

Seasonal influence on biochemical blood parameters in males of Californian rabbit breed

Y.V. Duda¹, M.P. Prus², R.S. Shevchik¹, L.V. Koreyba¹, R.V. Mylostyvyi¹, V. V. Samoiliuk¹

¹ Dnipro State Agrarian and Economic University, 25, S. Efremov St, Dnipro, 49600, Ukraine,

² National University of Life and Environmental Sciences of Ukraine, Kiev

Corresponding author E-mail: dudajulia1976@gmail.com

Received: 15.08.2020. Accepted 29.09.2020

Biochemical parameters of rabbit blood are important for the control of animal diseases and the impact on meat productivity of diets and various supplements. The biochemical profile of blood changes under the influence of various factors, including temperature and humidity, which are associated with seasonality. The aim of the study was to determine the influence of seasonality on the biochemical analysis of blood of meat rabbits. The average annual temperature in the rabbitry was 15.9 ± 1.10 °C during the observation period, with minimum values in winter $+5.30$ °C, maximum values in summer $+27.70$ °C and an average relative humidity of $68.3 \pm 1.2\%$. The seasonal fluctuations (within physiological values) of some serum indicators of protein-nitrogen metabolism in males of Californian rabbits breed have been established. In spring, we observed a significant increase in the concentration of total protein by 7.0-12.0% and albumin – by 9.7-9.8%. At the same time, the lowest values of urea, creatinine and uric acid were equal or less than the average annual physiological indicators. Deviations of temperature from comfortable indicators in winter and summer led to the adaptive changes in rabbit organisms. We revealed the changes in the creatinine content in summer, uric acid content in summer and winter and increased activity of ALAT enzyme in winter and summer. We suggested that in case of rabbit keeping in rooms without artificial regulation of the microclimate, it is necessary to consider the influence of seasonal temperatures on blood serum biochemical parameters.

Key words: protein metabolism, enzyme activity, physiological norm, seasons, rabbits.

Introduction

Californian rabbit breed is very popular among the meat rabbit breeds of rabbits in Ukraine and was imported in the seventies of last century (Skabal, 2014). Biochemical parameters of rabbit blood are important for determining the impact of commercial diets, immunomodulators, biologically active substances and feed additives on rabbit growth, development and resistance to diseases (Gumerov, 2015; Molina et al., 2017; Daader et al., 2018; Salisu et al., 2018; Habeeb et al., 2018). The basic values of blood serum components are necessary for the study, development and testing of rabbit disease controls in veterinary medicine (Gbore & Akele, 2010; Dontas et al. 2011; Freitas et al. 2011; Guo et al., 2017; Duda et al., 2018; Oohashi et al., 2019). Physiological state and biochemical profile of rabbit blood are subject to change under external factors influence (Yaqub et al., 2013). Some authors reported the fluctuations in rabbit blood biochemical parameters depending on their sex, breed and age (Gattaniet al., 2016; Dailidenok, & Noreyko, 2014; Çetin et al., 2009; Shaobo et al., 2019); the others indicated the influence of climate, seasonality, growing conditions and feeding ration (Al-Eissa, 2011; Molina et al., 2017; Okab et al., 2008; Ondruska et al., 2018). Proper interpretation of animal biochemical profile contributes to effective control of rabbit health (Etim et al., 2014; Duda, 2019). However, differences in regulatory values and the impact of external environmental factors complicate the use of such indicators, so the scientists determined the baseline values for certain conditions. (Özkan et al., 2012).

According to the State Statistics Service of Ukraine from 2015-2017, in Ukrainian households (private sector) about of 93.5% from total number of rabbits were kept, whereas in the Zaporozhye region it was 100%. At the same time, according to the traditional conditions of rabbit breeding, in the southern region of Ukraine more than 95% of rabbit farms do not have an artificial microclimate regulation (Gonchar et al., 2020). Lack of information about the seasonal influence on the biochemical profile fluctuations in the rabbit blood requires the relevant study.

Therefore, the aim of our study was to determine the influence of seasonality on blood biochemical parameters in male rabbits of the California breed with dual keeping in the southern region of Ukraine.

Material and methods

The work was performed during 2015–2017 in a private farm in the Zaporizhye region with Californian rabbits, which used cage keeping in compliance with all zoohygienic requirements and balanced diet. Laboratory tests were conducted in the test center of the Zaporozhye Regional State Laboratory of the State Service for Food Safety and Consumer Protection, which is s accredited

in accordance with the requirements ISO/IE 17025:2006, certificate of accreditation No 2H305, National Accreditation Agency of Ukraine.

Considering that most of euthanized animals were males (females mostly are used to restore the parent flock), we selected the male rabbits of the Californian breed for our research. The first (I) group were formed from rabbits that were kept in the rabbitry with a climate control system (CC+) during the year (temperature 16-20 °C, relative humidity 65-70%); the second (control, II, 510) group was kept in the rabbitry with natural ventilation (CC-). The biochemical profile of rabbit blood was determined in different seasons: winter (W), spring (Sp), summer (Su) and autumn (A). We selected 25 healthy 2-month-old male rabbits adapted after the weaning from mothers after 10 days. The animals were kept in the experiment for one month, after which they were euthanized. According to the results of post-mortem veterinary and sanitary examination, the data of blood biochemical parameters from animals with pathologies were removed from the experiment. Each month, the following analogous groups of males from one parent herd were formed. Rabbits were kept in conventional cages made of wire mesh and equipped with a feeder and a nipple drinker, controlled by 12L:12D (12 h light:12 h dark) lighting schedule with automatic lighting.

