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РЕЗЮМЕ

Ген BoLA-DRB3, входящий в главный комплекс гистосовместимости (ГКГ), играет ключевую роль в организации иммунного ответа у крупного рогатого скота и обладает высокой полиморфностью. В связи с этим он рассматривается как кандидатный ген в исследованиях генетической устойчивости к заболеваниям, включая вирус лейкемии крупного рогатого скота (BLV), и используется в популяционном генетическом анализе. Полиморфизм BoLA-DRB3 может определять восприимчивость или устойчивость животных к различным заболеваниям, в том числе к персистентному лимфоцитозу (PL) — клинической форме проявления BLV-инфекции.

В данной работе изучена генетическая изменчивость гена BoLA-DRB3 у местных пород крупного рогатого скота в Казахстане. Для идентификации аллелей использован метод ПЦР-ПДРФ с рестриктазами RsaI, BstYI и HaeIII. Были рассчитаны аллельные частоты и отклонения. Анализ 54 идентифицированных аллелей выявил различия в их распределении между породами, что свидетельствует о генетической неоднородности изученных популяций.

Высокий уровень полиморфизма BoLA-DRB3, наличие аллелей, ассоциированных с устойчивостью к заболеваниям, а также его связь с продуктивными признаками делают данный ген перспективным объектом для селекционных программ, направленных на улучшение устойчивости скота к инфекционным заболеваниям. Метод ПЦР-ПДРФ, используемый для анализа локуса BoLA-DRB3.2, позволяет эффективно выявлять генетическую изменчивость, различать гетерозиготные и гомозиготные аллели, что делает его ценным инструментом в генетических и эволюционных исследованиях крупного рогатого скота.

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THE EFFECT OF KETONE BODIES ON SOME BLOOD PARAMETERS IN PREGNANT SHEEP

ANNOTATION

One of the main issues currently in veterinary medicine is the problem of obtaining high fertility and increasing the longevity of queens in animal husbandry. Currently, in sheep farming, we observe the tension of the functional systems of the sheep's body, which work at the peak of a pathological condition that contributes to the development of new diseases.

Due to disorders in the body of pregnant animals, the syndrome of feto placenta insufficiency develops, which triggers changes in the fetus / fetuses in the uterus of the mother.

In our study, the experimental group of pregnant animals with signs of latent ketosis included sheep in which, when studying the composition of urine, we found ketone bodies in urine of more than 0.5 mmol/l.

We have found that when the indicators of ketone bodies increase by 2.3 times more than physiological norms, both the indicators of the fraction — acetoacetic acid with acetone and β -hydroxybutyric acid also increases 5.9 and 1.5 times, respectively, buffer base values decrease to 18.41 ± 1.53 mmol/L, glucose concentrations in animals also decrease to 2.25 ± 0.16 mmol/L, as well as a decrease in the coefficient of acetoacetic acid with acetone and β -hydroxybutyric acid to 1.53 ± 0.28 indicates that we have studied pregnant animals with changes in metabolism in the body, which corresponds to signs of latent ketosis.

The results of our study also show that the proportionality of the fraction of the studied ketone bodies of VN to AcAs was reduced by 1.9:1 and we take these data into account as a marker of fatty liver dystrophy, which does not depend on the limit of total ketone bodies in the blood.

Changes in the metabolism of the body in pregnant animals disrupt the function of the fetoplacental complex, which leads to stimulation of the metabolic process.

We have confirmed an increase in the proportion of ketodiene products and trione binding in the blood of pregnant animals with latent ketosis by 1.75 times. malondialdehyde values in latent ketosis were in the range of 1.125 ± 0.34 mmol/l.

Accordingly, markers of metabolism that are commonly used in veterinary medicine for diagnostic purposes in pregnant animals with latent ketosis differ in that they show low sensitivity and have low specificity in comparison with these systems of lipid peroxidation and antioxidant protection. In the future, the results of these studies should be taken into account when studying diseases such as latent and clinical ketosis of pregnant animals as a concept of the occurrence of a change in the function of lipid peroxidation – antioxidant protection in this metabolic pathology.

Key words: *ketone bodies, sheep, latent ketosis, metabolic disorders, blood.*

Introduction. Among the many fundamental problems of modern veterinary medicine, the problem of increasing fertility and maintaining productive longevity of the breeding stock in sheep breeding is put forward in one of the first places.

In modern conditions of sheep farming, excessive functional stress of the animal's body, its various organs and systems is noted, 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, fetoplacental insufficiency syndrome develops, which is the main mechanism of fetal/fetal development disorders in the prenatal period.

