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BIOCHEMICAL PARAMETERS IN THE BLOOD OF PREGNANT SHEEP AGAINST THE BACKGROUND OF KETONURIA

Abstract

The metabolic parameters traditionally used in the diagnostic algorithm in pregnant sheep in subclinical ketosis are in many cases less sensitive and specific than the «lipid peroxidation-antioxidant protection» system parameters. In the future, the material obtained in this study should be considered when studying the problem of subclinical and clinical ketosis of pregnant sheep as the concept of development of dysfunction «LPO-AOD» system for this metabolic disease.

***Key words:** pregnant sheep, blood, «LPO-AOD» system, subclinical ketosis, metabolic processes.*

Among the many fundamental problems of modern veterinary medicine, one of the first places is the problem of increasing fertility and preserving the productive longevity of the broodstock in sheep breeding. In the current conditions of sheep breeding, there is an excessive functional strain of the animal's organism, its various organs and systems, in some cases functioning «on the verge of pathology», which leads to the evolution of old and the emergence of new diseases. As a result of changes in the body of pregnant sheep, there is a development of the syndrome of fetoplacental insufficiency, which is the main mechanism of impaired fetal development during the intrauterine period.

Currently, many issues of the functioning of the system «lipid peroxidation - antioxidant protection» according to V.S. Avdeenko, S.A. Migaenko [1] and V.S. Avdeenko, A.V. Molchanov, R.N. Bulatov [2] concerning the state of metabolic processes in the organism of pregnant sheep in the presence of subclinical ketosis have not yet been studied.

The mechanism of development of subclinical ketosis in pregnant sheep in the context of metabolic disorders is considered in scientific publications as a factor in the destabilization of homeostasis in pregnant animals and, at the present time, according to the analysis of the studies conducted by V.S. Avdeenko [3] and I.V. Kireev [4], is in the stage of accumulation of factual material. At present [5,6,7], selenium is involved in reducing the level of lipid peroxidation and binding of free radicals, which optimizes the immunobiological reactions in the organism.

In the works of E.W. Edens [8] and K.A. Jacques [9] showed that selenium metabolism, absorbed in the animal's tissue, is fixed by protein globulins. At the same time, according to J.Kohrle, [10] and J.A. Johannigman [11], with the low content of selenium in the ration of small cattle, the work of the proventriculus is disrupted, as a result, the metabolism in the rumen is disrupted with the formation of insoluble forms of the microelement, that are excreted with excrements, which leads to a significant accumulation of free radicals and disruption of the «LPO-AOD» system.

Purpose of article. Determination of the change in the status of the system «lipid peroxidation - antioxidant protection» in pregnant sheep with subclinical ketosis.

The experimental group of pregnant sheep was made up of animals with symptoms of subclinical ketosis. For hematological studies, blood was taken before morning feeding. Biochemical blood tests were performed on a CIBA-CORING 288 BLOOD GAS SYSCEM analyzer (manufactured in the USA).

In addition, the primary and intermediate products of lipid peroxidation were determined in the blood of sick animals, which were estimated from the content of isolated double bonds, ketodienes and conjugated trienes (KDCT) and diene conjugates (DC), secondary ones by the content of malondialdehyde (MDA).

The obtained data were expressed in mmol/l, KDCT – in conventional units. The total antioxidant activity was evaluated using a model system, which is a suspension of egg yolk lipoproteins, allowing one to assess the ability of blood serum to inhibit the accumulation of TBA-active products in suspension. Antioxidant activity was expressed in conventional units. The determination of α -tocopherol was carried out by the fluorimetric method.

As a standard, we used D, L, α -tocopherol from «Serva». The content of α -tocopherol was expressed in mmol/l. The determination of retinol is carried out simultaneously with α -tocopherol. In this case, α -tocopherol and retinol with intense fluorescence with a maximum excitation at $\lambda = 350$ nm and radiation at $\lambda = 420$ nm. The retinol content was expressed in mmol/l.

Determination of reduced glutathione (GSII), oxidized glutathione (GSSG) by fluorimetric method (Hissin, Hilf, 1976).

The determination of GSSG was carried out in an alkaline medium (pH = 12). In addition, to prevent the oxidation of GSH in GSSG, N-ethylmalenite is added to the samples. The measurements were carried out on a Shimadzu spectrophotometer (RT-5000). The content of GSII and GSSG was expressed in mmol/l.