Rabbits of both groups received the same diet throughout the seasons. The diet of rabbits was commercial granular feed (PEDomashenko D.I.) was made according to the recipe No K-94-22 for fattening of young animals (alfalfa hay 35%, wheat 20%, corn 12.5%, sunflower meal 10.5%, barley bran 10%, limestone 10.5% and premix 1.5%). The temperature and relative humidity in the rooms were measured using an automatic thermohygrometer Walcom HT-350. The average daily temperature and relative humidity were determined by daily maximum and minimum values, the monthly average values were calculated, followed by the calculation of seasonal average.

Rabbit blood was sampled every two weeks in a state of calm from the jugular vein to test tubes. In total, more than 1.000 rabbits were used in our study for two years. Serum biochemical studies were performed using reagent kits from "Filisit-Diagnostyka" (Ukraine, Dnipro). Benchtop 722(n) lab digital vis-spectrophotometer was used to determine the total protein content by biuret method (Nowotny, 1979), albumins were determined with bromocresol green indicator (Doumas et al., 1972), globulins (calculated index equal to the difference between the content of total protein and albumin) and globulin fractions – by the precipitation (Vlizlo et al., 2012).

Protein ratio (calculated index) was calculated as the ratio of albumin to globulin, urea content – by the reaction with diacetyl monooxime (Tietz, 1986), uric acid – by phosphorus-tungsten method (Kamyshnikov, 2003), creatinine – by Jaffe-Popper method (Men'shikova, 1997); activity of alanine aminotransferases (ALAT) and aspartate aminotransferases (ASAT) – by the Reitman-Frenkel method (Reitman & Frankel, 1957), and gamma-glutamyltranspeptidase (GGT) – by method with substrate γ -L - (+) - glutamyl-4-nitroanilide (Barnes et al., 2007). De Ritis index was calculated as the indicator equal to the ratio of ASAT and ALAT activity.

Keeping, feeding, care and all animal manipulations were carried out in accordance with the requirements of the European Convention for the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes (Strasbourg, 18.03.1986), "General ethical principles of animal experiments" approved at the First National Congress of Bioethics (Kyiv, September 20, 2001), Article 26 of the Law of Ukraine №5456-VI from 16.10.2012 "On the protection of animals against cruel treatment" and EU Directive 86/609 / EEC of 24.11.1986. Results were obtained within two years; their seasonal averages were calculated using Statistica v. 10 software (StatSoft Inc., USA). The reliability of the difference between the season parameters was evaluated by Student's t-test. The numbers in tables and charts were presented like arithmetic means and standard errors.

Results

The average annual temperature values in the cages with group I (CC+) and group II (CC-) were 18.6±0.04 and 16.9±0.25 °C, average annual relative humidity – 67.8±0.06% and 69.0±0.4% respectively during the period of observation (Table 1).

Table 1. Average seasonal temperature and relative humidity in rabbitry (n=730)

Parameters	I group (CC+)				II group (CC-)			
	Winter (a)	Spring (b)	Summer (c)	Autumn (d)	Winter (e)	Spring (f)	Summer (g)	Autumn (h)
Temperature, °C								
Mean	18.1	18.8	19.4	18.3	11.9 ^a	16.7 ^b	23.3 ^c	15.7 ^d
Range	16.3-19.6	17.5-19.8	17.9-20	16-19.9	5.3-14.3	13.0-21.1	19.7-27.7	8.5-23.4
Cv (%)	4.42	2.76	2.54	4.95	13.05	11.74	8.63	25.78
SE	0.08	0.05	0.06	0.09	0.16	0.21	0.21	0.42
Relative humidity, %:								
Mean	67.8	67.8	67.4	68.3	76.3 ^a	67.7	61.6 ^c	70.3
Range	65.8-70.0	65.0-70.0	62.7-69.9	66.3-70.0	68.9-84.1	55.1-80.7	50.3-71.4	58.3-84.1
Cv (%)	1.54	1.70	1.81	1.36	5.35	8.73	7.01	9.88
SE	0.11	0.12	0.13	0.10	0.43	0.21	0.45	0.73

Cv (%) - coefficient of variation; SE - standard error, ^{a,f} - significant (p<0.05) difference with the specified group, ^{aa, ee, ff, gg} - significant (p<0.01) difference with the specified group, ^{aaa, eee, fff, ggg} - significant (p<0.001), difference with the specified group

We collected the results of microclimate monitoring (temperature-humidity regime) in rabbitry towards the seasons with and without a climate control system (Table 1). The lowest average winter air temperature in the room for keeping II group rabbits was +5.3 °C, the highest summer temperature was +27.7 °C, whereas in spring-autumn the temperature was more moderate without sharp deviations from comfortable values (+16.7 and +15.7 °C respectively). The average relative humidity in the rabbitry of group II (CC-) ranged from 50.3 to 84.1% during the year. At the same time, the average values were the lowest in summer: 61.6% and the largest were in winter – 76.3%. In spring and autumn, the average values of relative humidity in the room had a small difference (67.7 and 70.3%).

Comparisons of microclimate in rabbitry of groups I and II show that the average temperature differed ($p < 0.001$) in all four seasons. The relative humidity had a significant difference between the groups only in winter and summer seasons ($p < 0.001$). Therefore, we identified a significant difference in temperature and relative humidity in winter and summer for the rabbits with and without the microclimate control. Our results indicated the probable changes in some biochemical parameters of rabbit serum blood from group I (CC+) and group II (CC-) in different seasons (Table 2). Seasonal fluctuations in serum parameters concentrations were within the physiological limits (Makarova & Makarova, 2013; Vlizlo et al., 2012; Melillo, 2007; Suckow et al., 2002; Gumerov, 2015).

