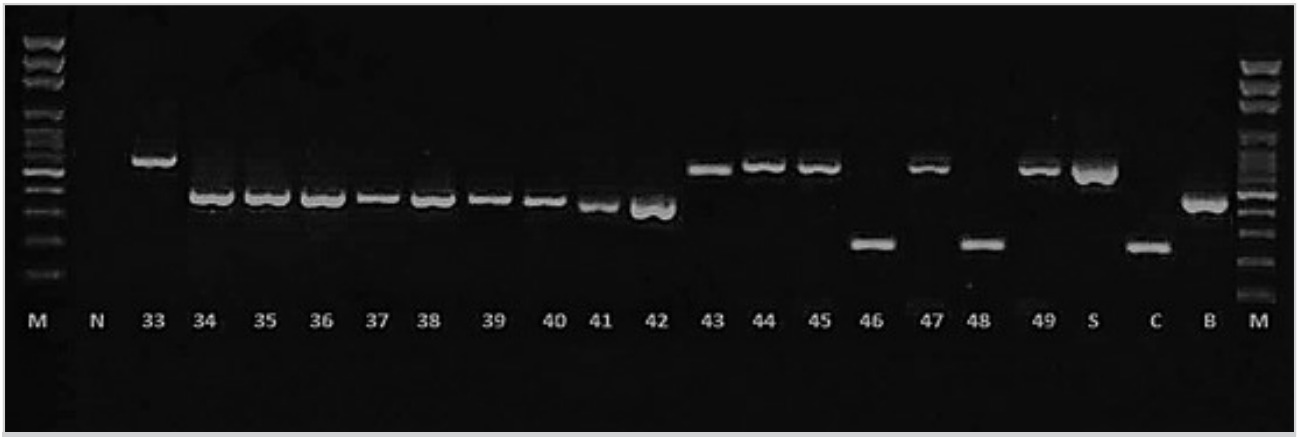

Figure 2. M: Marker, N: Negative Control, B: *A.butzleri* Positive Control, C: *A.cryaerophilus* Positive Control, S: *A.skirrowii* Positive Control, 33-49: Samples

Table 3. Antibiotic sensitivity test results of *Arcobacter* strains

		***A.butzleri* (n)**			***A.cryaerophilus* (n)**			***A.skirrowii* (n)**		
	Antimicrobial Agent	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**
Penicillin	Penicillin G 10 unit/disk	9	0	0	9	0	0	29	0	2
	Ampiciline + Sulbactam 1:120 mg	3	3	3	5	3	1	5	14	12
	Amoxicillin 25 µg	2	5	2	5	3	1	11	12	8
	Methicillin 30 µg	0	9	0	8	0	1	17	4	10
	Amoxicillin + Clavulanic acid 2:1 30 µg	3	5	1	4	4	1	10	21	0
Tetracyclines	Tetracycline 30 µg	2	6	1	0	9	0	0	31	0
	Oxytetracycline 30 µg	4	5	0	0	7	2	0	25	6
	Doxycycline 30 µg	1	6	2	0	9	0	1	30	0
Quinolones	Ofloxacin 5 µg	8	1	0	9	0	0	9	5	17
	Ciprofloxacin 5 µg	4	0	5	4	1	4	7	6	18
	Enrofloxacin 5 µg	2	2	5	3	3	3	2	22	7
	Nalidixic Acid 30 µg	9	0	0	6	1	2	8	8	15
Aminoglycosid	Amikacin 30 µg	0	7	2	0	9	0	1	29	1
	Gentamisin 10 µg	0	9	0	0	8	1	2	28	1
Macrolides	Erythromycin 15 µg	0	0	9	0	1	8	1	0	30
Rifamycin	Rifampicin 30 µg	9	0	0	9	0	0	30	1	0
Cephalosporins	Cephalothin 30 µg	8	1	0	8	0	1	28	1	2
Nitrofurantoin	Nitrofurantoin 300 µg	8	1	0	4	4	1	1	30	0
Vancomycin	Vancomycin 30 µg	9	0	0	9	0	0	29	1	1

R: resistant; S: sensitive; I: intermediate

(Oxoid, Basingstoke, UK), and all *A. cryaerophilus* strains were susceptible to tetracycline (30 µg) (Oxoid, Basingstoke, UK), doxycycline (30 µg) (Oxoid, Basingstoke, UK), and Amikacin (30 µg) (Oxoid, Basingstoke, UK), while all *A. skirrowii* strains were found to be susceptible to tetracycline.

All of *A. butzleri* strains had intermediate sensitivity to erythromycin (15 µg) (Oxoid, Basingstoke, UK), whereas all *A. butzleri* strains had resistance to penicillin G (10 Unit/disk) (Oxoid, Basingstoke, UK), rifampicin (30 µg) (Oxoid, Basingstoke, UK), vancomycin (30 µg) (Oxoid, Basingstoke, UK), and nalidixic acid (30 µg) (Oxoid, Basingstoke, UK). And all *A. cryaerophilus* strains showed resistance to ofloxacin (5 µg) (Oxoid, Basingstoke, UK), vancomycin, and rifampicin. Other strains showed variable results as shown in Table 3.

Statistical findings

The effects of season, age, gender, and sample type on isolation rate were examined. The effect of season was found statistically significant ($p<0.05$) for *A. cryaerophilus* and *A. skirrowii`s* isolation rates. The effect of age was statistically significant ($p<0.05$) on *A. butzleri* and *A. skirrowii*'s isolation rates. The effect of gender was not statistically significant for none of these three *Arcobacter* species` isolation ratios. The sample type was found 99% statistically significant ($p<0.05$) related to *A. butzleri, A. cryaerophilus,* and *A. skirrowii`s* isolation rates.

Discussion

Arcobacters have been accepted as "important zoonotic pathogenic" bacterial strains (Cardoen et al., 2009). The non-human sources of arcobacters have also been reported to be healthy or have affected animals of various species, various foods, and water (Kabeya et al., 2003).

In a study carried out in Turkey (Sürmeli, 2006), 104 sheep stool samples were taken under microaerobic conditions using membrane filtration technique and only one *A. cryeraophilus* was identified by mPCR method as reported. In Belgium, Van Driessche et al. (2003) collected 62 healthy sheep feces from slaughterhouses and performed a direct isolation from the feces samples. According to their report, they identified *A. butzleri* only in 3 (4.8%) samples, whereas 10 *Arcobacter* species (16%) were isolated after pre-enrichment.

In our study, 31 of 49 were identified as *A. skirrowii* (63.3%), 9 of 49 as *A. butzleri* (18.3%), while 9 of 49 as *A. cryaerophilus* (18.3%). Using the arcobacter broth medium-mCCDA-Columbia agar (defibrinated horse blood supplement) as an isolation technique may account for the increase in the isolation rate. Another explanation for the differences found in the rates may be the use of stool sample instead of swab. In this study, 8 *A. butzleri* (16%), 1 *A. cryaerophilus* (2%), and 1 *A. skirrowii* (2%)

were identified from 50 sheep carcass swab samples. Any information with regard to whether the animals having enteritis could not be obtained.

In a study carried out in Turkey (Ertaş and Doğruer, 2009), *Arcobacter* spp. were isolated from 85 (42.5%) of 200 minced meat samples. *A. butzleri* was detected from 39% and *A. skirrowii* was detected from 4% of minced sheep meat samples, while *A. butzleri* was detected from 40% and *A. skirrowii* was detected from 2% of minced cattle meat. The prevalence rate of arcobacters in minced meat obtained from sheep in their study was higher than the prevalence rate we had from swab sample in our thesis study. The difference in prevalence suggested a possible increase in *Arcobacter* contamination as the meat samples were processed by equipment like mincer. Further, the microorganisms on the surface of the meat might have spread all over it during the course of grinding and mixing the mincemeat leading to food spoilage.

In Japan, Kabeya et al. (2003) found 12 (3.6%) *Arcobacter* species from 332 healthy cattle feces samples, whereas Van Driessche et al. (2003) identified 39.2% *Arcobacter* species from healthy bovine feces samples and 16.1% *Arcobacter* species from healthy ovine feces samples. Nachamkin et al. (2008) reported that they identified *A. butzleri* from feces samples of pig, cattle, horse, ostrich, turtle, and *A. skirrowii* from sheep and cattle with diarrhea and hemorrhagic colitis. *A. skirrowii* was detected from sheep having enteritis and most *A. butzleri* strains were isolated from diarrheic feces of humans and animals (Vandamme et al., 1992a). In our study, 34 (68%) *Arcobacter* species were detected from 50 feces samples belonged to sheep with enteritis and we found 25 (50%) *A. skirrowii*, 8 (16%) *A. cryaerophilus,* and 1 (2%) *A. butzleri*.

It was reported that season, climate, geographical location, sampling type, and isolation method had an effect on the prevalence values to be obtained (Merga et al., 2011). Golla et al. (2002) stated that there was a direct correlation between increased age and increased prevalence of arcobacters in their studies. In our study, the incidence of *A. cryaerophilus* increased in proportion to the increase in age from 1 month to 3 years, but the graph showed a reverse slope for *A. butzleri*. The incidence of *A. skirrowii* reached the highest rate between 1 and 3 years.

Researchers reported that there was no significant difference in the rates of arcobacters between goats and sheep ($p>0.05$) (Van Driessche et al., 2003; Van Driessche et al., 2005). In our study, the effect of gender difference on the incidence of *Arcobacter* species was statistically insignificant ($p>0.05$). The effect of seasons on the incidence of *A. cryaerophilus* ($p<0.05$) and *A. skirrowii* ($p<0.001$) in this study was also statistically significant.

It was also reported in other research (Van Driessche et al., 2003; 2005), as reported in this study, that the difference in season and farm management could have an effect on the rates found. The heavy conditions of the winter season in Turkey and keeping the animals in narrow and unhygienic shelters may be another cause of the seasonal effect. The effect of the presence of diarrhea was found to be statistically significant on the isolation rates ($p<0.001$) of *A. cryaerophilus* ($p<0.01$) and *A. skirrowii*.

The most commonly prescribed drugs as antibiotics are erythromycin or a fluoroquinolone such as ciprofloxacin (Luber et al., 2003). Tetracycline, doxycycline, and gentamicin are sometimes listed as alternative drugs for treatment (Houf et al., 2004). Pérez-Cataluña et al. (2017) stated that the resistance to ciprofloxacin, one of the antibiotics recommended for the treatment of intestinal infections of *Arcobacter*, detected 10.7% of the strains, and indicated the importance of selecting the most effective treatment. In our study as well, most of the *Arcobacter* strains showed a resistance or an intermediate profile against some of the fluoroquinolones antibiotics, such as enrofloxacin, ofloxacin, and ciprofloxacin. The resistance to erythromycin and quinolone antibiotics found in this study can be considered as a worrisome condition, because these antimicrobial agents have been reported to be the first-line antibiotics used in the treatment of infections caused by members of the Campylobacteracea family (Houf et al., 2004).

In Milesi's research (2011), it was reported that all *Arcobacter* spp. isolates from animal originated food were detected as resistant to cephalothin, sulfamethoxazole / trimethoprim, and nalidixic acid. The majority of *A. cryaerophilus* and *A. skirrowii* strains were found as sensitive to tetracyline and amikacin, which is compatible with some other research results (Abay et al., 2012; Collado and Figueras, 2011; Son et al., 2007; Ünver et al., 2013). However, *A. butzleri*'s strains were detected to have started to gain some resistance against to these 2 antimicrobial agents.

The results have exhibited that *A. skirrowii* may also show a significant diarrhea effect in sheep, such as in human and other animals. Considering the increase in the prevalence of *Arcobacter spp.* isolates from sheep in autumn (Grove-White et al., 2014), another reason for the high incidence of *A. skirrowii* isolates in our research may be the gathering of diarrhea cases in November and December.

Although the results of the studies on fluoroquinolones indicate that it is the most effective antibiotic group in the treatment of *Arcobacter* infections, most of the *Arcobacter* strains detected in this research show resistance or half sensitivity to enrofloxacin, ofloxacin, and ciprofloxacin, which are the antibiotics of this group. In this study arcobacters cause diarrhea in sheep and these animals may play a very important reservoir role for humans. Hence, as with human beings, of the analyses of diarrhea cases should take into consideration the existence of *Arcobacter* species in sheep. As a result, the study suggests, in line with many other researches, that these varying sensitivity and resistance profiles to the antibiotics may be due to the

lack of a standard antimicrobial susceptibility test developed for *Arcobacter* species (Vandenberg et al., 2006).

In conclusion, *A. skirrowii A. butzleri* and *A. cryaerophilus* were identified with the rate of (63.3%), (18.3%) and (18.3%) respectively. *A. skirrowii* was found more than other *Arcobacter* species in fecal samples with diarrhea. Regarding the effect of season, age, sample type and gender, only the effect of gender was not found statistically significant for none of these three *Arcobacter* species` isolation ratios whereas the effect of season was found statistically significant for *A. cryaerophilus* and *A. skirrowii`s* isolation rates and the effect of age was statistically significant on *A. butzleri* and *A. skirrowii*'s isolation rates. The sample type was found also statistically important regarding *A. butzleri, A. cryaerophilus,* and *A. skirrowii`s* isolation rates. In terms of the medication of *Arcobacter* infections, it was observed that they started to gain resistance against to fluoroquinolones which was known as the best antibiotic groups for arcobacters.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of İstanbul University Faculty of Veterinary Medicine (2013/74).

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – S.İ., C.Ç.; Design – S.İ., C.Ç.; Supervision – S.İ.; Resources – C.Ç.; Materials – C.Ç.; Data Collection and/or Processing – C.Ç.; Analysis and/or Interpretation – C.Ç., S.İ.; Literature Search – C.Ç.; Writing Manuscript – C.Ç.; Critical Review – C.Ç., S.İ.

Conflict of Interest: The authors have no conflicts of interest to declare.

Financial Disclosure: This study was supported by Scientific Research Projects Coordination Unit of İstanbul University (Project number: 35968).

References

Abay S., Kayman T., Hizlisoy H., Aydin F., 2012. In vitro antibacterial susceptibility of *Arcobacter butzleri* isolated from different sources. Journal of Veterinary Medical Science 74, 613-616. [CrossRef]

Aski H.S., Tabatabaei M., Khoshbakht R., Raeisi M., 2016. Occurrence and antimicrobial resistance of emergent *Arcobacter* spp. isolated from cattle and sheep in Iran. Comparative Immunology, Microbiology and Infectious Diseases 44, 37-40. [CrossRef]

Assanta M.A., Roy D., Lemay M.J., Montpetit D., 2002. Attachment of *Arcobacter butzleri*, a new waterborne pathogen, to water distribution pipe surfaces. Journal of Food Protection 65, 1240-1247. [CrossRef]

Atabay H.I., Corry, J.E., 1998. Evaluation of a new arcobacter enrichment medium and comparison with two media developed for enrichment of *Campylobacter* spp. International Journal of Food Microbiology 41, 53-58. [CrossRef]

Cardoen S., Van Huffel X., Berkvens D., Quoilin S., Ducoffre G., Saegerman C., Speybroeck N., Imberechts H., Herman L., Ducatelle R., Dierick K., 2009. Evidence-based semiquantitative methodology for prioritization of foodborne zoonoses. Foodborne Pathogens and Disease 6, 1083-1095. [CrossRef]

Collado L., Figueras M.J., 2011. Taxonomy, epidemiology and clinical relevance of the genus *Arcobacter*. Clinical Microbiology Reviews 24, 174-192. [CrossRef]

Ertaş N., Doğruer Y., 2009. Prevalence of *Arcobacter* species in ground meat from cattle and sheep using multiplex PCR techniques. Fırat University Medical Journal of Health Sciences 23, 95-100.

European Committee on Antimicrobial Susceptibility Testing, 2015. European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for Interpretation of MICs and zone diameters ,Version 5.0

Fernandez H., Villanueva M.P., Mansilla I., Gonzalez M., Latif F., 2015. *Arcobacter butzleri* and *A. cryaerophilus* in human, animals and food sources, in southern Chile. Brazilian Journal of Microbiology 46, 145-147. [CrossRef]

Golla S.C., Murano E.A., Johnson L.G., Tipton N.C., Cureington E.A., Savell J.W., 2002. Determination of the occurrence of *Arcobacter butzleri* in beef and dairy cattle from Texas by various isolation methods. *Journal of Food Protection* 65, 1849-1853. [CrossRef]

Grove-White D.H., Leatherbarrow A.J.H., Cripps P.J., Diggle P.J., French N.P., 2014. Temporal and farm-management-associated variation in faecal pat prevalence of *Arcobacter* spp. in ruminants. Epidemiology & Infection 142, 861-870. [CrossRef]

Houf K., Devriese L.A., De Zutter L., Van Hoof J., Vandamme P., 2001. Development of a new protocol for the isolation and quantification of *Arcobacter* species from poultry products. International Journal of Food Microbiology 71, 189-196. [CrossRef]

Houf K., Devriese L.A., Haesebrouck F., Vandenberg O., Butzler J.P., Hoof J.V., Vandamme P., 2004. Antimicrobial susceptibility patterns of *Arcobacter butzleri* and *Arcobacter cryaerophilus* strains isolated from humans and broilers. Microbial Drug Resistance 10, 243-247. [CrossRef]

Houf K., De Zutter L., Van Hoof J., Vandamme P., 2002. Assessment of the genetic diversity among Arcobacters isolated from poultry products by using two PCR- based typing methods. Applied Enviromental Microbiology 68, 2172-2178. [CrossRef]

Houf K., Tutenel A., de Zutter L., Hoof J.V., Vandamme P., 2000. Development of a multiplex PCR assay for simultaneous detection and identification *of Arcobacter butzleri, Arcobacter cryaerophilus* and *Arcobacter skirrowii*. FEMS Microbiology Letters 193, 89-94. [CrossRef]

Kabeya H., Maruyama S., Morita Y., Kubo M., Yamamoto K., Arai S., Izumi T., Kobayashi Y., Katsube Y., Mikami T., 2003. Distribution of *Arcobacter* species among livestock in Japan. Veterinary Microbiology 93, 153-158. [CrossRef]

Kemp R., Leatherbarrow A.J., Williams N.J., Hart C.A., Clough H.E., Turner J., Wright E.J., French N.P., 2005. Prevalence and genetic diversity of *Campylobacter* spp. in environmental water samples from a 100-square-kilometer predominantly dairy farming area. Applied and Environmental Microbiology 71, 1876-1882. [CrossRef]

Lau S.K., Woo PC, Teng J.L., Leung K.W., Yuen K.Y., 2002. Identification by 16S ribosomal RNA gene sequencing of *Arcobacter butzleri* bacteraemia in a patient wit[CrossRef]

Luber P., Bartelt E., Genschow E., Wagner J., Hahn H., 2003. Comparison of broth microdilution, E Test, and agar dilution methods for an-

tibiotic susceptibility testing of *Campylobacter jejuni* and *Campylobacter coli*. Journal of Clinical Microbiology 41, 1062-1068. [CrossRef]

Merga J.Y., Leatherbarrow A.J., Winstanley C., Bennett M., Hart C.A., Miller W.G., Williams N.J., 2011. Comparison of *Arcobacter* isolation methods, and diversity of *Arcobacter* spp. in Cheshire, United Kingdom. Applied and Environmental Microbiology 77, 1646-1650. [CrossRef]

Milesi, S., 2011. Emerging pathogen *Arcobacter* spp. in food of animal origin. Doctoral thesis, University of Milan, Milan.

