Adaptation potential of imported Lacaune rams evaluated by haematological parameters

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Abstract

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The adaptation potential and resistance to stress of Lacaune rams, imported from southern France to environmental and climatic conditions in Bulgaria were investigated. The following blood parameters were assayed on day 15, month 2 and one year after the import: complete blood counts, total cholesterol, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), gamma-glutamyltransferase (GGT), alkaline phosphatase, total protein, albumin, globulins, cortisol, triiodothyronine (T_3) and thyroxine (T_4). Blood serum concentrations of hormones were determined by enzymatic microimmune assay on an AccuBindTM ELISA Test System. It was found out that adaptation potential of rams was good – the values of most of analysed parameters were regained with period of adaptation that lasted more than 2 months after the import and ended within a year after that. Adaptation was accompanied by metabolic changes – reduction of blood serum glucose ($p<0.001$), cholesterol ($p<0.01$), albumins ($p<0.01$) and alkaline phosphatase ($p<0.001$), and increase in ALAT ($p<0.01$) and GGT ($p<0.05$) activities. The elevation ($p<0.001$) in serum cortisol, triiodothyronine (T3) and thyroxine (T4) corresponded to presence of idiopathic stress, while the lack of negative health and behavioural signs indicated good resistance to stress, making Lacaune sheep breed appropriate for raising in conditions specific for the Republic of Bulgaria.

Keywords: Lacaune; rams; adaptation; blood parameters; cortisol; triiodothyronine; thyroxine

Introduction

In intensive husbandry systems, animals are constantly suffering stress which has a negative impact on their welfare, natural behaviour and physiological status. The animals undergo a successful adaptation by virtue of their natural defense potential but also with the help of men who provide favourable environmental conditions in order to reduce stress to minimum and to achieve good health and high productive performance.

The animal body possesses several mechanisms of adaptation to the wide array of environmental changes. Recently, studies on welfare evaluation take into consideration a series of responses, which are usually termed "parameters" of adaptation: behavioural (data from ethograms and behavioural tests showing abnormal reactions, pathological (injuries, diseases), physiological (hormonal levels, heart rate etc.) and productive (growth and fertility). A complete assessment and analysis of adaptation status and welfare of animals and identification of problems influencing animal performance and reproduction could be attained only with parallel utilisation of these parameters. Therefore, the registration of a number of physiological variables and abnormal behaviour allows assessing the adaptive response of animals (Canali, 2008). In general, every state of discomfort in animals and men caused by numerous factors followed by physiological and behavioural alterations, is termed stress. Stress is responsible for impairment of normal physiological processes in the body and induces changes in blood parameters. Studies on blood parameters are used for evaluation of animal health and identification of stressors. Haematological, blood biochemical and oxidative stress parameters are commonly used as an important part of physical examination in order to pose a specific differential diagnosis. It is well acknowledged that physiological and haematological parameters are influenced by a lot of factors including breed, age, sex, reproduction status, feeding, environmental factors, stress and transportation (Arfuso et al., 2016; Piccione et al., 2016).

The hypothalamo-pituitary-adrenal axis is essential for systemic stress reactions and for restoration of body normal homeostasis (O'Connor et al., 2000).

At the time of stress, attention is enhanced; the brain is focused on potential stress and initiates various endocrine reactions to improve the physiological state of the individual. The cardiac activity, respiratory rate and catabolism become enhanced, the brain is focused on the potential threat and blood is redistributed to provide energy to excited organs and tissues – brain, heart and muscles (Tsigos & Chrousos, 2002; Gudev et al. 2014). Hormones released in the body to overcome stress situations are glucocorticoids and catecholamines. The glucocorticoid secretion is the classic endocrine response to stress (Kannan et al., 2000). Glucocorticosteroids provide the initial integration signal and together with other hormones and paracrine secretions determined specific behavioural. Physiological and biochemical responses allow the body a good adaptation to various environmental conditions (Wingfield & Kitaysky, 2002).

The level of fear sensitivity in sheep changes with age. Young animals (Peeva et al., 2009) and sheep at various ages exhibit different fear sensitivity during transportation, milking, feeding and other routine manipulations (Peeva et al., 2011). According to Dimitrov et al. (2012) the proportion of animals with clear fear reactions is reduced as age advances, while that of animals with calm temperament increases. This is an evidence for the role and importance of environmental factors on sheep adaptation.

The increasing use of sheep from the Lacaune breed in Bulgaria and the lack of sufficient national male breeding material is the cause for the continuous import of pedigree rams from abroad. The present study therefore aimed to investigate the adaptation potential and stress resistance level of rams from the imported high-yielding dairy breed Lacaune on the basis of changes in their blood throughout one year after the import.

