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Effect of season and housing system on hematochemical attributes in rabbits of Southern Punjab, Pakistan

[*Efeito da estação do ano e do sistema de alojamento nos atributos hemato-químicos em coelhos do Punjab do Sul, Paquistão*]

I. *Ahmad*, M.H. *Lashari*

The Islamia University Bahawalpur, Department of Zoology, Baghdad-ul-Jadeed Campus, Pakistan

ABSTRACT

The present study was conducted to investigate the effect of season and housing system on hematochemical attributes of rabbits in Southern Punjab, Pakistan. Adult healthy rabbits (n=30) were divided into two groups of equal size (n=15). Group I was housed in a modern cage system and group II in traditional colony system for a period of one year. Blood samples of rabbits from both groups were collected in the summer and the winter seasons and analyzed for various hematochemical attributes. Hematochemical attributes including red blood cell (RBC) count, hematocrit (HCT), mean corpuscular volume (MCV), total white blood cell (WBC) count, mixed cells (MID), granulocytes (GRA), glucose and alanine aminotransferase (ALT) were lowered significantly (P<0.05) in the summer in relation to the winter, while mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), creatinine, and cholesterol level were significantly (P<0.05) higher during the summer season. Biochemical attributes as cholesterol, triglycerides and globulin were significantly (P<0.05) lower in the caged rabbits. In conclusion, the summer season caused a significant decrease in most of the hematochemical attributes whereas housing system did not affect most of the hematochemical attributes of the rabbits under the climatic conditions of Southern Punjab, Pakistan.

Keywords: rabbit, summer, housing, hematochemical

RESUMO

O presente estudo foi realizado para investigar o efeito da estação do ano e do sistema de alojamento nos atributos hemato-químicos dos coelhos no Sul do Punjab, Paquistão. Os coelhos adultos saudáveis (n=30) foram divididos em dois grupos de mesmo tamanho (n=15). O grupo I foi alojado em um moderno sistema de gaiolas e o grupo II em sistema de colônia tradicional por um período de um ano. Amostras de sangue de coelhos em ambos os grupos foram coletadas no verão e no inverno e analisadas para vários atributos hemato-químicos. Os atributos hemato-químicos, incluindo contagem de hemácias, hematócrito (HCT), volume corpuscular médio (MCV), contagem total de leucócitos, células mistas (MID), granulócitos (GRA), glicose e alanina aminotransferase (ALT) foram significativamente reduzidos (P<0.05) no verão comparado ao inverno, enquanto a hemoglobina corpuscular média (HGM), a concentração média de hemoglobina corpuscular (HGM), creatinina e nível de colesterol foram significativamente (P<0,05) mais altos durante a estação do verão. Atributos bioquímicos como colesterol, triglicerídeos e globulina foram significativamente (P<0,05) mais baixos em coelhos engaiolados. Em conclusão, a estação do verão causou uma diminuição significativa na maioria dos atributos hemato-químicos, enquanto o sistema de alojamento não afetou a maioria dos atributos hemato-químicos dos coelhos sob as condições climáticas do Punjab do Sul, Paquistão.

Palavras-chave: coelho, verão, carcaça, hemato-química

INTRODUCTION

Animal welfare is an intensifying concern in livestock production and to evaluate the welfare state, it is desirable to consider healthiness of an animal. Alterations in hematochemical constituents that serve as a prime tool to investigate the physiological and stress status of an animal may be affected by several factors like sex, seasons, housing systems and management

Corresponding author: irshadahmad.zoologist@gmail.com
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procedures (Cetin *et al*., 2009) which normally spoil animal welfare at different levels. The extreme seasonal conditions during the summer in tropical regions play a negative role in rabbit physiology and production. Rabbits possess remarkable behavioral pattern, bodily conditions, and communal relationships, which are not parallel to other livestock species, leading to particular needs in terms of housing and management.

Rabbits, the third leading species in terms of numbers (about 922 million), are becoming popular for meat production in tropical and subtropical climatic regions of the world (Food…, 2018) either under the deep litter colony or modern cage system of housing. Rabbits naturally live in the form of colonies in underground burrows. This system of production has limitations like no control on breeding, higher rate of communicable diseases and little access to the ill rabbits for treatment. The modern rabbit farming is being practiced in form of cage system under well-managed controlled sheds, but several inherent welfare evils, mainly the lack of right of choice, has intensely questioned the animal production in the last years (Webster, 2005; Arantxa, 2020). For that reason, rearing of rabbits must adjust to these concerns and re-evaluation and re-structuring of husbandry systems, appear needed.