Table 2. Seasonal distribution of protein metabolism parameters in rabbits (n=1010)

Parameters	Norm	I group (CC+)				II group (CC-)			
		Winter(a)	Spring(b)	Summer(c)	Autumn(d)	Winter(e)	Spring(f)	Summer (g)	Autumn (h)
Total protein, g/L	30-82 ² 54-75 ³	65.11±1.30	65.71±1.24	59.44±2.13	59.86±1.99	61.77±3.01	66.44±1.19	60.80±1.95 ^f	58.46±2.22 ^{ff}
Albumin, g/L	15-56.6 ² 27-50 ³	35.87±0.72	36.47±0.60	33.84±0.56	33.66±0.55	33.17±0.89 ^{aa}	36.76±0.58	36.24±0.65	33.18±0.55 ^{fff}
Globulin, g/L	9.3-41.0 ² 15-27 ³	29.24±1.13	29.32±1.17	26.61±2.10	26.20±1.98	28.60±2.39	29.68±1.19	24.55±1.83 ^f	25.28±2.23
Protein ratio	1.4-1.6 ² 1-3.3 ³	1.23±0.08	1.24±0.08	1.27±0.13	1.29±0.12	1.16±0.13	1.24±0.08	1.48±0.18	1.31±0.14
Urea, mmol/L	10.0 ¹ 2.2-4.9 ² 3.3-7.5 ⁴	6.69±0.30	5.98±0.31	6.56±0.58	6.73±0.53	7.44±0.48	5.60±0.27 ^{ee}	6.69±0.71	7.80±0.61 ^{ff}
Uric acid, µmol/L	59.5-255.8 ⁵	107.92±11.53	103.21±11.97	118.00±9.75	108.81±7.26	174.19±15.40 ^{aaa}	103.42±12.21 ^{ee}	171.11±10.07 ^{fff}	102.92±8.15 ^{eee, ggg}
Creatinine, µmol/L	44-223 ²	85.63±10.18	87.40±10.48	96.22±10.40	96.85±10.04	89.05±13.81	80.54±10.64	152.03±14.2 ^{eee, fff}	111.23±9.02 ^{f, ggg}
ALT, U/L	36-59 ² 45-80 ³ 25-60 ⁶ 22-80 ²	41.59±4.86	43.17±3.81	42.96±3.82	42.66±3.21	59.90±4.54 ^{aaa}	41.59±4.86	57.87±2.88	34.04±3.90
AST, U/L	35-130 ³ 5-31 ⁶	26.68±3.04	25.99±2.77	25.39±2.08	24.82±1.89	23.12±3.61	26.69±3.06	26.30±1.83	25.33±2.04
The de Rithis index	1.0-2.6 ²	0.64±0.05	0.59±0.05	0.59±0.06	0.58±0.05	0.39±0.04	0.64±0.07	0.45±0.05	0.74±0.08
GGT, U/L	1-14 ² 0-7 ³	12.79±0.60	12.94±0.71	12.64±1.05	12.84±0.99	10.42±0.79 ^a	14.21±0.80 ^{eee}	7.86±1.08 ^{fff}	12.72±1.03 ^{gg}

^{a, f} – significant ($p < 0.05$) difference with the specified group, ^{aa, ee, ff, gg} – significant ($p < 0.01$) difference with the specified group, ^{aaa, eee, fff, ggg} – significant ($p < 0.001$) difference with the specified group, ¹Makarova & Makarova (2013), ²Vlizlo et al. (2012), ³Veterinary Manual <https://www.msdrvvetmanual.com/special-subjects/reference-guides/serum-biochemical-reference-ranges>, ⁴Melillo (2007), ⁵Suckow et al. (2002), ⁶Gumerov (2015).

Significant seasonal dynamics of the biochemical profile was determined in rabbits kept indoors and with significant temperature fluctuations. During the observation period, the content of total protein in the blood of rabbits was within the above average physiological values for two groups (Vlizlo et al., 2012). The total protein in the blood of rabbits of both groups increased in spring by 7.03-12.01% and only in group II the difference was determined between spring and summer ($p < 0.05$), as well as spring and autumn seasons ($p < 0.01$). The growth of this indicator in spring was caused by an increase in the content of albumin and globulin fractions (36.76±0.58 and 29.68±1.19 g/l). The concentration of serum albumin in rabbits of group II was higher by 6.6% ($p < 0.01$) in summer and less by 7.5% ($p < 0.01$) in winter than in rabbits of group I. The highest values of globulin fraction in rabbit blood from both groups were observed in winter and spring. In summer and autumn this indicator gradually decreases. However, the content of albumin and globulin in rabbit blood was in the average regulatory range for both groups (Vlizlo et al., 2012). The protein coefficient in group II rabbits was highest in summer (1.48) due to a decrease in the globulin fraction and the lowest – in winter (1.16) due to increase in globulins, whereas in group I the protein coefficient had minimal seasonal fluctuations (1.23-1.29). Thus, the seasonal fluctuations of total protein, albumin and globulin in the blood of rabbits were periodicals. Important indicators of nitrogen metabolism, such as urea, uric acid and creatinine in both groups of rabbits were at physiological levels during the observation period (Makarova & Makarova, 2013; Vlizlo et al., 2012; Suckow et al., 2002), except for the value of urea, which in our studies exceeded some norms (Vlizlo et al., 2012).