Nachamkin, I., Szymanski, C. M., Blaser, M. J., 2008. Campylobacter (No. Ed. 3). ASM Press.

Neill S.D., Ellis W.A., O'Brien J.J., 1978. The biochemical characteristics of *Campylobacter*-like organisms from cattle and pigs. Research in Veterinary Science 25, 368-372. [CrossRef]

Neubauer C., Hess M., 2006. Detection and identification of foodborne pathogens of the genera *Campylobacter, Arcobacter* and *Helicobacter* by multiplex PCR in poultry and poultry products. Journal of Veterinary Medicine Series B 53, 376-381. [CrossRef]

On S.L., Jensen T.K., Bille-Hansen V., Jorsal S.E., Vandamme P., 2002. Prevalence and diversity of *Arcobacter* spp. isolated from the internal organs of spontaneous porcine abortions in Denmark. Veterinary Microbiology 85, 159-167. [CrossRef]

Pérez-Cataluña A., Tapiol J., Benavent C., Sarvisé C., Gómez F., Martínez B., Terron-Puig M., Recio G., Vilanova A., Pujol I., Ballester F., 2017. Antimicrobial susceptibility, virulence potential and sequence types associated with *Arcobacter* strains recovered from human faeces. Journal of Medical Microbiology 66, 1736-1743. [CrossRef]

Rahimi E., 2014. Prevalence and antimicrobial resistance of *Arcobacter* species isolated from poultry meat in Iran. British Poultry Science 55, 174-180. [CrossRef]

Ramees T.P., Dhama K., Karthik K., Rathore R.S., Kumar A., Saminathan M., Tiwari R., Malik Y.S., Singh R.K., 2017. *Arcobacter*: an emerging food-borne zoonotic pathogen, its public health concerns and advances in diagnosis and control–a comprehensive review. Veterinary Quarterly 37, 136-161. [CrossRef]

Sekhar, M.S., Tumati, S.R., Chinnam, B.K., Kothapalli, V.S., Sharif, N.M., 2017. Virulence gene profiles of *Arcobacter* species isolated from animals, foods of animal origin, and humans in Andhra Pradesh, India. Veterinary World 10, 716. [CrossRef]

Snelling W.J., Matsuda M., Moore J.E., Dooley J.S., 2006. Under the microscope: *Arcobacter*. Letters in Applied Microbiology 42, 7-14. [CrossRef]

Son I., Englen M.D., Berrang M.E., Fedorka-Cray P.J., Harrison M.A., 2007. Antimicrobial resistance of *Arcobacter* and *Campylobacter* from broiler carcasses. International Journal of Antimicrobial Agents 29, 451-455. [CrossRef]

Sürmeli P., 2006. Kars ili ve çevresinde yetiştirilen koyunlardan alınan dışkı örneklerinde *Arcobacter* türlerinin prevalansının araştırılması. Master thesis, Kafkas University, Faculty of Veterinary Medicine, Department of Microbiology, Institute of Health Sciences, Kars.

Ünver A., Atabay H.I., Şahin M., Çelebi Ö., 2013. Antimicrobial susceptibilities of various *Arcobacter* species. Turkish Journal of Medical Sciences 43, 548-552. [CrossRef]

Van Driessche E., Houf K., Hoof J.V., De Zutter L., Vandamme P., 2003. Isolation of *Arcobacter* species from animal feces. FEMS Microbiology Letters 229, 243-248. [CrossRef]

Van Driessche E., Houf K., Vangroenweghe F., De Zutter L., Van Hoof J., 2005. Prevalence, enumeration and strain variation of *Arcobacter* species in the faeces of healthy cattle in Belgium. Veterinary Microbiology 105, 149-154. [CrossRef]

Vandamme P., De Ley J., 1991. Proposal for a new family, Campylobacteraceae. International Journal of Systematic and Evolutionary Microbiology 41, 451-455. [CrossRef]

Vandamme P., Pugina P., Benzi G., Van Etterijck R., Vlaes L., Kersters K., Lauwers S., 1992b. Outbreak of recurrent abdominal cramps associated with *Arcobacter butzleri* in an Italian school. Journal of Clinical Microbiology 30, 2335-2337.

Vandamme P., Vancanneyt M., Pot B., Mels L., Hoste B., Dewettinck D., Vlaes L., Van Den Borre C., Higgins R., Hommez J., Kersters K., 1992a. Polyphasic taxonomic study of the emended genus *Arcobacter* with *Arcobacter butzleri* comb. nov. and *Arcobacter skirrowii* sp. nov., an aerotolerant bacterium isolated from veterinary specimens. International Journal of Systematic and Evolutionary Microbiology 42, 344-356. [CrossRef]

Vandenberg O., Houf K., Douat N., Vlaes, L., Retore P., Butzler J.P., Dediste A., 2006. Antimicrobial susceptibility of clinical isolates of non-jejuni/coli campylobacters and arcobacters from Belgium. Journal of Antimicrobial Chemotherapy 57, 908-913. [CrossRef]

Diseases and Mortality Incidences of Calves Born from Imported Brown Swiss and Simmental Heifers in Western Anatolian Conditions#

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Cite this article as: Küçük Baykan, Z., Özcan, M., 2019. Diseases and Mortality Incidences of Calves Born from Imported Brown Swiss and Simmental Heifers in Western Anatolian Conditions. Acta Vet Eurasia 45, 50-55.

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Abstract

The purpose of this study was to find the disease and mortality incidences of calves born from pregnant Brown Swiss and Simmental heifers imported from Austria in newly established dairy cattle farm in Manisa province and determine the effect of breed, sex, calving type and calving position on calf health. The study material consisted of the 180-days health records of 62 Brown Swiss and 266 Simmental calves born alive from the pregnant Brown Swiss and Simmental heifers imported from Innsbruck Austria. The first calving of heifers was between June and December. Calves' mortality rates in 180-days period were 3.23% for Brown Swiss and 4.89% for Simmentals; 5.39% for males and 3.73% for females; 3.14% for assisted born, 9.38% for difficult born and 3.81% for spontaneous born calves. Calves' most common health problems were digestion, respiratory and foot diseases. It was found that calving position had a significant effect on digestion problems and dystocia complications. Treatment was more successful in Brown Swiss, female, twin and spontaneous born calves.

Keywords: Brown swiss, calf, disease, mortality rate, simmental

Introduction

The sustainability of a cattle enterprise is related to health and high production capacity that is only possible with a healthy and regular birth of calves. The first condition of a healthy calf has a genetically high production capacity and is resilient to changing environmental conditions. Proper care and feeding methods should be implemented under the responsibility of enterprise owners and technical personnel. Taking necessary measures before the herd gets sick, conducting regular health screening when there is a disease and implementing eradication programs ensure the protection of herd health and continuity of income.

Calves might have many diseases in their early periods. Septicemia, pneumonia and chronic diarrhea in those periods are among the most important reasons for losing calves. Some diseases in the neonatal period might increase calf mortality rate and development disorders in the recovering calves, and even tough recovery happens calves is affected negatively in the years ahead and breeding value decreases (Donovan et al., 1998).

Calves are quite sensitive against various disease factors in their first months. Proper care, feeding and hygiene practices can prevent early period calf losses. Calf mortality rate below 5% until weaning has been considered normal for cattle enterprises (Oğan et al., 2011).

Factors effecting the calf mortality and morbidity rates in cattle enterprises include calf's breed, sex, birth season, calving position, location, herd size and different care and feeding con-

#This study was arranged from a part of the first author's PhD thesis.

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Received Date: 22 October 2018 • **Accepted Date:** 17 June 2019 • DOI: 10.26650/actavet.2019.18020
Available online at actaveteurasia.istanbulc.edu.tr

ditions practiced (Azizzadeh et al., 2012; Lombard, et al., 2007; Lundborg et al., 2005; Parker, 1980).

A study made in 73 enterprises producing beef cattle reported that calf mortality rate until weaning was 4.5%, and the death of a cow cost 215 dollars to the enterprise. Reported death causes included dystocia (17.5%), stillbirth (12.4%), hypothermia (12.2%), diarrhea (11.5%) and respiratory diseases (7.6%) (Wittum et al., 1993).

A field study covering 100 cattle enterprises in Switzerland reported that the calf mortality rate before weaning was 5%. Of those deaths, 52.3% was due to the respiratory system, 15.9% was due to the digestive system, 11.4% was due to the circulatory system, 11.4% was due to the nervous system, 4.6% was due to the musculoskeletal system, and 2.3% was due to the urogenital system diseases. In that study, 33% of calves were treated for diarrhea, 27% were treated for pneumonia, 16% were treated for omphalitis, 1% was treated for septicemia, and 22% were treated for other diseases. It was found that diseases were more common during November, December and January in the enterprises (Busato et al., 1997).

Calving season and the hygiene and ventilation conditions of calves' location effect the incidences of digestion and respiratory system diseases, which are most common in their early periods. Being born during extremely cold weather, in dirty locations or having insufficient or extreme airflow were reported as the main factors increasing the disease incidence (Donovan et al., 1998; Lombard et al., 2007; Lundborg et al., 2005; Meganck et al., 2015).

The purpose of this study was to identify the disease incidences of the calves born from pregnant Simmental and Brown Swiss heifers imported from Austria in their first 180-day period, their treatment success rates, and mortality rates due to those diseases according to breed, sex, calving type and calving position, and determine the adaptation capacity of the calves, which were born from the cows under transport effect, to the location.

Materials and Methods

The research protocol of the current study was approved by the Ethic Committee of the İstanbul University (Approval number: 2012/31).

Material description

The study was conducted in a private dairy cattle enterprise, which was located in Manisa and which has recently started livestock operations. Manisa is located in Western Anatolia Region that has Mediterranean/Continental climate. Precipitation generally occurs in winter, and summer is dry (Municipality of Manisa, 2017).

The first animal material of the enterprise comprised of 70 Brown Swiss and 282 Simmental (Fleckvieh) pregnant heifers imported from Innsbruck/Austria by the highway. Imported pregnant heifers had births between June and December in 2011 and had 328 (62 Brown Swiss, 266 Simmental) live calves.

The study material consisted of the first 180-days health records of 62 Brown Swiss and 266 Simmental calves that were born alive in the enterprise.

Material care and feeding

The imported pregnant heifers calved between the months of June and December. Umbilical cord disinfection and septicemia serum were applied to the calves. Calves were separated from their mothers right after birth and put into individual standard calf boxes where they were fed with colostrum via feeding bottles. Colostrum quality was measured by colostrometer, and only the colostrum with high specific weight (1035> mg/mL) was given.

Calves consumed 10 liters of colostrum in the first 24 hours, and they were fed with milk two times a day in the following days. Calves were fed 4 liters of milk per day at the first week, consumed by the milk is gradually reduced by 60 days of age. They were given calf feed and water when milk feeding started. Calves' appetites, body temperatures and stools were checked every day.

Healthy calves after 60 days of age were taken into wider individual calf cabins in the open, and fed with pelleted calf grower feed. Calf grower feed was composed of a premix, which included 2600 kcal/kg metabolic energy and enriched with A, D, E vitamins and various minerals. They were weaned around 60-days of age. Dry alfalfa was started one week before weaning. After weaning, calves were grouped according to sex, age and weight. They were taken into a semi-open young animal barn and raised in groups of 15. Young animal shelters were open, airy, dry base floor.

Calves passed to coarse fodder after 60 days, and they were given TMR (Total Mix Ration) in addition. TMR included: 8% hay, 12% clover, 30% corn silage, 26% vetch, 6% seasonal pulp and 18% young animal feed. After 60 days, calves were introduced to silage and also given young animal ration which had: net energy maintenance-NEm: 1.57 Mcal/kg, net energy gain-NEg: 0.96 Mcal/kg, crude protein-CP: 21%DM (dry matter), acid detergent fiber-ADF: 24%DM, neutral detergent fiber-NDF: 36%DM, starch 24%DM.

Disease incidences

Calves' health status was followed daily since their birth, and the diagnosis and treatment processes were recorded in the herd management program. Diseases were diagnosed by the veterinarian of the enterprise based on calves' body temperature, auscultation, palpation, urine and stool examination, observation and ultrasound data.

It was grouped the diagnosed disease cases as: (a) digestive diseases (*diarrhea, timpani and constipation*); (b) respiratory diseases *(pneumonia presenting with mucous or seromucous nasal flow and frequent abdominal respiration*); (c) foot diseases *(lameness in or not standing on one or more foot; swelling bleeding in joints; fractures or cracks in extremities);* (d) premature birth complications; (e) dystocia complications; and (f) anomalies. It was expressed the disease incidences, treatment success and disease-based deaths in tables.

It was evaluated the calf diseases according to breed (Simmental, Brown Swiss), sex (male, female), calving type (single, twin), and calving style (spontaneous, assisted, difficult). Calving position definitions were: (a) spontaneous calving: heifer gives birth by itself, without requiring any intervention, (b) assisted calving: calving occurs with the assistance of one or two people, (c) difficult calving: calving occurs with the help of three or more people, by using a jack or requires surgical intervention (C-section).

Statistical analysis

It was used "Pearson Chi–Square Test" and "Fisher's Exact Test" for intergroup comparisons. SPSS, 1999 (Statistical Package for the Social Sciences) program package for all statistical analysis were used.

Results

It was grouped the disease incidence, treatment success and disease-based deaths of calves in their first 6-months according to breed, sex, calving type and calving position. It was indicated the numerical and proportional values in Table 1, Table 2, and Table 3.

The disease incidences for Brown Swiss and Simmental calves were 25.81% and 25.56%, treatment success rates were 87.50% and 80.88% respectively. Both Brown Swiss and Simmental calves had digestive, respiratory and foot diseases intensively. While the treatment success rates for respiratory and foot problems were over 94.74%, the treatment success rates for digestive problems were 83.78% and 88.89% (Table 1). The highest mortality rate was in Brown Swiss calves due to digestive system diseases and difficult labor complications. Simmental calves died due to all system diseases except for foot diseases. The highest mortality rate in Simmental calves was due to digestive system diseases (Table 3).

It was found that disease incidence and treatment success rates were 28.74% and 81.25% in male calves and 22.36% and 83.33% in female calves. Twins were born only in Simmental calves in the first production year in the enterprise. It was determined that disease incidence and treatment success rates were 26.52% and 81.93% in single born calves and 6.67% and 100.00% in twin born calves (Table 2). When It was evaluated calves' system diseases according to sex and calving type, there was no statistically significant difference between male and

Table 1. Proportional values and significance controls of diseases in 0-180 days according to breed and sex (%)*

	Breed								Sex						Overall			
	Incidence				Treatment Success				Incidence				Treatment Success				Incidence		Treatment Success	
	Brown Swiss (N:62)		Simmental (N:266)		Brown Swiss		Simmental		Male (N:167)		Female (N:161)		Male		Female		(N:328)			
Diseases	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Digestive diseases	9	14.52	37	13.91	8	88.89	31	83.78	24	14.37	22	13.66	18	75.00	21	95.45	46	14.02	39	84.78
Respiratory diseases	5	8.06	19	7.14	5	100	18	94.74	16	9.58	8	4.97	16	100	7	87.50	24	7.32	23	95.83
Foot diseases	1	1.61	6	2.26	1	100	6	100	5	2.99	2	1.24	5	100	2	100	7	2.13	7	100
Premature birth complication	0	0	2	0.75	0	0	0	0	1	0.60	1	0.62	0	0	0	0	2	0.61	0	0
Dystocia complication	1	1.61	2	0.75	0	0	0	0	2	1.20	1	0.62	0	0	0	0	3	0.91	0	0
Anomaly birth	0	0	2	0.75	0	0	0	0	0	0	2	1.24	0	0	0	0	2	0.61	0	0
Overall	16	25.81	68	25.56	14	87.50	55	80.88	48	28.74	36	22.36	39	81.25	30	83.33	84	25.61	69	82.14

*Difference between breed and sex groups is not significant (p>0.05)

Table 2. Proportional values and significance controls of diseases in 0-180 days according to calving type and calving position (%)

Diseases	Calving Type								Calving Style									
	Incidence				Treatment Success				Incidence						Treatment Success			
	Single (N:313)		Twin (N:15)		Single		Twin		Assisted (N:159)		Difficult (N:64)		Spontaneous (N:105)		Assisted		Difficult		Spontaneous	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Digestive diseases	46	14.70	0	0	39	84.78	0	0	14	8.81[b]	14	21.88[a]	18	17.14[a]	11	78.57	12	85.71	16	88.89
Respiratory diseases	23	7.35	1	6.67	22	95.65	1	100	10	6.29	4	6.25	10	9.52	9	90.00	4	100	10	100
Foot diseases	7	2.24	0	0	7	100	0	0	3	1.89	2	3.13	2	1.90	3	100	2	100	2	100
Premature birth complication	2	0.64	0	0	0	0	0	0	0	0	0	0	2	1.90	0	0	0	0	0	0
Dystocia complication	3	0.96	0	0	0	0	0	0	0	0[b]	3	4.69[a]	0	0[a]	0	0	0	0	0	0
Anomaly birth	2	0.64	0	0	0	0	0	0	1	0.63	1	1.56	0	0	0	0	0	0	0	0
Overall	83	26.52	1	6.67	68	81.93	1	100	28	17.61[b]	24	37.5[a]	32	30.48[a]	23	82.14	18	75.00	28	87.50

a, b: Differences between the means marked with different letters in the same line are significant ($p<0.05$)

Table 3. Mortality rates and significance controls of diseases in 0-180 days (%)

	Breed				Sex				Calving Type				Calving Style							
	Brown Swiss (N:62)		Simmental (N:266)		Male (N:167)		Female (N:161)		Single (N:313)		Twin (N:15)		Assisted (N:159)		Difficult (N:64)		Spontaneous (N:105)		Overall (N:328)	
Causes of Death Reason	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Digestive system diseases	1	1.61	6	2.26	6	3.59	1	0.62	7	2.24	0	0	3	1.89	2	3.13	2	1.90	7	2.13
Respiratory system diseases	0	0	1	0.38	0	0	1	0.62	1	0.32	0	0	1	0.63	0	0	0	0	1	0.31
Dystocia complication	1	1.61	2	0.75	2	1.20	1	0.62	3	0.96	0	0	0	0[b]	3	4.69[a]	0	0[b]	3	0.92
Premature birth complication	0	0	2	0.75	1	0.60	1	0.62	2	0.64	0	0	0	0	0	0	2	1.90	2	0.61
Anomaly	0	0	2	0.75	0	0	2	1.24	2	0.64	0	0	1	0.63	1	1.56	0	0	2	0.61
Foot diseases	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

a, b: Differences between the means marked with different letters in the same line are significant ($p<0.05$)

female, or single and twin calves (p>0.05). It was found that the mortality rates of male and female calves were similar. Calf deaths occurred only in single born calves. Twin calves' number was 15 because one of the twins was stillborn.