Rabbits have several good reproductive and behavioral qualities such as a very short gestation period (Ghosh *et al.*, 2008), a very short inter-kindling interval (Hassan *et al*., 2012), and feed largely on low-cost fibrous roughage including household waste organic materials (Irlbeck, 2001). Furthermore, they don't compete with humans for cereal crops as compared to other livestock species. Such facts provide sense to take advantage of the rabbit farming for meat production, especially in developing countries with low-income such as Pakistan.

In Pakistan, most common practice of rabbit keeping is backyard colony system in burrows in rural areas whereas intensive production of the rabbits under modern cage system is scarce (Khan *et al*., 2014). Literature regarding effect of seasons and housing conditions on hematochemical attributes of rabbits is not available in Pakistan. Hence, the present study was planned with an aim to evaluate the effect of season and housing system on various hematochemical attributes of local rabbits being reared in Southern Punjab, Pakistan. The study may also perhaps furnish baseline information that may help the researchers and veterinarians in future.

MATERIAL AND METHODS

The current investigative study was performed at Tehsil Khanpur, District Rahim Yar Khan a city of South Punjab Pakistan (27°40′-29°16′ N latitude, 60°45′-70°01′ E longitude) from March 2020 to February 2021. Rahim Yar Khan is an agricultural area with fertile soil. The rural population of this district is mainly associated with agriculture and livestock. The climate of Rahim Yar Khan is arid with, less rainfall, high evaporation rates and humidity. The mean annual precipitation is 900mm (Farooq *et al*., 2007).

An ethical consideration about the use of study animals was approved from the ethical committee of faculty of sciences, the Islamia University of Bahawalpur. Adult (>8 months old) apparently healthy indigenous rabbits (n=30) with average weight of 1500 grams were purchased from local rabbit farmers in District Rahim Yar Khan who kept the rabbits under traditional backyard colony system. The age of rabbits was estimated by the apophyseal line method (Watson and Tyndale-Biscoe, 1953). Rabbit health was evaluated by examining their look, coat shine, movements, feeding behavior and absence of ectoparasites. These rabbits were randomly divided into two groups (n=15/group). Group I was housed in modern cage system and group II in colony system for a period of one year from March 2020 to February 2021. In the cage system the rabbits were kept in a brick-build shed of 18′ ×18′ × 10′ (length, width, and height) with two closed and two wire-meshed open sides for ventilation. The roof of the shed was made up of plant materials. Two open sides of the shed were covered with curtains during peak warm days and cold nights of summer and winter season respectively. Welded wire mesh cages with wire mesh floor (30″ length, 30″ width and 18″ height) were arranged in the shed about 18 inches high from ground. One rabbit was kept in a single cage. In the colony system, the rabbits were kept in burrows in soil within the same shed separated from caged rabbits by a

