

## **Changes in haematological, cholesterol and cortisol values in Syrian Awassi rams and ewes during winter and summer seasons**

Mazen Alomar, Moutaz Zarkawi

<sup>1</sup> *Division of Animal Production, Department of Agriculture, Atomic Energy Commission, P.O. Box 6091, Damascus, Syria*

\*E-Mail: [ascientific15@aec.org.sy](mailto:ascientific15@aec.org.sy)

Received July 16, 2024

In the present study, the effects of temperature changes during summer and winter seasons on haematological parameters, total cholesterol, and cortisol level in male and female Syrian Awassi sheep were studied. Blood samples were collected weekly from ten animals (five rams and five ewes) during August and January months (2022-2023), and these samples were analyzed by a Veterinary Hematology Analyzer to determine the haematological parameters, and by enzyme-linked immunosorbent assay technique (ELISA) to determine the level of cortisol and total cholesterol. The results showed that there were significant differences in white blood cells (WBC), monocytes (MON) and granulocytes (GRA), as well as in haemoglobin (HGB) between August and January for both females and males. No significant difference was recorded between the two months for both sexes regarding platelet count (PLT) and mean platelet volume (MPV). There were significant differences ( $P < 0.001$ ) between the months of August and January in females for the level of cortisol and cholesterol, while no significant difference ( $P > 0.05$ ) was recorded for males between these two months. It was concluded that there were significant differences in the values of some blood components between summer and winter for males and females Awassi sheep, but the differences in the level of total cholesterol and cortisol were clearer in the females than in males between summer and winter. In general, this study showed the ability of the Syrian Awassi sheep to adapt higher temperature degrees than the lower ones in semi-arid area where Syrian Awassi flocks are raised.

*Key words: Awassi sheep, Blood components, Temperature, Cholesterol, Cortisol*

It is well known that environmental factors such as ambient temperature, solar radiation and relative humidity have direct and indirect effects on animals (Collier *et al.*, 1982; Collier and Zimbelman, 2007). In this respect, high or low ambient temperature was one of the major concerns that challenges the animal's ability to maintain energy, water, hormonal and mineral balance (Silanikove, 1992; Al-Dawood, 2017; Tüfekci and Sejian, 2023). On the other hand, recent developments in housing and management practice of farm animals under intensive system reflected the increase in moral concerns about animal welfare. For that, there was much interest in the effects of the interaction between temperature changes and livestock physiology and productivity under intensive and extensive management systems.

Sheep are homeothermic animals, as they can maintain near constant body temperature under a wide range of environmental conditions (Lefcourt and Adams, 1998). In this respect, thermoneutral zone is the range of temperature when the animal needs no more energy to maintain its body temperature (Kadzere *et al.*, 2002). However, above the upper or below the lower thermoneutral zone, the animal will be stressed and its production process will be affected (Da Silva *et al.*, 2002). For sheep, the thermoneutral zone is about 12–32 °C (Taylor, 1992), but this zone will clearly be different between the different sheep breeds around the world. It must be noted that, animal exposure to temperature changes or heat stress may change their biological functions and these include: blood metabolites, hormones profiles, enzymatic reactions, feed intake and the disturbance in water metabolism (Habeeb *et al.*, 1992; Marai *et al.*, 2000).

Blood profile, haematological and biochemical parameters of animals are sensitive to changes in the environmental temperature and they are very important indicators of physiological responses to any stressing agent (Okoruwa and Ikhimioya, 2014). Moreover, determination of blood parameters may be important in establishing the effect of the changes in temperature. It must be stressed out that the values of haematological

parameters were affected by several factors including season, age, environment, reproductive status, husbandry, hormonal treatments and most importantly the animal breed (Al-Samarai *et al.*, 2017; Habibu *et al.*, 2017; Ahmed *et al.*, 2018; Zarkawi and Soukouti, 2022). Moreover, total cholesterol and LDL cholesterol are major metabolites of energy metabolism and these transported in the circulation by lipoprotein particles (Tall, 1990). However, the concentration of total cholesterol varies widely in animal's blood and it was associated with nutrition, age and with the physiological and production states (Katia *et al.*, 2019). On the other hand, the reactivity of the hypothalamic – pituitary – adrenal (HPA) axis was considered as indicator of stress response, which was frequently used in the animal welfare studies (Hennessy, 2013). The most reliable and widely used biomarker of HPA axis was cortisol (Hennessy, 2013). In this respect, cortisol has long been used as a reliable physiological measure of the stress response in domestic mammals such as pig (Turner *et al.*, 2005), cow (Christison and Johnson 1972), goat (Aoyama *et al.*, 2008) and sheep (Smith and Dobson 2002; Alomar *et al.*, 2016).