Material and Methods

Study area: The study was carried out in the Nucleus farm for pedigree Lacaune sheep in Saedinenie town, Plovdiv district. In order to investigate the adaptation potential and stress resistance of imported animals to local climatic conditions and intensive husbandry system, 6 Lacaune rams were studied. They were imported by the end of 2017 from southern France (Aveyron region). The geographic location of Aveyron and climate are the same as in Bulgaria.

Collection of blood samples and analysis: Blood samples were collected from *v. jugularis externa* on post import day 15, month 2 and by the end of the first year. Samples were obtained by a licensed veterinarian in vacutainers. Blood for analysis of morphology was anticoagulated with EDTA.K2. Samples were transported in cooling bag and assayed in licensed labs. Complete blood counts were analysed in the clinical lab to the Faculty of Veterinary Medicine, Trakia University – Stara Zagora. Blood biochemical and hormonal indices were analysed in the licensed lab of the National Centre for Professional Training and Competence "America for Bulgaria" – Trakia University. Complete blood counts were assayed on automated 4-Diff haematological analyser „EXIGO EOS Vet", and biochemical ones – on automated biochemical analyser „BS-120". Blood serum concentrations of hormones were determined by enzymatic microimmune assay on an AccuBindTM ELISA Test System. The following morphological parameters considered as essential for the study of adaptation potential and stress resistance in imported rams were: white blood counts (WBC), lymphocytes (LYM), granulocytes (GRAN), monocytes (MONO), red blood cells (RBC), haemoglobin (HGB), haematocrit (HCT), platelets (PCT). Studied blood biochemical indices comprised blood glucose, total cholesterol, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), gamma-glutamyltransferase (GGT), alkaline phosphatase, total protein, albumin, globulins. Adaptation potential and resistance to stress of Lacaune rams was also evaluated on the basis of cortisol, triiodothyronine (T_3) and thyroxine (T_4).

Statistical analysis: Data were analysed by own-made standard software created in Microsoft Excel environment. All data were presented as means with standard errors of means.

Results and Discussion

Morphological, blood biochemical and hormonal parameters referring to the adaptation potential and resistance to stress of imported rams from the high-yielding dairy sheep breed Lacaune are presented on Tables 1, 2 and 3.

White blood cell counts (Table 1) varied from 6.22.109/l to 8.32.109/l during the three sampling periods, with insignificant differences. Nevertheless, there was a clear tendency towards reduction by the 2nd month after import – by nearly 22% compared to baseline and restoration of counts by the end of the first year. The cause was the change of diet along with other environmental factors, to which rams became adapted. This was also supported by data about granulocytes and agranulocytes that followed generally the same tendency. At the same time, the reduction in lymphocyte counts by the 2nd month was by 35%, that of monocytes – by 17%, and granulocytes – by 9%. The lack of statistically significant differences was attributed to the small number of experimental subjects and individual variations, yet the described tendency was nevertheless seen.

Along with specific normal variations of these parameters associated with specificity of metabolism depending on body physiological state, an increase (nutritional leukocytosis) or reduction (leukopaenia) of total white blood cell counts could be observed in inflammations, intoxications, irradiation, stress etc. or infectious diseases, starvation, suppression of bone marrow etc. respectively (Fishman & Hofman, 2004; Harris, 2006).

Specifically in sheep, apart data for heritability of the studied haematological parameter (Semerdjiev, 1999; Mostaghni et al., 2005; Tibbo et al., 2005), a limited body of evidence is available about the sum of influences of biotic and abiotic paratype factors, and seasonal variations of the parameter (Petrova et al., 1990; Mot et al., 2011).

Erythrocytes are an important part of blood building blocks. Their counts depend on food, climate, physiological state, productivity etc. In our studies, RBC of Lacaune rams by the 15th day post import were on the average $9.98.10^{12}$/l, and decreased two months after the import – $8.1.10^{12}$/l, while one year post import the counts were close to initial ones – $10.05.10^{12}$/l (Table 1). The trend was similar to that observed for leukocyte counts – decrease by 18%. Our data could serve as reference for determining normal ranges of this index, although in other sheep breeds, an effect associated to milk yield was found out – erythrocyte counts in high-yielding animals were higher (Petkov et al., 2000).

Blood haemoglobin content presented in Table 1, was in line with the trend described for WBC and RBC, yet the decrease was almost insignificant (by 8%) and could be attributed to be within the limits of random error for such type of studies. Fifteen days after import, haemoglobin was 122 g/l, by the 2nd month it was 112.63 g/l and one year after the import – 117.4 g/l.

Unchanged haemoglobin content and RBC during the three studied periods were the reason for similar haematocrit values – they varied from 32.6% to 36.7%. The higher level by the 2nd post import month proved the compensatory nature of the adaptation, which helped preserving the respiratory function of the blood despite the reduction in erythrocyte counts.

