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Pregnant Cows and Heifers Blood Profile Comparison

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***Abstract*—This research is aimed to establish stress intensity in pregnant cows and compare it with metabolism load in heifers. This issue has been important due to significant intensification of dairy production in recent decades. The organism of a dairy cow is under a constant load, especially in heavy-yielding animals. Considering animal's needs and demands in high production environments is beneficial not only for continuous dairy and offspring production, but also for animal health and wellbeing. This study established that during the course of several lactations there is increase of an endogenous and exogenous stress level. It is important to monitor and reduce metabolic load by appropriate treatment and / or environment management. It is essential to monitor, prevent and when possible control metabolic distress, infectious diseases and high yield aftermaths in order to gain healthy and long lasting highly yield dairy herd and reduce culling.**

Keywords—heifer; cow; dairy; biochemistry; stress; mycotoxin

I. INTRODUCTION

According to some researchers, the high yield and continuous animal reproduction is the key to profitable dairy enterprise [5]. Nowadays environment of dairy production animals husbandry becomes increasingly severe. Amassment and strict specialization of dairy herds are growing as well. Several harmful factors for the health and productivity of the dairy cattle are possible to outline feeding rules and routines disregard, technical and herd management errors, low qualification of the staff [7]. Some authors notice a high level of culling – 25-30% by 3rd year in some farms, also the reducing of calving (by 25-30 calves per 100 cows) [9]. The reason for such drastic results is low feeds quality, lack of insolation, feeding regimes breach. Physiological condition of dairy cow has a direct impact on dairy yield and offspring productivity [14].

Nowadays there are numerous researches in the field of dairy yield increasing [7] and offspring productivity, health and wellbeing improvement [8, 12]. But because of modernization and industrialization of dairy cattle husbandry, there is insufficient control and monitoring of the in-farm feeds production and storage [18]. Because of that the probability of fungi and mycotoxins infestation in meat and milk is very high. This is dangerous not only because of direct damaging impact, but also due to indirect cumulative effect. It is established that most of the mycotoxicosis in humans is arising due to mycotoxins contamination in milk [11]. That is why it is important to control health and wellbeing in dairy herd thorough its lifetime [2, 4, 10].

The research was conducted in «Plemennoy Zavod Prinevskoye», the Northwestern region of the Russian Federation, in department of biochemistry and physiology laboratory of FGBOU VO «SPbSAVM» on white-and-black cattle. Experimental group included 10 heifers and 10 pregnant cows in 3rd lactation. The blood samplings were taken four times – at the 6th, 7th, 8th and 9th month of pregnancy. Mean heifer weight is 450 kg, mean cow weight – 540 kg. An average daily yield is 26 kg.

The "Plemennoy Zavod Prinevskoe" is a farm, located within the boundaries of Saint-Petersburg – city with the

developed infrastructure and the high level of industrial pollution. The access to the farm is restricted, only authorized staff and visitors received permission are permitted.

The activity of serum aspartate aminotransferase (AST) and serum alanine aminotransferase (ALT) was defined by method of Raitman and Frenckel by using the "Abris" commercial kit. The activity of serum alkaline phosphatase was estimated by photometric method, based on hydrolysis of nitrophenylphosphate disodium salt which involved using the "Mizar" commercial kit. The level of serum total protein was estimated by the colorimetric method based on using the biuretic reagent. The level of serum urea was estimated by the colorimetric method involving using the "Abris" commercial kit, this method is based on color reaction with diacetylmonoxime. The level of serum creatinine was estimated by the photometric method by using picric acid; this method is based on the Jaffe method. The level of serum bilirubin was estimated by the method of Jendrassik-Grof using the "Kliny Test-Bil" commercial kit. The level of serum carotene was estimated by the photometric method, based on the reaction of carotene extraction by petroleum ether.

II. Results

The results are displayed in tables 1-4.