three feet high wire meshed fence. Fresh seasonal local fodders like berseem (*Trifolium alexandrinum*), alfalfa (*Medicago sativa*), jantar (*Sesbania bispinosa*), jowar (*Sorghum bicolor*), makai (*Zea mays*), and different vegetables were offered according to season as basal diet in morning and evening times daily. The Basal diet was also supplemented with crushed maize and wheat grains (1:3 ratios by weight) @ 40 g/animal per day at evening time. Freshwater was provided to all rabbits all the times in clay pots. The temperature and relative humidity of the shed was determined with the help of a digital thermometer and hygrometer. Temperature humidity index (THI) calculation was made by (Marai *et al.,* 2001) equation *i.e* THI = db°C–[(0.31–0.31(RH)] × (db°C−14.4)] and db°C was dry bulb temperature measured in degree Celsius and RH was the relative humidity in percentage form. The THI values thus obtained were graded in following classes: <26.2 = no heat stress; 26.2–27.3 = moderate heat stress; 27.4–28.5 = severe heat stress; and >28.5 as extremely severe heat stress. Blood (5 mL) of both caged and colony rabbits was collected by jugular vein puncture using sterilized syringe during January (winter season) and July (summer season). The 2 mL blood was immediately transferred into the tubes with K3 EDTA (tri potassium ethylene diamine-tetra acetic acid) as anticoagulant and remaining 3 mL was transferred into blood clot activator gel tubes. Blood was allowed to clot in gel tubes for half an hour then it was centrifuged for 10 minutes at 3000 rpm for serum separation. Serum was transferred into 1.5 mL serum cups and frozen at -20°C until analyzed further. Different hematological attributes *viz.* red blood cell (RBC) count, hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), hematocrit (HCT), mean corpuscular hemoglobin concentration (MCHC), white blood cell (WBC) count, lymphocytes (LYM), mixed cells (MID) and granulocytes (GRA) were determined within 4 hours of blood collection by using fully automated human hematology analyzer (EDAN H30, Germany). Biochemical parameters *viz.* glucose, creatinine, cholesterol, triglycerides, total protein, globulin, albumin, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined by using automatic human biochemical analyzer Micro Lab 300 (Merck, Germany) with chemical kits of same company (Merck, Germany) as per recommended protocol. All data were presented in the form of mean ± standard error (SE). Statistical package Graph pad prism Version 8.0.2(263) was used for statistical analysis. T-test was applied to determine the effect of season (summer and winter) and housing system (cage and colony).

RESULTS

Average values for meteorological parameters in the rabbit shed during the winter and the summer months are illustrated in Table 1. Seasonal effects on different hematological and biochemical attributes of rabbits are given in Table 2 and 3 while Table 4 and 5 show the values of hematological and biochemical attributes in rabbits kept under cage and colony systems of housing.

Table 1. Average values of meteorological parameters in the rabbit shed during the winter and the summer months

Months	Temperature {^{o}C}	Humidity {%}	THI
December	18.48	56.55	17.91
January	17.29	58.94	16.89
February	22	50.25	20.82
Overall average (Winter)	19.17	55.41	18.47
June	32.83	39.4	29.38
July	31.32	55.10	28.95
August	28.06	61.29	26.44
Overall average (Summer)	30.72	52.07	28.24

THI = temperature-humidity index

Table 1 shows that there was very sever heat stress during June and July (THI=29.38 and 28.95) months and moderate heat stress during August (THI =26.44). Rabbits experienced no heat stress during December to February months of the winter.

Table 2. Mean±SE values for hematological attributes of local rabbits during the winter and the summer season

Parameters	Winter season	Summer season
Red blood cell (10^6/µL)	5.5±0.1^A	4.8±0.1^B
Hemoglobin (g/dL)	9.9±0.1	10± 0.2
Hematocrit (%)	33.1±0.4^A	27.0±0.6^B
Mean corpuscular volume (fL)	59.7±0.5^A	57.3±0.6^B
Mean corpuscular hemoglobin (Pg)	18±0.2^B	21± 0.2^A
Mean corpuscular hemoglobin concentration (g/dL)	30±0.1^B	37± 0.3^A
White blood cell (10^3/µL)	7.5±0.4^A	5.8±0.2^B
Lymphocytes (10^3/µL)	3.6±0.3	3.8±0.2
Mixed cells (10^3/µL)	1.8±0.1^A	1.5±0.1^B
Granulocytes (10^3/µL)	2.1±0.1^A	0.5±0.04^B

Significant difference in the same row is indicated by A and B superscripts at P<0.05

Table 2 shows the results of various hematological attributes in winter and summer seasons. During summer, most of the hematological attributes *viz.* RBC count, HCT, MCV, total WBC count, mixed cells (MID) and granulocytes (GRA) were significantly (P<0.05) lower while few (MCH and MCHC) were significantly (P<0.05) higher than that of the winter season.

Table 3. Mean±SE values for biochemical parameters of local rabbits during the winter and the summer season

Parameters	Winter season	Summer season
Glucose (mg/dL)	144±12^A	105±3.2^B
Creatinine (mg/dL)	0.72±0.02^B	0.97±0.03 ^{A}
Cholesterol (mg/dL)	38±0.9^B	52±2.8 ^{A}
Triglycerides (mg/dL)	115±7.5	106±7.1
Total protein (g/dL)	7.0±0.3	6.6±0.2
Albumin (g/dL)	3.7±0.07	3.6±0.07
Globulin (g/dL)	3.3±0.2	3.0±0.2
Alanine aminotransferase ALT (U/L)	80±5.1^A	53±4.3^B
Aspartate aminotransferase AST (U/L)	57±4.1	51±5.5

Significant difference in the same row is indicated by A and B superscripts at P<0.05

Table 3 shows the results of biochemical parameters of rabbits in winter and the summer seasons. It is clear from this table that glucose and ALT of rabbits were significantly (P<0.05) higher in the winter while creatinine and cholesterol in the summer.