Platelets have an important protective function, mainly for blood clotting, but are also important for the general immunity. In the literature, reference ranges for sheep platelets are rather broad – from 170 to 980.10^{9}/l. Values found in this study: $229.6.10^{9}$/l 15 days post import, $275.17.10^{9}$/l by the second month and $183.4.10^{9}$/l by the end of the first year after the import are within the said physiological range, while our data could serve as reference for the specific breed in Bulgaria.

Table 1. Blood morphology in Lacaune rams

Parameter	Rams (n=6)					
	15 days post import		2 months post import		1 year post import	
	x	$\pm S_x$	x	$\pm S_x$	x	$\pm S_x$
WBC 10^{9}/l	7.94	94	6.22	0.99	8.32	1.07
LYM 10^{9}/l	3.76	0.38	2.45	0.22	3.62	0.39
MONO 10^{9}/l	0.58	0.08	0.48	0.08	0.58	0.07
GRAN 10^{9}/l	3.6	0.25	3.29	0.76	4.12	0.61
RBC 10^{12}/l	9.89	0.25	8.1	1.39	10.05	0.57
HGB g/l	122	3.14	112.63	3.61	117.4	5.79
HCT %	32.92	0.9	36.7	1.84	32.6	1.48
PCT 10^{9}/l	292.6	56.42	275.17	61.92	183.4	70.29

Blood glucose concentrations are presented in Table 2. During the first sampling period, blood glucose of Lacaune rams was 3.55 mmol/l, during the second period – 3.43 mmol/l, while one year after the import – it was statistically significantly reduced to 2.02 mmol/l. Data showed clearly that one of the main energy sources underwent considerable changes. The extensive and statistically significant change was rather due to intensive use of rams and impossibility for compensation by the diet. This hypothesis of ours would be either supported or rejected very soon by the terms of use of rams as breeders on the basis of productive parameters. Accumulation of more data is necessary in order to formulate a justified suggestion for physiological reference ranges accounting for the load exerted on rams. A seasonal effect decreasing blood glucose levels in sheep has been already confirmed (Antunovic´ et al., 2002).

The same tendency as for blood sugar was also found out for total cholesterol concentrations. Fifteen days after the import, blood serum cholesterol was 1.55 mmol/l, and decreased progressively to 0.86 mmol/l at the end of the study period (Table 2) with moderate significance of differences. These data supported our confidence about the occurrence of important metabolic changes in studied subjects, seeking the causes either in their diet or load they suffered. Having studied the total cholesterol concentrations in yearling rams, Slavov (2013) demonstrated that ration II with addition of coconut oil increased total cholesterol and HDL cholesterol in blood two and half hours after feeding (p<0.001).

Cellular enzymes are numerous and clinically relevant. They occur in the blood when cellular membrane permeability is impaired or after destruction of cells and tissues, causing hyperenzymemia. Aminotransferases have particularly important clinico-diagnostic relevance .

Data about enzyme activities of L–aspartate 2–oxoglutarate aminotransferase (ASCAT), L-alanine 2–oxoglutarate aminotransferase (ALAT), gamma-glutamyltransferase (GGT) and alkaline phosphatase (AP) in Lacaune rams are presented in Table 2. The level of L–aspartate 2–oxoglutarate aminotransferase 15 days after the import was 90.17 U/I, two months after the import was 93.68 U/I and increased (p<0.01- p<0.05) up to 161.4 U/I one year after import compared to previous sampling intervals. ALAT in blood followed the same tendency, with increased blood activities (p<0.05) up to 30.2 U/I one year after the import in Lacaune rams compared to levels measured 15 days and 2 months respectively. Serum gamma-glutamyltransferase in Lacaune rams at the three studied periods varied from 60.55 U/I to 83.8 U/I one year after import, but the elevation was statistically insignificant. Alkaline phosphatase (Table 2) in Lacaune rams was the highest by the 2nd month post import – 278.5 U/I, and this increase remained statistically significant one year after the import (p<0.001). Regardless of the sampling period, blood serum enzymes in rams from the studied breed remained within the respective physiological ranges, yet additional research is necessary to explain the observed differences.

Slavov (2013) found out a substantial reduction of ASAT, ALAT and AP, which is an indirect evidence for normal function of the liver, despite the higher lipid content of rations.

Plasma proteins are the most important among organic blood constituents. Our results (Table 2) showed no statistically significant differences in total protein levels for the three sampling periods – the values were very close (76-77 g/l) and within reference ranges. After addition of coconut oil to three different sheep rations, Slavov (2013) found out considerable increase of the studied parameter.

Albumins are the main source for protein synthesis in the organs. Their blood concentrations 15 days after the import was the highest and attained 40.93 g/l, with relevant differences at the 2nd month (p<0.01) and 1st year (p<0.05) after the import. According to Caldeira et al. (2007) albumins and urea are the best indicators of the level of protein metabolism in the body of ruminants.

