Haematological parameters and production characteristics of novel Romanov × Hissar crossbred sheep adapted to European steppe climate

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Abstract

The study presented some haematological parameters and production characteristics of novel crossbred Romanov × Hissar and purebred Romanov sheep. A number of biochemical and haematological tests were performed to assess the level of adaptation of F1 crossbred progeny to local rearing conditions and evaluate its potential economic benefits for industrial production. The newly created crossbreed demonstrated significant increase in carcass production characteristics, with pre-slaughter weight increased by 29.2%, slaughter weight - by 39.7%, cooled carcass weight - by 40.5% and total yield – by 8.2% compared to Romanov purebreed. The meat quality was also increased: fat content remained at the same level (6.6-6.8%), carcasses of F1 crossbreed lambs had higher relative weight of muscle tissue (78.8% vs. 73.3%), increased content of the 1st grade meat (94.6% vs. 91.6%), and higher caloric value of the meat (by 8.7%) compared to the parent purebreeds. An increased erythrocyte (by 3.1%) and leukocyte (by 6.45%) count was noted in the F1 rams. Haemoglobin level in F1 lambs was increased by 4.2%. Protein metabolism parameters were improved: blood serum total protein level – by 11.2%, albumin – by 11.4%, globulin – by 10.4%, suggesting intensified protein metabolism in F1 lambs; this was also evidenced by the increased activities of aspartate transaminase (by 3.9%) and alanine transaminase (by 16.6%) in F1 lambs over Romanov purebred animals. Blood serum of crossbred lambs had higher bactericidal (by 1.92%), lysozyme (by 3.53%), and phagocytic activity. The increased haematological and biochemical parameters and enhanced antibacterial properties of serum indicate the enhanced metabolic activity in F1 crossbreed sheep and underlay the improved meat production parameters. Obtained results demonstrate increased adaptation of the novel crossbred to industrial rearing conditions and local microclimate of steppe zone of Ukraine, which is undergoing gradual aridization.

Key words: *Sheep breeding; Romanov × Hissar crossbreed; meat quality; haematology; animal resistance; blood enzymes*

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Introduction

The economic and social significance of agricultural products of sheep industry is determined by the needs of the country, the possibility of efficient production, the ability to use available natural and logistical resources. One of the main problems of modernity, faced by the agro-industrial complex of Ukraine, is the search for the ways and methods to increase food production as a basement for the food security of the country (Pokhyl and Mykolaichuk, 2020). Modern lamb production technologies are mainly focused at the breeding of young sheep with a life cycle less than one year. Such approach ensures cost-effective lamb production, as young sheep use forage with the highest efficiency and quickly accumulate muscle weight (Pokhyl and Mykolaichuk, 2019; Turkyilmaz and Esenbuga, 2019). Animal protein is the most valuable component of meat, and it is being deposited with the highest activity during the first eight months of lamb's life (Bórnez et al., 2009; Santo da Cruz et al., 2017). At older age, the increase in sheep carcass weight is mainly due to the deposition of fats, which reduces both the nutrient value of meat and the economic efficiency of its production (Hernandez et al., 2020).

Studies weight (Rasali et al., 2006; Pokhyl and Mykolaichuk, 2019; Turkyilmaz and Esenbuga, 2019) had confirmed that an efficient way to increase lamb production, and to improve meat quality is the use of composite two-breed and three-breed industrial crossbreeding of Dams of different breeds to a service Sires of intensive meat breed. In this case, the offspring lambs demonstrate increased meat productivity.

At the same time, the efficient stud breeding strategies have to evolve a set of marker parameters enabling optimal selection of the offspring for the further breed improvement. Proper chose of marker parameters is pivotal for obtaining fast and reliable results to guide the process of crossbreeding. Such markers must informatively reflect physiological condition of sheep to direct the selection of animals for further cross breeding.

Blood is an internal medium and the central communication system of the body, it have vital functions and maintains the integrity and functionality of animal organism. As one of the body tissues, blood is also the most informative interior indicator (Bórnez et al., 2009). Blood performs multiple functions, such as respiratory, transport, trophic, protective, homeostatic (Kaneko et al., 2008), so its parameters can be used to quantitatively estimate physiological condition of the body, as well as animal production characteristics (Braun et al., 2010). Blood composition largely depends on the species, breed, age, gender (Santo da Cruz et al., 2017), feeding and housing conditions of animals (Novoselec et al., 2021). Haematological indicators provide valuable and objective data to assess the internal environment of the body, the level and the direction of metabolic processes, and the activity of body protective systems, therefore they are of exceptional importance in the evaluation of farm animals (Wang et al.; 2015 Rahman et al., 2018). An essential role in the assessment of sheep internal homeostasis plays the content of blood cells, as a complex indicator of the body's physiological state (Agar, 2009). During the creation of the meaty breeds, protein metabolism should be assessed, as it underlies the capacity of animals to gain weight quickly and efficiently as per unit of the forage. A range of serum parameters are used to assess the level of protein metabolism in animals, such as total protein content, ratio between different globulin fractions, and enzymatic activity (Turkyilmaz and Esenbuga, 2019).