Syrian Awassi sheep are the local sheep breed raised in Syria. This breed is used for different purposes such as meat, milk and wool. Moreover, Syrian Awassi sheep are known to be well-adapted to the harsh environment (Salhab *et al.*, 2003). Since blood parameters may vary due to different causes, the extrapolation of information from elsewhere and from other breeds would hardly be meaningful for this local sheep breed. Anyhow, to date, there has been no extensive report on the effect of temperature changes in summer and winter on the vital haematobiochemical parameters of both male and female Awassi sheep. Therefore, this study aimed to determine the effect of the changes of temperature degrees during summer and winter seasons on haematological parameters, cholesterol and cortisol level of Awassi rams and ewes.

## MATERIAL AND METHODS

### Location, experimental animals and ethical approval

This study was carried out at the Animal Production Division, in Deir Al-Hajar area, about 33 km south-east

of Damascus. This area is considered a dry area with an annual rainfall of about 120 mm occurring mainly in December and January, and it is a part of the Syrian steppe region where the majority of sheep population is raised.

A total of 10 Awassi sheep including 5 rams and 5 ewes were used. The animals aged between 2 and 3 years and weighing  $71.4 \pm 7.9$  kg for the rams and  $36.4 \pm 2.8$  kg of ewes. All experimental animals were housed in individual metal pens in a semi-open barn and fed the same diet twice daily. Throughout the experimental period, the animals were kept under clinical observation. The experiment lasted for 4 weeks in summer in August (this month normally has the highest temperature degrees throughout the year) and 4 weeks in winter in December (the month which characterized by the lowest temperature degrees throughout the year). This study was approved by the Local Scientific and Ethical Committee of the Atomic Energy Commission of Syria (AECS), Damascus, Syria (Permit Number 36/Z/M1-2022).

#### **Climatic Data**

Ambient temperature and relative humidity were weekly measured at 2.00 pm (afternoon) using a thermometer (Hygrometer testo- 608-H) throughout the study. Table 1 shows the average temperature and humidity inside and outside the barn during the study period from August 2022 to January 2023.

#### **Haematological, cholesterol and cortisol analysis**

In August and December, blood samples were taken from all animals from the jugular vein (one week interval) in the afternoon at 2.00 pm. The samples were placed into two different 5 mL vacuum tubes containing anti-coagulant (18 mg  $K_2E$ ). The first part of the blood samples was immediately and directly transferred to the lab for determination of blood components using a Veterinary Hematology Analyzer (Mythic<sup>TM</sup> Vet, Orphée, Geneva, Switzerland). The following haematological parameters were measured: White Blood Cells (WBC), Red Blood Cells (RBC), Monocytes (MON), Granulocytes (GRA), Haemoglobin (HGB), Platelet Counts (PLT) and Mean Platelet Volume (MPV).

The second part of blood tubes were centrifuged for

20 minutes at 3500 rpm to collect plasma and stored at -20 °C for cortisol and total cholesterol analysis. The levels of cortisol and total cholesterol in the preserved samples were analyzed by enzyme-linked immunosorbent assay (ELISA) technology, using cortisol analysis kits (cortisol, ELISA kit DIA.METRA-DK 001, 06038, Spello, Italy) and cholesterol analysis kits (General CH/cholesterol ELISA kit E0012Ge, Sunlong Biotech, Co. Ltd, Zhejiang Province, China). The measurements were conducted using an ELISA reader system (Human Reader-HS-Human – Germany).

#### **Statistical analysis**

Results were statistically analyzed and the differences among the means were calculated by ANOVA using Statview-IV programme on IBM system.