There was no significant difference between rabbits from groups I and II in the content of blood urea in same season. Thus, in the II group, the maximum values of this indicator was noted in autumn-winter (7.80±0.61 and 7.44±0.48 mmol/l, respectively), and the minimum was in spring (5.60±0.27 mmol/l) and summer (6.69±0.71 mmol/l). In the rabbit blood from group II we found a certain ($p < 0.001$) increase for uric acid in winter (by 38%) and in summer (by 31%) and for creatinine by 36.7% in summer compared with group I (Fig. 1). Uric acid in the blood of animals without the correction of temperature and humidity had high concentrations in winter and summer, which was almost 40% more than in spring and autumn seasons: in winter and summer–

174.19±15.40 and 171.11±10.07 µmol/l, respectively, in spring and autumn– 103.41±12.21 and 102.92±8.15 µmol/l. In the blood of rabbits, there was a significant increase in creatinine (by 27-47%) in the summer. In autumn, winter and spring, this indicator decreased by 1.37 times ($p<0.001$), 1.25 and 1.11 times, respectively. Therefore, the main peaks in creatinine and uric acid occurred in summer. In addition, the maximum values of uric acid were recorded in winter, whereas the minimum values of both indicators were detected in spring.

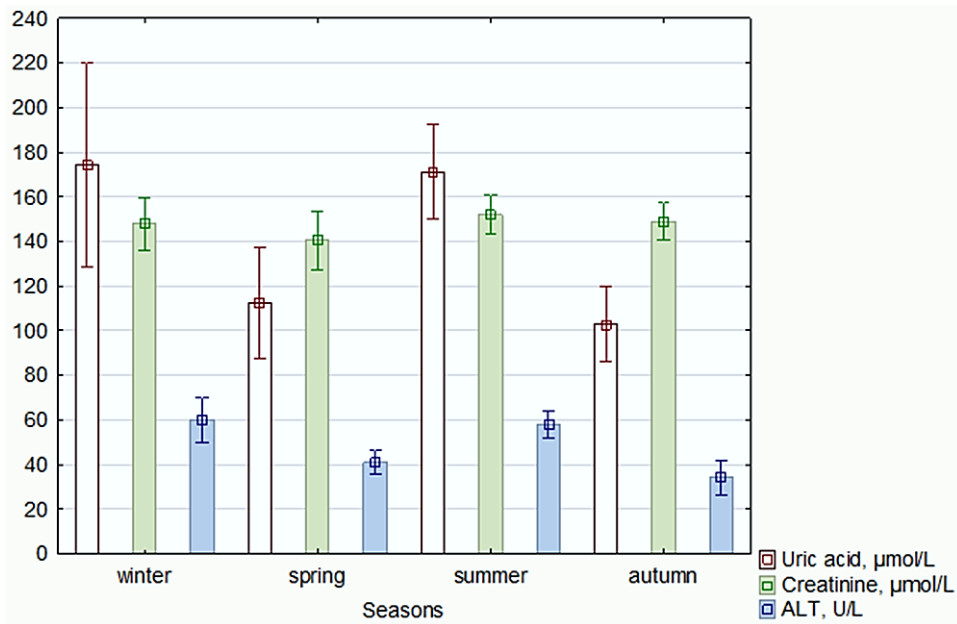


Fig. 1. Seasonal fluctuations of uric acid, creatinine and ALT in the blood of group II rabbits

The activity of enzymes ASAT and ALAT, which participate in the synthesis of amino acids, carry out their decomposition into keto acids and serve as an indicator system of liver functional was within the average physiological values in rabbit blood from both groups (Vlizlo et al., 2012; Gumerov, 2015). No significant difference in ASAT activity was found between groups I and II in same season. The ALAT activity in rabbit blood of group II increased by 24.44% ($p<0.001$) in comparison with group I in summer, reaching the upper physiological range of 57.87 U/l. In rabbits of both groups, the calculated ASAT-ALAT ratio did not correspond the normative interval and was less than one (0.39-0.74) for the entire observation period.

The value of gamma-glutamyltranspeptidase activity in the blood of rabbits from both groups was in the upper limits above physiological values during the observation period (Vlizlo et al., 2012). The CGT activity in rabbit blood from group II was significantly higher in spring and autumn months (14.21±0.80 and 12.72±1.03 U/l respectively) compared with its activity in winter (10.42±0.79 U/l) and summer (7.86±1.08 U/l).

Table 3. Seasonal distribution of blood protein globulin fractions in rabbits (%)

Parameters	Norm	I group				II group			
		Winter (a)	Spring (b)	Summer (c)	Autumn (d)	Winter (e)	Spring (f)	Summer (g)	Autumn (h)
Albumin, %	50-69 ²	55.65±1.14	56.27±1.16	60.21±1.79	59.39±1.70	54.71±2.23	56.02±1.18	61.18±2.20 ^f	60.23±1.91
Globulin, %	31-50 ²	43.35±1.14	43.73±1.15	39.79±1.79	40.61±1.70	45.29±2.23	43.98±1.18	38.82±2.20 ^f	39.77±1.91
α ₁ -globulins	7-13 ²	4.89±0.53	4.68±0.81	4.19±0.38	4.78±0.37	6.51±0.64 ^a	4.76±0.40	6.67±0.67 ^{cc}	3.70±0.37 ^{e,g}
α ₂ -globulins		7.78±0.73	7.58±0.71	5.84±0.41	6.02±0.40	8.12±0.93	8.20±0.72	7.63±0.82	5.52±0.40 ^{e,ff,g}
β-globulins	7-14 ²	5.30±0.77	5.76±0.45	5.17±0.64	5.62±0.61	8.98±1.27 ^a	4.68±0.48 ^{ee}	7.54±0.75 ^{ff}	5.61±0.63
γ-globulins	17-23 ²	25.35±1.59	25.69±1.59	24.62±1.56	24.22±1.52	21.65±1.32	26.31±1.60	16.99±1.35 ^{ccc,fff}	24.93±1.57 ^{gg}

a, e, f, g – significant ($p<0.05$) difference with the specified group, cc, ee, ff, gg – significant ($p<0.01$) difference with the specified group, ccc, fff, – significant ($p<0.001$) difference with the specified group, ² – Vlizlo et al. (2012)