Table 4. Mean±SE values for hematological attributes of local rabbits kept under modern cage and colony systems

Parameters	Cage system	Colony system
Red blood cell (10^6/µL)	5.3±0.2	5.0±0.1
Hemoglobin (g/dL)	10±0.2	9.7±0.2
Hematocrit (%)	31±0.8	29±0.8
Mean corpuscular volume (fL)	58±0.6	59±0.7
Mean corpuscular hemoglobin (Pg)	19±0.5	20±0.4
Mean corpuscular hemoglobin concentration (g/dL)	33±0.9	33±0.9
White blood cell (10^3/µL)	6.6±0.4	6.7±0.3
Lymphocytes (10^3/µL)	3.6±0.3	3.8±0.2
Mixed cells (10^3/µL)	1.7±0.09	1.6±0.1
Granulocytes (10^3/µL)	1.3±0.2	1.3±0.2

Significant difference in the same row is indicated by A and B superscripts at P<0.05

Results of hematological attributes of caged and colony rabbits are shown in Table 4 which indicates non-significant (P>0.05) change in any hematological attributes due to housing system. Apparently higher (P>0.05) RBC count, hemoglobin (Hb), hematocrit (HCT) and mixed cells (MID) were seen in caged rabbits. Colony rabbits had apparently higher (P>0.05) values of MCV, MCH, total WBC counts, and lymphocytes (LYM) as compared to the caged rabbits.

Table 5. Mean±SE values for biochemical parameters of local rabbits kept under modern cage and colony systems

Parameters	Cage system	Colony system
Glucose (mg/dL)	116±8.3	134±10.7
Creatinine (mg/dL)	0.86±0.04	0.84±0.04
Cholesterol (mg/dL)	42 ± 1.7^{B}	49 ± 3.1^{A}
Triglycerides (mg/dL)	97 ± 5.3^{B}	124 ± 7.8^{A}
Total protein (g/dL)	6.6±0.2	7.1±0.2
Albumin (g/dL)	3.8±0.07	3.6±0.07
Globulin (g/dL)	2.9 ± 0.2^{B}	3.5 ± 0.2^{A}
Alanine aminotransferase (U/L)	60±4.4	73±6.4
Aspartate aminotransferase (U/L)	48±4.6	61±4.7

Significant difference in the same row is indicated by A and B superscripts at P<0.05

Table 5 shows a significant (P<0.05) higher difference in the values of cholesterol, triglycerides, and globulin in colony rabbits than that of caged rabbits. Non-significant (P>0.05) changes in glucose, creatinine, total protein, albumin, and AST of rabbits were seen due to housing system.

DISCUSSION

The present study is the first of its kind being reported with an aim to determine the effect of season and housing system on hematochemical attributes in local rabbits of Southern Punjab Pakistan This region has an arid environment with four seasons but the winter and the summer being two main and prominent seasons.

Results of the present study revealed that the summer season imposed stress (Average THI= 28.24) on the rabbits as most of the hematochemical attributes of rabbits including red blood cell (RBC) count, hematocrit (HCT), mean corpuscular volume (MCV), total white blood cell (WBC) count, granulocytes (GRA), mixed cells (MID), alanine aminotransferase (ALT) and glucose were significantly lowered during the summer season. Some hematochemical attributes like mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), creatinine and cholesterol were significantly increased in the summer season.

RBC count of rabbits was significantly decreased due to the summer season which in agreement to the findings of Ayoub *et al.* (2007) who stated a negative result of summer on RBC count in Egyptian Baladi Red rabbits. Similarly, HCT value of the rabbits also significantly decreased in the summer. Raouf *et al*. (2021) stated a significant decrease in the HCT in the summer season in Algerian local rabbits which agrees with our investigation. This drop in erythrocytic indices is due to lower oxygen inhale for reducing the production of metabolic heat during hot summer season (Ashour, 2001) So, rabbits were less energetic in the summer due to lower energy production by cellular aerobic respiration.