## **RESULTS**

In the present study and during the experimental period, the average temperature inside the barn was 25.3 °C and average relative humidity was 52.87 %, while the average temperature outside the barn was 26.7 °C and average relative humidity was 50.7%. However, low relative humidity levels were recorded, whether in the summer or winter months, and the relative humidity did not exceed 44 % inside the barn in August and 57 % in January (Table 1).

Table 2 shows the average values of the blood components (including haematological parameters, total cholesterol and cortisol) studied in Awassi rams and ewes in both August and January months. The results show that there was a significant difference between white blood cells (WBC) between the months of August and January for both males and females, with the highest values recorded for both sexes in January. Significant differences were also recorded for monocytes (MON) and granulocytes (GRA), as well as for haemoglobin (HGB) between August and January for both sexes. On the other hand, no significant difference was recorded between the two months for both sexes with regard to the two platelet indicators, namely platelet count (PLT) and mean platelet volume (MPV).

Regarding cortisol level, there were clear significant differences between August and January for females ( $P = 0.0017$ ), while despite the difference in the level of the

hormone between August and January months for males, the difference was not significant. The average value of the cortisol for females was higher in January, 40.30 nmol/L compared to males, 16.56 nmol/L during this month of the year, with a significant difference ( $P < 0.001$ ) for the general average of the cortisol hormone between males and females. The data from this study

also show that there were clear significant differences between the two months for total cholesterol in females, with a higher average value for females of 14.93 nmol/L in January compared to 7.8 nmol/mL for males during this month. No significant difference was recorded between August and January for males.

**Table 1.** Average temperature (°C) and humidity (%) inside and outside the barn throughout six months period in the study site.

Month	Average Temperature Inside Barn	Average Temperature Outside Barn	Average Humidity Inside Barn	Average Humidity Outside Barn
August	2.59±34.55	1.68±37.2	3.74±43.95	2.81±38.68
September	0.96±32.1	2.59±34.55	6.44±44.3	6.43±40.37
October	2.28±28.8	3.1±29.13	5.38±44.87	4.49±39.85
November	1.19±23.22	1.95±23.7	7.63±59.5	7.83±58
December	2.26±17.65	1.90±17.78	7.40±67.98	9.14±68
January	2.09±15.45	2.29±14.48	8.46±56.6	9.25±57.9
<b>Average</b>	<b>25.3</b>	<b>26.17</b>	<b>52.87</b>	<b>50.46</b>

**Table 2.** Overall means ( $\pm$  SDs) for the haematological parameters, total cholesterol and cortisol in Syrian Awassi rams and ewes during August and January months.

Parameter	Rams		<i>P value</i>	Ewes		<i>P value</i>	Rams	Ewes	<i>P value</i>
	August	January		Average	Average				
<b>WBC</b> (mm <sup>3</sup> /x10 <sup>3</sup> )	3.53±1.42	21.22± 7.97	$P < 0.001$	4.62±1.49	22.81± 8.26	$P < 0.001$	13.93± 10.79	14.74 ± 11.06	NS
<b>PLT</b> (mm <sup>3</sup> /x10 <sup>3</sup> )	1107± 220	1062 ±603	NS	1211 ±556	1003 ±550	NS	1083 ±464	1097 ±557	NS
<b>MON</b> (%)	0.4± 0.217	2.98±1.13	$P < 0.001$	0.63±0.33	3.08±1.74	$P < 0.001$	1.22±1.36	1.47±1.56	NS
<b>RBC</b> (x10 <sup>3</sup> /mm <sup>3</sup> )	13.87± 0.65	8.92±3.67	$P < 0.001$	13.71±0.78	10.35±3.23	$P < 0.001$	11.84±3.5	13.0±2.05	NS
<b>GRA</b> (%)	0.32 ± 0.19	4.56±1.41	$P < 0.001$	0.5± 0.29	5.21± 2.50	$P < 0.001$	1.70± 2.16	2.15±2.71	NS
<b>HGB</b> (g/dL)	12.36±1.96	17.50± 2.52	$P < 0.001$	10.99±1.22	18.65±1.93	$P < 0.001$	14.76±3.41	14.82±4.18	NS
<b>MPV</b> (fL)	3.97± 0.26	4.38±1.30	NS	3.92±0.24	4.14±0.98	NS	4.12±0.91	4.01±0.66	NS
<b>Cholesterol</b> (nmol/L)	3.76±2.12	7.8±1.4	NS	8.03± 6.15	14.93±10.27	$P = 0.0017$	5.83±5.87	11.58± 9.07	$P = 0.003$
<b>Cortisol</b> (nmol/L)	10.35±5.85	16.56 ±9.05	NS	20.73±13.94	40.30± 26.72	$P = 0.0004$	13.25±8.06	31.05±23.93	$P < 0.001$