Thus, the blood of rabbits showed the highest content of total protein, against the background of low levels of urea, uric acid and creatinine in spring. There was a decrease in total protein due to a decrease in globulin content and a sharp increase in uric acid and creatinine in summer, which probably caused by high temperatures. These indicators do not change significantly in winter compared to autumn, except for uric acid. Analyzing the fractional spectrum of globulins in the serum of rabbits, we found that the percentage of globulins throughout the year was within physiological norms (Vlizlo et al., 2012). We observed the alternate seasonal changes in the content of α₁-globulins and β-globulins (Fig. 2). At the same time, their rises were observed

in winter and summer and their declines – in spring and autumn. The percentage of γ -globulins, in contrast, decreased in winter and summer, and increased in autumn and spring

The lowest percentage of α_1 -globulins and α_2 -globulins in group II rabbits was found in autumn; it was $3.70 \pm 0.37\%$ and $5.52 \pm 0.40\%$, respectively, which was differed from other seasons.

Discussion

In our studies, the average annual temperature and humidity (15.9 ± 1.1 °C and $68.3 \pm 1.2\%$) of the microclimate in the room for rabbits were close to the indicators (16 °C, 72.5%) identified as the most favorable (Habeeb et al., 2018). Therefore, comfortable mean values of temperature and relative humidity in the rabbitry were observed in spring ($+15.3 \pm 0.19$ °C and $68.2 \pm 0.61\%$) and autumn ($+14.1 \pm 0.05$ °C and $71.3 \pm 1.31\%$). In hot and cold periods of the year we registered significant deviations from the optimum temperature: summer ($+27.7$ °C) and winter ($+5.3$ °C). Temperature from $+27$ to $+28$ °C is characterized by researchers as critical with the effects of heat stress and reduced growth in rabbits (Okab et al., 2008). In contrast, Habeeb et al. (2018) reported that the heat stress in rabbits was absent at a temperature less than $+27.8$ °C and moderate heat stress was observed in the temperature range of 27.8 - 28.9 °C.

The average values of total protein in the blood of Californian rabbits in our studies ranged from 58.46 to 66.44 g/l, which is less than the values established by Dailidenok & Noreyko (2014). We found an increase in protein metabolism in rabbits in spring compared to summer and autumn, as evidenced by a significant increase in total protein ($p < 0.05$ and $p < 0.01$, respectively), albumin compared with autumn ($p < 0.001$) and globulins ($p < 0.005$) compared with summer. Comparison of similar in the most optimal temperature indicators of the spring and autumn shows an increase in the concentration of total protein ($p < 0.01$) and albumin ($p < 0.001$) in the spring. Changes in serum albumin concentration as one of the indicators of liver function (Meineriet al., 2017) indicate an increase in the protein-synthesizing ability of the liver in the spring and a decrease in the autumn. We found that decrease of total protein ($p < 0.05$) and globulin ($p < 0.05$) in the blood of rabbits in summer coincides with the data of scientists who explain this decrease in feed intake in the hot season (Marai et al., 2008). In contrast, the increase of total protein, albumin, and globulin in rabbits blood in summer compared to spring, some researchers explained by the reaction of rabbits to heat stress and maintenance of osmotic pressure by blood proteins and fluid flow control between blood and tissues (Okab et al., 2008; Çetin et al., 2009; Ondruska et al., 2011).

We did not find significant changes in the protein coefficient due to the increase in total protein in albumin and globulin fractions in spring, while Okab et al. (2008) recorded an increase in total protein in summer due to corresponding decrease in the protein ratio.

Nitrogen metabolism products, urea, uric acid and creatinine in spring-autumn were within the physiological norms with slight fluctuations. Compared with other seasons, an increase in uric acid in summer ($p < 0.001$) and winter ($p < 0.01$) was found, but its levels remained within the physiological limits. Probably, it was explained by temperature stress factors: temperature decrease to the minimum value of $+5.3$ °C in winter, and increase to $+27.7$ °C in the summer. Plank et al. (2017) confirmed an increase of uric acid (the final compound of purine catabolism) in the blood of newborn rabbits under the action of stress factor. The concentration of creatinine in the blood significantly increased in summer ($p < 0.001$ and $p < 0.01$), which coincides with the research of Marai et al. (2005), who shows a significant increase in creatinine in the blood of Californian rabbits in the hot period due to dehydration. In contrast, Okab et al. (2008) had noted a slight increase in creatinine in the blood of New Zealand rabbits regardless of the season. Certain seasonal patterns in urea content fluctuations in the rabbit blood corresponded to the normal range were not detected.

We have found wave-like seasonal fluctuations in the levels of globulin fractions. The lowest percentages of α_1 -, α_2 -globulins were observed in autumn, and β -globulins – in spring. At the same time, in the critical temperature seasons, winter and summer, there is an increase in α_1 -globulins and β -globulins in the blood of rabbits against the background of a decrease in these seasons of γ -globulins, which indicates a negative temperature effect on rabbit immune system. The increase in the percentage of γ -globulins obtained by us in spring and autumn coincides with the report on these in spring by Gromadzka-Ostrowska & Zalewska (1985).