Serum enzymes activity of cows and heifers of the same physiological state was compared. AST activity in cows was higher than in heifers – by 14% at 7th month of pregnancy ($p \leq 0.05$), by 4% at 8th month of pregnancy, by 7% at 9th month of pregnancy ($p \leq 0.05$). ALT activity in cows was higher than in heifers – by 4% at 6th month of pregnancy ($p \leq 0.05$), by 16% at 9th month of pregnancy ($p \leq 0.05$). Also there is alkaline phosphatase elevation during the course of the pregnancy in heifers and cows altogether. Alkaline phosphatase activity in cows was higher than in heifers – by 21% at 6th month of pregnancy, by 36% at 7th month of pregnancy, by 39% at 8th month of pregnancy, by 43% at 9th month of pregnancy.

Total protein serum level was higher in cows than heifers - by 18% at 6th month of pregnancy ($p \leq 0.05$), by 16% at 7th month of pregnancy ($p \leq 0.05$), by 20% at 8th month of pregnancy ($p \leq 0.05$), by 10% at 9th month of pregnancy ($p \leq 0.05$). The serum urea nitrogen level in cows also was higher than in heifers by 11% at 6th month of pregnancy, by 21% at 7th month of pregnancy, by 43% at 9th month of pregnancy ($p \leq 0.05$).

Serum creatinine level in heifers was higher, than in cows – by 4% at 6th month, by 8% at 7th month of pregnancy, by 7% at 8th month of pregnancy ($p \leq 0.05$).

Bilirubin level in heifers was higher, than in cows – by 6% at 6th month, by 5% at 7th month of pregnancy, by 15% at 9th month of pregnancy ($p \leq 0.05$).

The carotene level in cows was higher than in heifers by 13% at 6th month ($p \leq 0.05$), by 11% at 7th month ($p \leq 0.05$), by 9% at 9th month of pregnancy. It is notable that at 9th month of pregnancy, the carotene level was higher in heifers by 5% ($p \leq 0.05$).

III. Discussion

Nowadays there are assessments of familiar and new methods of dairy productivity increase [16]. One of the numerous harmful factors for the health and productivity of the dairy cattle is low quality feed and mycotoxins [11]. This results in not only low dairy productivity, culling and reduced calving, but also increase in feeds cost.

TABLE I. Comparing analysis of heifers and cows blood profiles during the 6TH month of pregnancy (M±m)

	Units	Heifers (n=10)	Cows (n=10)
ALT	IU/l	25.15 ± 0.23*	26.17 ± 0.25
AST	IU/l	68.24 ± 1.22*	75.12 ± 2.10
Alkaline phosphatase	IU/l	65.34 ± 5.23	51.39 ± 2.31
Total protein	g/l	62.41 ± 1.59*	73.72 ± 1.62
Urea nitrogen	mmol/l	4.63 ± 0.21	5.15 ± 0.31
Creatinine	umol/l	92.24 ± 1.35	88.57 ± 3.99
Bilirubin	umol/l	1.74 ± 0.21	1.63 ± 0.17
Carotene	umol/l	12.47 ± 0.2*	14.08 ± 0.15

a. * p<0.05, comparing heifers group with cows group

TABLE II. Comparing analysis of heifers and cows blood profiles during the 7TH month of pregnancy (M±m)

	Units	Heifers (n=10)	Cows (n=10)
ALT	IU/l	30.93 ± 0.27*	29.85 ± 0.29
AST	IU/l	73.54 ± 2.12*	83.75 ± 3.16
Alkaline phosphatase	IU/l	75.24 ± 6.64	48.39 ± 3.35
Total protein	g/l	60.47 ± 0.94*	70.12 ± 0.94
Urea nitrogen	mmol/l	3.85 ± 0.27	4.64 ± 0.22
Creatinine	umol/l	98.24 ± 2.16	90.60 ± 4.85
Bilirubin	umol/l	1.89 ± 0.11	1.80 ± 0.18
Carotene	umol/l	12.23 ± 0.2*	13.58 ± 0.2

b. * p<0.05, comparing heifers group with cows group

TABLE III. Comparing analysis of heifers and cows blood profiles during the 8TH month of pregnancy (M±m)