NS: Not significant, White Blood Cells: (WBC), Red Blood Cells (RBC), Monocytes (MON), Granulocytes (GRA), Haemoglobin (HGB), Platelet Counts (PLT) and Mean Platelet Volume (MPV).

## DISCUSSION

In many countries around the world there is a great public pressure concerning farm animal welfare (Broom,

1993). From welfare point of view, the animals ideally should be raised in the zone of optimal thermal well being. For that, temperature changes during both summer and winter seasons and their diverse effects are very important factors related to animal welfare. The

temperature of the present study inside our semi-open barn ranged from 15.5 °C to 34.5 °C and the humidity from 67.9% to 43.9%. Clearly, these degrees are within the thermoneutral zone for sheep as described by Taylor (1992). However, such information was necessary to define the climatic space for our local Awassi breed inside the barn, and as a first approximation in evaluating its susceptibility to exposure, the conditions that may affect Awassi breed welfare.

Generally, the values of haematological parameters in this study were within the normal range for Awassi sheep. According to Zarkawi and Soukouti study (2022) which was conducted using Syrian Awassi rams during one year period where the overall mean values for WBC:  $8.92 \pm 5.05 \times 10^3/\text{mm}^3$ , MON:  $1.00 \pm 0.45\%$ , RBC:  $7.94 \pm 0.85 \times 10^3/\text{mm}^3$ , MCV:  $38.55 \pm 3.22 \text{ fL}$ , HGB:  $10.24 \pm 1.16 \text{ g/dL}$ , PLT:  $577.3 \pm 179.8 \times 10^3/\text{mm}^3$  and for MPV:  $3.99 \pm 0.49 \text{ fL}$ . It must be stressed out that the differences of our study from Zakawi and Soukouti report in 2022 are that we introduced both cortisol and total cholesterol analysis and also we directly compared the different haematological values between rams and ewes during the summer and winter season.

In this study, the season has the most important effect on the haematological values and cortisol level. In Nigeria, the haematological parameters (WBC, RBC, HGB, and LYM) of the local sheep species were significantly affected by season (Njidda *et al.*, 2014; Nafisat *et al.*, 2021). In the previous reports, the authors suggested that the increase in environmental temperature caused an increase in haematological values and that seasonal variation might be due to high environmental temperature in dry than in wet season, while Fagiolo *et al.* (2004) noted higher HGB in lactating buffaloes during the summer season than in the winter season. On the other hand, WBC showed an insignificant change in heat-stressed group compared to the control one. Al-Haidary (2004) reported higher value of RBC and haemoglobin in heat stressed sheep group than in control group. In our study and in contrast to all previous reports, it was clear that low temperature degrees in winter had higher effects on the different parameters than the high degrees in summer. Indeed, in winter one of the biggest stress factors for animals is the

exposure to cold (Tüfekci and Sejian, 2023). However, it must be stressed out that in summer and during the year 2022 the temperature degrees did not exceed the 35 °C inside the barn and for that the animals were not heat stressed during this season. Anyhow, it was very interesting to record the effects of temperature changes, which are in the range of the thermoneutral zone for the Syrian Awassi sheep.