Biochemical profile of blood, such as the level of total protein, the activity of serum transferases: ASAT, and ALAT, has diagnostic value in various diseases and represent the state of protein metabolism (Hristev et al., 2008; Prasad, 2008). An increase in ALAT activity with values close to the upper physiological limits was observed in winter ($p < 0.05$) and summer ($p < 0.01$; $p < 0.001$). At the same time, no significant changes in seasonal ASAT activity were found, which coincides with the report of Okab et al. (2008). The increase in serum ALAT levels in summer and winter registered in our experiments corresponds to the data of Nakyinsige et al. (2013) and the tendency to increase ALAT levels in winter in humans as an adaptive response to cold stress (Miyake et al., 2009). The decrease in ALAT activity in autumn ($p < 0.05$; $p < 0.001$) in our experiment coincides with a decrease of total protein and albumin in the same season which may indicate a decrease in protein-synthesizing function of the liver.

The ASAT-ALAT ratio (de Ritis index) is recommended as valuable prognostic and diagnostic information (Botros & Sikaris, 2013). In our studies the de Ritis index for all seasons was lower than one, which does not coincide with the normative indicators (Vlizlo et al., 2012). Botros and Sikari (2013) reported the lack of generally accepted reference intervals of de Ritis index and the difficulty in determining its physiological limits due to the need to take into account relatively large individual variations.

In our studies, the serum GGT levels in spring and autumn reached the upper physiological limits, which was also defined by Vlizlo et al. (2012) and exceeded the regulatory intervals indicated in Veterinary Manual. By Susan E. Fielder). However, the obtained values of GGT concentration in rabbit blood (from 127.43 to 236.43 nmol/(s•l)) were lower than in Özkan et al. (2012), who established GGT levels in the range of 150 - 516.7 nmol/(s•l) in healthy male rabbit. Therefore, due to the different regulatory ranges of CGT concentration, we can not testify the deviation of our data from physiological limits. In our opinion, more detailed researches are necessary for the establishment of seasonal fluctuations in enzymatic activity of rabbit blood.

Conclusions

The seasonal fluctuations in serum indicators of protein-nitrogen metabolism in male rabbits of the Californian breed were established and ranged within the physiological values. There was a significant increase in the concentration of total protein by 7.0-12.0% and albumin by 9.7-9.8% in spring. At the same time, the lowest values of urea, creatinine and uric acid were annyaly less than the average physiological interval.

The total protein in the blood of males from Californian rabbit breed was set by us at a level slightly higher than the average physiological values: from 58.46±2.22 to 66.44±1.19 g/l. Deviations of temperature from comfortable indicators in winter and summer led to adaptive changes in rabbit organisms. As a result, there were the jumps in the values of creatinine in summer, uric acid: in summer and winter. We also registered increased activity of ALAT enzyme in winter and summer.

Our resuts can be useful for veterinarians, producers, and researchers. In case of keeping rabbits in rooms without artificial thermoregulation, it is necessary to consider the effect of uncomfortable temperatures on blood serum biochemical profile.