The causes of ketonuria in pregnant sheep are multifactorial and complex to this day. The timing has not yet been fully determined. Studies by various authors have shown that this complication is the basis. The reason for this is a decrease in blood circulation in the kidneys and the development of metabolic disorders associated with incomplete and unbalanced nutrition.

This has now been convincingly proven. Selenium has antioxidant properties and protects the reproductive health of animals suffering from many diseases. The researchers were interested in the question: do animals need selenium, who received sufficient amounts of vitamin E.

The results of subsequent studies have shown the need to introduce the trace element selenium into the body. Insufficient intake of selenium trace elements into the animal body leads to selenium

deficiency diseases affecting the main physiological systems and internal organs. Therefore, it is important to normalize the selenium content in the animal's body, in accordance with its functional

capabilities, conditions for homeostasis correction.

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Highlighting previously unsolved parts of a common problem. 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 body of suyag sheep in the presence of subclinical ketosis have not yet been studied.

Analysis of recent research and publications. The mechanism of development of subclinical ketosis in pregnant sheep in the context of metabolic disorders is considered in scientific publications as a factor of homeostasis destabilization in pregnant animals and currently, according to the analysis of studies conducted by V.S. Avdeenko [3] and I.V. Kireev [4], is in the stage of accumulation of factual material. Currently, [5,6,7] the participation of selenium in reducing the level of lipid peroxidation and binding of free radicals has been established, which optimizes immunobiological reactions in the body.

In the works of E.W. Edens [8] and K.A. Jacques [9], it was shown that the metabolism of selenium absorbed into animal tissues is fixed by protein globulins. At the same time, according to J.Kohrle, [10] and J.A.Johannigman [11], with a low content of selenium in the diet of small cattle, the work of the pancreas is disrupted, as a result, its metabolism in the rumen is disrupted with the formation of insoluble forms of the trace element, which are excreted in faeces, which leads to a significant accumulation of free radicals and disruption the "POL-AOZ" system.

The purpose of the article. Determination of changes in the status of the "lipid peroxidation – antioxidant protection" system in pregnant sheep against the background of subclinical ketosis.

Materials and methods. The experimental group of sheep consisted of animals with symptoms of subclinical ketosis. For hematological studies, blood was taken before morning feeding. Biochemical blood tests were performed on a CIBA - CORNING 288 BLOOD GAS SYSTEM analyzer (manufactured in the USA).

A total of 491 cases, or 22.00% of sheep, were recorded between the inspection and planning stages. The molecular signs of this disease "OPG-gestosis" are characterized by an

increase in dorsal skin, croup, anogenital area, enlargement of the nasal cavity, tapping movements, lordosis of the stem. A decrease in blood pressure in the pancreas of the studied mammals was found, which took the entire duration of the study, and the effect of blood and urine Annalysis gave a positive result for subcutaneous ketosis.

The manifestation of "OPG-gestosis" in pregnant pigments against the zone of subcutaneous ketosis, which is associated with depressive states, loss of appetite, blooming of the mucous membrane of vision and sudden disorders of the main nervous system, as well as protein content in urine.

In addition, primary and intermediate products of lipid peroxidation were determined in the blood of sick animals, which were evaluated by the content of isolated double bonds, ketodienes and conjugated trienes (CDiST) and diene conjugates (DC), secondary — by the content of manol dialdehyde (MDA).

The data obtained were expressed in mmol/l, CDiST - in units. The total antioxidant activity was evaluated using a model system representing a suspension of egg yolk lipoproteins, which allows to assess the ability of blood serum to inhibit the accumulation of TBK-active products in suspension. The antioxidant activity was expressed in units. The determination of α -tocopherol was carried out by the fluorimetric method. D, L, α -tocopherol from Serva was used as a standard.

The content of a-tocopherol was expressed in mmol/L. Retinol is determined simultaneously with α -tocopherol. At the same time, α -tocopherol and retinol have intense fluorescence with a maximum of excitation at $\lambda = 350$ nm and radiation at $\lambda = 420$ nm. The retinol content was expressed in mmol/L. Determination of reduced glutathione (GSH), oxidized glutathione (GSSG) by the fluorimetric method (Hissin, Hilf, 1976).

GSSG was determined in an alkaline medium (pH = 12). In addition, N-ethylmalienite was added to the samples to prevent the oxidation of GSH in GSSG. The measurements were carried out on a Shimadzu spectrofluorophotometer (RT-5000).