According to calving style, It was found that calves with difficult and spontaneous labor had a higher disease rate compared to ones with assisted labor, while the difference between groups was not statistically significant (p>0.05). Calves with assisted labor had lower rates in all diseases except for anomaly complications. There was no statistically significant difference between calving position groups in terms of disease treatment success (p>0.05).

Differences between groups' mortality rates based on breed, sex and calving type were not statistically significant both in general and in specific system diseases (p>0.05). Although the mortality rate of calves with difficult labor was more than the ones with assisted and spontaneous labor, this difference was not statistically significant. The calf mortality rate due to difficult labor complication was 4.69%, and there was no calf death due to other calving positions (p<0.05).

Discussion

Calves' overall mortality rate for the first 180-days was 4.57%; it was 4.89% and 3.23% for Simmental and Brown Swiss respectively. Considering that the generally-targeted calf mortality rate for dairy cattle enterprises is below 5% (Oğan et al., 2011), the values found for the first lactation calves were within normal acceptable limits for both breeds.

Mortality rates until weaning for Simmental and Brown Swiss calves were lower than the rates reported by Klein-Jöbstl et al. (2015) for calves in Austria, by Silva Del Rio et al. (2007) for Holstein, Fuerst-Waltl et al. (2012) for Brown Swiss, and by Stanton et al. (2011) for Holstein; and similar to the rates reported by Gürcan et al. (2014) for Simmental hybrid, by Torsein et al. (2011) for various cows and by Fuerst-Walt et al. (2010) for Simmental. Calf mortality rates were at reasonable levels because the studied enterprise was new, and the technical personnel practiced good care, feeding and veterinarian services for the cows and calves during and after labor.

The effect of breed, sex, calving type and calving position were generally insignificant for the first term calf deaths. This can be explained with the fact that calves were born in the same months and subjected to an attentive care and feeding program. In this period, all of the calf deaths were among single born calves. Twin born calves did not have any deaths in the first period which were probably due to the fact that their numbers were few (4.5% of all calves were twin born).

Deaths were more in calves with difficulty labor than those with spontaneous and assisted labor. This might be because labor complications adversely affected calves' survival and resilience and increased in mortality rates particularly in first weeks. It was found that Brown Swiss and Simmental calves mainly had digestive (14.02%), respiratory (7.32%) and foot disease (2.13%) problems in similar rates in their first 180-days. Calves' the digestive and respiratory system diseases were higher than other disease rates, which was consistent with literature (Busato et al., 1997; Gulliksen et al., 2009; Klein-Jöbstl et al., 2015; Svensson et al., 2003; Ünal et al., 2001). Calves' respiratory and digestive problems were at reasonable levels (14.02% and 7.32%), and this was because births occurred collectively in summer and autumn, and shelters had good hygiene and ventilation conditions.

While breed, sex and calving type did not affect disease incidence, calving position (assisted, difficult, spontaneous) had a statistically significant effect on digestive and difficult labor complications. Disease incidence was higher in calves with spontaneous and difficult labor. After the medical intervention for the calves' diseases, treatment success was above 80% for all sub-groups. In general, whole foot disease cases succeeded treatment. The treatment success rate was 95.83% for the respiratory system diseases and 84.78% for the digestive system diseases. None of the labor complication or anomaly cases had treatment success. Labor difficulty and twin births have been reported as significant risk factors for both cow and calf health in the cases of heifers giving birth for the first time (Busato et al., 1997; Lombard et al., 2007; Meganc et al., 2015). The findings of our study were consistent with the reports that the disease incidence of such calves were higher different breeds.

In our study, It was found very lower values than the ones (diarrhea 29%, naval infections 29%, pneumonia 25%) reported for the most common diseases for dairy cattle enterprises by Oğan et al. (2011) and calculated in literature. Simmental and Brown Swiss calves had no problem in adapting to the location, which indicated that reasonable levels are attainable in calf disease control with the help of rational calf care and veterinarian practices in the enterprise.

The mortality rates of the calves born from pregnant Brown Swiss and Simmental heifers were at normal and acceptable levels in the first 180-days. Calves mostly had digestive, respiratory and foot diseases. It was found that disease incidence was not affected by breed, sex and calving type, while calving position had a significant impact on digestive problems and difficult labor complications. Calves with spontaneous and difficult labor had a higher disease incidence. The treatment success rate of the medical interventions for calves' diseases was high. Treatment success rate was higher in Brown Swiss, female, twin and spontaneous born calves.

Both breeds had no serious problems in adapting to local conditions; the calves of both breeds responded positively to treatments in emerging diseases with the help of rational herd management and health practices except for the cases of difficult labor complications and anomalies; and much attentive care

was needed for the cows and calves involved in such labors in order to minimize difficult labor complications. In order to reduce the calf losses in the dairy cattle farms in the western Anatolian region, it is recommended primarily taking preventive health precautions for the importance of highlighted diseases in this study. This type of studies with more animals, breeds and regional scales will support these research findings.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of the İstanbul University (Approval number: 2012/31).

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – Z.K.B., M.Ö.; Design – Z.K.B., M.Ö.; Supervision – M.Ö.; Resources – Z.K.B.; Materials – Z.K.B.; Data Collection and/or Processing – Z.K.B., M.Ö.; Analysis and/or Interpretation – Z.K.B., M.Ö.; Literature Search – Z.K.B.; Writing Manuscript – Z.K.B.; Critical Review – M.Ö.

Conflict of Interest: The authors have no conflicts of interest to declare.

Financial Disclosure: The present study was supported by Research Fund of İstanbul University (Project No: 23141).

References

Azizzadeh, M., Shoorokia, H.F., Kamalabadia, A.S., Stevenson, M.A., 2012. Factors affecting calf mortality in Iranian Holstein dairy herds. Preventive Veterinary Medicine 104, 335-340. [CrossRef]

Busato, A., Steiner, L., Martin, S.W., Shoukri, M.M., Gaillard, C., 1997. Calf health in cow- calf herds in Switzerland. Preventive Veterinary Medicine 30, 9-22. [CrossRef]

Donovan, G.A., Dohoo, I.R., Montgomery, D.M., Bennett, F.L., 1998. Calf and disease factors affecting growth in female Holstein calves in Florida, USA. Preventive Veterinary Medicine 33, 1-10. [CrossRef]

Fuerst–Waltl, B., Fuerst, C., 2010. Mortality in Austrian dual purpose Fleckvieh calves and heifers. Livestock Science 132, 80-86. [CrossRef]

Fuerst-Waltl, B., Fuerst, C., 2012. Effect of inbreeding depression on survival of Austrian Brown Swiss calves and heifers. Journal of Dairy Science 95, 6086-6092. [CrossRef]

Gulliksen, S.M., Lie, K.I., Osteras, O., 2009. Calf health monitoring in Norwegian dairy herds. Journal of Dairy Science 92, 1660-1669. [CrossRef]

Gürcan, İ.S., Özen, D., Yamaç, A., Güllü, Ö., 2014. Investigation of effect of year and season factors on calving difficulty, using Poisson regression model in Simmental×South Anatolian Red crossbred cattle. Ankara Üniversitesi Veteriner Fakültesi Dergisi 61, 55-58. [CrossRef]

Klein-Jöbstl, D., Arnholdt, T., Sturmlechner, F., Iwersen, M. Drillich, M., 2015. Results of an online questionnaire to survey calf management practices on dairy cattle breeding farms in Austria and to estimate differences in disease incidences depending on farm structure and management practices. Acta Veterinaria Scandinavica 19, 57-44. [CrossRef]

Lombard, J.E., Garry, F.B., Tomlinson, S.M., Garber, L.P., 2007. Impacts of dystocia on health and survival of dairy calves. Journal of Dairy Science 90, 1751-1760. [CrossRef]

Lundborg, G.K., Svensson, E.C., Oltenacu, P.A., 2005. Herd-level risk factors for infectious diseases in Swedish dairy calves aged 0-90 days. Preventive Veterinary Medicine, 68, 123-143. [CrossRef]

Meganck, V., Hoflack, G., Piepers, S., Opsomer, G., 2015. Evaluation of a protocol to reduce the incidence of neonatal calf diarrhea on dairy herds. Preventive Veterinary Medicine, 118, 64-70. [CrossRef]

Municipality of Manisa. Available from: http://www.manisa.bel.tr/s23_manisa-cografyasi.aspx) (Date of access: 20.05.2015).

Oğan, M., Türkmen, İ.İ., Seyrek İntaş, K., Şentürk, S., Orman, A., 2011. Temel Sürü Sağlığı Yönetimi Kitabı. T.C. Anadolu Üniversitesi Yayını, No:2333, Syf: 6-146.

Parker, W.H., 1980. Health and Disease in Farm Animals. 3rd ed., Pergamon Press, ISBN: 0-08-025900-6.

Silva Del Rio, N., Stewart, S., Rapnicki, P., Chang, Y.M., Fricke, P.M., 2007. An observational analysis of twin births, calf mortality in Holstein dairy cattle. Journal of Dairy Science 90, 1255-1264. [CrossRef]

Stanton, A.L., Kelton, D.F., Leblanc, S.J., Wormuth Leslie, K.E., 2011. The effect of respiratory disease and a preventative antibiotic treatment on growth, survival, age at first calving, and milk production of dairy heifers. Journal of Dairy Science 95, 4950-4960. [CrossRef]

Svensson, C., Lundborg, K., Emanuelson, U., Olsson, S., 2003. Morbidity in Swedish dairy calves from birth to 90 days of age and individual calf-level risk factors for infectious diseases. Preventive Veterinary Medicine 58, 179-197. [CrossRef]

Torsein, M., Lindberg, A., Sandgren, C.H., Waller, K.P., Törnquist, M., Svensson, C., 2011. Risk factors for calf mortality in large Swedish dairy herds. Preventive Veterinary Medicine 99, 136-147. [CrossRef]

Ünal, N., Ertuğrul, O., Alpan, O., 2001. Growth and survival of Simmental calves raised outdoors in individual hutches. Turkish Journal of Veterinary and Animal Sciences 25, 789-795.

Wittum T.E., Salman M.D., Odde K.G., Mortimer R.G., King M.E., 1993. Causes and costs of calf mortality in Colorado beef herds participating in the National Animal Health Monitoring System. Journal of the American Veterinary Medical Association 203, 232-236.

Effects of Lemon Juice on Performance, Egg Quality Trait, and Some Blood Parameters of Laying Hens in the Late Phase of Production

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Cite this article as: Gültepe, E.E., Iqbal, A., Çetingül, İ.S., Uyarlar, C., Özçınar, Ü., Bayram, İ., 2019. Effects of Lemon Juice on Performance, Egg Quality Trait, and Some Blood Parameters of Laying Hens in the Late Phase of Production. Acta Vet Eurasia 45, 56-62.

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Abstract

A total of 120 laying hens (57 weeks old) were randomly assigned to 5 groups and lemon juice (LJ) was daily added to drinking water (0%, 0.5%, 1%, 2.5%,5%) during 4 weeks. Egg production increased ($p<0.05$) in 1% and 2.5% LJ groups. The LJ had no effect on feed and water intake, live weight, feed conversion ratio, egg mass and weight. Haugh unit increased in 1% LJ group and egg yolk color was lighter in 5% LJ group ($p<0.05$). The LJ had no effect on eggshell thickness, albumin and yolk index. Serum HDL levels increased and Total Antioxidant Status decreased in 0.5% LJ group ($p<0.05$). The LJ juice had no effect on serum AST, ALT, LDL, Cholesterol, Glucose, Total Protein, IgG levels and Oxidant Status. Blood lymphocyte decreased ($p<0.05$) in 2.5% LJ over control, however, red blood cell numbers ($p<0.01$) increased in 1% LJ group. The LJ had no effect on other hematology parameters. In conclusion, the water supplementation of LJ showed positive effects on production without adverse effects on egg quality traits and health of late-phase laying hens. However, the positive responses may be more relevant to acidity of water.

Keywords: Aged laying hens, *Citrus lemon,* water supplement

Introduction

The production of good quality eggs with maintaining health of laying hens is one of the most critical goals of the industry. Low egg quality, shorter egg production period and metabolic diseases relevant to age are big challenges in late-phase aged laying hens. Although earlier stage of growth and peak period have great importance for sustainable production, late-phase should also be well-managed before molting or culling period of layers. Gradual decrease of egg production and low egg quality with an increase of feed intake in late-phase of the production cycle of aged laying hens has given rise to high expenses and low profitability in the industry until culling the birds (Roberts, 2004). Although egg size increases with age, Elaroussi et al. (1994) reported that ratio of shell weight to egg weight gradually decreases. Due to the decreasing eggshell quality with age, egg loss may increase in late phase of laying cycle (Roberts, 2004). In last decade, some researchers have focused to improve production and quality of eggs from aged laying hens via dietary manipulations and supplements (Catli et al., 2012; Mabe et al., 2003; Min et al., 2018; Molnar et al., 2018; Zhang et al., 2017).

The different supply route of same supplement or nutrient may cause different responses in poultry. For instance, Noy and Sklan (1999) concluded that providing nutrients via drinking water to earlier stage of life improved weight gain in poultry rather than supply of same nutrients via diet. Some evidence suggests that supplementation of probiotics via drinking water may have more beneficial rather than in-feed route on broilers (Karimi Torshizi et al., 2010; Ritzi et al., 2014). Although Virden et al. (2009) concluded that feeding supplemental water-sucrose to stressed broilers resulted no beneficial response under stress condition, but it had a

Presented in: The results of the study was partially presented in the 5th International Conference on Sustainable Agriculture and Environment 2018 as an oral presentation, 08 October 2018, Hammamet, Tunisia

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Received Date: 28 March 2019 • **Accepted Date:** 22 May 2019 • DOI: 10.26650/actavet.2019.19005

Available online at actaveteurasia.istanbulc.edu.tr

good response on feed conversion ratio (FCR) during post-stress recovery period. Recently, Karadağoğlu et al. (2018) concluded that supplementing drinking water with different concentrations of an essential oil blend (peppermint, oregano, anise) improved egg production and weight, Haugh Unit, eggshell thickness, and yolk height of laying hens from week 20 to week 36 of age. Even though some evidence has been reported on broilers and laying hens, research data on effects of nutrient supplementation via drinking water on late-phase laying hens is still limited.

Lemon (*Citrus limon* L.) is an evergreen native plant of Asia and belongs to family Rutaceae. The juice of lemons are commonly used for cleaning and culinary purposes throughout the world. The lemon juice (LJ) is sour in taste and contains 5% to 6% citric acid (Yapo, 2009). Lemon fruits are rich in Vitamin C and contain different phytochemicals such as tannins, polyphenols, terpenes and flavonoids. The concentration of citric acid in LJ is twice than grapefruit juice and about five times higher than orange juice (Penniston et al., 2008). Although citrus fruits have different flavonoids such as narirutin, hesperidin, naringin and neohesperidin, only hesperidin transmission could be detected from fruit to juice (Xu et al., 2008). Hesperidin had significant benefits on poultry, such as anti-inflammatory, anti-stress, antioxidant, growth promoting, anticancer and immunological properties (Yatao et al., 2018). LJ in drinking water increased the immunity of broiler chickens (Behboudi et al., 2016; Kadam et al., 2009) and eggshell quality of layers (Tavakkoli et al., 2014) under heat stress. Furthermore, Farghly et al. (2018) concluded that supplying with water containing 10% LJ during the growing period of turkey chicks improved growth performance, immune response, antioxidant status and economic efficiency.

Keeping in view the above points, LJ may be used as potent water supplement to improve health and extend performance of late-phase layer hens. Therefore, the present study was aimed to explore the effects of different level of LJ on performance, egg quality and serological parameters in late-phase laying hens.