	Units	Heifers (n=10)	Cows (n=10)
ALT	IU/l	33.38 ± 0.53*	31.20 ± 0.51
AST	IU/l	82.20 ± 0.98*	85.69 ± 1.16
Alkaline phosphatase	IU/l	87.19 ± 7.25	52.79 ± 4.32
Total protein	g/l	60.31 ± 1.97*	72.61 ± 1.14
Urea nitrogen	mmol/l	4.98 ± 0.28*	3.40 ± 0.2
Creatinine	umol/l	103.52 ± 1.84*	95.80 ± 2.25
Bilirubin	umol/l	2.06 ± 0.11	2.10 ± 0.08
Carotene	umol/l	11.75 ± 0.2	12.83 ± 0.7

c. * p<0.05, comparing heifers group with cows group

TABLE IV. Comparing analysis of heifers and cows blood profiles during the 9TH month of pregnancy (M±m)

	Units	Heifers (n=10)	Cows (n=10)
ALT	IU/l	28.92 ± 1.24*	33.54 ± 1.12
AST	IU/l	83.26 ± 1.98*	89.13 ± 1.57
Alkaline phosphatase	IU/l	96.38 ± 10.66	54.62 ± 5.64
Total protein	g/l	61.15 ± 0.91*	67.34 ± 0.73
Urea nitrogen	mmol/l	2.50 ± 0.10*	2.96 ± 0.18
Creatinine	umol/l	107.41 ± 2.25	106.90 ± 1.84
Bilirubin	umol/l	3.05 ± 0.2*	2.60 ± 0.19
Carotene	umol/l	7.03 ± 0.2*	6.7 ± 0.05

d. * p<0.05, comparing heifers group with cows group

Female body is bearing the heaviest stress during pregnancy – all systems and organs endure overload not only during pregnancy, but also after – during calving and ongoing lactation [2, 13]. Dairy yield and the calf condition depend on health and wellbeing of the cow [15]. That is why it is essential to pay attention to the cows and heifers during the last trimester of pregnancy.

After analyzing the received data of serum, enzyme activity in heifers and cows during the last trimester of pregnancy the specific trend was revealed. There was elevation of serum ALT activity in heifers – from 30.93±0.27 IU/l to 33.38±0.53 IU/l during 7th and 8th month of pregnancy. In cows serum ALT activity elevated from 29.85±0.29 IU/l to 31.20±0.51 IU/l during the same period of pregnancy. During 8th and 9th month of pregnancy activity of serum, ALT in heifers has declined (from 33.38±0.53 IU/l to 28.92±1.24 IU/l). In the cows, activity of serum ALT elevated between 8th and 9th months of pregnancy – from 31.20±0.51 IU/l to 33.54±1.12 IU/l. These tendencies are estimated as stress increasing in pregnant females and indicate the greater load in older animals in comparison with heifer.

Activity of serum AST in heifers and cows changed similarly into serum ALT activity. During 7-8 months of pregnancy there was elevation – from 73.54±2.12 IU/l to 82.20±0.98 IU/l in heifers and from 83.75±3.16 IU/l to 85.69±1.16 IU/l in cows. During 8-9 month of pregnancy, there was elevation – from 82.20±0.98 IU/l to 83.26±1.98 IU/l in heifers and from 85.69±1.16 IU/l to 89.13±1.57 IU/l in cows.

AST and ALT activity reduced in heifers and cows is possibly caused by systemic slowdown of central and peripheral metabolism in order to save amino acids and glucose for lactation [14].

Also there was tendency to increase the serum alkaline phosphatase activity during the course of pregnancy in heifers and in cows. Alkaline phosphatase activity elevated from 75.24±6.64 IU/l at 7th month of pregnancy to 96.38±10.66 IU/lat 9th month of pregnancy in heifers. In cows alkaline phosphatase activity elevated from 87.19±7.25 IU/l at 7th month of pregnancy to 96.38±10.66 IU/lat 9th month of pregnancy. The reason for increasing the serum alkaline phosphatase activity is due to growing intensity of dephosphorylation processes in pregnant females and due to bones formation in fetus [6]. Figures 1-3 present visual graphs for serum enzymes activity in cows and heifers during the last trimester of pregnancy.