Determination of superoxide dismutase activity (SOD). The method is based on the ability of SOD to inhibit the autooxidation reaction of adrenaline at pH = 10.2. Measurement of the activity of SOD was carried out on a spectrofluorophotometer at $\lambda = 320$ nm. SOD was expressed in conventional units.

For morphological studies of the liver were used standard histological techniques, liver samples were taken from killed animals. Statistical analysis of data was carried out using standard programs like Microsoft Excel 2000 SPSS 10.0.5 for Windows.

The results of a biochemical blood test in pregnant sheep, positively reacting to ketone bodies in the urine, are presented in the data of Table 1.

Table 1 – Biochemical studies of blood of pregnant sheep, positively reacting to ketone bodies in the urine

Researched parameters	Actual blood content	Reference values
Glucose, mmol/l	2,25 ± 0,16	2,22 - 3,33
Total protein, g/l	82,0 ± 6,1	72 - 86
Alkaline reserve, mmol/l	18,41 ± 1,53	19 - 27
Common ketone bodies (CKB), mmol/l	2,38 ± 0,22**	0,18 - 1,03
Acetoacetic acid with acetone (), mmol/l	0,94 ± 0,09**	0,03 - 0,24
β -hydroxybutyric acid (), mmol/l	1,44 ± 0,16*	0,48 - 0,79
The BH/AcAc ratio	1,53 ± 0,28	-

Note: hereafter * $p < 0,05$, ** $p < 0,01$

Analysis of the obtained materials indicates that an increase in the level of ketone bodies above physiological limits by 2,3 times and their fractions – AcAc (acetoacetic acid with acetone, mmol /l) and BH (β -hydroxybutyric acid, mmol /l), respectively 5,9 times and 1,5 times, reduction of buffer bases to $18,41 \pm 1,53$ mmol /l, glucose concentration to $2,25 \pm 0,16$ mmol / l, and the ratio coefficient BH/AcAc to $1.53 \pm 0,28$. These changes indicate a metabolic disorder in pregnant sheep that is characteristic of subclinical ketosis.

From the materials presented in Table 2 it follows that the highest values of CKB (common ketone bodies, mmol/l), BH and BH/AcAc were observed in pregnant sheep with no significant fatty infiltration of hepatic tissue and were $3,2 \pm 0,31$, $2.53 \pm 0,23$ mmol/l, and $3,8 \pm 0.6$ mmol/l.

In this case, a more intensive lesion of the liver is accompanied by a decrease in these parameters and an increase in AcAc.

Table 2 – Dependence of fatty liver infiltration on concentration ketone bodies in the blood (mmol/l)

The parameter of ketogenesis	Large-droplet fatty degeneration of centrolobular localization	The absence of visible (with light microscopy) fatty degeneration
CKB	$2,49 \pm 0,12^*$	$3,22 \pm 0,11$.
	$0,97 \pm 0,07^*$	$0,62 \pm 0,07$
	$1,82 \pm 0,05^{**}$	$2,53 \pm 0,03$
/	$1,9 \pm 0,43^{**}$	$3,8 \pm 0,6$

Thus, with large-droplet fatty degeneration of centrolobular localization, which is more characteristic of subclinical ketosis, the concentration of CKB, BH and BH/AcAc were $2,79 \pm 0,22$, $1,82 \pm 0,15$ mmol /l and $1,9 \pm 0,43$ mmol /l, respectively, the level of AcAc in the blood of these animals, in contrast, was higher and was $0,97 \pm 0,07$ mmol /l.

Based on the above, it can be concluded that fatty liver infiltration is accompanied by an increase in the blood level of the most toxic fraction of ketone bodies - AcAc, a decrease in the concentration of CKB, BH and the BH/AcAc coefficient.

Therefore, the ratio of the fractions of ketonic bodies of β -hydroxybutyric acid (BH) to acetone with acetoacetic acid (AcAc) is lower than 1,9:1, should be considered as a non-specific marker of fatty liver dystrophy regardless of the level of CKB in the blood.

For the study of the state of lipid peroxidation processes in patients with subclinical ketosis of pregnant sheep were determined the concentrations of primary, intermediate and final products of lipid peroxidation (table 3).