Materials and Methods

Experimental design and management

A total of 120 Babcock white laying hens (57 weeks old) were divided into 5 groups (n=24) with 4 replication groups containing 6 hens in each subgroup. LJ was added to the drinking water of the experimental groups with 0%, 0.5%, 1%, 2.5%, and 5% respectively during 4 weeks. The LJ levels were determined according to reported positive effects of LJ in literature (Kadam et al., 2009). Sixteen hours light and eight hours dark were applied and also feed and water were supplied *ad libitum*. In this study, all treatment groups were fed a basal diet, which was prepared according to requirement of the birds (Table 1) (NRC, 1994). In 0.5% LJ group, one bird has been moved out of the study due to sudden death. No mortality was recorded during the study except for the mentioned animal. LJ was poured on daily bases in fresh drinking water. Lemon fruits were provided from a commercial local trader.

Automatic nipple drinking system was used and each group have separated water tank where different concentration of LJ was added in their water tank. Graduated cylinder glass was used for scaling of LJ. Then, the LJ was mixed with water at the mentioned ratio in 20 liter water box between 1 p.m. to 2 p.m.

Table 1. Ingredient composition and chemical composition of basal diet

Ingredients	**%, as fed basis**	
Corn	54.90	
Vegetable oil	0.34	
Sunflower meal (32% CP)	16.92	
Full fat soya	10.00	
Soybean meal (44% CP)	7.39	
Limestone	7.87	
Dicalcium phosphate	1.73	
Common salt	0.40	
Vitamin-mineral premix[1]	0.25	
L-Lysine HCl	0.10	
DL-Methionine	0.10	
Calculated values[2]		
CP, %	17.00	
ME, kcal/kg	2750	
Ca	3.71	
Av.P	0.38	
Na, %	0.20	
Met+Sis	0.71	
Lysine, %	0.83	
Treonin, %	0.61	
Triptophane, %	0.20	
Linoleic acid, %	2.36	
pH levels of drinking water	$\bar{X}$	SEM
0% Lemon	7.07^{a}	0.23
0.5% Lemon	3.22^{b}	0.07
1% Lemon	3.13^{bc}	0.10
2.5% Lemon	3.02^{cd}	0.01
5% Lemon	2.89^{d}	0.03
p	0.0001	

[1]Provided per kg of diet: Vitamin A:12.000.000 IU, Vitamin D3:3.000.000IU, Vitamin E:35.000 IU, Vitamin K3:3.500 IU,Vitamin B1:2.750IU, Vitamin B2:5.500IU, Nicotinamid: 30.000IU,Ca-D-Panthotenate:10.000IU,Vitamin B6: 4.000IU, Vitamin B12: 15IU, Folic acid:1.000IU, D-Biotin: 50IU,Cholin clorid:150.000IU, Manganese: 80.000mg, Iron: 60.000 mg, Zinc:60.000 mg, Copper:5.000 mg, Iodine:2.000 mg, Cobalt: 500 mg, Selenium: 150 mg, Antioxidant:15.000 mg.
[2]NRC (1994)
Values with different superscripts differ significantly ($p<0.05$)
HCl: Hydrochloride; DL: Dextrorotation & Levorotation; CP: Crude protein; ME: Metabolisable energy; Ca: Calcium; Av.P: Available phosphorus; Na: Sodium; Met: Methionine; Sis: Sistein; The different superscripts (a, b, c, d) represent significant differences between the values ($p<0.05$).

every day. The LJ could be solved easily in the water and homogenity was confirmed visually. During the study, water consumption (L) was measured by total water consumption per each groups after 24 hours interval.

Data collection and analyses

Hens were weighted at the beginning and at the end of the study to determine their live weights. Egg production was recorded daily and was expressed percent of hen-day egg production (HDEP). Feed intake and egg weight of hens was recorded weekly. Mortality was recorded daily while eggs were weighed once a week. Egg mass was calculated as follows: Egg Mass = Percent of HDEP x average egg weight in grams. FCR values were calculated as follows: FCR = feed intake (g) / egg mass (g).

Eggs were delivered to the laboratory at the end of the 4th week as three egg samples from each subgroup to determine egg quality parameters. Eggs were kept for 24 hours at room temperature before the egg trait analyses. Egg weight, breaking strength, and eggshell thickness were determined in these eggs. Egg breaking strength was measured by using ORKA Egg Force Reader (EF 0468-2011; Orka Food Tech. Ltd., Hong Kong, China) and Haugh Unit were calculated by measuring albumen height (Digital micrometer). Egg yolk color was determined by using Yolk Color Fan (DSM; Basel, Switzerland) and comparing the color of yolks with 15 bands of the color fan.

At the end of the trial, 3 hens were randomly selected from each replication group and blood was collected from the heart, then, the samples were transferred into two separate tubes (vacutainer tubes without anticoagulant and with ethylenediaminetetraacetic acid-EDTA, Becton Dickinson; Franklin Lakes, NJ, USA). Blood samples were immediately arrived in the laboratory under a cold chain. For serum biochemical analyses, the samples in vacutainer tubes were centrifuged at 5000 rpm for 10 minutes. Supernatants were transferred to Eppendorf tubes and stored at -20°C till biochemical analyses. Serum glucose, total cholesterol (CHO), high density lipoprotein (HDL), low density lipoprotein (LDL), aspartate aminotransferase (AST), alanine aminotransferase (ALT), Gamma-Glutamyl Transpeptidase (GGT), total protein (TPRO), phosphorus, calcium and Immunoglobulin G (IgG) concentrations were determined by automated ELISA analyzer (Elisys Uno; Human mbH, Wiesbaden, Germany). The effect of LJ on pH levels of drinking water was determined with a portable bench-top digital pH meter at 0 h and 23rd h.

Statistical analysis

The model assumptions of normality and homogeneity of variance were examined by Shapiro-Wilk and Levene tests, respectively. The statistical analysis was performed with MedCalc (MedCalc Software bvba, Oostend, Belgium, v.18). General Linear Model was used for group comparison followed by Tukey-Kramer for post-hoc (Neter et al., 1996). The statistical model used to test the effects of treatment on variables was:

$$Yij = \mu + \alpha i + eij$$

where Yij = the response variable, μ = the general mean, αi = the effect of dietary treatments and eij = the random error. The significance level was considered as $p<0.05$ and all data were expressed as $\bar{X} \pm SEM$.

Results

In this study, pH levels of drinking water in treatment groups were significantly decreased according to control group ($p<0.05$). Low pH was observed in 2.5% and 5% LJ supplemented groups. However, the control group had a neutral pH (7.07±0.23, Table 1).

The result of recent study indicates that HDEP significantly ($p<0.05$) increased in 1% and 2.5% LJ supplemented groups; however, re-

Table 2. Effect of lemon juice given in drinking water on performance parameters of laying hens from week 57 to week 61 of age (Mean ± SEM; n=24)

	0% Lemon		0.5% Lemon		1% Lemon		2.5% Lemon		5% Lemon		
Item	$\bar{X}$	**SEM**	$\bar{X}$	**SEM**	$\bar{X}$	**SEM**	$\bar{X}$	**SEM**	$\bar{X}$	**SEM**	**p**
Feed intake, g/b/d	115.0	1.58	113.8	2.72	117.5	2.5	117.5	3.48	115.0	2.89	0.819
Water intake, L/b/d	0.40	0.008	0.39	0.007	0.41	0.007	0.38	0.008	0.40	0.008	0.072
HDEP[1], %	83.17^{b}	1.61	86.07^{ab}	1.77	88.54^{a}	1.51	89.88^{a}	1.25	86.45^{ab}	1.67	0.032
Egg weight, g	64.81	0.56	65.36	0.77	66.02	0.68	66.82	0.50	66.13	0.67	0.240
Egg mass, g	54.31	1.91	56.26	1.52	58.36	1.31	60.08	1.29	57.19	1.45	0.096
FCR[2]	2.15	0.08	2.03	0.05	2.02	0.05	1.97	0.08	2.04	0.07	0.504
Initial BW, g	1587.5	19.3	1602.0	33.3	1624.3	35.1	1630.8	33.4	1623.1	35.2	0.861
Final BW, g	1535.5	26.6	1448.2	97.1	1626.1	31.0	1616.5	28.9	1571.2	35.6	0.104

[1]Hen day egg production

[2]Feed conversion ratio, feed intake/egg mass

Values with different superscripts (a, b) in same row differ significantly ($p<0.05$)

HDEP: hen day egg production; FCR: feed conversion ratio; BW: body weight; SEM: standart error of mean

sults from feed intake, water intake, egg mass, FCR and egg weight were not significantly different (p>0.05) among all LJ supplemented groups as compared with control group. Similarly, hens live weight of all LJ supplemented groups were not significantly different (p>0.05) during the whole period of the trial (Table 2).

Regarding egg quality parameters, Haugh unit significantly (p<0.05) increased in 1% LJ supplemented group. In contrast egg yolk color significantly (p<0.05) decreased in 5% LJ supplemented group. Moreover, differences in eggshell thickness, albumin index, and yolk index were not significant (p>0.05) in all treatment groups as compared with control group (Table 3).

For serological parameters, HDL level significantly increased (p<0.05) however, TAS level significantly decreased (p<0.05) in 0.5% group as compared with other treatment and control group. Moreover, glucose, AST, LDL, CHO, ALT, TPRO, TOS, and IgG level remained unaffected (p>0.05) in all LJ supplemented group over control (Table 4).

Regarding blood parameters, lymphocyte count decreased significantly (p<0.05) in 2.5% LJ treatment group over control and red blood cell (RBC) significantly (p<0.01) increased in 1% supplemented group compared to 5% LJ group. Moreover, total leukocyte, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin concentration, platelet, lymphocyte, neutrophil and monocyte counts were unaffected by LJ supplemention (p>0.05) (Table 5).

Discussion

In our knowledge, this is the first report on effects of drinking water mixed with LJ on late-phase aged layer hens. Phenolic compounds of citrus species are flavonoids and phenolic acids. Dominant flavonoids of citrus fruits are flavanone glycosides such as narirutin, hesperidin, naringin, and neohesperidin. Although Xu et al. (2008) reported no presence of naruritin, naringin, and neohesperidin in LJ, they just determined trans-

Table 3. Effect of lemon juice given in drinking water on egg trait parameters of laying hens from week 57 to week 61 of age (Mean ± SEM; n=24)

	0% Lemon		0.5% Lemon		1% Lemon		2.5% Lemon		5% Lemon		
Item	$\bar{X}$	**SEM**	$\bar{X}$	**SEM**	$\bar{X}$	**SEM**	$\bar{X}$	**SEM**	$\bar{X}$	**SEM**	**p**
Eggshell thickness, mm	40.01	0.01	38.89	0.02	40.04	0.06	37.43	0.03	38.88	0.01	0.513
Haugh unit	92.49^{b}	1.37	94.77^{ab}	1.06	97.30^{a}	1.40	92.64^{b}	1.27	95.46^{ab}	1.00	0.036
Yolk color score	11.3^{a}	0.15	10.9^{ab}	0.10	11.3^{a}	0.12	11.2^{a}	0.13	10.7^{b}	0.15	0.018
Albumen index, %	10.99	0.41	11.10	0.30	12.06	0.49	10.77	0.38	11.69	0.31	0.142
Yolk index, %	54.31	1.91	56.26	1.52	58.36	1.31	60.08	1.29	57.19	1.45	0.195

Values with different superscripts (a, b) in same row differ significantly (p<0.05)
SEM: standart error of mean

Table 4. Effect of lemon juice given in drinking water on serum biochemical parameters of laying hens from week 57 to week 61 of age (Mean ± SEM; n=24)

	0% Lemon		0.5% Lemon		1% Lemon		2.5% Lemon		5% Lemon		
Item	$\bar{X}$	**SEM**	$\bar{X}$	**SEM**	$\bar{X}$	**SEM**	$\bar{X}$	**SEM**	$\bar{X}$	**SEM**	**p**
Glucose, mg/dL	163.31	9.23	175.08	6.17	169.93	8.04	172.25	8.28	179.47	8.64	0.753
CHO[1], mg/dL	88.94	10.36	128.91	14.56	98.27	10.77	109.18	9.35	92.60	7.80	0.116
HDL[2], mg/dL	15.38^{b}	0.51	17.36^{a}	0.51	16.83^{ab}	0.53	15.31^{b}	0.38	16.93^{ab}	0.41	0.004
LDL[3], mg/dL	27.69	4.52	39.50	6.37	30.20	3.68	34.44	3.38	30.73	3.10	0.782
AST[4], U/L	155.53	11.05	161.50	24.79	183.47	8.77	191.31	13.84	193.21	15.43	0.113
ALT[5], U/L	2.39	0.30	3.31	0.66	3.04	0.45	3.64	0.47	3.43	0.43	0.410
TPRO[6], g/dL	5.28	0.30	6.78	0.63	6.49	0.39	6.39	0.38	5.82	0.47	0.157
TOS[7], µmol/dL	58.60	10.89	101.90	10.39	94.56	18.99	76.24	12.83	66.06	12.03	0.146
TAS[8], mmol/L	1.20^{a}	0.05	0.87^{b}	0.02	1.28^{a}	0.11	1.00^{ab}	0.10	1.28^{a}	0.05	0.002
IgG, mg/dL	166.80	6.23	145.54	12.38	143.14	6.42	150.76	6.37	149.60	9.23	0.296

[1] CHO: total cholesterol; [2] HDL: high-density lipoprotein; [3] LDL: low-density lipoprotein; [4] AST: aspartate aminotransferase; [5] ALT: alanine aminotransferase; [6]TPRO: total protein; [7] TOS: total oxidant status; [8] TAS: total antioxidant status
Values with different superscripts (a, b) in same row differ significantly (p<0.05)
SEM: standart error of mean

Table 5. Effect of lemon juice given in drinking water on hematological parameters of laying hens from week 57 to week 61 of age (Mean ± SEM; n=24)

	0% Lemon		0.5% Lemon		1% Lemon		2.5% Lemon		5% Lemon		
Item	$\bar{X}$	**SEM**	$\bar{X}$	**SEM**	$\bar{X}$	**SEM**	$\bar{X}$	**SEM**	$\bar{X}$	**SEM**	**p**
TLC[1], 109/L	2.73	0.19	2.41	0.20	2.47	0.25	2.55	0.26	2.18	0.18	0.510
LC[2], 109/L	1.74[a]	0.03	1.75[a]	0.04	1.77[a]	0.03	1.62[b]	0.04	1.77[a]	0.04	0.031
NC[3], 109/L	0.70	0.04	0.76	0.05	0.77	0.03	0.77	0.04	0.72	0.04	0.690
MC[4], 109/L	0.043	0.001	0.045	0.001	0.044	0.002	0.044	0.001	0.044	0.003	0.100
RBC[5], 1012/L	2.70[ab]	0.03	2.66[ab]	0.04	2.74[a]	0.03	2.61[ab]	0.04	2.58[b]	0.04	0.009
Hemoglobin. g/L	10.75	0.23	10.42	0.22	10.44	0.17	10.20	0.18	10.81	0.20	0.212
Hematocrit. %	34.54	0.27	34.78	0.26	34.71	0.31	34.37	0.33	35.45	0.37	0.162
MCV[6]. fL	107.87	0.58	109.39	0.41	109.26	0.48	107.96	0.49	107.95	0.48	0.053
MCH[7]. pg	30.99	0.44	31.27	0.23	30.94	0.50	31.34	0.46	31.04	0.46	0.934
MCHC[8]. g/L	30.84	0.45	30.81	0.49	30.65	0.46	30.43	0.49	31.66	0.35	0.353
Platelet. 10^9/L	27.09	0.43	27.03	0.39	27.65	0.37	26.84	0.34	27.81	0.40	0.337
MPV[9]. pg	6.48	0.08	6.47	0.06	6.52	0.08	6.56	0.07	6.52	0.07	0.908

[1] TLC: Total leukocyte count; [2] LC: Lymphocyte count; [3] NC: Neutrophil count; [4] MC: Monocyte count; [5] RBC: Red Blood Cell count; [6] MCV: Mean Corpuscular Volume; [7] MCH: Mean Corpuscular Haemoglobin; [8] MCHC: Mean Corpuscular hemoglobin concentration; [9] MPV: Mean platelet
Values with different superscripts (a, b) in same row differ significantly ($p<0.05$)
SEM: Standart error of mean

mission of hesperidin from lemon fruit to juice (237.96±0.12 mg/L). Hesperidin supplementation improved immun response, antioxidant capacity, HDL percentage of total blood cholesterol pool and growth parameter in human and animal models (Yatao et al., 2018). Castillo et al. (2000) reported that freshly squeezed LJ had bactericidal activity *in vitro* against *Vibrio cholerae (V. cholerae)*. Also, supplementation of LJ has shown significantly better giardiacidal activity *in vitro* than unsupplemented group (Sadjjadi et al., 2006). The mentioned effects may relevant with hesperidin. Despite *in vitro* potential effects on pathogens, LJ had no constant effect on the immune response of bird's, except for a significant decrease of blood lymphocyte in this study. *In vivo* conditions such as a gut environment with a huge diversity of microorganism and nutrients in diet may have inhibited expected effects of LJ. On the other hands, the supplementation through drinking water may have led to inadequate intake of active ingredients with well-known positive effects on the immune response due to dilution effect (Del Toro-Arreola et al., 2005). Furthermore, the lack of expected antioxidant effect may also be due to the same reason.

Lemon juice had no significant effect on immune response in our study inconsistent with other studies which were determined an improved immune response with LJ supplementation via drinking water (Behboudi et al., 2016; Farghly et al., 2018; Kadam et al., 2009; Tavakkoli et al., 2014). Since Kadam et al. (2009), Behboudi et al. (2016) and Tavakkoli et al. (2014) studied under experimental heat stress conditions, their results may be differ from our results. Also, Farghly et al. (2018) used turkey chicks in earlier stage of their life as an experimental model. In our study, we had late-phase laying hens (57 week aged) as an experimental model and it is a first study on effects of LJ on late-phase laying hens in our knowledge. Due to different experimental animal model, the results may differ from the previous studies.