In addition to the quantitative and qualitative production parameters, another pivotal trait in sheep breeding is animal disease resistance (Sotirov et al., 2011). Such characteristics as serum bactericidal activity (Pal and Chakravarty, 2020), lysozyme and phagocytic activity (Taylor, 1983), presence of immunoglobulins (Miglio et al., 2018) and other antimicrobial peptides (Katsafadou et al., 2018) are important indicators of the potential resistance in crossbreed animals, and strongly affect their economic value. Thus, to evaluate the production potential of the novel breeds and the level of their adaptation to local environment, in addition to carcass measurements, in present study we used a set of blood biochemical and haematological parameters, due to their highly informative value.

Romanov breed remains one of the most popular sheep breeds in European countries (Dýrmundsson and Niżnikowski, 2010). With that, more than 30 years had passed since the breed was introduced in Ukraine (Suharl'ov, 2012). During this period, the climate in Europe had changed due to the global warming, as many areas in the world are affected by aridization process (Ceglar et al., 2019), when local climate become dryer (Jacob et al., 2018). Steppe zones of Eurasia are at special risk, and this trend is continuing and even aggravating during recent decades (Joy et al., 2020). To ensure sustainable sheep husbandry, new breeds adapted to hot and dry climate of specific regions are needed (Sejian et al., 2018; Azhimetov et al., 2020). Therefore we aimed our research at the creation of a novel sheep crossbreed through the integration of selected traits of Hissar breed into Romanov breed. Hissar sheep breed was created on the basis of Central Asian autochthonous sheep (Aziz, 2020), thus it is well adapted to dry arid climate. However, meat production characteristics of Hissar sheep in the conditions of most European areas are not optimal.

In this article, the results of the cross-breeding of purebred Romanov Dams to local Ukrainian crossbred Sires of Hissar breed was presented, which resulted in a new F1 crossbreed with qualitative and quantitative characteristics superior to the parent breeds. Better adaptation of crossbreed F1 animals to local environment, and their high production characteristics were confirmed by carcass morphometric measurements, meat quality tests, and haematological and biochemical indicators. The proposed approach to the directed crossbreeding and complex evaluation of its results based on haematological, biochemical and pathogen-resistance tests can be useful in the creation of new sheep breeds better adapted to the continental climate, enabling sustainable animal husbandry in changing environments across the Eurasia and other world areas suffering from aridization.

Materials and methods

The experiment was conducted in the sheep breeding enterprise "Terra Rich" LLC located in Polohivsky district, Zaporizhia region, Ukrainian steppe zone. Purebred Romanov Dams were crossed to local Ukrainian crossbreed Sires of Hissar breed (Pokhyl and Mykolaichuk, 2020). Study groups included 10 rams (8-months old) of the resulted new F1 crossbreed (experimental arm), and 10 purebred Romanov rams of the same age (control arm). Test animals were sampled from the respective flocks randomly. Sheep productivity and meet quality was studied by control slaughter of lambs according to common methods (Thomas, 2010; Turkyilmaz and Esenbuga, 2019). Carcasses were weighted to determine pre-slaughter and slaughter weight, cooled carcass weight, and content of fats. Primal cuts of the carcasses were made to determine the content of meat of the 1st and 2nd grade. The meatiness ratio was calculated as a ratio of yield (meat+fat) to the bone weight.

The morphological composition of the carcasses was studied after 24 hours of cooling to determine the yield of meat pulp, bone and fat. The caloric content (CC) of meat was calculated from the actual protein (P) and fat (F) content of the product:

$$CC = (P \times 4{,}1) + (F \times 9{,}3),$$

where CC = energy value of meat, kcal / 100 g of product;

P = amount of protein, g / 100 g of meat;

F = amount of fat, g / 100 g of meat.

The functional condition of sheep was estimated by the morphological and biochemical parameters of blood. Blood samples were collected from jugular vein of animals in the morning before feeding. Red blood cells (RBC, erythrocytes) and white blood cells (WBC, leukocytes) were counted in the Goryaev counting chamber (Wang et al., 2015).

Haemoglobin level was measured by the colorimetric Sahli's method. Total blood protein level was measured by refractometry, and its albumin and alpha-, beta- and gamma-globulin fractions were assessed by colorimetric methods (Kaneko et al., 2008). The activities of alanine transaminase (ALT) and aspartate transaminase (AST) in sera were measured by the Reitman-Frankel assay with α-ketoglutaric acid and phenylhydrazone. Optical density of the reaction product was measured at 510 nm, enzymatic activity was expressed in units/litre (U/L).