Globulin concentrations ranged between 36.25–39.6 g/l and followed trends described for total protein (Table 2). They comprise alpha-, beta- and gamma globulins and their primary role in immunity buildup and systemic defense are long acknowledged. Obtained values are within reference ranges for sheep (35-55 g/l). Similar values were reported in previous studies of ours in other sheep breeds (Tosev, 1975; Varlyakov & Radev, 1998; Sivkova et al., 2007; Slavov, 2013; Radev et al., 2011).

Table 2. Blood biochemical parameters in Lacaune rams

Parameter	Rams (n=6)					
	15 days post import		2 months post import		1 year post import	
	x	±S$_x$	x	±S$_x$	x	±S$_x$
GLU mmol/l	3.53[aaa]	0.16	3.43[bbb]	0.12	2.02	0.08
CHOL mmol/l	1.55[aa]	0.12	1.17	0.1	0.86	0.08
ASAT U/L	90.17	10.57	93.68	4.45	161.4[a/bb]	13.81
ALAT U/L	16.67	0.56	17.33	1.74	30.2[a/b]	3.62
GGT U/L	60.55	8.54	65.67	3.48	83.8	6.8
ALP U/L	245	63.45	278.5[bbb]	27.6	112.6	18.22
TP g/l	77.18	0.57	76.42	0.98	77.32	1.72
ALB g/l	40.93[**/a]	0.29	37.77	0.72	37.72	0.89
GLB g/l	36.25	0.71	38.65	1.2	39.6	1.74

[*]: statistically significant differences between 15 days post import and 2 months post import
a: statistically significant differences between 15 days post import and 1 year post import
b: statistically significant differences between 2 months post import and 1 year post import
*, a, b - p<0.05; **, aa, bb - p<0.01; ***, aaa, bbb - p<0.001

Blood serum concentrations of cortisol, T_3 and T_4 of imported Lacaune rams are presented in Table 3. They were analysed because the thyroid and adrenal glands have a key role in the mechanism of adaptation. Our goal was to confirm our work hypothesis that animals would adapt well, as well as that already adapted, they would react rapidly to environmental changes with appropriate physiological correction.

The observed tendency regardless of numeric values was clear – increase of serum concentrations with clear statistically significant differences.

A continuous increase in cortisol levels from 1.12 µg/dl (15th day after import) to 3.09 µg/dl one year after import (p<0.001) was observed. Being a hormone most specific for the presence of stress, changes in cortisol levels should be sought in relation to three factors: stress caused by temperature changes, breeding campaign and changes in feeding regimen.

Serum triiodothyronine (T_3) and thyroxine (T_4) followed the same tendency with more obvious elevation of levels by the 2nd month and 1st year after import (p<0.001). These data, along with results for blood glucose and cholesterol, suggest the occurrence of metabolic changes in studied animals.

Serum hormonal concentrations indicative of thyroid and adrenal glands' status varied significantly under the influence of intrinsic or extrinsic factors: stress, ambient temperature, seasonal and circadian rhythms, even differences in metabolic activity. Most obviously, additional studies are necessary to provide a clear answer to this specific case.

Table 3. Blood hormones in Lacaune rams

Parameter	Rams (n=6)					
	15 days post import		2 months post import		1 year post import	
	x	±S$_x$	x	±S$_x$	x	±S$_x$
Cortisol µg/dl	1.12	0.28	2.09	0.28	3.09[aaa]	0.25
Triiodothyronine (T_3) ng/ml	0.77	0.07	2.21***	0.06	2.73[aaa]	0.32
Thyroxine (T_4) µg/dl	1.06	0.21	3.42***	0.42	5.15[aaa]	0.43

[*]: statistically significant differences between 15 days post import and 2 months post import
a: statistically significant differences between 15 days post import and 1 year post import
[***], aaa - p<0.001

Conclusions

It was found out that adaptation potential of Lacaune rams after their introduction from France was good – the values of most of analysed parameters were regained with period of adaptation that lasted more than 2 months after the import and ended within a year after that. Values of total and differential leukocyte counts, serum total protein and erythrocyte counts were restored. The initial decline in RBC was compensated and haemoglobin content was preserved.

Adaptation was accompanied by metabolic changes – reduction of blood serum glucose (p<0.001), cholesterol (p<0.01), albumins (p<0.01) and alkaline phosphatase (p<0.001), and increase in ALAT (p<0.01) and GGT (p<0.05) activities.

The elevation (p<0.001) in serum cortisol, triiodothyronine (T3) and thyroxine (T4) corresponded to presence of idiopathic stress, while the lack of negative health and behavioural signs indicated good resistance to stress, making Lacaune sheep breed appropriate for raising in the conditions specific for the Republic of Bulgaria.

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