The pH of pure LJ was classified as strong acid (pH 2.39±0.05) characteristic. Citric acid is the predominant acid type (6% of the total juice weight) and providing approximately 95% of the overall acidity (Yapo, 2009). Due to the strong acidic capacity of LJ, the pH levels of drinking water significantly decreased as expected in our study. Recently, Shihab et al. (2019) have focused effects of using ionized water on performance of Japanese quails from week 6 to week 18 of age. They concluded that acidic drinking water (pH 5) has led to greater HDEP than water with neutral pH. Although there is no significant effect of treatment on immune response with insufficient active ingredients as a potential dilution effect, higher HDEP in treatment groups may be the result of acidic water caused by strong acidity effect of LJ in our study. Recently, Palamidi and Mountzouris (2018) concluded that dietary supplementation of an organic acids-based blend was increased expression of genes associated with gut barrier and health of broilers. Samanta et al. (2010) concluded that organic acids have led to a decreasing in pH of gizzard and selective promotion of beneficial bacteria species in the gut. Since dietary hesperidin has no effect on HDEP in laying hens (Goliomytis et al., 2014), improvement with LJ treatments in our study may be arise from acidity rather than direct effect of active ingredients of juice. Moreover, Ezzat et al. (2017) concluded that acidic water (pH 5) did not change egg traits (yolk diameter, yolk height, yolk index, albumen height)

of Japanese quails compared to control group and these results are inconsistent with our results. However, the researchers reported no effect of acidic water on Haugh Unit while we determined significant effects of 1% LJ supplementation on Haugh Unit compared to the control and other supplemented groups. Disagreement between studies probably due to a result of different water pH levels in both studies.

Goliomytis et al. (2014) concluded that dietary hesperidin supplementation improved egg yolk oxidative stability, however, the supplementation has shown no effect on egg quality parameters which is consisted with our results on egg quality parameters. In a recent study, the same researchers focused effects of dietary orange pulp on egg yolk traits (Goliomytis et al., 2018) and hesperidin was the most abundant in the orange pulp at a concentration of 8.52±0.78 mg/g among the flavonoids. Although dietary supplementation of a synthetic hesperidin+naringenin combination did not change egg yolk color properties (DSM Yolk Fan score, lightness – L, redness – a, yellowness – b), supplementation of orange pulp included abundant levels of Hesperidin significantly decreased all egg yolk color properties compared to control group. The observed results suggested that hesperidin led to lighter egg yolks even though synergetic effects with a combination with naringenin. In our study, lighter egg yolks in treatment groups than control group may be result of LJ supplementation which has just hesperidin among the other citrus flavonoids (Xu et al., 2008).

The inhibition of 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase improves LDL receptors and increases plasma HDL concentration (Liang et al., 2005). Also, some evidence suggested that hesperetin acts as a cholesterol-lowering agent through decreasing activity of hepatic HMG-CoA reductase (Choi et al., 2004; Kim et al., 2003). In our study, we observed increase of serum HDL levels in 0.5% LJ supplemented group which can be explained by a potential HMG-CoA reductase inhibition of LJ via hesperidin. In contrast with the results of Kim et al. (2003) and Choi et al. (2004), we observed no effect of hesperetin on serum CHO and LDL levels. Differences may be caused by using hesperetin, which is an aglycone form of hesperidin, in mentioned studies. Goliomytis et al. (2014) concluded that dietary hesperidin did not affect plasma CHO levels in laying hens and this finding is consistent with our result. Moreover, hesperidin had a beneficial effect on human red blood cells (Allegra et al., 1995). Although there is no constant dose-dependent effect of treatment, significant changes in RBC levels in some treatment groups might be explained by mentioned effects of hesperidin. However, further evidence need to fully explain its potential mechanism.

In conclusion, LJ showed positive effects on HDEP without any adverse effects on the egg quality traits and health status of late-phase laying hens. However, the positive responses of LJ may be more relevant to acidity of water rather than active ingredients of juice, due to the dilution effect. Further side-by-side researches need the dose-controlled hesperidin and acidic water as well as higher concentration of LJs to explore accurate mechanism of their action and its positive effects on late-phase of laying hens.

Ethics Committee Approval: The current study was performed at the Animal Research Center of Afyon Kocatepe University Faculty of Veterinary Medicine after the approval of the Local Ethics Committee on the ethical use of animals under approval (Case No: 49533702/22, Date: 16/02/2017).

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – E.E.G., C.U., İ.S.Ç., İ.B.; Design - E.E.G., C.U., İ.S.Ç., İ.B.; Supervision - E.E.G., C.U., İ.S.Ç., İ.B.; Resources – İ.S.Ç., İ.B., A.I., Ü.Ö.; Materials – E.E.G, İ.S.Ç., İ.B., A.I., Ü.Ö.; Data Collection and/or Processing – E.E.G., A.I., C.U., İ.S.Ç., İ.B., Ü.Ö.; Analysis and/or Interpretation – E.E.G., A.I., İ.S.Ç., İ.B.; Literature Search – E.E.G., A.I., İ.S.Ç., İ.B.; Writing Manuscript – E.E.G., A.I., İ.S.Ç., İ.B.; Critical Review – E.E.G., A.I., C.U., İ.S.Ç., İ.B., Ü.Ö.

Acknowledgements: The authors thank the staff of Afyon Kocatepe University Faculty of Veterinary Medicine Animal Teaching & Research Center for their assistance with this study.

Conflict of Interest: The authors have no conflicts of interest to declare.

Financial Disclosure: This project was supported by Scientific Research Projects Commission of Afyon Kocatepe University (Afyonkarahisar, Turkey) with project no: 17.KARIYER.153.

References

Allegra, C., Bartolo, M.J., Carioti, B., Cassiani, D., 1995. An original microhaemorheological approach to the pharmacological effects of Daflon 500 mg in severe chronic venous insufficiency. International Journal of Microcirculation, Clinical and Experimental 15, 50-54. [CrossRef]

Behboudi, H., Esmaeilipour, O., Mirmahmoudi, R., Mazhari, M., 2016. The influence of drinking water containing lemon juice and thyme supplemented diet on performance and some blood parameters of broilers under heat stress. Iranian Journal of Applied Animal Science 6, 169-174.

Castillo, M.C., de Allori, C.G., de Gutierrez, R.C., de Saab, O.A., de Fernandez, N.P., de Ruiz, C.S., Holgado, A.P., de Nader, O.M., 2000. Bactericidal activity of lemon juice and lemon derivatives against *Vibrio cholerae*. Biological and Pharmaceutical Bulletin 23, 1235-1238. [CrossRef]

Catli, A.U., Bozkurt, M., Kucukyilmaz, K., Cinar, M., Bintas, E., Coven, F., Atik, H., 2012. Performance and egg quality of aged laying hens fed diets supplemented with meat and bone meal or oyster shell meal. South African Journal of Animal Science 42, 74-82. [CrossRef]

Choi, G.S., Lee, S., Jeong, T.S., Lee, M.K., Lee, J.S., Jung, U.J., Kim, H.J., Park, Y.B., Bok, S.H., Choi, M.S., 2004. Evaluation of hesperetin 7-O-lauryl ether as lipid-lowering agent in high-cholesterol-fed rats. Bioorganic and Medicinal Chemistry 12, 3599-3605. [CrossRef]

Del Toro-Arreola, S., Flores-Torales, E., Torres-Lozano, C., Del Toro-Arreola, A., Tostado-Pelayo, K., Guadalupe Ramirez-Dueñas, M., Daneri-Navarro, A., 2005. Effect of D-limonene on immune

response in BALB/c mice with lymphoma. International Immunopharmacology 5, 829-838. [CrossRef]

Elaroussi, M.A., Forte, L.R., Eber, S.L., Biellier, H.V., 1994. Calcium homeostasis in the laying hen. 1. Age and dietary calcium effects. Poultry Science 73, 1581-1589. [CrossRef]

Ezzat, H.N., Shihab, I.M., Hussein, M.A., 2017. Effects of ionized water on certain egg quality traits and the levels of proteins and enzymes in the blood of the Japanese quail *Coturnix japonica*. International Journal of Poultry Science 16, 69-80. [CrossRef]

Farghly, M.F.A., Ahmad, E.A.M., Alagawany, M., Abd El-Hack, M.E., Ali, R.A.M., Elnesr, S.S., Taha, A.E., Salah, A.S., 2018. Use of some nutritional supplements in drinking water of growing turkeys during 1st month of age and their effect on performance, meat quality, blood profile and antioxidant status. Journal of Animal Physiology and Animal Nutrition 102, 1625-1633. [CrossRef]

Goliomytis, M., Kostaki, A., Avgoulas, G., Lantzouraki, D.Z., Siapi, E., Zoumpoulakis, P., Simitzis, P., Deligeorgis, S.G., 2018. Dietary supplementation with orange pulp (*Citrus sinensis*) improves egg yolk oxidative stability in laying hens. Animal Feed Science and Technology 244, 28-35. [CrossRef]

Goliomytis, M., Orfanou, H., Petrou, E., Charismiadou, M.A., Simitzis, P.E., Deligeorgis, S.G., 2014. Effect of hesperidin dietary supplementation on hen performance, egg quality and yolk oxidative stability. British Poultry Science 55, 98-104. [CrossRef]

Kadam, A.S., Lonkar, V.D., Patodkar, V.R., Kolangath, S.M., Bhosale, T.A., 2009. Comparative efficacy of supplementation of natural (*Citrous limon* juice), herbal and synthetic Vitamin C on the immune response of broiler chicken during summer stress. Asian Journal of Poultry Science 3, 57-62. [CrossRef]

Karadağoğlu, Ö., Özsoy, B., Ölmez, M., Durna Aydın, Ö., Şahin, T., 2018. The effects of drinking water supplemented with essential oils on performance, egg quality and egg yolk fatty acid composition in laying hens. Acta Veterinaria Eurasia 44, 85-92.

Karimi Torshizi, M.A., Moghaddam, A.R., Rahimi, S.H., Mojgani, N., 2010. Assessing the effect of administering probiotics in water or as a feed supplement on broiler performance and immune response. British Poultry Science 51, 178-184. [CrossRef]

Kim, H.K., Jeong, T.S., Lee, M.K., Park, Y.B., Choi, M.S., 2003. Lipid-lowering efficacy of hesperetin metabolites in high-cholesterol fed rats. International Journal of Clinical Chemistry 327, 129-137. [CrossRef]

Liang, K., Kim, C.H., Vaziri, N.D., 2005. HMG-CoA reductase inhibition reverses LCAT and LDL receptor deficiencies and improves HDL in rats with chronic renal failure. American Journal of Physiology. Renal Physiology 288, F539-544. [CrossRef]

Mabe, I., Rapp, C., Bain, M.M., Nys, Y., 2003. Supplementation of a corn-soybean meal diet with manganese, copper, and zinc from organic or inorganic sources improves eggshell quality in aged laying hens. Poultry Science 82, 1903-1913. [CrossRef]

Min, Y.N., Liu, F.X., Qi, X., Ji, S., Ma, S.X., Liu, X., Wang, Z.P., Gao, Y.P., 2018. Effects of methionine hydroxyl analog chelated zinc on laying performance, eggshell quality, eggshell mineral deposition, and activities of Zn-containing enzymes in aged laying hens. Poultry Science 97, 3587-3593. [CrossRef]

Molnar, A., Maertens, L., Ampe, B., Buyse, J., Zoons, J., Delezie, E., 2018. Effect of different split-feeding treatments on performance, egg quality, and bone quality of individually housed aged laying hens. Poultry Science 97, 88-101. [CrossRef]

Neter, J., Kutner, M.H., Nachtsheim, C.J., Wasserman, W., 1996. Applied Linear Statistical Models. Fourth Edition, McGraw-Hill/Irwin, New York, USA

NRC, 1994. Nutrient Requirements of Poultry.Nineth Revised Edition, National Academy Press, Washington, DC, USA

Noy, Y., Sklan, D., 1999. Different types of early feeding and performance in chicks and poults. The Journal of Applied Poultry Research 8, 16-24. [CrossRef]

Palamidi, I., Mountzouris, K.C., 2018. Diet supplementation with an organic acids-based formulation affects gut microbiota and expression of gut barrier genes in broilers. Animal Nutrition 4, 367-377. [CrossRef]

Penniston, K.L., Nakada, S.Y., Holmes, R.P., Assimos, D.G., 2008. Quantitative assessment of citric acid in lemon juice, lime juice, and commercially-available fruit juice products. Journal of Endourology 22, 567-570. [CrossRef]

Ritzi, M.M., Abdelrahman, W., Mohnl, M., Dalloul, R.A., 2014. Effects of probiotics and application methods on performance and response of broiler chickens to an Eimeria challenge. Poultry Science 93, 2772-2778. [CrossRef]

Roberts, J.R., 2004. Factors affecting egg internal quality and egg shell quality in laying hens. The Journal of Poultry Science 41, 161-177. [CrossRef]

Sadjjadi, S.M., Rostami, J., Azadbakht, M., 2006. Giardiacidal activity of lemon juice, vinifer and vinegar on Giardia intestinalis cysts. The Southeast Asian Journal of Tropical Medicine and Public Health 37, 24-27.

Samanta, S., Haldar, S., Ghosh, T.K., 2010. Comparative efficacy of an organic Acid blend and bacitracin methylene disalicylate as growth promoters in broiler chickens: effects on performance, gut histology, and small intestinal milieu. Veterinary Medicine International 645150. [CrossRef]

Shihab, I.M., Ezzat, H.N., Hussein, M.A., 2019. Effect of using ionized water on some productive and physiological performance of Japanese quails. Poultry Science DOI: 10.3382/ps/pey590, in press. [CrossRef]

Tavakkoli, H., Masallanejad, S.S., Salandari, S., 2014. Effect of lemon juice on the egg shell quality of layers subjected to heat stress. International Journal of Advance Biological Biomedical Research 2, 847-853.

Virden, W.S., Dozier, W.A., Corzo, A., Kidd, M.T., 2009. Physiological stress responses in broilers as affected by drinking water supplements or dietary corn particle size. The Journal of Applied Poultry Research 18, 244-251. [CrossRef]

Xu, G., Liu, D., Chen, J., Ye, X., Ma, Y., Shi, J., 2008. Juice components and antioxidant capacity of citrus varieties cultivated in China. Food Chemistry 106, 545-551. [CrossRef]

Yapo, B.M., 2009. Lemon juice improves the extractability and quality characteristics of pectin from yellow passion fruit by-product as compared with commercial citric acid extractant. Bioresource Technology 100, 3147-3151. [CrossRef]

Yatao, X., Saeed, M., Kamboh, A.A., Arain, M.A., 2018. The potentially beneficial effects of supplementation with hesperidin in poultry diets. World`s Poultry Science Journal 74, 265-276. [CrossRef]

Zhang, Y.N., Zhang, H.J., Wang, J., Yue, H.Y., Qi, X.L., Wu, S.G., Qi, G.H., 2017. Effect of dietary supplementation of organic or inorganic zinc on carbonic anhydrase activity in eggshell formation and quality of aged laying hens. Poultry Science 96, 2176-2183. [CrossRef]

ISTANBUL UNIVERSITY CERRAHPASA

Maintenance of Thermal Homeostasis with Special Emphasis on Testicular Thermoregulation

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Cite this article as: Sarfraz, A., Qureshi, A.S., Shahid, R.U., Hussain, M., Usman, M., Umar, Z., 2019. Maintenance of Thermal Homeostasis with Special Emphasis on Testicular Thermoregulation. Acta Vet Eurasia 45, 63-72.

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Abstract

The testicular thermoregulation is maintained by a complex anatomical structures and physiological phenomena including convection, conduction, radiation and evaporation. The testicular temperature variation has a deleterious outcome on the reproductive performance of male reproductive system, though, duration and intensity of the insult is the pivot point. By regulating the altered thermal offense, the reproductive performance can be improved, however, with a consistent increase in the global temperature, there is a need of extensive investigations to undermine the thermoregulatory mechanism, its performance, anatomical and physiological adaptations, in the upcoming hot and humid environment.

Keywords: Heat stress, scrotum, testes, testicular cone, thermal balance

Introduction

The maintenance of internal milieu is a basic phenomenon observed in human and other warm blooded mammals to cope with the temperature fluctuations in the external environment (Nakamura and Morrison, 2007). The central nervous system orchestrates a repertory neuronal machinery to constantly tackle the environmental thermal challenges. A variety of mechanisms are exercised including the blood circulation to the skin, metabolism of the brown adipocytes, thermogenesis by striated muscles, and some species-specific apparatuses like panting and sweating to shield the thermal offence (Morrison and Nakamura, 2011). Afferent and efferent neuronal pathways are converged in the brain thermal clock to collect and convey thermal information and to potentiate appropriate regulatory strategy in the response tissue (Morrison and Nakamura, 2011). For instance, the sympathetic branch of the autonomic nervous system (ANS) activates along with a tachycardia when the cutaneous temperature falls. The mechanism is fast enough to generate the required amount of heat that the core and brain temperature remains unaltered (Nakamura and Morrison, 2007). So, there is only a slight or no change in the core temperature of the body (Figure 1) (Guyton et al., 2006) as well as the brain tissues with the exposure of an individual to the cold environmental conditions (Bratincsák and Palkovits, 2005). This is because of rapid elicit response mechanism. On the skin, a family of transient receptor potential (cation channels) is supposed to sense the temperature over a wide range of temperature, however, none of these channels are convincingly proven to be the thermoreceptor that may be involved in initiating a molecular thermoregulatory response (McKemy et al., 2002).

The cutaneous temperature data is transmitted to the spinal dorsal horn via somatosensory nerve strands, which have some of the best known ascending thermosensory pathways that synapse with the thalamic neurons, for the judgment of the skin temperature (Craig, 2002). However, there are at least three classes of such afferent neurons involved in the sensation of heat, cold and mechanical changes to the skin.