Animal resistance level was assessed by lysozyme activity, bactericidal activity and phagocytic activity in serum (Taylor, 1983). Serum lysozyme activity was measured by turbidimetric assay. Micrococcus Lysodeikticus culture was prepared, grown for 1 day, diluted with 0.5% NaCl and standardised to contain 109 cells per 1 mL. This standardized culture was diluted 1:1 with 0.5% NaCl, and 2 ml of the culture was added to 1 mL of blood serum sample, pre-diluted with 0.5% NaCl (1:10, pH 7.2). 2 ml of 0.5% NaCl + 1 mL of standardised culture was used as a control. Optical density of sera and control samples were measured by photoelectrocolorimeter FEC-56 (ZOMZ, RF) using 10 mL cuvettes and green filter. After incubation at 37°C, the optical densities were measured again, and the % of lyzis was calculated by the formula:

$$\text{LYZ\%} = \frac{(D0-D1)\ \text{x}\ 100}{D0} - \frac{(Dc0-Dc1)\text{x}\ 100}{Dc0},$$

where LYZ% = % of lyzis, D0 = optical density of the sample sera before incubation, D1 = optical density of the sample sera after incubation, Dc0 = optical density of the control sample before incubation, Dc1 = optical density of the control sample after incubation.

To measure bactericidal activity of blood serum, 0.8 mL of blood was added to sterile cuvettes with 0.2 mL of 2.5% sodium citrate solution and added 0.1 mL of 1-day agar culture of *Staphylococcus aureus* (strain № 279), diluted with saline to the equal concentrations of *staphylococcus* per 1 mL. The control cuvette contained the same volume of saline. After adding *S. aureus*, the content of the cuvettes was thoroughly mixed with a platinum loop, and optical densities of the test (D0) and control cuvettes (Dc0) were measured against distilled water on FEC-56 photoelectrocolorimeter with a green filter.

Then all the cuvettes were placed in a thermostat for 3 hours at 37°C, and optical densities of the test (D1) and control cuvettes (Dc1) were measured again. Percentage of bactericidal activity (BCA%) of blood serum was calculated by the formula:

$$\text{BCA\%} = 100 - \frac{(D1-D0)\text{x }100}{Dc1-Dc0}$$

To quantify blood phagocytic activity, the sterile centrifuge tubes were filled with 0.2 ml of 2% sodium citrate, 0.1 ml of test blood and 0.05 ml of microbial sample was added, which contained 25×10^6 microbial cells (i.e., 500×10^6 microbial cells in 1 mL) according to the optical standard of turbidity. The microbial culture was *S. aureus* strain № 997. The tubes with the mixture was gently shaken, placed for 30 minutes in a thermostat at 37°C, and then centrifuged at 2000 rpm for 15 min until the separation of the 3 layers: upper yellow layer of plasma, lower layer of erythrocytes and the middle silver film between them, a layer of leukocytes. This middle layer was aspirated with a Pasteur pipette, and 3-5 smears were made from it, which were fixed with methyl alcohol and stained by the Romanovsky-Giemsa method (Kaneko et al., 2008). Up to 200 leukocytes (neutrophils) were counted in each smear. Phagocytic number (PHA) was determined as a proportion of leukocytes involved in phagocytosis (in %).

All quantitative parameters were processed statistically by calculating their means and standard deviations. The differences between mean values were tested by Student's t-test and considered significant at P<0.05.

During experimentation, we adhered to international requirements of the "The European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes" (Strasbourg, 1986) and to the relevant Law of Ukraine No 3447-IV "On Protection of Animals from Cruel Treatment". The protocol of the study was approved by the ethic committee of Dnipro State Agrarian and Economic University.

Results

It was established that Romanov × Hissar crossbred lambs (F1) at 8 months of age, under appropriate conditions of rearing and fattening, had higher pre-slaughter, slaughter and cooled carcass weight compared to purebred Romanov sheep (Table 1).

Table 1. Carcass measurements of experimental lambs with different genotypes (8 months of age, *n* = 5)

Parameter	$\bar{X} \pm S\bar{x}$	Cv, %	$\bar{X} \pm S\bar{x}$	Cv, %
Breed	R		F_1	
Weight, kg:				
Pre-slaughter	32.2 ± 1.03	7.12	41.6 ± 1.18**	6.36
Slaughter	14.1 ± 0.383	6.06	19.7 ± 0.78***	8.83
Cooled carcass	13.1 ± 0.45	7.65	18.4 ± 0.61***	7.34
Internal fat	0.89 ± 0.03	7.88	1.22 ± 0.033***	6.10
Resulting yield, %	43.8		47.4	

Note. R = Romanov pure breed, F_1 = Romanov × Hissar cross breed, generation 1. The table shows mean values ($\bar{X}$) ± standard deviations ($S\bar{x}$) of mean. Cv = coefficient of variation ($S\bar{x}$*100%/$\bar{X}$). ** *P*<0.01, *** *P*<0.001.