In analyzing the concentration of double bonds in the blood, it should be noted that in pregnant sheep with subclinical ketosis observed an increase of 20,46%.

Table 3 - Oscillations of primary, intermediate and final products of lipid peroxidation in blood of pregnant sheep

Parameters	Subclinical ketosis (n = 15)	Clinically healthy (n = 15)
Isolated double bonds (conv.units)	$1, 866 \pm 0,12^*$	$1,194 \pm 0,21$
Diene conjugates (mmol/l)	$0,527 \pm 0,04^*$	$0,930 \pm 0,09$
Ketodienes and conjugated trienes (conv.units)	$0,168 \pm 0,05^*$	$0,161 \pm 0,05$
-tocopherol (mmol /l)	$6, 61 \pm 0,26^*$	$8,75 \pm 0,32$
Retinol (mmol /l)	$1,351 \pm 0,25^*$	$2,578 \pm 0,19$
Glutathione reduced (mmol /l)	$1, 684 \pm 0,06^{**}$	$1,436 \pm 0,04$
Glutathione oxidized (mmol /l)	$2,913 \pm 0,13^*$	$2,565 \pm 0,16$
Superoxide dismutase (conv.units)	$1,613 \pm 0,23^{**}$	$1,832 \pm 0,19$

The level of diene conjugates in the blood of sheep with the development of subclinical ketosis in comparison with clinically healthy animals was statistically increased 1,87 times ($p < 0,01$).

The concentration of intermediate products of ketodienes and conjugated trienes in the blood of sheep with subclinical ketosis was significantly increased by 1,75 times in comparison with the parameters of clinically healthy animals ($p < 0,01$).

Conclusion. As follows from the presented data, the metabolic parameters that are traditionally used in the diagnostic algorithm in animals with subclinical ketosis at the end of pregnancy of animals are less sensitive and specific than the parameters of the system «peroxide oxidation of lipids - antioxidant protection».

Therefore, an increase in the level of intermediate products of lipid peroxidation (KDCT) has a comparable sensitivity and greater specificity in comparison with a decrease in the metabolic parameters of the blood.

The mechanism of development of subclinical ketosis in pregnant sheep is disclosed, since the parameters of the system «peroxide oxidation of lipids - antioxidant protection» have a significantly greater diagnostic value in subclinical ketosis in pregnant sheep;

Among the studied parameters, the concentration of isolated double bonds in the blood of pregnant sheep with subclinical ketosis was increased by 20,46%, and the level of diene conjugates was 1,87 times;

The concentration of intermediate products of ketodienes and conjugated trienes in the blood of sheep with subclinical ketosis is significantly increased by 1,75 times in comparison with the parameters of clinically healthy animals.

In the long term, the material obtained in this work should be taken into account when studying the problem of subclinical and clinical ketosis in pregnant sheep and its relationship with fetoplacental insufficiency in domestic animals as a concept of the development of a malfunction of the «LPO-AOD» system in these metabolic pathologies.

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HEMATOLOGICAL INDICATORS OF CHICKEN WHO RECEIVED FEED ADDITIVES ON THE BASIS OF CHANCANAY ZEOLITES OF AKJAR BENTONITES AND KOKSUIC SHUNGYTS

Abstract

It has been studied for about the state of general physiological parameters of the blood of the broilers who received dietary supplements and «Ceobenth» and «Tseoshun» Getting e Chakanayskih based on zeolites, Akzhar bentonites and Koksuschungites.

The study was conducted on the total protein content, hemoglobin meters morphological indicators of blood glucose wells, calcium and phosphorus in the background feeding the birds the two formulations of natural minerals.

As a result of the conducted studies it was established that the application of «Ceobenth» and «Tseoshun» has a beneficial effect on the body of broiler chickens.

Keywords: *chicken -broilers, glucose, hemoglobin, erythrocytes, leukocytes, leukogram.*

Meat production of chickens depends on the rate of growth, meat precocity, live weight of the bird, payment of feed growth, quality of meat.

According to Masliev O.I. and Stollar T.A., the value of meat, its composition, juiciness, color and taste are largely dependent on the feeding of the bird. Improperly balanced diets without age, poultry cross, fodder quality can reduce the grade and taste of poultry meat.