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Received Date: 10 September 2018 • **Accepted Date:** 5 July 2019 • DOI: 10.5152/actavet.2019.18017

Available online at actaveteurasia.istanbulc.edu.tr

Apart from the skin, many other core body temperature information centers exist including the abdomen, spinal cord and the brain. Among them, the abdominal thermal information is quite complex to study as its nerves fibers also contain the information of appetite, taste, thirst and other gastric influxes along with the thermal information. It is suggested that the thermal informations, before reaching the preoptic area, are amended in the lateral parabrachial nucleus (Geerling and Loewy, 2008; Gupta et al., 1979). The studies have shown that the spinal cord thermal activation affects the neurons in the preoptic region suggesting that the spinal cord might have some thermal receptors similar to the cutaneous thermal receptors that are, somehow, involved in evoking the thermogenic response. It is still not clear that how these fibers may be interacting with those of the preoptic region, however, it is supposed that they may be co-involved in the cutaneous thermal input as the deep body temperature is not directly affected by the change in the environmental temperature (Bratincsák and Palkovits, 2005; Guieu and Hardy, 1970; Lomax et al., 1964).

With the change in the environmental temperature, a number of neurons in the central nervous system (CNS) show altered activity indicating their thermal response but the most concentrated area for thermoregulatory neurons is the preoptic area. After their discovery, they became the area of interest for the thermoregulatory studies especially after the findings that the loss of the neurons in this area leads to disturbed regulatory responses. Their dischargeability is markedly increased with the increase in local and skin temperatures (Boulant and Hardy, 1974; Nakayama et al., 1961; Nakayama et al., 1963).

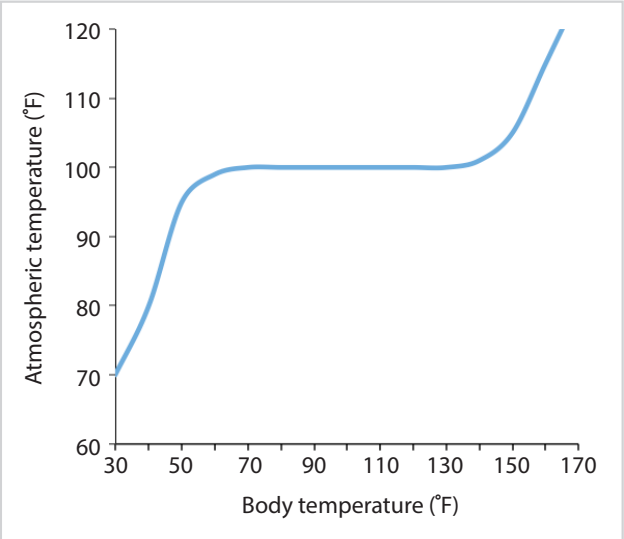

Figure 1. Effect of high and low atmospheric temperatures of several hours' duration on the internal body "core" temperature. Note that the internal body temperature remains stable despite wide changes in atmospheric temperature

There is a need to keep the core temperature of the body in a narrow range of temperature for which the hypothalamus has temperature sensitive neurons. A recent study reveals that there are the two types of nuclei in the hypothalamus; preoptic and dorsomedial, which have body temperature modulating activity and alterations in these areas lead to alterations in the core temperature (Zhang et al., 2017). Both the areas have an effective regulatory molecular connections for thermogenesis (Liedtke, 2017). Even now, there is a major study required to pin point the receptors for the thermosensitivity in the preoptic area on the neurons as well as specific markers for such neurons need further investigation.

Since skin is the first organ to sense the threats in the environmental temperature, it has the key role in triggering a thermoregulatory response although some animals, including human, have thermoregulatory responses even on the non-thermal signs like lightening, thunder or even news. It is a matter of great interest that some of the thermoregulatory responses are not processed by the preoptic area (Nakamura and Morrison, 2008). The cutaneous blood flow is routed to control the heat loss to the environment especially in the area of skin devoid of hair. During thermic intimidations, the decreased and increased blood flow is observed via sympathetic vasoconstriction and vasodilation (Wallin and Charkoudian, 2007). The contraction of the smooth muscle related to the vasomotor action of the cutaneous blood vessels is largely considered to be influenced by acetylcholine, a neurochemical mediator. Fewer studies support the role of nitric oxide via nor-epinephrine, another neurochemical mediator. These difference of findings may be due to the local and general thermal status of an individual (Hodges et al., 2008; Holowatz et al., 2005; Kellogg et al., 2007). However, during the process of cutaneous vasodilation, the visceral vasoconstriction of unknown mediation is observed.

The thermogenic response of heart through the activation of sympathetic mediation along with tachycardia is reported responses to the cold challenges to the body. The cardiac output is also maintained through the process of tachycardia. These processes are in accord with the shivering and production of heat from brown fat tissues (Nakamura and Morrison, 2007). The shivering of the skeletal muscle has long been recognized essential response to cope up with the cold environment, however, the mechanism and the neuromediators are hardly explored except the rhythmic gust of the alpha neurons are involved in the activation of the associated skeletal muscles and the intensity of shivering response (Schäfer and Schäfer, 1973).

Another predominant response to hyperthermia is sweating that helps to keep the core body temperature with in an ambient temperature range. The evaporative cooling is efficiently mediated by the skin, especially bare one. It provides a huge surface area in all the mammals with the exception of a few species which rely on the respiratory system through panting and salivation. The mechanism that evokes the release of sweat from

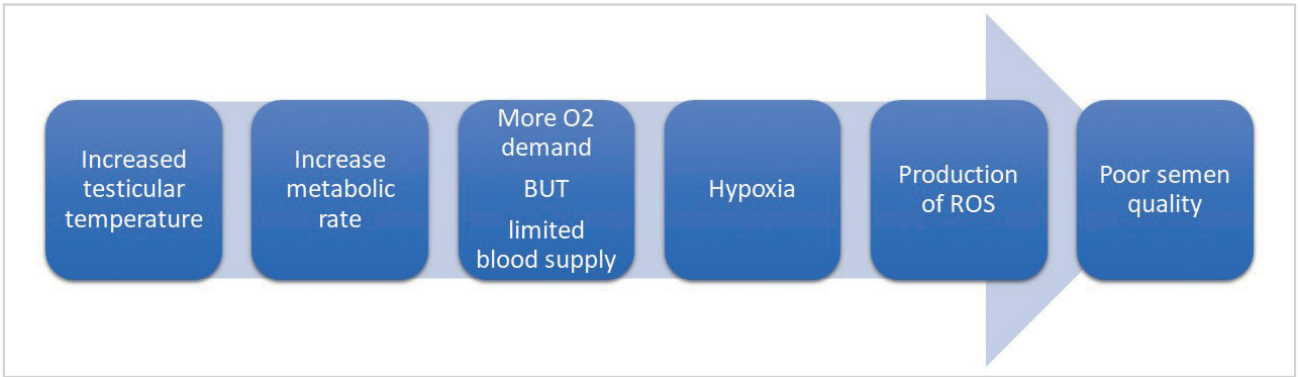

Figure 2. Schematic diagram to show the mechanism of thermal offense on the semen quality. ROS: Reactive Oxygen Species

the glands is still not known but it is supposed that the heat sensitive receptors of the skin are possible pathway to the spinal neurons (Morrison and Nakamura, 2011). The saliva, on the other hand, has inconstant nature as it is devoid of the proteins and is secreted and dispersed on the body. A similar mechanism may be present for the salivation as that of the sweat glands as very little is published in this connection. As a large volume of blood is diluted through evaporative cooling, there must exist a very efficient and quick mechanism between the cutaneous and circulatory systems (Whyte and Johnson, 2005).

Testicular thermoregulation

Although all the body organs are susceptible to heat damage but the testicles are unique in a sense that they are damaged even at a temperature optimal for the other organs. The reason, why the testicular temperature is kept lower than the body temperature is a mystery. The idea of scrotal thermoregulation was first floated by Moore and Quick (1924). There are few theories like; lower temperature may have role in lower mutation rate of the spermatozoa (Eheenberg et al., 1957), minimize physical damage to the testicles (Chance, 1996), decorative purpose (Portmann, 1952), to limit the blood supply to growing sperm cells and to enhance the mitochondrial oxidative enzymatic activity (Freeman, 1990) and optimum sperm production with minimum damage to DNA, mutation and better storage (Bedford, 2004; Werdelin and Nilsonne, 1999). Externalization of the testes does not seem necessary but the lower testicular temperature, however, is required as some species have internal testes with lower testicular temperature than core temperature (Hansen, 2009) and some others show seasonal externalization behaviour (Atkinson, 1997). The theories of the evolution of the scrotum revolve around the requirement of an ideal temperature, lower than the body temperature, for optimum spermatogenesis (Stanfield and Germann, 2008; Wallage et al., 2017).

The scrotum is a key organ in thermoregulation that has been extensively studied for its attachment with the ventral body wall, the amount of subcutaneous fat, number of hair follicles, extent of blood and lymph vasculature, the composition of the tunics, structural and functional details of the sweat glands (Artyukhin, 2007b; Cividini, 2017; Kastelic, 2014a; Setchell, 1978; Setchel, 2006; Souto et al., 2017). Thermoregulation of the scrotum is maintained by a complex combination of anatomical, physiological and behavioural adaptations and mechanisms. The scrotum is a skin extension that is attached to ventral body wall through the pendulous neck. The skin is thin in most of the mammals, devoid of sebaceous glands and richly supplied with blood vessels (Kastelic et al., 1997a).

A study has shown that the anatomical structure of the scrotum, testicular vascular cone and testicular morphology has a significant influence on the testicular thermoregulatory capabilities and hence affects the sperm quality. In their comprehensive study, they established that semen quality is positively correlated with the testicular volume and subcutaneous temperature of the scrotum and negatively correlated with the scrotal surface and testicular temperature. The temperature gradient, volume of the testes and testicular artery are positively associated with the increased sperm production, scrotal surface and testicular circumference and negatively associated with wall thickness of the vessels (Brito et al., 2004).

Thermal stress: Mechanism and effects

Failure to maintain the optimum testicular temperature increases the metabolic rate of the organ, which in consequence demand more oxygen. But as far as testes are concerned, their blood supply is limited and unaltered, therefore, an increase in the metabolic rate leads to hypoxia and production of reactive oxygen species, which reduce the quality of semen (Setchell, 1998) as shown in Figure 2. The amount of blood supply increases only when the testicular temperature is well above the body temperature (Setchell et al., 1995), but does not increase anymore on further increase on body temperature (Mieusset et al., 1992). At the same time as the testicular weight decreases with the heat exposure, so the overall blood supply to the organ stays the same (Setchell et al., 1991).

The major cause of the thermal damage is oxidative stress (Paul et al., 2008; Paul et al., 2009; Pérez-Crespo et al., 2008). The ther-

mal stress alone has adverse effects only on the semen volume and mass motility of the sperms and the thermal stress in combination with nutritional stress decreases the volume along with scrotal width and length as well as testosterone level (Maurya et al., 2016).

It has been shown that local heating of the scrotum has general effects on the body like panting and sweating behaviour without altering the core body temperature (Robertshaw and Vercoe, 1980a). An interesting phenomenon is a rise in rectal temperature on cooling the scrotum to a certain extent (Vash et al., 2002). The scrotum has heat and cold detecting receptors that activate the ventromedial hypothalamic nucleus (Li and Thornhill, 1996; Li and Thornhill, 1998). The cold receptors are not activated until the local temperature is between 10-20°C, however, cooling the scrotum does not cause shivering or thermogenesis by the metabolism of brown fat which suggests that there is a different mechanism of activating the center which causes shivering and fat metabolism (Li and Thornhill, 1993; Li and Thornhill, 1998). The physiological basis of this singularity still needs further clarifications but the scrotal heating cause panting and rise in respiratory frequency and lower the body temperature that indicates some activation of preoptic neuron (Maloney et al., 2003).

The blood flow pattern in the testicles does not depend on the heart and respiration rate but their frequency increases and the amplitude decreases with the increase in the testicular temperature and returns to normal after removing the stress (Setchell et al., 1995). There is no effect on the fluid flow in the rete testes or on the blood-testes barrier with the temperature (Setchell et al., 1996) except slight disintegration after seven days of stress (Turner et al., 1982). There is an increase in flow of anions and lysine following the stress (Main and Waites, 1977).

The testicular insulation has been studied in detail with a wide variety of experimentation on both, the testes and epididymis, collectively as well as independently. The experimental models are of quite diverse in nature but the results concluded that there are fewer pathological changes in the sperm count, however, pronounced morphological deformities and mobility incapacities of varying nature depending upon the time and nature of insulation are observed when there is improper heat dissipation and the raised testicular temperature. Most common of them are the testicular weight loss on heating and recovery on removing the heat stress (Bartlett and Sharpe, 1987; Galil and Setchell, 1988; Jannes et al., 1998; McLaren et al., 1994; Sailer et al., 1997; Van Zelst et al., 1995), increased apoptosis in spermatocytes (Lue et al., 1999), the pyriform heads (Barth and Bowman, 1994; Lagerlof, 1938), decreased motility (Austin et al., 1961; Vogler et al., 1993), longer meiotic phases, elongated spermatids which are easily injured (Austin et al., 1961), abnormal acrosomes, abnormal tails and presence of protoplasmic droplets (Wildeus and Entwistle, 1983), decapitated sperm, droplets and sperm tail abnormalities (Wildeus and Entwistle, 1986), absence of tail, knobbed acrosome, Dag defect and nuclear vacuolation (Barth and Bowman, 1994; Vogler et al., 1993), distal mid-piece reflex, detached head, mitochondrial sheet defect, microcephalic head, teratoid, coiled principal piece and abnormal DNA (Barth and Bowman, 1994), mid piece and head defects (Kastelic et al., 1996a), poor development of the embryo obtained from heat stress sperm (Paul et al., 2008) and underweight fetus and placenta (Jannes et al., 1998; Paul et al., 2009).

The heat stressed individuals, on recovery, show better semen motility characteristics, however, the sperm abnormalities of acrosome and the mid piece persist even after removing the stress for some time (Saab et al., 2011). Some infertile individuals are reported to improve the sperm quality on long term cooling of the testes, even, (Jung et al., 2001) and on ligation of the dilated veins (Pasqualotto et al., 2003).

Factors affecting scrotal thermoregulation

The testicular temperature always remains lower than the core body temperature by a difference of 2-7°C irrespective of the ambient temperature, still, the temperature gradient depends upon the regions of the scrotum; proximal, middle or distal. So the testicular surface vary in the temperature dynamics, the bottom is the coolest and top, being close to the body is the hottest area while the middle area stands between the two extremes (Kastelic et al., 1995). The more the distance from the body, the higher is the temperature gradient (Gábor et al., 1998; Kastelic, 2014b; Lunstra and Coulter, 1997; Silva et al., 2017; Skinner and Louw, 1966). A varying degree of temperature suggests that every part of the testicles require a unique temperature to operate. The temperature is particularly low in the region of the epididymis signifying a cool storage temperature in the tail because an increased storage temperature of the spermatozoa causes a significant rise in the embryonic mortality (Blazquez et al., 1988). The tail is cooler than the rest of the epididymis may be owing to its closer association with the vascular cone (will be discussed later in this article) as well as its physiological role in sperm storage (Kastelic et al., 1995).

Testicular vascular cone

Traditionally, the testicular vascular cone containing the pampiniform plexus is the site for effective exchange of heat across the vessels during the process of counter-current flow of blood resulting in lowering the testicular arterial blood temperature and raised venous blood temperature. The average efficacy of the cone is 91% for the transfer of heat under *in vivo* and 100% *in vitro* (Figure 3) (Glad Sørensen et al., 1991) indicating no loss of heat during transfer via passive transfer termed as counter-current transfer process (Figure 4). The cone is also the area of highest temperature close to the scrotum with a network of blood capillaries and the heat is lost through convection and radiation (Coulter and Kastelic, 1994). The morphological study of the angioarchitecture of the testicular artery especially its length, convolution and association with the vein have a significant role in the elu-

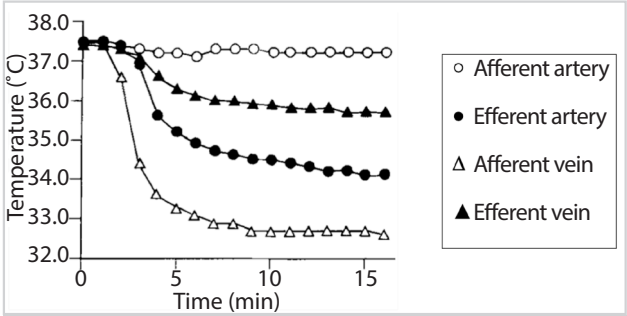

Figure 3.Temperature changes (ΔT ˚C) in the blood vessels of the spermatic cord in an in-vitro experiment with a bull testis. The pampiniform plexus was perfused with citrate plasma at 32˚C, and the testicular artery with citrate plasma at 37˚C (Glad Sørensen et al., 1991- Figure courtesy of Glad Sørensen – used with permission)

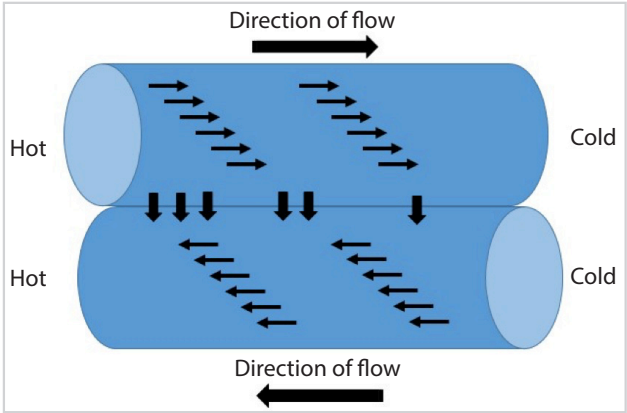

Figure 4. Model of counter-current transfer of heat or a substance between two tubes

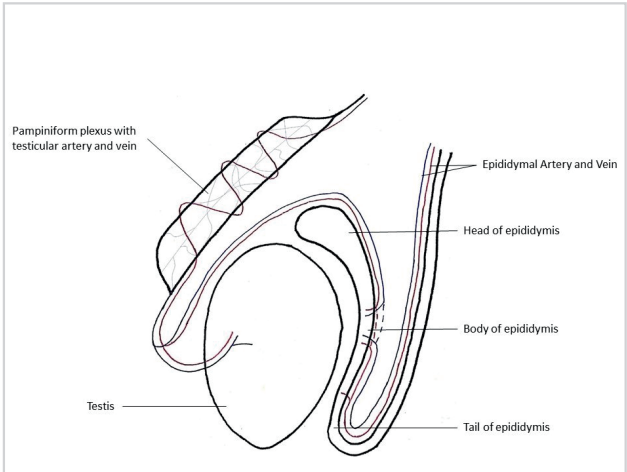

Figure 5. Model of counter-current transfer of heat or a substance from the venous blood in the pampiniform plexus to the blood in the testicular artery. The testicular artery also supplies the caput and part of the corpus of the epididymis

cidation of thermoregulation (Khalifa and Noor, 2017). The testicular artery occurs singly in most of the cases with a few exceptions in man (Asala et al., 2001; Mostafa et al., 2008; Raman and Goldstein, 2004), bull (Amselgruber and Sinowatz, 1987) and buffalo (El-Gaafary et al., 1980). The artery may be as long as 240 cm convoluted in 20 cm (Osman et al., 1979). Its wall thickness also depletes (Hees et al., 1984) along the length, starting from the abdominal aorta to the testes. It is highly coiled around the testicular vein in the entire testicular vascular cone (Figure 5) (Einer-Jensen and Hunter, 2005) in most of the species, however, the degree of convolutions varies; highly coiled in camel (Osman et al., 1979), with moderate convolutions in goat and buffalo (Abu-zaid and Gad, 1999; Borthakur and Dhingra, 1979; Dhingra, 1979) and least in donkey (Fehlings and Pohlmeyer, 1978). The testicular vessels arrangement is classified into four types of pattern; pattern I in ram and buffalo, pattern II in camelids, pattern III in donkey and pattern IV in rabbit (Elayat et al., 2014; Khalil, 2013). Briefly, in addition to other differences, tunica vasculosa and arteriosa of marginal artery in addition to the fashion of its termination determines the type of pattern. The density of the tunica vasculosa in type I pattern is prominent in the distal one-third of the testis while, proximally, it appears flimsy, while in type II pattern, the tunica arteriosa testis is poorly dense. In type III pattern the density of the tunica arteriosa testis is quite opposite to that of type I as it more pronounced in the distal two third of the testis. The density is poor and less distinct in type IV pattern.