Table 2. Blood haematological parameters of experimental lambs with different genotypes (8 month of age, *n* = 10)

Breed	Haemoglobin, g/L	RBC, $\times 10^{12}$/L	WBC, $\times 10^{9}$/L
R	102.84 ± 0.61^{a}	8.45 ± 0.34^{a}	8.67 ± 0.44^{a}
F_1	107.18 ± 1.78^{b}	8.71 ± 0.35^{b}	9.23 ± 0.51^{b}

Note. R = Romanov purebreed, F1 = Romanov × Hissar crossbreed, generation 1. The table shows mean values ± standard deviations of mean. RBC = red blood cells, WBC = white blood cells

In terms of pre-slaughter live weight, F1 lambs outperformed the animals from the control arm by 29.2%, and by slaughter weight – by 39.7%. Post-slaughter yield in F1 lambs was also higher: 47.4% vs. 43.8% in the control arm. F1 crossbreed lambs had higher weight of carcass primal cuts: meat of the first grade comprised 94.6% of the carcass weight, the second grade – 5.4%, against 91.6% and 8.4%, respectively, in the control animals. Carcasses of F1 crossbreed lambs had the relative weight of muscle tissue 78.8%, and bones – 21.2%, against 73.3% and 26.7% in purebred animals, respectively. The meatiness ratio in F1 crossbreed lambs was also higher: 3.7 vs. 2.7 in Romanov lambs. The advantage of crossbred over purebred Romanov lambs in terms of caloric content of muscle tissue was 8.7%.

The obtained morphometric results reflect increased level of assimilative metabolic processes, which can be monitored by haematological parameters (Table 2). F1 crossbreed sheep had a higher count of red blood cells compared to the purebred Romanov sheep (increase by 3.1%). Since blood is involved in delivering oxygen to the body, the intensity of respira-

Table 3. Blood protein content and transaminating activity in experimental lambs with different genotypes (8 months of age, *n* = 10)

Parameter	Breed	
	R	F_1
Total protein, g/L	62.66 ± 0.59^{a}	69.32 ± 0.58^{b}
Albumin, g/L	23.81 ± 0.49[illegible]	26.44 ± 0.52^{b}
Total globulins, g/L	38.85 ± 0.39^{a}	42.88 ± 0.48^{b}
α-globulin, g/L	13.16 ± 0.33^{a}	14.51 ± 0.39^{b}
β-globulin, g/L	8.15 ± 0.27^{a}	9.36 ± 0.29^{b}
γ-globulin, g/L	17.54 ± 0.29^{a}	19.01 ± 0.37^{b}
ALT, U/L	1.26 ± 0.02^{a}	1.31 ± 0.03^{b}
AST, U/L	0.42 ± 0.01^{a}	0.49 ± 0.01^{a}

Note. R = Romanov purebreed, F1 = Romanov × Hissar crossbreed, generation 1. The table shows mean values ± standard deviations of mean. AST = aspartate transaminase, ALT = alanine transaminase tion is primarily determined by the level of haemoglobin in erythrocytes. Haemoglobin level in F1 crossbreed lambs was higher than in lambs of Romanov breed by 4.2%.

Analysis of the content of white blood cells revealed some differences between the study groups (+6.5% increase in F1 crossbreed), while their count was within the range of physiological norm for sheep of this age (5.8-10.6 × 10^9/L).

The level of resistance of the animal organism to environmental conditions, i.e. feeding and housing, the level of productive traits, including meatiness and wooliness of sheep is largely defined by the content of proteins in the blood, as they are involved in complex physiological processes in the body. Guided by this, we studied total content of protein and its proportion of albumin-globulin fractions (Table 3).

The total protein concentration in blood serum of F1 crossbreed lambs was significantly higher (by 11.2%) than in purebred Romanov flock. Genotypic variability in albumin content in experimental sheep blood had the same patterns as the total protein.

The crossbreed lambs had 11.04% higher levels of blood albumin; globulin was also increased by 10.4% compared to Romanov breed. Globulin fractions in the blood serum of experimental lambs were distributed unevenly: α- and γ-globulins accounted for 78-79% of the total serum globulin level.

With that, β-globulins in serum comprised 21-22% of total globulin fraction, depending on breed genotype, and were increased (by 14.8%) in crossbred compared to purebred lambs. These data suggest improved resistance potential of the crossbreed sheep.