Despite the general average values for the male and female were not significantly different for the haematological parameters, there were big differences concerning the animal sex in the present study. In Iraq, Awassi rams had significantly higher mean values of PCV, HGB, and RBC as compared with corresponding means of females while on the contrary, Awassi females means were significantly higher for MCH and MCV as compared with the means of males (Al-Samarai and Al-Jbory, 2017). In contrast, Oramari and co-workers (2014) demonstrated that sex has no significant effect on blood parameters PCV and HGB in three Iraqi breeds of sheep (Awassi, Naimy and Karadi). However, the contradicts between the different studies and ours could be due to hormonal differences (Njidda *et al.*, 2014). In fact, the female hormone was associated with high metabolic activities resulting in higher values of haematological parameters (Shumaila *et al.*, 2012). On the other hand, the differences between the results could be related to other important factors. In this respect, not only the temperature degree or the animal sex may affect haematological parameters. Abdel-Lattif and Al-Muhja (2021) showed that the age and body weight of Awassi sheep had significantly affected HGB and WBC values, while no effects were recorded for the other blood and biochemical parameters such as PVC, neutrophils and monocytes. Moreover, in Iraq, the data of Al-Samarai and Al-Jbory (2017) showed that the region where Awassi sheep was located had significantly affected haematological parameters.

In our study, and as the other haematological parameters, total cholesterol was affected by the season in both Awassi rams and ewes and it had higher values in winter compared to summer. In goats, several studies showed that in a high-temperature environment, the levels of glucose and cholesterol in the blood decrease,

which was an indicator of failure in homeostasis (Ribeiro *et al.*, 2016; 2018). Joo and co-workers (2021) noted that total cholesterol, LDL cholesterol, glucose and albumin were decreased by heat stress in both Holstein and Jersey cows. The results of the previous study indicated that such reductions may be a common metabolic alteration for dairy cows, regardless of breed, in a high-temperature environment. However, higher cholesterol concentration in stressed cows reflected a lower secretion of thyroxine that is a common finding in animals suffering heat stress as a mechanism for reduction of metabolic heat production (West, 2003).

Cortisol plays an important role in all types of stress, as it is a classic endocrine response to stress (Kannon *et al.*, 2000). Moreover, this hormone was the most important indicator in studying stress in animals (Wang *et al.*, 2015; Smith & Dobson 2002). In our previous study using Awassi rams, we noted that there was a clear significant difference in the levels of cortisol during the different months of the year, where the highest level was recorded in December (11.30 nmol/L), while the lowest level was in the month of June (0.01 nmol/L; Alomar *et al.*, 2016). In the same previous study, an elevated level was recorded associated with the copulation of animals inside and outside the reproductive season, and during semen collection using an electrical ejaculation method (Alomar *et al.*, 2016). In goats, plasma cortisol concentration increased significantly in both winter and summer season but within normal range in spring (Shukla *et al.*, 2021). Anyhow, according to the previous authors, the higher concentration of plasma cortisol level in summer was mainly due to cope up with stress conditions. In fact, temperature changes and heat stress are the most factors affecting cortisol level. In this regard, plasma cortisol increased within 20 min of exposure to acute heat stress, and reach a plateau within 2 h (Christison and Johnson, 1972). However, the decline in plasma cortisol activity under chronic heat stress indicated the adaptation to the stress (Silanikove, 2000). Tilbrook and co-workers (2000) stressed in the fact that sex differences need to be taken into account when studying the effect of stress on reproduction. Nevertheless, in *in vivo* study no differences in cortisol levels were found

between gonadectomised male and female sheep (Tilbrook *et al.*, 2000). While in an *in vitro* study and by the evaluation of different parts of HPA axis, Canny and co-workers (1999) found an increase in cortisol secretion from adrenocortical cells of female in response to ACTH compared to the secretion from male adrenocortical cells. However, the *in vivo* results of Van Lier *et al.* (2003) confirmed sex differences in ACTH induced cortisol secretion in intact sheep.

The most important observation that we can record from this study is that Syrian Awassi sheep seem to be more adaptable in summer with high temperatures degrees than in winter with low temperatures. It is clear that some sheep breeds have the ability to adapt to the surrounding climatic conditions. In this respect, studies conducted in the dry and arid regions of northwestern Mexico during the summer months showed that the productive and reproductive parameters of local breeds did not decrease significantly (Macías-Cruz *et al.*, 2013). Activation of physiological and metabolic thermoregulation mechanisms was partly responsible for their ability to avoid hyperthermia (Macías-Cruz *et al.*, 2016). Moreover, some sheep breeds were characterized by genetic and phenotypic characteristics that allowed them to be more tolerant to heat stress (McManus *et al.*, 2011). Syrian Awassi rams showed a clear adaptation to the high temperature degree than low degrees (Zarkawi and Soukouti, 2022). In fact, the animal being homeotherms can resist heating stress up to some extents depending on the species, breed and productivity (Al-Tamini, 2007). Anyhow, the productive and reproductive impact generated by heat stress in animals varies among species and breeds, with small ruminants showing the best adaptation to these environmental conditions (Al-Dawood, 2017).