The contents of GSII and GSSG were expressed in mmol/L. Determination of the activity of superoxide dismutase (SOD). The method is based on the ability of SOD to inhibit the reaction of autoxidation of adrenaline at pH = 10.2. The measurement of SOD activity was carried out on a spectrofluorophotometer at $\lambda = 320$ nm. SOD was expressed in conl.ed.

Standard histological techniques were used for morphological studies of the liver, liver samples were taken from slaughtered animals.

Statistical data analysis was performed using standard Microsoft Excel 2000 SPSS 10.0.5 for Windows programs.

Results and discussion. The results of a biochemical blood test in suyag sheep that respond positively to ketone bodies in urine are presented in the data in Table 1.

Table 1 — Biochemical studies of the blood of suyag sheep that respond positively to ketone bodies in urine

The studied indicator	Reference values	The actual content in the blood
Glucose, mmol/l	2,23 - 3,34	2,26 $\pm$ 0,17
Total protein, g/l	72 - 86	82,0 $\pm$ 6,1
Alkaline reserve, mmol/l	19 - 27	19,11 $\pm$ 2,13
Common Ketone bodies mmol/l	0,18 - 1,03	2,38 $\pm$ 0,22**
Acetoacetic acid with acetone (AcAc), mmol/l	0,03 - 0,24	0,94 $\pm$ 0,09**
β -hydroxybutyric acid (VN), mmol/l	0,48 - 0,79	1,44 $\pm$ 0,16*
Relationship VN / Acetoacetic acid with acetone	-	1,62 $\pm$ 0,31

Note: hereafter * $p < 0.05$, ** $p < 0.01$

The analysis of the obtained materials indicates that there is 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 VN (β -hydroxybutyric acid, mmol/l) by 5.9 times and 1.5 times, respectively, a decrease in buffer bases to 19.11 ± 2.13 mmol/l, glucose concentrations up to 2.26 ± 0.17 mmol/l, as well as the coefficient of the HH/AcAc ratio up to 1.62 ± 0.31 .

The changes obtained indicate a violation of metabolic metabolism in sheep of the type characteristic of subclinical ketosis.

It follows from the materials presented in Table 2 that the highest values of total ketone bodies, mmol/l, VN and VN/AcAs were observed in pregnant sheep with no pronounced fatty infiltration of liver tissue and amounted to 3.2 ± 0.31 , 2.53 ± 0.23 mmol/l and 3.8 ± 0.6 mmol/l, respectively.

At the same time, more intense liver damage is accompanied by a decrease in these indicators and an increase in AcAs.

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

The indicator of ketogenesis	Absence of visible (with light microscopy) fatty degeneration	Large-drop fatty dystrophy of centrolobular localization
Common Ketone bodies	$3,22 \pm 0,11$.	$2,49 \pm 0,12^*$
Acetoacetic acid with acetone AcAc	$0,62 \pm 0,07$	$0,96 \pm 0,08^*$
VN	$2,53 \pm 0,03$	$1,82 \pm 0,05^{**}$
Relationship VN / Acetoacetic acid with acetone	$3,8 \pm 0,6$	$1,9 \pm 0,43^{**}$

Thus, in large-drop fatty dystrophy of predominantly centrolobular localization, which is most characteristic of subclinical ketosis, the concentration of OCT, VN and VN/AcAs amounted to 2.79 ± 0.22 , 1.82 ± 0.15 mmol/l and 1.9 ± 0.43 mmol/l, respectively, the level of AcAs in the blood of these animals, on the contrary, was higher and amounted to 0.97 ± 0.07 mmol/l.

Table 3 – Fluctuations of primary, intermediate and final products of lipid peroxidation in the blood of sick sheep

Indicators	Clinically healthy (n = 15)	Subclinical ketosis (n = 15)
Isolated double bonds (cont. units)	$1,203 \pm 0,22$	$1,901 \pm 0,13^*$
Diene conjugates (mmol/l)	$0,898 \pm 0,11$	$0,532 \pm 0,05^*$
Ketodienes and conjugated trienes (conl. units)	$0,159 \pm 0,06$	$0,171 \pm 0,06^*$
<i>A-tocopherol</i> (mmol/l)	$8,69 \pm 0,32$	$6,62 \pm 0,27^*$
<i>Retinol</i> (mmol/l)	$2,581 \pm 0,21$	$1,361 \pm 0,26^*$
Reduced glutathione (mmol/l)	$1,438 \pm 0,05$	$1,685 \pm 0,07^{**}$
Oxidized glutathione (mmol/l)	$2,567 \pm 0,17$	$2,915 \pm 0,14^*$
<i>Superoxide dismutase</i> (conl. units)	$1,834 \pm 0,18$	$1,609 \pm 0,21^{**}$

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 — AcAs, a decrease in the concentration of OCT, VN and the VN/AcAs coefficient.