The activities of alanine transaminase (ALT) and aspartate transaminase (AST) enzymes in the both breeds. F1 crossbreed had 4.0% increase in AST, activity, and 16.6% increase in ALT activity.

The adaptive capabilities of experimental lamb genotypes and their non-specific resistance to various diseases were further investigated by the activity of serum lysozyme (LYZ), bactericidal (BCA) and phagocytic (PHA) activities in experimental animals (Table 4).

Crossbred sheep had increased indicators of humoral immunity compared to Romanov lambs. Lysozyme activity demonstrated the highest differences (by 3.53 absolute per cent units). Bactericidal activity was also higher in crossbreed lambs.

The cellular link of nonspecific immunity was characterized by phagocytic activity in blood serum. Overall, high levels of this parameter was found in the both genotypes; however, phagocytic activity

Table 4. Serum nonspecific resistance parameters of experimental lambs with different genotypes (8 month of age, *n* = 10)

Breed	Activity, %		
	LYZ	BCA	PHA
R	38.16± 0.63[a]	52.72 ± 0.62[a]	45.75 ± 0.41[a]
F_1	41.69 ± 0.74[b]	54.64 ± 0.52[a]	46.29 ± 0.62[a]

Note. R = Romanov purebreed, F1 = Romanov × Hissar crossbreed, generation 1. The table shows mean values ± standard deviations of mean. LYZ = lysozyme activity, BCA = bactericidal activity, PHA = phagocytic activity was slightly higher in crossbreed lambs, which suggest good potential of newly created breed in terms of nonspecific resistance. Thus, comparative study of both humoral and cellular links of non-specific immunity showed that the serum of crossbreed lambs had higher resistance potential than in purebred animals.

Based on the data obtained, F1 crossbred lambs outperformed the Romanov purebred animals in production characteristics. This improvement is based on the increased metabolic activity and non-specific resistance of animals. Intensification of metabolism during breeding process could be efficiently monitored by blood parameters, which reflect a higher level of redox and catabolic processes in crossbreed animals; this could be due to the heterosis effect.

Discussion

Historically, sheep breeding has always been an integral part of the national economy in Ukraine, due to a number of socio-economic and national reasons. It is essential, and in some cases indispensable source of such materials as wool, lamb, mutton, milk, cheese, lambskin, fleece, fur, and leather. However, the recent trend is the breeding of sheep for meat production, due to the highest profitability of meat breeds. Increasing the production of marketable sheep products and improving their quality is possible through breeding programs, where crossbreeding takes a leading role, along with optimizing housing and feeding conditions of the animals (Pokhyl and Mykolaichuk, 2019).

Romanov breed of sheep originates from North-European short tail sheep (Dýrmundsson and Niżnikowsk, 2010). It has good production parameters, high prolificacy and is well adapted to moderate European climate. Romanov breed is widely used in the crossbreeding programs for improving the traits of other sheep breeds across Europe (Dýrmundsson and Niżnikowsk, 2010), Asia (Turkyilmaz and Esenbuga, 2019) and Northern America (Đuričić et al., 2019). However, the early studies noted a depressive effect of excessive summer heat on male activity and female fertility in Romanov breed. Since its introduction in Ukraine in 1991, the breeding areal of Romanov sheep was limited to moderate forest-steppe zone and not included southern arid steppe and Crimean zones (Suharl'ov, 2012). It is well established now that air temperature and precipitations greatly affect Romanov sheep productivity (Al-Dawood, 2017).

With that, environmental conditions for sheep husbandry are being changing globally. Some regions of the world already experience dramatic shifts in agro-climatic conditions, including heat stress, aridization and desertification; those processes will continue in upcoming decades (Ceglar et al., 2019). According to available data and current prognoses, Southern and Eastern Europe, and Mediterranean zone will be mostly affected (Joy et al., 2020). Due to global climate changes, the historical European agro-climatic zones are moving northward (Jacob et al., 2018), decreasing the area of forest zone. Thus, local climate in many regions of Europe, including Ukraine, had already changed: southern areas of steppe zone undergo aridization process, which affects the production environment for sheep breeding. In such conditions, the classical sheep breeds lose their productivity, as dry hot climate is not optimal for them. For sustainable sheep husbandry, the novel approaches to sheep breeding are needed, to obtain new breeds, which would be better adapted to the changing environment (Sejian et al., 2014), including heat stress (Bhat et al., 2014; Azhimetov et al., 2020) and climate aridization. In this article we present our results on the development of a new sheep crossbreed, which is based on Romanov breed but has increased production characteristics. This was achieved by introducing genotypic features from Hissar sheep breed, which originate from Central Asia and carries the traits of adaptation to arid and semi-arid environment (Aziz, 2020). To address this aim, we developed both the strategy of targeted cross-breeding, and a set of quantitative parameters, to guide such a breeding process, and to quantitatively asses its results.