## CONCLUSION

The major findings of this study provide key information regarding the different effects of temperature changes in both rams and ewes Syrian Awassi sheep. Most of the haematological parameters were affected by season especially in female. Regarding the thermoneutral zone, Awassi sheep is more sensitive to low temperature degree than high ones. The results

obtained from this study could serve as reference values for Syrian Awassi sheep. However, further studies are needed to explore more information to minimize the effect of changes of temperature in summer and winter on blood parameters for both qualitative and quantitative approaches of sheep production.

## ACKNOWLEDGEMENTS

The authors would like to thank the Director General and the Head of the Department of Agriculture of the Atomic Energy Commission of Syria for their encouragement. Thanks are also to Mr. A. Soukouti, Mr. R. Dayoub, Ms. G. Maklad, Ms. N. Adriass, Ms S. Al-Saeed for the lab assistance of this work.

## CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

## REFERENCES

- Abdel-Lattif F.H. and R.K. Al-Muhja. (2021). Some Blood and Biochemical Parameters and Body Dimensions in Awassi Sheep. IOP Conf. Ser. *Earth Environ Sci*, 735 012010.
- Ahmed M.N., A.O. Humide and M. J. Muhadi. (2018). Hematological state of ewes injected with some mediators during postpartum and lactation period. *J Pharm Sci*, 10, 1921-1924.
- Alomar M., A. Soukouti, M. Alzobi and M. Zarkawi. (2016). Testosterone and cortisol patterns and the effects of elector –ejaculation and copulation in Awassi rams. *Arch Anim Breed*, 2, 1-6.
- Al-Dawood A. (2017). Towards heat stress management in small ruminants—a review. *Ann Anim Sci*, 17, 59-88.
- Al-Haidary A. (2004). Physiological responses of naimey sheep to heat stress challenge under semi-arid environments. *Int J Agric Biol*, 6, 2004.
- Aoyama M., A. Negishi A. Abe, R. Yokoyama T. Ichimaru, and S. Sugita. (2008). Physiological and behavioural effects of an intracerebroventricular injection of corticotropin releasing hormone in goats. *Vet J*, 177, 116–123.
- Al-Samarai F.R. and W.A. Al-Jbory. (2017). Effect of some environmental factors on hematological parameters in apparently healthy Iraqi Awassi sheep. *J Entomol Zool Stud* 5, 1668-1671.
- Al-Tamini H. (2007). Thermo regulatory response of goat kids subjected to heat stress. *Small Rumin Res* 7, 280-285.
- Broom D.M., and K.G., Johnson. (1993). Stress and Animal Welfare. Publisher: Chapman & Hall, London, 1993.
- Canny B.J., K.A., O'Farrell, I.J., Clarke, and A.J., Tilbrook (1999). The influence of sex and gonadectomy on the hypothalamo-pituitary–adrenal axis of the sheep. *J Endocrinol* 162, 215–225.
- Christison G.I. and H.D., Johnson. (1972). Cortisol turnover in heat stressed cows. *J Anim Sci*, 53, 1005–1010.
- Collier R.J., D.K., Beede, W.W., Thatcher, L.A. Israel and C.J. Wilcox. (1982) Influences of environment and its modification on dairy animal health and production. *J Dairy Sci*, 65, 2213-2227.
- Collier R.J. and R.B. Zimbelman. (2007). Heat stress effects on cattle: what we know and what we Don't Know. 22nd Annual Southwest Nutrition & Management Conference Proceedings, February 22-23, Tempe, AZ.
- Da Silva R.G., Jr N., LaScala, A.E., Lima-Filho and M.C. Catharin. (2002). Respiratory heat loss in the sheep: a comprehensive model. *Int J Biometeorol*, 46, 136–140.
- Fagiolo A., O. Lai, L. Alfieri, A. Nardon and R. Cavallina (2004). Environmental factors and different managements that influence metabolic, endocrine and immuno responses in water buffalo during lactation, p. 24-26. In Proceedings of the 7th World Buffalo Congress, Manila, Philippines.
- Habeeb A.A.M., I.F.M. Marai and T.H., Kamal. (1992). Heat stress. In: Phillips, C., Pigginn, D. (Eds.), Farm Animals and the Environment. CAB International, Wallingford, UK, pp. 27–47.
- Habibu B., M. Kawu H. Makun T. Aluwong L. Yaqub, T. Dzenda & H. Buhari, (2017). Influences of breed, sex and age on seasonal changes in haematological variables of tropical goat kids. *Arch Anim Breed*, 60, 33– 42.