Therefore, the ratio of fractions of ketone bodies of β -hydroxybutyric acid (BH) to acetone with acetoacetic acid (Ac) is lower than 1.9:1, should be considered as a non-specific marker of fatty liver dystrophy regardless of the level of OCT in the blood.

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

When analyzing the concentration of double bonds in the blood, it should be noted that in pregnant sheep with subclinical ketosis, their increase is observed by 20.46%.

The level of diene conjugates in the blood of sheep with the manifestation of subclinical ketosis in comparison with clinically healthy animals was statistically significantly increased by 1.87 times ($p < 0.01$).

The concentration of ketodiene intermediates and conjugated trienes in the blood of sheep with subclinical ketosis was significantly increased by 1.75 times compared with those of clinically healthy animals ($p < 0.01$).

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 indicators of the "lipid peroxidation – antioxidant protection" system.

Therefore, an increase in the level of lipid peroxidation intermediates (Cysts) has a comparable sensitivity and greater specificity compared with a decrease in blood metabolic parameters.

Conclusion. The mechanism of development of metabolic stress as a developmental factor in pregnant sheep revealed eclampsia, since during this period the indicators of the system of "lipid peroxidation" were revealed the diagnostic value of "antioxidant protection" is quite high.

The mechanism of development of subclinical ketosis in suag sheep has been revealed, since the indicators of the system "lipid peroxidation – antioxidant protection" have a significantly high diagnostic value in subclinical ketosis in suag sheep; among the studied indicators, the concentration of isolated double bonds in the blood of pregnant sheep with subclinical ketosis.

The molecular signs of this disease "OPG-gestosis" are characterized by an increase in dorsal skin, croup, anogenital area, enlargement of the nasal cavity, tapping movements, lordosis of the stem. A decrease in blood pressure in the pancreas of the studied mammals was found, which took the entire duration of the study, and the effect of blood and urine Annalysis gave a positive result for subcutaneous ketosis.

Was increased by 20.46%, and the level of diene conjugates was 1.87 times higher; the concentration of ketodiene intermediates and conjugated trienes in the blood of sheep with subclinical ketosis was significantly increased by 1.75 times compared with those of clinically healthy animals. Therefore, an increase in the level of lipid peroxidation intermediates (Cysts) has a comparable sensitivity and greater specificity compared with a decrease in blood metabolic parameters.

In the future, 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 for the development of a violation of the functioning of the "HALF-AOZ" system in these metabolic pathologies.

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ТҮЙІН

Қазіргі кезеңде ветеринариядағы негізгі мәселелердің біріне жоғары төлділікті алу және мал шаруашылығында аналықтардың ұзақ өмір сүруін ұзарту мәселесі жатады. Қазіргі уақытта қой шаруашылығындағы жаңа аурулардың дамуына ықпал ететін патологиялық жағдайдың шыңында пайда болатын қой ағзасындағы функционалды жүйелерінің ауытқуын байқаймыз. Буаз жануарлардың денесіндегі бұзылуларға байланысты, фетоплацентарлық жеткіліксіздігі синдромы дамиды, бұл аналықтың жатырындағы ұрықтың даму механизмінің өзгеруін тудырады.

Біздің зерттеуіміздің тәжірибелі тобына жасырын кетоз белгілері бар буаз жануарлар енгізілді, олардан біз зәрдің құрамын зерттеу кезінде несепте 0,5 ммоль/л-ден астам кетон денелерін таптық.

Біз кетон денелерінің көрсеткіштері физиологиялық нормалардан 2,3 есе артқан кезде, фракция көрсеткіштерінің де артқанын байқадық, яғни ацетосірке қышқылы ацетонмен фракциясы 5,9 есе көбейген, β-оксимай қышқылы фракциясы 1,5 есе артқан, ал буферлік негіздердің көрсеткіштері $18,41 \pm 1,53$ дейін ммоль / л дейін төмендеген, жануарлардағы глюкоза концентрациясының көрсеткіштері де $2,25 \pm 0,16$ ммоль / л дейін төмендеген, сондай-ақ ацетосірке қышқылы ацетонмен және β-оксимай қышқылы коэффициентінің көрсеткіштері $1,53 \pm 0,28$ дейін төмендеді, бұл көрсеткіштер біз зерттеген буаз жануарлардың жасырын кетоз белгілеріне сәйкес келетін организмдегі метаболизмнің өзгеруі бар екенін көрсетеді.