Numerous studies (Rasali et al., 2006; Pokhyl and Mykolaichuk, 2019) had confirmed that the increased lamb production, and the improved meat quality could be achieved by composite two-breed and three-breed crossbreeding of Dams of different breeds to service Sires of intensive meat breed. In this case, the offspring lambs usually have increased meat productivity. To that end, we mated Dams of purebred Romanov sheep breed to Sires of local Ukrainian Hissar crossbreed, according to our previous results (Pokhyl and Mykolaichuk, 2020).

Increasing the levels of animal meatiness and wooliness during sheep breeding requires comprehensive research of their physiological and biochemical indicators important in establishing viability, resistance, productivity and adaptability of animals to the appropriate conditions of commercial production. Obtained F1 crossbreed lambs at 8 month of age demonstrated better growth performance and weight gain under the same feeding conditions: their live weight was higher by 29%, as compared to Romanov purebreeds (Table 1). The weight of cooled carcass of crossbreeds was increased, in average, by 41%. This trend suggests the manifestation of the heterosis effect in industrial sheep crossbreeding. With that, the meat quality remained high, as the relative fat content was not increased in the crossbreed compared to the purebred lambs (6.6 and 6.8%, respectively). Carcasses of F1 crossbreed lambs had higher relative muscle/bones ratio. Primal cuts demonstrated that crossbreed lambs yielded more meat of the 1st grade. Also, meat of F1 crossbreeds had better meatiness ratio and caloric value. These results clearly demonstrate superior production characteristics of newly created Romanov × Hissar crossbreed, which outperform Romanov purebreed in the same rearing conditions. Thus, crossbreed animals are much better adapted to the local environmental and rearing conditions. To investigate the physiological basis of those differences between the animals with different genotypes, we studied several key markers of their metabolism. Adequate assessment of the internal environment of the body, the activity and the direction of metabolic processes and performance of immune system could be achieved through the monitoring of morphological and biochemical haematological indicators. The morphological composition of blood is one of the most important diagnostic parameters, as it is directly related to the level of general metabolism in the body during the formation of productive traits in postnatal ontogenesis (Bórnez et al., 2009).

The results demonstrated specific differences between purebred and crossbred genotypes in their haematological parameters, i.e. blood morphological composition (Tables 2). Thus, increased number of erythrocytes (red blood cells, RBCs) was found in the blood of F1 crossbred lambs over the purebred Romanov lambs (Table 2). It is well known that RBC constitute the majority of blood cells and have essential role in oxygen/CO2 transport in the organism. Also they are directly involved in regulating the acid-base balance in the body.

Crossbred lambs also had higher haemoglobin content in the blood than purebred Romanov rams (Table 2). The increased content of erythrocytes and haemoglobin in the crossbreeds indicates an increased oxygen capacity of their blood, and better respiratory-oxidative capacity, which supports the intensified level of metabolic processes in crossbreed animals.

The crossbreed lambs also showed increased number of leukocytes (white blood cells, WBCs) (Table 2). This could be a sigh of more active cellular immunity of the animals and improved resistance to the diseases. WBC increase can indicate the adaptation to unfavourable environmental factors (Suharl'ov, 2012). Since leukocytes have numerous protective functions, they can be involved in the formation of cellular immunity by phagocytosis, participate in inflammation reactions and the repair process in damaged tissues, capture and digest bacteria and other pathogens. Determination of WBC in blood is essential in studying of young sheep reactivity under conditions of industrial breeding, as WBC content is indicative of the responses to various environmental factors.

An essential parameter of animal metabolism is a total blood protein content, which indicates the balance between the synthesis and catabolism of proteins in the body (Kaneko et al., 2008; Braun et al., 2010). Blood protein content is a reliable indicator of the body provision with nutrient monomers, especially proteinogenic amino acids, because they are in constant exchange with proteins during body's active growth, and perform numerous functions. Crossbred lambs had increased blood total protein content compared to Romanov purebreeds (Table 3). Such increase indicates the elevated proportion of proteins in peripheral tissues and the higher intensity of protein metabolism (Pokhyl and Mykolaichuk, 2019). The proportion between the major blood serum protein classes, i.e. albumins and globulins, is of particular interest, as it provides information about the metabolic processes in animal organism, affecting the formation of productive traits in postnatal ontogenesis. Albumins constitute the majority of blood serum proteins in sheep and play a leading role in regulating the osmotic pressure, acid-base balance; they are the universal binding substances, and comprise the reserve of proteins in the body. Albumins are essential in the maintaining of the growth and muscle tissue gain (Rahman et al., 2018), thus their increased content is a quantitative measure of a higher protein metabolism in Romanov × Hissar F1 crossbreeds.