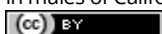
References

- Al-Eissa, M. S. (2011). Effect of Gestation and Season on the Haematological and Biochemical Parameters in Domestic Rabbit (*Oryctolagus cuniculus*). *British Biotechnology Journal*, 1(1), 10-17.
- Barnes, I.H., Bagnall, M.C., Browning, D.D., Thompson, S.A., Manning, G. & Newell, D.G. (2007). Gamma-glutamyl transpeptidase has a role in the persistent colonization of the avian gut by *Campylobacter jejuni*. *Microbial Pathogenesis*, 43(5-6), 198-207. DOI: 10.1016/j.micpath.2007.05.007.
- Botros, M., & Sikaris, K. A. (2013).The deritis ratio: the test of time. *The Clinical biochemist. Reviews*, 34(3), 117–130. PMID: PMC3866949
- Çetin, N., Bekyürek, T., & Çetin, E. (2009). Effects of Sex, Pregnancy and Season on some Haematological and Biochemical Blood Values in Angora Rabbits. *Scandinavian Journal of Laboratory Animal Sciences*, 36 (2), 155-162. doi.org/10.23675/sjlas.v36i2.180
- Couch, C. E., Movius, M. A., Jolles, A. E., Gorman, M. E., Rigas, J. D., & Beechler, B. R. (2017). Serum biochemistry panels in African buffalo: Defining reference intervals and assessing variability across season, age and sex. *PLoS One*, 12(5). doi: 10.1371 / journal.pone.0176830
- Daader, A., Al-Sagheer, A., Gabr, H., & Abd El-Moniem, E. (2018).Alleviation of heat-stress-related physiological perturbations in growing rabbits using natural antioxidants. *Spanish Journal of Agricultural Research*, 16(3), e0610. doi:http://dx.doi.org/10.5424/sjar/2018163-13184
- Dailidenok, V. N., & Noreyko, A. Yu. (2014). Morphological and biochemical indicators of the blood of rabbits of different breeds bred in the Republic of Belarus. *Bulletin Zootechnical science of Belarus*, 49, 76-84. (in Russian)
- Dontas, I. A., Marinou, K. A., Iliopoulos, D., Tsantila, N., Agrogiannis, G., Papalois, A., & Karatzas, T. (2011).Changes of blood biochemistry in the rabbit animal model in atherosclerosis research; a time- or stress-effect. *Lipids in health and disease*, 10, 139. doi:10.1186/1476-511X-10-139
- Doumas, B. T., Biggs, H. G., Arends, R. L., & Pinto, P. V. C. (1972). Determination of Serum Albumin. *Standard Methods of Clinical Chemistry*, 7, 175–188. https://doi.org/10.1016/B978-0-12-609107-6.50022-2
- Duda, Y. (2019). Nonspecific reactivity of the rabbits organism when exposed to cysticercosis. *Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies. Series: Veterinary Sciences*, 21(94), 132-135. https://doi.org/10.32718/nlvvet9424 (in Ukrainian).
- Duda, Yu. V., Prus, M. P., Kunieva, L. V., Shevchyk, R. S., & Blyskavka, K. Yu. (2018). Protein metabolism and enzyme activity during spirochaetosis of rabbits. *Mizhvidomchyi tematychnyi naukovyi zbirnyk: veterynarna medytsyna*, Kharkiv, 104, 254-257. (in Ukrainian).
- Enciklopediya klinicheskikh laboratornyh testov (1997). Moscow. Labinform (in Russian).
- Etim, N. N., Williams, M. E., Akpabio, U., & Offiong, E. E. A. (2014). Haematological parameters and factors affecting their values. *Agricultural Science*, 2(1), 37-47. https://doi.org/10.12735/as.v2i1p37
- Da Freitas, F. L.C., Yamamoto, B. L., de Freitas, W. L.C., Fagliari, J. J., de Almeida, K.S., Machado, R. Z., & Machado, C. R. (2011). Systemic inflammatory response indicators in rabbits (*Oryctolagus cuniculus*) experimentally infected with sporulated oocysts of *Eimeria stiedaei* (Apicomplexa: Eimeriidae). *Revista Brasileira de Parasitologia Veterinária*, 20(2), 121-126. https://dx.doi.org/10.1590/S1984-29612011000200006
- Gattani, A., Pathak A., Kumar, A., Mishra, V., & Bhatia, J. S. (2016). Influence of season and sex on hemato-biochemical traits in adult turkeys under arid tropical environment. *J Veterinary World*, 9(5), 530–534. doi: 10.14202 / vetworld.2016.530-534
- Gonchar A., Boyko A. & Gavrish O. (2020).Modern trends in the development of rabbit breeding in Ukraine. *Livestock today*, 1, 74-79
- Gbore, F. A., & Akele, O. (2010). Growth performance, haematology and serum Growth performance, haematology and serum biochemistry of female rabbits (biochemistry of female rabbits (*Oryctolagus cuniculus* rycctolagus cuniculus) fed dietary fumonisin. *Vet. Arhiv*, 80, 431-443.
- Gromadzka-Ostrowska, J., & Zalewska, B. (1985). Seasonal fluctuations in plasma protein fraction levels of chinchillas (*Chinchilla laniger*, M.). *Comparative Biochemistry and Physiology Part A: Physiology*, 80(2), 215-224. https://doi.org/10.1016/0300-9629(85)90544-4
- Gumerov, V. G. (2015). Kliniko-biohimicheskie pokazateli krovi krolikov pri sovместnom primenenii associirovannoj inaktivirovannoj vakciny s immunomoduljatorami. *Uchenye zapiski Kazanskoj gosudarstvennoj akademii veterinarnoj mediciny im. N.Je. Baumana*, 221, 56-59. (in Russian)
- Guo, M., Wu, F., Hao, G., Qi, Q., Li, R., Li, N., Chai, T. (2017). *Bacillus subtilis* Improves Immunity and Disease Resistance in Rabbits. *Frontiers in immunology*, 8, 354. doi:10.3389/fimmu.2017.00354
- Habeeb, A. A., Sharoud, M. N., Basuony, H. A., & Michael, M. I. (2018). Effect of Environmental Climatic Conditions on levels of Some Hormones, Vitamins and Trace Elements in Blood and Seminal Plasma of Rabbits. *Int J Biotechnol Recent Adv*; 1(1), 18-23. doi: 10.18689/ijbr-1000104