Total protein in serum is an important marker not only for dairy productivity, but also for hepatic condition, metabolism intensity and feeding quality. In heifers serum total protein levels were in the range from 60.31±1.97 g/l to 61.15±0.91 g/l. In cows serum total protein levels were declining – from the range 70.12±0.94 to 72.61±1.14 g/l at 7-8 months of pregnancy to 67.34±0.73 g/l during the 9th month of pregnancy. This tendency is supposedly arisen due to feeding changing during the interlactating period; also it is possible because of metabolic disturbances (e.g. hepatic diseases in older animals). Figure 4 depicts graphs for the serum total protein level in heifers and cows.

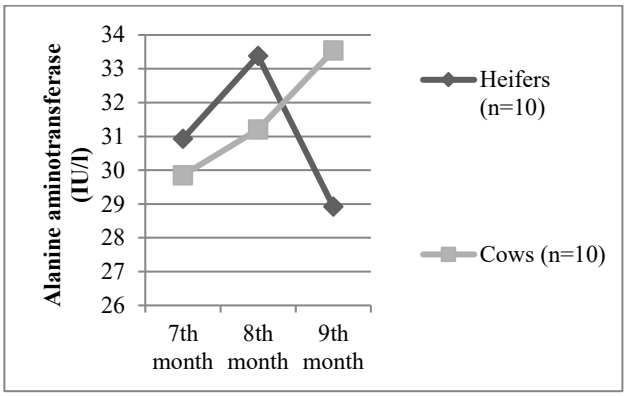

Fig. 1. Serum ALT activity in heifers and cows during the last trimester of pregnancy.

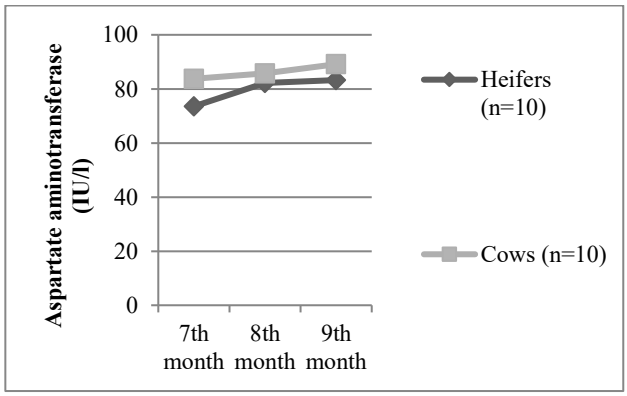

Fig. 2. Serum AST activity in heifers and cows during last trimester of pregnancy.

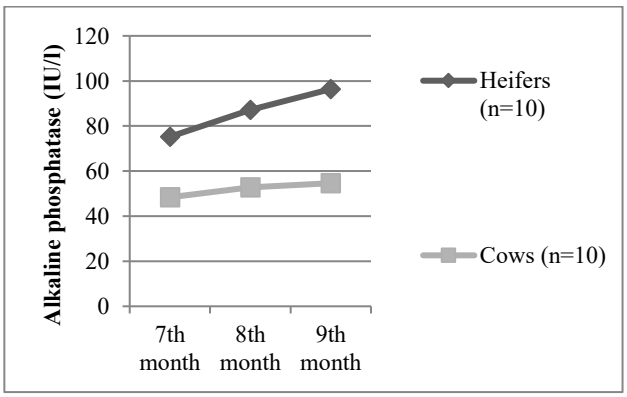

Fig. 3. Serum alkaline phosphatase activity in heifers and cows during last trimester of pregnancy.

Urea nitrogen is an important marker for kidneys status, protein metabolism and hepatic function [3]. Urea nitrogen is also a significant element for energetic metabolism for protozoa in the rumen [17]. The serum urea nitrogen level in heifers increased during 7-8 months of pregnancy from 3.85±0.27 mmol/l to 4.98±0.28 mmol/l and then during 9th month of pregnancy declined to 2.50±0.10 mmol/l. The serum urea nitrogen level declined during the last trimester of pregnancy – from 4.64±0.22 mmol/l to 2.96±0.18 mmol/l in cows. This is

related to increasing load for the liver and kidneys, but also may be referred to feeding changing during the interlactating period at the 9th month of pregnancy. Figure 5 shows the graph for the serum urea nitrogen level in heifers and cows.