Gamma-globulins are essential for the humoral immune processes. Alpha- and beta-globulins take part in fat metabolism, iron transport, lipid, carbohydrate metabolism, and play roles in regulating steroid hormones, vitamin A and D (Braun et al., 2010). Notably, the crossbreed animals had higher values of both albumin and globulin content (Table 3). All studied classes of globulins, i.e. α-, β- and γ-globulins, were uniformely elevated in the crossbreed animals. Such increase indicates more intensive protein metabolism in F1 crossbreeds, which constitute the basis for the increased meat productivity.

Recent studies show that the improvement of growth, development, and productivity in newly developed animal breeds requires comprehensive research of the underlying biochemical processes (Novoselec et al., 2021). Considering the biological significance of transaminating enzymes (aminotransferases), their leading role in regulating the intensity of redox processes, transamination, activation of nitrogen metabolism, synthesis of urea and creatinine, those enzymes support the active growth and development in highly-productive meat breeds at the postnatal stage of ontogenesis. Alanine transaminase (ALT) and aspartate transaminase (AST) have low specificity to liver, and rather indicate the overall process of transamination and protein metabolism in the body (Kaneko et al., 2008). The differences in transaminase activity found between the sheep breeds (Table 3) reflect the role of intensified transamination in F1 crossbred animals in maintaining their protein metabolism and regulating the intensity of redox processes necessary for the young organism's growth and development. Noteworthy, both ALT and AST participate in the adaptation to heat stress and drought stress in sheep and goats (Azhimetov et al., 2020). Higher activity of the both enzymes in crossbred over purebred sheep further indicates more intensive protein metabolism in the crossbreed, and suggest increased capacity to adaptation to hot rearing conditions (Bhat et al., 2014; Sejian et al., 2018). This may partially explain better adaptation of Romanov × Hissar crossbreed over Romanov sheep to the local conditions of steppe zone of Ukraine. We supposed that ALT/AST activities could characterize assimilative processes of the postnatal ontogenesis in animals obtained through industrial crossbreeding and under the influence of the heterosis effect. The identified patterns of biochemical parameters for crossbred vs. purebred lambs probably indicate the high potential of Romanov × Hissar crossbred animals in terms of their production characteristics and increased level of natural resistance. The improved balance between feeding and growth of the novel crossbreed suggest its higher economic benefits for industrial husbandry (Hernandez et al., 2020).

Not only high productivity and growth performance, but also disease resistance is one of the key traits which direct modern animal breeding strategies (Sotirov et al., 2011; Pal and Chakravarty, 2020). When the data on white blood cells (Table 2) and the content of gamma-globulins, which possess immune functions (Table 3), are analysed together, one can suggest that both cellular and humoral immunity in the crossbred lambs has greater capacity than in the Romanov purebreeds. To test this hypothesis, we studied in details the non-specific resistance in lambs of the both genotypes.

Crossbred lambs had improved serum bactericidal activity (by 3.6%) (Table 4). Analysis of the total bactericidal activity of blood serum in experimental young sheep deserves special attention, being an integral parameter in the formation of nonspecific natural resistance (Taylor, 1983). Higher bactericidal activity in the blood of crossbred lambs is associated with the presence of numerous soluble substances (complement, antibodies, lysozyme, properdin and antimicrobial peptides) in ovine plasma, capable of neutralizing and dissolving microbial cells. Bactericidal activity may demonstrate significant variations depending on the physiological condition of the animal and the influence of numerous environmental factors (Miglio et al., 2021). Thus, bactericidal activity of the whole blood could be an informative marker of animal nonspecific resistance.

A pivotal role in the formation

of natural nonspecific immunity belongs to lysozyme (Sotirov et al., 2011). Crossbred lambs had increased serum lysozyme activity (by 9.3%), whereas phagocytic activity demonstrated only minor improvement (by 1.2%) (Table 4), as compared to the purebred Romanov lambs. Lysozyme has hydrolytic, bacteriostatic, and bactericidal activity and is used as an indicator of antimicrobial defence in animals (Kaneko et al., 2008). In addition, lysozyme contributes to the regulation of inflammatory response, amelioration of oxidant stress, and participates in the immune homeostasis by activating regulatory T-lymphocytes. Increased serum lysozym activity in crossbred lambs (Table 4) provides an adequate representation of their improved defence processes, which in part can explain their better adaptation to the local environment, which is reflected by higher weight gain (Table 1). However, lysozyme is efficient only in combination with other defence mechanisms, e.g. phagocytosis, antibodies, complement and lactoferrin, increasing susceptibility of bacteria (Katsafadou et al., 2019). Thus different defence mechanisms and systems taken in their complexity may be highly informative in assessing natural resistance of newly created sheep breeds.