MCH and MCHC were increased significantly in the summer which is also in agreement with Raouf *et al*. (2021). Okab *et al.* (2008) reported a significantly lower MCV value in New Zealand White rabbits in summer which is parallel to the results of current study.

Total WBC count and GRA were significantly lowered during the summer. Parallel to our findings, Al-Eissa M. (2011) also reported a significant decrease in WBC count in rabbits in the summer season. Higher WBC count in the winter shows a better immunity status in this season as compared to the summer.

According to our findings, the glucose level was significantly lowered in the summer season.

These results match with the findings of Ayoub *et al*. (2007) and Ismail *et al*. (2002) showing a significantly lower blood glucose level during the summer season as compared to the winter in rabbits and rams respectively. Increase in respiration rate in the summer increases utilization of blood glucose by the muscles of respiratory system causing a decrease in blood glucose concentration (Hassan and Roussel, 1975).

The present study results show that creatinine was significantly higher during the summer season which is in accordance with Ayoub *et al*. (2007) reporting a significant increase in creatinine during the summer in Baladi Red rabbits. Creatinine is an anhydride of creatine which is a reflector of filtration rate in kidney tubules (Khalil *et al*., 2002) and its increased concentration in summer may be an effect of kidney function alterations due to seasonal change (Soliman *et al*., 2000).

There was a non-significant effect of season on total protein, albumin, and globulin. Ondruska *et al*. (2011) reported a significant decrease in serum total protein value due to high ambient temperature in male New Zealand White rabbits which is opposite to results of current study. A significantly higher cholesterol level was calculated during the summer which matches the findings of Okab *et al*. (2008) and Ayoub *et al*. (2007).

ALT concentration was significantly lowered in summer and AST concentration was non-significantly affected due to the season. Results regarding ALT and AST match to that of Okab *et al*. (2008) reporting significantly decreased ALT concentration in the summer and non-significant effect of season on AST concentration in New Zealand White rabbit. In contrast to our findings, Ayoub *et al*. (2007) found a significantly higher ALT concentration in the summer season with respect to other seasons. AST transfers amino group of aspartate to glutamine and ALT is involved in sugar synthesis and ATP production in the liver. ALT and AST concentrations are indicators of change in glucose linked metabolism of liver (El-Maghawry *et al*., 2000). Decrease in ALT concentration in the summer caused a lower metabolic energy production in rabbits due to lower glucose production in the liver and muscles.

The most common practice of keeping rabbits in Southern Punjab, Pakistan is traditional backyard colony system in the burrows, mostly in rural areas. Modern cage system of farming is just starting in a few urban areas of Pakistan. However, colony system of production has limitations like no control on breeding, higher rate of communicable diseases and little access to the ill rabbits for treatment.

Most of the hematochemical attributes *viz*. RBC, Hb, HCT, MCV, MCH, MCHC, WBC, LYM, MID, GRA, glucose, creatinine, total protein, albumin, AST, and ALT of rabbits were non-significantly affected due to housing system. But significantly higher values of cholesterol, triglycerides and globulin were recorded in the colony rabbits as compared to the cage rabbits. Ayo-Ajasa *et al.* (2015) also reported results similar to the current study.

Likewise, Salama *et al*. (2015) found non-significant difference in various hematological and biochemical parameters of rabbits kept under different housing conditions. Amaravadhi *et al.* (2012) also reported no-significant difference in RBC count, total WBC count, Hb and HCT for New Zealand White, Flemish Giant and Grey Giant rabbits kept under cage and backyard colony systems.

Chandra *et al.* (2015) reported non-significant effect of different housing systems on the biochemical indices such as cholesterol, glucose, ALT, AST, total protein, and albumin of rabbits which are in line with the results of present study.

CONCLUSION

According to current investigation, the summer season negatively affected the hematochemical attributes of the rabbits, whereas housing system caused no change in most of the hematochemical attributes of the rabbits in Southern Punjab, Pakistan. It is concluded that winter season is better as compared to summer season for rabbit health and welfare whereas modern cage system of housing can be adopted without any rabbit health and welfare issues in Southern Punjab, Pakistan.

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