The diameter of the testicular artery shows some drastic changes over the phase from prepubertal to post-puberty. As the individual reaches towards it puberty, the association of the testicular artery with that of vein becomes stronger, their walls become thinner and the diameter increase dramatically to facilitate an effective exchange of material and heat with fewer defects in sperms (Brito et al., 2012; Cook et al., 1994).

The testicular vascular cone is not only responsible for heat exchange but also for transportation of other macro and micro-molecules including testosterone. Testosterone recirculates from testicular vein back into the testicular artery resulting in ten times higher concentration of the hormone in the gland, responsible for the maturation of the spermatocytes (Einer-Jensen and Hunter, 2005).

A unique feature of the intratesticular thermoregulation is the opposing temperature gradients of the scrotal surface and the testicular parenchyma. The testes are vascularized from bottom to top and scrotum from top to bottom. As a consequence, the testicles are cooler at the top while scrotum at the bottom and vice versa for converse situation (Gunn and Gould, 1975; Kastelic et al., 1997b). However, the temperature of the blood in the testicular artery post-testicular cone is similar at the bottom of the testis but significantly cools down just before entering the parenchyma (Kastelic et al., 1996b).

Environmental temperature

The increased ambient temperature has an indirect effect on the scrotal temperature as individuals having covered scrotum, with dense hair or fleece, show a significant rise in the scrotal surface temperature as well as the scrotal subcutaneous temperature, however, a non-significant rise in the intratesticular temperature (Kastelic et al., 1999). A morphometric study of the testis concluded that variations in seasonal temperature markedly influences morphology of the testicular tissue and activity of Leydig cells (Pasha et al., 2011). In another comprehensive study, a total 29 parameters of male reproductive system were studied out of which 16 were markedly influenced by natural environment (Qureshi et al., 2015). Nevertheless, there is a positive linear relationship between the scrotal surface temperature and the internal testicular temperature (Kastelic et al., 2001). As the ambient temperature rises, the temperature difference between the scrotum and the environment decreases which leads to decreased ability of the scrotum to dissipate heat into the environment which, in return, raises the temperature of blood in the testicular vein. It results in poor exchange of heat between the testicular artery and vein and finally there is the supply of warmer blood to the scrotum and raised testicular temperature (Garcia, 2013; Marai et al., 2008; Sealfon and Zorgniotti, 1991). So, it seems quite appropriate to monitor the ambient temperature of the animal's environment otherwise it is a thermal stress on the testes.

Scrotal circumference

The individuals with larger scrotal circumference tend to have lower scrotal temperature on account of having a larger surface area but there may also be involvement of amount of blood supply variations. Moreover, the scrotal temperature is like wisely influenced by the behavioral and postural variations that depend on the housing practices (Wallage et al., 2017). The bipartite scrotum has a better reproductive performance on account of the greater surface area and thinner medial epidermis compared to partially bipartited or without bipartition at all; in spite of the fact that they have more number of apocrine sweat glands (Nunes et al., 2010).

Sweat glands and hair follicles

A neural reflex is responsible for sweating with at least two components; scrotal skin heat receptors and sympathetic nerves of adrenergic nature (Setchell, 1978). The sweat glands of the body and the scrotum are under the collective adrenergic neural control, however, the amount of sweat produced is as much as five times the thorax because of larger sized glands that lead to huge amount of moisture loss and cooling of the scrotum (Robertshaw and Vercoe, 1980a).

The sweat gland size, number and density on the cutaneous surface of the skin serve as a good determinant for the estimation amount of heat loss through evaporative cooling that was first measured by the amount of sweat evaporated per unit area during summer and other seasons (Robertshaw and Vercoe, 1980b). The number of secretory units of the scrotum is higher than that of the neck or abdomen of the same individual. The concentration of the glands gradually increases distally on the scrotum that supports the fact to cooler distal area of the scrotum (Blazquez et al., 1988). Irrespective of the size and number of the sweat glands, the evaporation rate is also higher in the scrotal region compared to the lumbosacral region (Blazquez et al., 1994). A significantly huge volume of vapours is evaporated compared to the rest of the body for which the considerably larger size of the scrotal sweat glands is responsible (Amakiri, 1974; Lyne and Hollis, 1968; Waites and Voglmayr, 1962; Weiner and Hellmann, 1960). The amount of the sweat is not only affected by the size and the number of the glandular units (Blazquez et al., 1988) but also by the capacity which may be as high as ten discharges per hour from them (Waites and Voglmayr, 1963).

Ultra-structural and immunohistochemical studies on the secretory cells of the sweat glands display the presence of abundant rough endoplasmic reticulum, network of golgi apparatus along with secretory vesicles involved in the secretion of beta-defensin. Thus, these glands not only regulate the temperature of the testicles with a narrow range but also provide non-specific immunity against the microbes (Yasui et al., 2007). The physical appearance of the scrotum also changes with breeding behavior i.e. smooth and fine at high breeding interims and rugose and thick at the low (Abdullahi et al., 2012).

The hair traps the air and creates a local mechanism to entrapping the convected heat and keeping the body warm, however, their absence on the scrotum facilitates an easy heat loss (Blazquez et al., 1988). The number of hair follicles also decreases from the young to an adult individual.

Vascularization, innervation and musculature

The male reproductive system is commonly attributed to be highly influenced by anatomical features, physiological conditions and nutritional status of an individual as well as temperature and humidity changes in the environment. A number of individual studies have emphasized the importance of each parameter. The themoregulatory center is supplied with sympathetic fibers of postganglionic part of lumber region, stimulation of which has vasoconstrictive response in the smooth muscles of cutaneous arterioles in the scrotum (Langley and Anderson, 1895). The vasoconstriction tone is lost during the direct local heating of the scrotal skin via sensing of the cutaneous scrotal receptors mediated by sympathetic pathway causing the dilatation of the arterioles. Contrarily, the total loss of tone is observed with the raise of core body temperature accompanied with the heat loss by radiation and sweat (Setchell, 1978).

However, recent findings suggest that it is the blood microvasculature of the scrotal integument which is involved in the testicular thermoregulation, and not the secretions of apocrine glands. The epidermis shows a dense network of vessels that is comparable to the skin vascular system. The underlying tunica dartos has a specific vascular system. It is attached to the blood

vessels (arterioles and venlues) from one end and to the rete dermis on the other end, so, it ensures a uniform nutrient supply to the whole layer (Artyukhin, 2007a; Godynicki et al., 2013).

Two types of muscles, smooth and skeletal muscles namely tunica dartos and cremaster, respectively, are responsible for contraction under the sympathetic innervations of lumber origin in response to the alterations in the optimum temperature so that an optimum distance from the body may be maintained. The former being a smooth muscle is capable for sustained contraction and keeping the testicles closer to body for longer periods of time but the later exhausts sooner on account of its skeletal nature (Gibson et al., 2002; Schwarz and Hirtler, 2017).

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – A.S.Q., A.S., M.U.; Design – A.S., R.U.S., M.U.; Supervision – M.H., Z.U., M.U.; Literature Search – Z.U., M.H., R.U.S.; Writing Manuscript – A.S.Q., A.S., Z.U.; Critical Review – A.S.Q., R.U.S., M.H.

Conflict of Interest: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

References

Abu-zaid RM, Gad MR, 1999. Goat testicular blood supply: A study by corrosion cast and angiography. Journal of the Egyptian Veterinary Medical Association 59.

Abdullahi IA, Musa HA, Jibril A, 2012. Scrotal circumference and testicular morphometric characteristics of the camel (*Camelus Dromedarius*) in the Semi-Arid Environment of Northern Nigeria. International Journal of Morphology 30, 1369-1372. [CrossRef]

Amakiri SF, **1974.** Sweat gland measurements in some tropical and temperate breeds of cattle in Nigeria. Animal Production 18, 285-291. [CrossRef]

Amselgruber W, Sinowatz F, **1987.** Zur Beziehung zwischen der Arteria testicularis und den Venen des Plexus pampiniformis beim Bullen. Anatomia, Histologia, Embryologia 16, 363-370.

Artyukhin AA, **2007a.** Anatomy and microanatomy of the venous system of scrotal organs and spermatic cord. Bulletin of Experimental Biology and Medicine 143, 99-104. [CrossRef]

Artyukhin AA, **2007b.** Vascular buffer system of the scrotal organs. Bulletin of Experimental Biology and Medicine 143, 642-647. [CrossRef]

Asala S, Chaudhary SC, Masumbuko-Kahamba N, Bidmos M, **2001.** Anatomical variations in the human testicular blood vessels. Annals of Anatomy - Anatomischer Anzeiger 183, 545-549. [CrossRef]

Atkinson S, **1997.** Reproductive biology of seals. Reviews of Reproduction 2, 175-194. [CrossRef]

Austin J, Hupp E, Murphee R, **1961.** Effect of scrotal insulation on semen of Hereford bulls. Journal of Animal Science 20, 307.

Barth AD, Bowman PA, **1994**. The sequential appearance of sperm abnormalities after scrotal insulation or dexamethasone treatment in bulls. The Canadian Veterinary Journal 35, 93-102.

Bartlett JM, Sharpe RM, **1987**. Effect of local heating of the rat testis on the levels in interstitial fluid of a putative paracrine regulator of the Leydig cells and its relationship to changes in Sertoli cell secretory function. Journal of Reproduction and Fertility 80, 279-287. [CrossRef]

Bedford JM, 2004. Enigmas of mammalian gamete form and function. Biological Reviews of the Cambridge Philosophical Society 79, 429-460. [CrossRef]

Blazquez NB, Long SE, Mayhew TM, Perry GC, Prescott NJ, Wathes C, **1994**. Rate of discharge and morphology of sweat glands in the perineal, lumbodorsal and scrotal skin of cattle. Research in Veterinary Science 57, 277-284. [CrossRef]

Blazquez NB, Mallard GJ, Wedd SR, **1988**. Sweat glands of the scrotum of the bull. Journal of Reproduction and Fertility 83, 673-677. [CrossRef]

Borthakur S, Dhingra LD, **1979**. Anatomical studies on the blood vessels of the testis in buffalo (Bubalus bubalis). Indian Journal of Animal Science 49, 266.

Boulant JA, Hardy JD, 1974. The effect of spinal and skin temperatures on the firing rate and thermosensitivity of preoptic neurones. The Journal of Physiology 240, 639-660. [CrossRef]

Bratincsák A, Palkovits M, **2005**. Evidence that peripheral rather than intracranial thermal signals induce thermoregulation. Neuroscience 135, 525-532. [CrossRef]

Brito LFC, Barth AD, Wilde RE, Kastelic JP, **2012**. Testicular vascular cone development and its association with scrotal temperature, semen quality, and sperm production in beef bulls. Animal Reproduction Science 134, 135-140. [CrossRef]

Brito LFC, Silva AEDF, Barbosa RT, Kastelic JP, **2004**. Testicular thermoregulation in Bos indicus, crossbred and Bos taurus bulls: relationship with scrotal, testicular vascular cone and testicular morphology, and effects on semen quality and sperm production. Theriogenology 61, 511-528. [CrossRef]

Chance MRA, **1996**. Reason for externalization of the testis of mammals. Journal of Zoology 239, 691-695. [CrossRef]

Cividini FG, **2017**. Characterization of the circulatory parameters of the testicular supra artery in young bulls of the aberdeen angus race. Unopar Universidade.

Cook RB, Coulter GH, Kastelic JP, **1994**. The testicular vascular cone, scrotal thermoregulation, and their relationship to sperm production and seminal quality in beef bulls. Theriogenology 41, 653-671. [CrossRef]

Coulter G, Kastelic J, **1994**. Testicular thermoregulation in bulls In: Proceedings of the 15th Technical Conference on Artificial Insemination Reproduction (pp. 28–34).

Craig AD, **2002**. How do you feel? Interoception: the sense of the physiological condition of the body. Nature Reviews Neuroscience 3, 655-666. [CrossRef]

Dhingra LD, **1979**. Angioarchitecture of the buffalo testis. Anatomischer Anzeiger 146, 60-68.

Eheenberg L, Ehrenstein GV, Hedgran A, **1957**. Gonad Temperature and Spontaneous Mutation-rate in Man. Nature 180, 1433-1434. [CrossRef]

Einer-Jensen N, Hunter RHF, **2005**. Counter-current transfer in reproductive biology. Reproduction 129, 9-18. [CrossRef]

El-Gaafary MA, Aly AE, El-Ayat MA, **1980**. Morphological studies on the testicular artery in the Egyptian water buffalo (Bos Bubalis L.). The Egyptain Veterinary Medical Journal 28.

Elayat MA, Khalil KM, Farag FM, Rizk HM, 2014. Gross anatomical studies on the pattern and density of the tunica vasculosa testis in some farm animals (buffalo, ram, camel, donkey and rabbit). Benha Veterinary Medical Journal 26, 1-9.

Fehlings K, Pohlmeyer K, 1978. Die Arteria testicularis und ihre Aufzweigung im Hoden und Nebenhoden des Esels (Equus africanus f. asinus): Korrosionsanatomische und röntgenologische Untersuchungen. Anat Histol Embryol 7, 74-78. [CrossRef]

Freeman S, 1990. The evolution of the scrotum: a new hypothesis. Journal of Theoretical Biology 145, 429-445. [CrossRef]

Gábor G, Sasser RG, Kastelic JP, Coulter GH, Falkay G, Mézes M, Szász F, 1998. Morphologic, endocrine and thermographic measurements of testicles in comparison with semen characteristics in mature Holstein-Friesian breeding bulls. Animal Reproduction Science 51, 215-224. [CrossRef]

Galil KA, Setchell BP, 1988. Effects of local heating of the testis on testicular blood flow and testosterone secretion in the rat. International Journal of Andrology 11, 73-85. [CrossRef]

Garcia AR, 2013. Estratégias para elevar a eficiência reprodutiva de búfalos em ambientes tropicais In: Proceedings of the 10th Brazilian Congress of Buiatrics (pp. 1–11). Belém: Brazilian Buiatrics Association.

Geerling JC, Loewy AD, 2008. Central regulation of sodium appetite. Experimental Physiology 93, 177-209. [CrossRef]

Glad Sørensen H, Lambrechtsen J, Einer-Jensen N, 1991. Efficiency of the countercurrent transfer of heat and 133Xenon between the pampiniform plexus and testicular artery of the bull under in-vitro conditions. International Journal of Andrology 14, 232-240. [CrossRef]

Gibson A, Akinrinsola A, Patel T, Ray A, Tucker J, McFadzean I, 2002. Pharmacology and thermosensitivity of the dartos muscle isolated from rat scrotum. British Journal of Pharmacology 136, 1194-1200. [CrossRef]

Godynicki SZ, Meyer W, Jackowiak H, Bollwein H, Pfarrer CH, 2013. Microvascularisation of the scrotal integument of Holstein-Friesian bulls. Folia Morphologica (Poland) 72, 132-136. [CrossRef]

Guieu JD, Hardy JD, 1970. Effects of heating and cooling of the spinal cord on preoptic unit activity. Journal of Applied Physiology 29, 675-683. [CrossRef]

Gunn S, Gould T, 1975. Vasculature of the testes and adnexa In *Handbook of Physiology* (pp. 117–142). Washington: American Physiological Society.

Gupta BN, Nier K, Hensel H, 1979. Cold-sensitive afferents from the abdomen. Pflugers Archiv: European Journal of Physiology 380, 203-204. [CrossRef]

Guyton AC, Hall JE, John E, 2006. Textbook of medical physiology. Elsevier Saunders.