- Hristev, H., Penkov, D., Hallak, A. K., Kirova, M., Baykov, B., & Bliznakov, A. (2008). Serum protein changes in rabbits after chronic administration of lead and cadmium. *Journal of Central European Agriculture*, 9(1), 141-145.
- Kamyschnikov, V.S. (2003). Textbook of kliniko-biohimicheskoy diagnostiki. Interpresservis, Ekoperspektiva, Belarus' (in Russian).
- Makarova, V. G. & Makarova, M. N. (2013). Fiziologicheskie, biohimicheskie i biometricheskie pokazateli normy jeksperimental'nyh zhivotnyh. Sankt-Peterburg: LEMA. (in Russian)
- Marai, I. F. M., Habeeb, A. A. M., & Gad, A. E. (2008). Performance of New Zealand White and Californian male weaned rabbits in the subtropical environment of Egypt. *Animal science Journal*, 79(4), 472-480. doi: 10.1111/j.1740-0929.2008.00552.x
- Marai, I. F. M., Habeeb, A. A. M., & Gad, A. E. (2005). Tolerance of imported rabbits grown as meat animals to hot climate and saline drinking water in the subtropical environment of Egypt. *Journals Animal Science*, 81(1), 115-123. <https://doi.org/10.1079/ASC41710115000>
- Meineri, G., Giacobini, M. & Forneris, G. (2017). Evaluation of physiological parameters of the plasma oxidative status in rabbits. *Journal of Applied Animal Research*, 45(1), 315-319. Doi: 10.1080/09712119.2016.1190734
- Melillo, A. (2007). Rabbit Clinical Pathology. *Journal of Exotic Pet Medicine*, 16, 135-145. doi.org/10.1053/j.jepm.2007.06.002
- Miyake, K., Miyake, N., Kondo, S., Tabe, Y., Ohsaka, A., & Miida, T. (2009). Seasonal variation in liver function tests: a time-series analysis of outpatient data. *Annals of Clinical Biochemistry*, 46(5), 377-384. <https://doi.org/10.1258/acb.2009.008203>
- Molina, E., González-Redondo, P., Moreno-Rojas, R., Montero-Quintero, K., Chirinos-Quintero, N., & Sánchez-Urdaneta, A. (2017). Evaluation of haematological, serum biochemical and histopathological parameters of growing rabbits fed *Amaranthus dubius*. *Journal of Animal Physiology and Animal Nutrition*, 102(2), 525-533. doi: 10.1111/jpn.12791
- Nakyinsige, K., Sazili, A. Q., Aghwan, Z. A., Zulkifli, I., Goh, Y. M., & Fatimah, A. B. (2013). Changes in Blood Constituents of Rabbits Subjected to Transportation under Hot, Humid Tropical Conditions. *Asian-Australasian Journal of Animal Sciences*, 26(6), 874-878. doi: 10.5713/ajas.2012.12652
- Nowotny, A. (1979). Protein Determination by the Biuret Method. In: *Basic Exercises in Immunochemistry*. Springer, Berlin, Heidelberg, 168-169. doi:10.1007/978-3-642-67356-6_50
- Okab, A.B., El-Banna, S.G., & Koriem, A. A. (2008). Influence of environmental temperatures on some physiological and biochemical parameters of New-Zealand rabbit males. *Slovak Journal of Animal Science*, 41(1), 12-19.
- Ondruska, L., Rafay, J., Okab, A. B., Ayoub, M. A., Al-Haidary, A. A., Samara, E. M. & Supuka, P. (2011). Influence of elevated ambient temperature upon some physiological measurements of New Zealand White rabbits. *Veterinarni Medicina*, 56(4), 180-186. doi.org/10.17221/3150-VETMED
- Oohashi, E., Kimura, Y., & Matsumoto, K. (2019). Pilot study on serum C-reactive protein in pet rabbits: clinical usefulness. *Vet Rec Open*, 6(1): e000272. doi: 10.1136/vetreco-2017-000272
- Özkan, C., Kaya, A., & Akgül, Y. (2012). Normal values of haematological and some biochemical parameters in serum and urine of New Zealand White rabbits. *World Rabbit Sci.*, 20, 253-259. doi.org/10.4995/wrs.2012.1229
- Plank, M. S., Boskovic, D. S., Tagge, E., Chrisler, J., Slater, L., Angeles, K. R., & Angeles, D. M. (2011). An animal model for measuring the effect of common NICU procedures on ATP metabolism. *Biological research for nursing*, 13(3), 283-288. doi:10.1177 / 1099800411400407
- Prasad, K. (2008). Serum biochemical changes in rabbits on a regular diet with and without flax lignan complex following a high-cholesterol diet. *The International journal of angiology: official publication of the International College of Angiology, Inc*, 17(1), 27-32. doi:10.1055 / s-0031-1278276
- Reitman, S., & Frankel, S. (1957). A Colorimetric Method for the Determination of Serum Glutamic Oxalacetic and Glutamic Pyruvic Transaminases, *American Journal of Clinical Pathology*, 28 (1), 56-63. <https://doi.org/10.1093/ajcp/28.1.56>.
- Salisu, I. B., Shahid, A. A., Ali, Q., Rao, A. Q., & Husnain, T. (2018). Nutritional Assessment of Dietary Bt and Cp4EPSPS Proteins on the Serum Biochemical Changes of Rabbits at Different Developmental Stages. *Frontiers in Nutrition*, 5(49). doi: 10.3389/fnut.2018.00049
- Shaobo Li, Zhifei He, Ying Hu & Hongjun Li (2019) Shotgun proteomic analysis of protein profile changes in female rabbit meat: the effect of breed and age, *Italian Journal of Animal Science*, 18(1), 1335-1344, DOI: 10.1080/1828051X.2019.1659107
- Skabal, V. I. (2014). Funkcionuvannya ta tendenciyi rozvitku galuzej tvarinnictva v regionah. *Ekonomichnij analiz*, 75-80.
- Suckow, V. A., Brammer, D.W., Rush, H. G., & Chrisp, C. C. (2002). *Biology and disease of rabbits*. In *Laboratory Animal Medicine*. Fox, J. G., Anderson, L. C., Loew, F. M., & Quimby, F. W. (Eds.). New York, NY: Academic Press.
- Tietz, N.W. (1986). *Textbook of Clinical Chemistry*, W.B.Saunders Company, Philadelphia. *Veterinary Manual*. By S.E. Fielder. Serum Biochemical Reference Ranges. Retrieved from <https://www.msdevetmanual.com/special-subjects/reference-guides/serum-biochemical-reference-ranges>
- Vlizlo, V. V., Fedoruk, R. S., & Ratych, I. B. (2012). Laboratorni metody doslidzhen'u biologii', tvarynnyctvi ta veterynarnij medycyni: dovidnyk. L'viv: Spolom. (in Ukrainian).
- Yaqub, L. S., Kawu, M. U. & Ayo, J. O. (2013). Influence of reproductive cycle, sex, age and season on haematologic parameters in domestic animals. *Journal of Cell Animal Biology*, 7(4), 37-43. doi.org/10.5897/JCAB12.063.

Citation:

Duda, Y.Y., Prus, M.P., Shevchik, R.S., Koreyba, L.V., Mylostyvyi, R. V., Samoiliuk, V.V. (2020). Seasonal influence on biochemical blood parameters in males of Californian rabbit breed. *Ukrainian Journal of Ecology*, 10(4), 262-268.



This work is licensed under a Creative Commons Attribution 4.0. License