- Hennessy, M. B. (2013). Using hypothalamic–pituitary–adrenal measures for assessing and reducing the stress of dogs in shelters: A review. *Appl Anim Behav Sci.*, 149, 1–12.
- Joo S.S., S.J. Lee, D.S. Park, D.H. Kim, B. H. Gu, Y.J. Park, C.Y. Rim, M. Kim & E.T. Kim. (2021). Changes in Blood Metabolites and Immune Cells in Holstein and Jersey Dairy Cows by Heat Stress. *Animals*, 11, 974.
- Kadzere C.T., M.R. Murphy, N. Silanikove and Maltz, E. (2002). Heat stress in lactating dairy cows: a review. *Livest Prod Sci*, 77, 59–91.
- Kannon G., T. Terril, B. Kouakou, O. Gazal, S. Gelaye, E. A. Amoah and S. Samaké (2000). Transportation of goats: effect on physiological stress responses and live weight loss. *J Anim Sci*, 78, 1450-1457.
- Katica M., A. Mukača, E. Saljic and K. Čaklovića, (2019). Daily milk production in cows: The effect on the concentration level of total cholesterol in blood serum. *Dairy Vet Sci J*, 12, 555841. DOI: 10.19080/JDVS.2019.12.55584
- Lefcourt A.M. and W.R. Adams. (1998). Radiotelemetric measurement of body temperature of feed lot steers during winter. *J Anim Sci*, 76, 1830–1837.
- Macías-Cruz U., F.D. Álvarez-Valenzuela, A. Correa-Calderón, R. Díaz-Molina, M., Mellado, and C.A., Meza-Herrera, (2013). Thermoregulation of nutrient-restricted hair ewes subjected to heat stress during late pregnancy. *J Therm Biol*, 38, 1-9.
- Macías-Cruz U., M. A. Lopez-Baca, R. Vicente, A. Mejia, F. D. Alvarez, A. Correa-Calderon, C. A. Meza-Herrera, M. Mellado, J. E. Guerra-Liera, and L. Avendano-Reyes. (2016). Effects of seasonal ambient heat stress (spring vs. summer) on physiological and metabolic variables in hair sheep located in an arid region. *Int J Biometeorol*, 60, 1279-1286.
- Marai I., L. Bahgat, T. Shalaby and M., Abdel-Hafez, (2000). Fattening performance, some behavioral traits and physiological reactions of male lambs fed concentrates mixture alone with or without natural clay, under hot summer of Egypt. *Ann Arid Zone*, 39, 449–460.
- McManus C., H. Louvandini, R. Gugel. L.C., Sasaki, and E. Bianchini, (2011). Skin and coat traits in sheep in Brazil and their relation with heat tolerance. *Trop Anim Health Prod*, 43, 121-126.
- Njidda A. A., A. A. Shuaibu., & E. C. Isidahomen, (2014). Haematological and serum biochemical indices of sheep in Semi-Arid environment of Northern Nigeria. *GJSFR*, 14, 2249-4626.
- Nafisat A., B. Boyi<sup>1</sup>, S. T. Mbap, T. Elizabeth, Y. Ibrahim, A. M. Ja'afar, and A. Shuaibu, 2021. Effect of breed, sex, age and season on the haematological parameters of sheep in Bauchi state, Nigeria. *Niger J Anim Sci*, 23, 28-37.
- Okoruwa, M.I. and I. Ikhimioya. (2014). Haematological indices and serum biochemical profiles of dwarf goats fed elephant grass and varying levels of combined plantain with mango peels. *Am J Exp Agric*, 4, 619-628.
- Oramari R.A.S., A.O. Bamerny, and H. M. H.Zebari. (2014). Factors affecting some hematology and serum biochemical parameters in three indigenous sheep breeds. *Adv life sci technol*, 21, 56-63.
- Ribeiro N.L., R.G. Costa, E.C. Pimenta Filho, M.N. Ribeiro, A. Croveti, E.P. Saraiva, & R. Bozzi. (2016). Adaptive profile of Garfagnina goat breed assessed through physiological, haematological, biochemical and hormonal parameters. *Small Rumin Res*, 144, 236–241.
- Ribeiro N.L., R.G. Costa, E.C. Pimenta Filho, M.N. Ribeiro, R. Bozzi, (2018). Effects of the dry and the rainy season on endocrine and physiologic profiles of goats in the Brazilian semi-arid region. *Ital J Anim Sci*, 17, 454–461.
- Salhab S.A., M. Zarkawi, M.F. Wardeh, M.R., Al-Masri, and R., Kassem. (2003). Characterization and evaluation of semen in growing Awassi ram lambs. *Trop Anim Health Prod*, 35, 455-463.
- Shukla S., A. Ludri, A. Parashar, K.V. Mehra, & Kumar, G. (2021). Effect of seasonal stress on cortisol level of goats. *Pharm Innov J*, 10, 2654-2656.
- Silanikove, N., (1992). Effects of water scarcity and hot environment on appetite and digestion in ruminants:



- a review. *Livestock Production Science*, 30, 175–194.
- Silanikove, N., (2000). Effects of heat stress on the welfare of extensively managed domestic ruminants. *Livest Prod Sci*, 67,1–18.
- Smith, R.F., & H. Dobson, (2002). Hormonal interactions within the hypothalamus and pituitary with respect to stress and reproduction in sheep. *Domest Anim Endocrinol*, 23, 75–85.
- Shumaila, K., A. M. Bhutta, B. A. Khan, S. Durrani, M. Ali, and Iqbal, F. (2012). Effect of age and gender on some blood biochemical parameters of apparently healthy small ruminants from Southern Punjab in Pakistan. *Asian Pac J Trop Biomed*, 2, 304-306.
- Tall A.R., (1990). Plasma high density lipoproteins. Metabolism and relationship to atherogenesis. *J Clin Invest*, 86, 379–384.
- Taylor R.E., (1992). Adaptation to the environment. In: *Scientific Farm Animal Production*, Macmillan Publishing Company, New York, pp. 326-332
- Tilbrook A.J., B.J. Canny, M.D. Serapiglia, T.J. Ambrose, and I.J., Clarke. (1999). Suppression of the secretion of luteinizing hormone due to isolation/restraint stress in gonadectomised rams and ewes is influenced by sex steroids. *J. Endocrinol*, 160, 469–481.
- Tilbrook, A.J., A.I. Turner, I.J. Clarke. (2000). Effects of stress on reproduction in non-rodent mammals. The role of glucocorticoids and sex differences. *Rev. Reprod*, 5, 105–113.
- Tüfekci, H., and V. Sejian. (2023). Stress factors and their effects on productivity in sheep. *Animals*, 13, 2769.
- Turner A.I., Hemsworth, P.H., Tilbrook A.J. (2005). Susceptibility of reproduction in female pigs to impairment by stress or elevation of cortisol. *Domest Anim Endocrinol*, 29(2), 398-410.
- Van Lier E., R. Pérez-Clariget, and M. Forsberg (2003). Sex differences in cortisol secretion after administration of an ACTH analogue in sheep during the breeding and non-breeding season. *Anim Reprod Sci*, 79, 81–92.
- Wang L., F. Liu, Y., Luo, L., G. Zhu Li, (2015). Effect of acute heat stress on adrenocorticotrophic hormone, cortisol, interleukin-2, interleukin-12 and apoptosis gene expression in rats. *Biomed Rep*, 3, 425-429.
- West, J.W., (2003). Effects of heat stress on production in dairy cattle. *J Dairy Sci*, 86, 2131–2144.
- Zarkawi, M., and A. Soukouti, (2022). Preliminary study regarding the effect of season on haematological parameters in Syrian Awassi rams. *Arch. zootech*, 25, 37-49.