According to numerous studies, the indicators of cellular and humoral immunity reflect the organism's viability under the conditions of modern industrial breeding and may be used to predict the economic value of novel sheep breeds (Hernandez et al., 2020). Among the studied animals, the crossbred lambs demonstrated higher expression of both cellular and humoral immunity, which positively affects the retention rates of lambs. This agrees with our previous studies, wherein the crossbred youngsters had better natural resistance of the organism, which was confirmed by survival rate of lambs during the period from birth to weaning (Pokhyl and Mykolaichuk, 2020).

Thus, the integral study of the hematological parameters, immunity status and key biochemical indicators, taken in their integrity, gives an idea of the animal's physiological condition with the possibility of predicting performance of the newly created sheep crossbreeds, and allows the breeder to control and guide the breeding processes toward the creation of novel sheep breeds adapted to hotter and drier climate.

Conclusions

Global climate changes require fast adaptation in the agricultural technologies. In this paper, was presented the novel approach to the targeted sheep breeding, which involves inclusion of the traits from Hissar sheep into Romanov breed to develop the highly productive sheep adapted to dry and hot aridizing climate. The developed Romanov × Hissar crossbreed sheep is highly adapted to local conditions of steppe zone of Ukraine; it has superior growth and production characteristics and significant increase in meat quantity and quality. The advanced exterior characteristics of the novel breed are based on the intensified metabolic processes and the increased non-specific resistance capacity of lambs. While testing the approach of the targeted breeding, we identified a set of key quantitative parameters, which enable the breeder to monitor and guide the selection process: haematological and biochemical parameters and the indicators of non-specific resistance of the animals. Taken together in their integrity, those parameters adequately measure basic metabolic processes and adaptive potential of animals, and could be used to direct the further breeding programs.

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Hematološki parametri i proizvodne osobine novog romanov × hissar križne pasmine ovce prilagođene europskoj stepskoj klimi

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U studiji su predstavljeni neki hematološki parametri i proizvodne karakteristike nove križne pasmine romanov × hissar i čistokrvne romanov ovce. Obavljen je niz biokemijskih i hematoloških testiranja u svrhu procjene razine prilagodbe F1 potomaka križane pasmine lokalnim uvjetima uzgoja i procjene potencijalne ekonomske koristi za stočarsku proizvodnju. Novostvorena križana pasmina pokazala je znatno povećanje proizvodnih karakteristika trupla, s masom prije klanja većom za 29,2 %, masom za vrijeme klanja za 39,7 %, masom ohlađenog trupla za 40,5 % i ukupnim prinosom većim za 8,2 % u usporedbi s čistokrvnom romanov ovcom. Povećala se i kvaliteta mesa; udio masnoća ostao je na istoj razini (6,6-6,8 %), trupla F1 janjadi križane pasmine imala su veću relativnu masu mišićnog tkiva (78,8 % u usporedbi sa 73,3 %), povećani udio mesa 1. razreda (94,6 % u usporedbi s 91,6 %) i veću kalorijsku vrijednost mesa (za 8,7 %) u usporedbi s čistokrvnim roditeljima. Povećani broj eritrocita (za 3,1 %) i leukocita (za 6,45 %) uočen je kod F1 ovnova. Razina hemoglobina u F1 janjadi bila je povećana za 4,2 %. Parametri metabolizma bjelančevina bili su poboljšani, tako se poboljšala i ukupna razina bjelančevina u krvnom serumu za 11,2 %, albumina za 11,4 %, globulina za 10,4 %, što ukazuje na intenzivniji metabolizam bjelančevina u F1 janjadi, a na to je ukazala i povećana aktivnost aspartat aminotransferaze (za 3,9 %) i alanin aminotransferaze (za 16,6 %) u F1 janjadi u usporedbi s čistokrvnim romanov ovcama. Krvni serum janjadi križane pasmine imao je i veću baktericidnu (za 1,92 %) aktivnost, aktivnost lizozima (za 3,53 %) i fagocitnu aktivnost. Pojačani hematološki i biokemijski parametri i poboljšana antibakterijska svojstva seruma ukazuju na veću metaboličku aktivnost u F1 križane pasmine ovaca i podupiru bolje parametre proizvodnje mesa. Dobiveni rezultati ukazuju na veću prilagodljivost nove križane pasmine uvjetima stočarskog uzgoja i lokalnoj mikroklimi stepske zone u Ukrajini, a koja se postupno isušuje.

Ključne riječi: *uzgoj ovaca, križana pasmina romanov × hissar, kvaliteta mesa, hematologija, otpornost životinja, enzimi u krvi*