Біздің зерттеу нәтижелеріне қарасақ, зерттелген кетон денелерінің фракциясының, яғни ацетосірке қышқылы ацетонмен және β-оксимай қышқылы пропорционалдылығы 1,9:1-ге төмендегенін көрсетеді және біз бұл деректерді бауырдың майлы дистрофиясының маркері ретінде қарастырамыз, бұл жалпы кетон шегіне тәуелді емес қандағы денелер болып саналады. Буаз жануарлардағы дене метаболизміндегі өзгерістер фетоплацентарлы кешеннің қызметін бұзады және бұл метаболизм процесін ынталандыруға әкеледі. Жасырын кетозбен ауыру кезінде буаз жануарлардың қанындағы кетодиен өнімдері мен триондарды байланыстыру үлесінің 1,75 есе артқаны расталды. Жасырын кетоздағы малон диальдегидінің көрсеткіштері $1,125 \pm 0,34$ мкмоль/л аралығында болды.

Тиісінше, жасырын кетоз кезінде буаз жануарларда диагностикалық мақсатта ветеринарияда жалпы бәрі жиі қолданылатын метаболизм маркерлері төмен сезімталдықты көрсететін және липидтердің тотығуы және антиоксидантты қорғаныс жүйелерінің деректерімен салыстырғанда төмен спецификалық айырмашылықтарға ие екені анықталды. Болашақта осы зерттеулердің нәтижелері буаз жануарлардың жасырын және клиникалық кетозы сияқты ауруларды зерттеу кезінде липидтердің тотығу функциясының өзгеруінің тұжырымдамасы, яғни метаболизмнің осы патологиясындағы антиоксидантты қорғанысы ретінде ескерілуі керек.

РЕЗЮМЕ

Одним из основных вопросов в настоящем времени в ветеринарии является проблема получения высокой плодовитости и увеличения долголетия маток в животноводстве. В настоящее время в овцеводстве наблюдаем напряжение функциональных систем организма овец, которые работают на пике патологического состояния, которое способствует развитию новых заболеваний. Из-за нарушений в организме беременных животных, развивается синдром недостаточности фетоплаценты, который запускает изменения у плода/плодов в матке у матери.

В нашем исследовании в опытную группу беременных животных с признаками скрытого кетоза были включены овцы, у которых мы при изучении состава мочи обнаружили в моче кетоновые тела более 0,5 ммоль/л.

Нами было выявлено, что когда показатели кетоновых тел увеличиваются больше физиологических норм в 2,3 раза и то и показатели фракции — ацетоуксусная кислоты с ацетоном и β -оксимасляной кислоты также увеличивается 5,9 и соответственно 1,5 раза, показатели буферных оснований снижаются до $18,41 \pm 1,53$ ммоль/л, показатели концентрации глюкозы у животных также понижаются до $2,25 \pm 0,16$ ммоль/л, а также уменьшение показателей коэффициента ацетоуксусная кислоты с ацетоном и β -оксимасляной кислоты до $1,53 \pm 0,28$ говорит о том что нами исследованных беременных животных имеются изменения метаболизма в организме, что соответствует признакам скрытого кетоза.

Результаты наших исследований также показывают, что пропорциональность фракции исследованных кетоновых тел ВН к АсАс было понижено на 1,9:1 и эти данные мы учитываем как маркер жировой дистрофии печени, который не зависит от предела общих кетоновых тел в крови. Изменения в метаболизме организма у беременных животных нарушает функцию фетоплацентарного комплекса что приводит к стимуляции процесса метаболизма. Нами было подтверждено увеличение доли межпродуктов из кетодиенов и связывания триенов в крови беременных животных при скрытом кетозе на 1,75 раза.

Показатели малонового диальдегида при скрытом кетозе были в пределах $1,125 \pm 0,34$ мкмоль/л. Соответственно, маркеры метаболизма которые общепринято применяются в ветеринарии в диагностических целях у беременных животных при скрытом кетозе имеют различия тем что показывают малую чувствительность и имеют низкую специфичность в сравнении с данными систем перекисное окисление липидов и антиоксидантной защиты. В будущем результаты данных исследований надо принимать во внимание при исследованиях таких болезней как скрытый и клинический кетоз беременных животных как концепцию возникновения изменения функции перекисного окисления липидов – антиоксидантная защита при данной патологии метаболизма.

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