Hansen PJ, 2009. Effects of heat stress on mammalian reproduction. Philosophical Transactions of the Royal Society B: Biological Sciences 364, 3341-3350. [CrossRef]

Hees H, Leiser R, Kohler T, Wrobel KH, 1984. Vascular morphology of the bovine spermatic cord and testis. Cell and Tissue Research 237, 31-38. [CrossRef]

Hodges GJ, Kosiba WA, Zhao K, Johnson JM, 2008. The involvement of norepinephrine, neuropeptide Y, and nitric oxide in the cutaneous vasodilator response to local heating in humans. Journal of Applied Physiology 105, 233-240. [CrossRef]

Holowatz LA, Thompson CS, Minson CT, Kenney WL, 2005. Mechanisms of acetylcholine-mediated vasodilatation in young and aged human skin. The Journal of Physiology 563, 965–973. [CrossRef]

Jannes P, Spiessens C, Van der Auwera I, D'Hooghe T, Verhoeven G, Vanderschueren D, 1998. Male subfertility induced by acute scrotal heating affects embryo quality in normal female mice. Human Reproduction (Oxford, England) 13, 372-375. [CrossRef]

Jung A, Eberl M, Schill WB, 2001. Improvement of semen quality by nocturnal scrotal cooling and moderate behavioural change to reduce genital heat stress in men with oligoasthenoteratozoospermia. Reproduction (Cambridge, England) 121, 595-603. [CrossRef]

Kastelic JP, 2014a. Thermoregulation of the Testes In R. M. Hopper (Ed.), *Bovine Reproduction* (1st ed., pp. 26–29). John Wiley & Sons, Inc. [CrossRef]

Kastelic JP, 2014b. Understanding and evaluating bovine testes. Theriogenology 81, 18-23. [CrossRef]

Kastelic JP, Cook RB, Coulter GH, 1996a. Contribution of the scrotum and testes to scrotal and testicular thermoregulation in bulls and rams. Journal of Reproduction and Fertility 108, 81-85. [CrossRef]

Kastelic JP, Cook RB, Coulter GH, 1997a. Contribution of the scrotum, testes, and testicular artery to scrotal/testicular thermoregulation in bulls at two ambient temperatures. Animal Reproduction Science 45, 255-261. [CrossRef]

Kastelic JP, Cook RB, Coulter GH, 1997b. Scrotal/testicular thermoregulation and the effects of increased testicular temperature in the bull. The Veterinary Clinics of North America. Food Animal Practice 13, 271-282. [CrossRef]

Kastelic JP, Cook RB, Coulter GH, 1999. Effects of ambient temperature and scrotal fleece cover on scrotal and testicular temperatures in rams. Canadian Journal of Veterinary Research = Revue Canadienne de Recherche Veterinaire 63, 157-160.

Kastelic JP, Cook RB, Coulter GH, Saacke RG, 1996b. Insulating the scrotal neck affects semen quality and scrotal/testicular temperatures in the bull. Theriogenology 45, 935-942. [CrossRef]

Kastelic JP, Cook RB, Pierson RA, Coulter GH, 2001. Relationships among scrotal and testicular characteristics, sperm production, and seminal quality in 129 beef bulls. Canadian Journal of Veterinary Research = Revue Canadienne de Recherche Veterinaire 65, 111-115.

Kastelic JP, Coulter GH, Cook RB, 1995. Scrotal surface, subcutaneous, intratesticular, and intraepididymal temperatures in bulls. Theriogenology 44, 147-152. [CrossRef]

Kellogg DL, Hodges GJ, Orozco CR, Phillips TM, Zhao JL, Johnson JM, 2007. Cholinergic mechanisms of cutaneous active vasodilation during heat stress in cystic fibrosis. Journal of Applied Physiology 103, 963-968. [CrossRef]

Khalifa EF, Noor NA, 2017. Anatomical study on the testicular artery of bull (Bos Taurus). International Journal of Advanced Research in Biological Sciences 4, 80-89.

Khalil KMHA, 2013. Comparative anatomical studies on the vascular architecture of the blood vessels of the testis of some farm animals (Thesis). Faculty of Veterinary Medicine - Department of Anatomy and Embryology, Cairo University.

Lagerlof N, 1938. Infertility in male domestic animals. Veterinary Medicine 33, 550.

Langley JN, Anderson HK, **1895**. The Innervation of the Pelvic and adjoining Viscera: Part II. The Bladder. Part III. The External Generative Organs. Part IV. The Internal Generative Organs. Part V. Position of the Nerve Cells on the Course of the Efferent Nerve Fibres. The Journal of Physiology 19, 71-139. [CrossRef]

Li Q, Thornhill J, **1993**. Neuronal activity changes of ventromedial hypothalamic neurons and associated temperature responses in rats following scrotal thermal stimulation. Canadian Journal of Physiology and Pharmacology 71, 604-610. [CrossRef]

Li Q, Thornhill J, **1996**. A functional medial preoptic nucleus (MPO) is required for scrotal thermal stimuli to alter the neuronal activity of thermoresponsive ventromedial hypothalamic (VMH) neurons. Brain Research 716, 134-140. [CrossRef]

Li Q, Thornhill J, **1998**. Thermoresponsiveness of posterior hypothalamic (PH) neurons of rats to scrotal and abdominal thermal stimulation. Brain Research 794, 80-87. [CrossRef]

Liedtke WB, **2017**. Deconstructing mammalian thermoregulation. Proceedings of the National Academy of Sciences 114, 1765-1767. [CrossRef]

Lomax P, Malveaux E, Smith RE, **1964**. Brain temperatures in the rat during exposure to low environmental temperatures. American Journal of Physiology-Legacy Content 207, 736-739. [CrossRef]

Lue YH, Sinha Hikim AP, Swerdloff RS, Im P, Taing KS, Bui T, Wang C, **1999**. Single Exposure to Heat Induces Stage-Specific Germ Cell Apoptosis in Rats: Role of Intratesticular Testosterone on Stage Specificity. Endocrinology 140, 1709-1717. [CrossRef]

Lunstra DD, Coulter GH, **1997**. Relationship between scrotal infrared temperature patterns and natural-mating fertility in beef bulls. Journal of Animal Science 75, 767-774. [CrossRef]

Lyne AG, Hollis DE, 1968. The skin of the sheep: a comparison of body regions. Australian Journal of Biological Sciences 21, 499-527. [CrossRef]

Main SJ, Waites GM, **1977**. The blood-testis barrier and temperature damage to the testis of the rat. Journal of Reproduction and Fertility 51, 439-450. [CrossRef]

Maloney SK, Bonomelli JM, DeSouza J, **2003**. Scrotal heating stimulates panting and reduces body temperature similarly in febrile and non-febrile rams (*Ovis aries*). Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 135, 565-573. [CrossRef]

Marai IFM, Darawany AAE, Fadiel A, Abdel-Hafez MAM, **2008**. Reproductive performance traits as affected by heat stress and its alleviation in sheep. Tropical and Subtropical Agroecosystems 8, 209-234.

Maurya VP, Sejian V, Kumar D, Naqvi SMK, **2016**. Impact of heat stress, nutritional restriction and combined stresses (heat and nutritional) on growth and reproductive performance of Malpura rams under semi-arid tropical environment. Journal of Animal Physiology and Animal Nutrition 100, 938-946. [CrossRef]

McKemy DD, Neuhausser WM, Julius D, **2002**. Identification of a cold receptor reveals a general role for TRP channels in thermosensation. Nature 416, 52-58. [CrossRef]

McLaren TT, Foster PM, Sharpe RM, **1994**. Identification of stage-specific changes in protein secretion by isolated seminiferous tubules from rats following exposure to short-term local testicular heating. Journal of Reproduction and Fertility 102, 293-300. [CrossRef]

Mieusset R, Sowerbutts SF, Zupp JL, Setchell BP, **1992**. Increased flow of testicular blood plasma during local heating of the testes of rams. Journal of Reproduction and Fertility 94, 345-352. [CrossRef]

Moore CR, Quick WJ, **1924**. The scrotum as a temperature regulator for the testes. American Journal of Physiology-Legacy Content 68, 70-79. [CrossRef]

Morrison SF, Nakamura K, **2011**. Central neural pathways for thermoregulation. Frontiers in Bioscience (Landmark Edition) 16, 74-104. [CrossRef]

Mostafa T, Labib I, El-Khayat Y, El-Rahman El-Shahat A, Gadallah A, **2008**. Human testicular arterial supply: gross anatomy, corrosion cast, and radiologic study. Fertility and Sterility 90, 2226-2230. [CrossRef]

Nakamura K, Morrison SF, **2007**. Central efferent pathways mediating skin cooling-evoked sympathetic thermogenesis in brown adipose tissue. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology 292, 127-36. [CrossRef]

Nakamura K, Morrison SF, **2008** A thermosensory pathway that controls body temperature. Nature-Neuroscience 11, 62-71. [CrossRef]

Nakayama T, Eisenman JS, Hardy JD, **1961**. Single unit activity of anterior hypothalamus during local heating. Science (New York, N.Y.) 134, 560-561. [CrossRef]

Nakayama T, Hammel HT, Hardy JD, Eisenman JS, **1963**. Thermal stimulation of electrical activity of single units of the preoptic region. American Journal of Physiology-Legacy Content 204, 1122-1126. [CrossRef]

Nunes AS, Filho MFC, Júnior AANM, Abreu-Silva AL, Júnior AMC, Souza JAT, Carvalho MAM, **2010**. Scrotum histological description in native goats from Piaui State, according to scrotal bipartition level. Ciência Rural 40, 1808-1813. [CrossRef]

Osman DI, Tingari MD, Moniem KA, **1979**. Vascular supply of the testis of the camel (Camelus dromedarius). Acta Anatomica 104, 16-22. [CrossRef]

Pasqualotto FF, Lucon AM, Hallak J, Goes PM, Saldanha LB, Arap S, **2003**. Induction of spermatogenesis in azoospermic men after varicocele repair. Human Reproduction 18, 108-112. [CrossRef]

Pasha RH, Qureshi AS, Lodhi LA, Jamil H, 2011. Biometric and Ultrasonographic evaluation of the testis of one-humped camel (*Camelus dromedarius*). Pakistan Veterinary Journal 31, 129-133.

Paul C, Murray AA, Spears N, Saunders PTK, **2008**. A single, mild, transient scrotal heat stress causes DNA damage, subfertility and impairs formation of blastocysts in mice. Reproduction 136, 73-84. [CrossRef]

Paul C, Teng S, Saunders PTK, **2009**. A single, mild, transient scrotal heat stress causes hypoxia and oxidative stress in mouse testes, which induces germ cell death. Biology of Reproduction 80, 913-919. [CrossRef]

Pérez-Crespo M, Pintado B, Gutiérrez-Adán A, **2008**. Scrotal heat stress effects on sperm viability, sperm DNA integrity, and the offspring sex ratio in mice. Molecular Reproduction and Development 75, 40-47. [CrossRef]

Portmann A, **1952**. *Animal forms and patterns : a study of the appearance of animals*. London.

Raman JD, Goldstein M, 2004. Intraoperative characterization of arterial vasculature in spermatic cord. Urology 64, 561-564. [CrossRef]

Robertshaw D, Vercoe J, 1980a. Scrotal thermoregulation of the bull (*Bos* sp.) Australian Journal of Agricultural Research 31, 401. [CrossRef]

Robertshaw D, Vercoe J, **1980b**. Scrotal thermoregulation of the bull (Bos sp.) Australian Journal of Agricultural Research 31, 401-407. [CrossRef]

Qureshi AS, Masood A, Ali, MZ, Shahid, RU, **2015**. Effects of natural environment conditions on reproductive histo-morphometric dynamics of male dromedary camel, in: Qureshi, M.S., Khan, S., Qureshi, I.U.H. (Eds.), 3rd International Workshop Dairy Science Park. Dairy Science Park, Peshawar, p. 13.

Saab Abi S, Fawwak TS, Najib K, Walid YD, Pauline YA, **2011**. Effect of adaptation and heat stress on reproductive performances of fattail awassi rams in eastern mediterranean. Lebanese Science Journal 12, 31-44.

Sailer BL, Sarkar LJ, Bjordahl JA, Jost LK, Evenson DP, **1997**. Effects of heat stress on mouse testicular cells and sperm chromatin structure. Journal of Andrology 18, 294-301.

Schäfer SS, Schäfer S, **1973**. The role of the primary afference in the generation of a cold shivering tremor. Experimental Brain Research 17, 381-393. [CrossRef]

Schwarz GM, Hirtler L, **2017**. The cremasteric reflex and its muscle - a paragon of ongoing scientific discussion: A systematic review. Clinical Anatomy 30, 498-507. [CrossRef]

Sealfon AI, Zorgniotti AW, **1991**. A theoretical model for testis thermoregulation. Advances in Experimental Medicine and Biology 286, 123-135. [CrossRef]

Setchell BP, **1978**. The Scrotum and Thermoregulation In *The Mammalian Testis* (pp. 90–108). New York: Cornell University Press.

Setchell BP, **1998**. The Parkes Lecture. Heat and the testis. Journal of Reproduction and Fertility 114, 179-194. [CrossRef]

Setchell BP, **2006**. The effects of heat on the testes of mammals Anim. Reprod. International Symposium on Animal Biology of Reproduction 3, 81-91.

Setchell BP, Bergh A, Widmark A, Damber JE, **1995**. Effect of testicular temperature on vasomotion and blood flow. International Journal of Andrology 18, 120-126. [CrossRef]

Setchell BP, Locatelli A, Perreau C, Pisselet C, Fontaine I, Kuntz C, Hochereau-de Reviers MT, **1991**. The form and function of the Leydig cells in hypophysectomized rams treated with pituitary extract when spermatogenesis is disrupted by heating the testes. The Journal of Endocrinology 131, 101-112. [CrossRef]

Setchell BP, Tao L, Zupp JL, **1996**. The penetration of chromium-EDTA from blood plasma into various compartments of rat testes as an indicator of function of the blood-testis barrier after exposure of the testes to heat. Journal of Reproduction and Fertility 106, 125-133. [CrossRef]

Silva LKX, Sousa JS, Silva AOA, Lourenço Junior JB, Faturi C, Martorano LG, Garcia AR, 2017. Testicular thermoregulation, scrotal surface temperature patterns and semen quality of water buffalo bulls reared in a tropical climate. Andrologia e12836. [CrossRef]

Skinner JD, Louw GN, **1966**. Heat stress and spermatogenesis in Bos indicus and Bos taurus cattle. Journal of Applied Physiology 21, 1784-1790. [CrossRef]

Souto PLG, McManus C, Zago FC, Martins E, Fonteque JH, Egito AA, Ramos AF, **2017**. Reproductive characteristics of Crioulo Lageano breed bulls (Bos taurus) at puberty. Animal Reproduction 14, 1034-1042. [CrossRef]

Stanfield C, Germann W, **2008**. *Principles of human physiology* (Third). San Francisco, Calif: Benjamin Cummings.

Turner TT, D'Addario DA, Forrest JB, Howards SS, **1982**. The Effects of Experimental Cryptorchidism on the Entry of [3 H]-Inulin and [3 H]-Horseradish Peroxidase into the lumen of the rat seminiferous tubules. Journal of Andrology 3, 178-183. [CrossRef]

Van Zelst SJ, Zupp JL, Hayman DL, Setchell BP, **1995**. X-Y chromosome dissociation in mice and rats exposed to increased testicular or environmental temperatures. Reproduction, Fertility, and Development 7, 1117-1121. [CrossRef]

Vash PD, Engels TM, Kandeel FR, Greenway F, **2002**. Scrotal cooling increases rectal temperature in man. Experimental Biology and Medicine (Maywood, N.J.) 227, 105-107. [CrossRef]

Vogler CJ, Bame JH, DeJarnette JM, McGilliard ML, Saacke RG, **1993**. Effects of elevated testicular temperature on morphology characteristics of ejaculated spermatozoa in the bovine. Theriogenology 40, 1207-1219. [CrossRef]

Waites GMH, Voglmayr JK, **1962**. Apocrine sweat glands of the scrotum of the ram. Nature 196, 965-967. [CrossRef]

Waites GMH, Voglmayr JK, **1963**. The functional activity and control of the apocrine sweat glands of the scrotum of the ram. Australian Journal of Agricultural Research 14, 839. [CrossRef]

Wallage AL, Johnston SD, Lisle AT, Beard L, Lees AM, Collins CW, Gaughan JB, **2017**. Thermoregulation of the bovine scrotum 1: measurements of free-range animals in a paddock and pen. International Journal of Biometeorology 61, 1381-1387. [CrossRef]

Wallin BG, Charkoudian N, **2007**. Sympathetic neural control of integrated cardiovascular function: Insights from measurement of human sympathetic nerve activity. Muscle & Nerve 36, 595-614. [CrossRef]

Weiner JS, Hellmann K, **1960**. The sweat glands. Biological Reviews 35, 141-186. [CrossRef]

Werdelin L, Nilsonne Å, **1999**. The Evolution of the scrotum and testicular descent in mammals: a phylogenetic view. Journal of Theoretical Biology 196, 61-72. [CrossRef]

Whyte DG, Johnson AK, **2005**. Lesions of the anteroventral third ventricle region (AV3V) disrupt cardiovascular responses to an elevation in core temperature. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 288, 1783-1790. [CrossRef]

Wildeus S, Entwistle KW, **1983**. Spermiogram and sperm reserves in hybrid Bos indicus X Bos taurus bulls after scrotal insulation. Journal of Reproduction and Fertility 69, 711-716. [CrossRef]

Wildeus S, Entwistle KW, 1986. Effects of scrotal insulation and unilateral vasoligation on ejaculate characteristics and sperm reserves in the bull. Animal Reproduction Science 10, 11-21. [CrossRef]

Yasui T, Fukui K, Nara T, Habata I, Meyer W, Tsukise A, **2007**. Immunocytochemical localization of lysozyme and β-defensin in the apocrine glands of the equine scrotum. Archives of Dermatological Research 299, 393-397. [CrossRef]

Zhang W, Zhou Q, Chen W, Ni X, Zhao Z, Yang WZ, Zhou Q, **2017**. A hypothalamic circuit that controls body temperature. Proceedings of the National Academy of Sciences 114, 1755. [CrossRef]