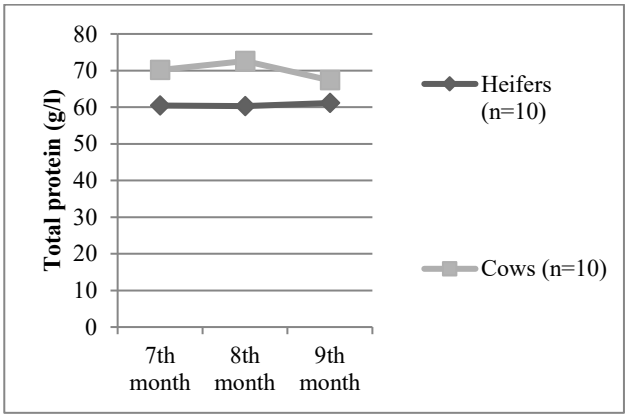

Fig. 4. Serum total protein level in heifers and cows during last trimester of pregnancy.

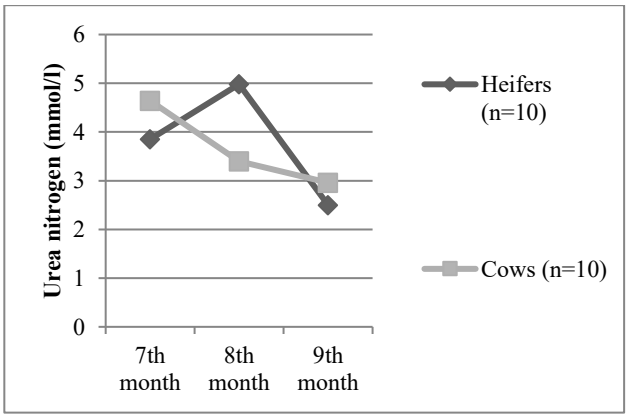

Fig. 5. Serum urea nitrogen level in heifers and cows during the last trimester of pregnancy.

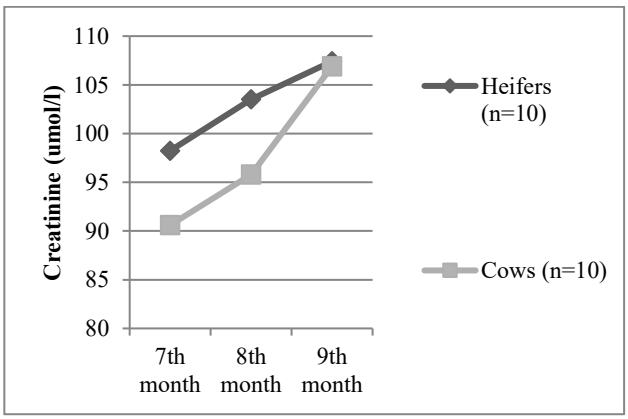

Fig. 6. Serum creatinine level in heifers and cows during last trimester of pregnancy.

Bilirubin is one of the main markers for the function of the hepatobiliary system. During the last trimester of pregnancy, the serum bilirubin level increased from 1.89±0.11 umol/l to 4.05±0.2 umol/l in heifers and from 1.80±0.18 umol/l to 2.60±0.19 umol/l in cows. This may indicate high stress of the liver in pregnant females during the last trimester of pregnancy. In figure 7 presents graphs for the serum bilirubin level in heifers and cows.

Carotene is a precursor for vitamin A. The main storage in the organism for vitamin A is the liver, so it is possible to evaluate hepatic function judging on serum carotene level. In heifers the serum carotene level declined during the last trimester of pregnancy from 12.23±0.12 umol/l to 7.03±0.07 umol/l. There is a similar tendency in cows - the serum carotene level declined from 13.58±0.16 umol/l to 6.7±0.05 umol/l. This tendency may indicate not only declining the liver function, but also reflect feeding changing during the 9th month of pregnancy. However, it is important to note that the serum carotene level was below reference levels in cows that is may be reflection of higher metabolism tension in older animals due to longer exogenous stress. Figure 8 presents graphs for the serum carotene level in heifers and cows.

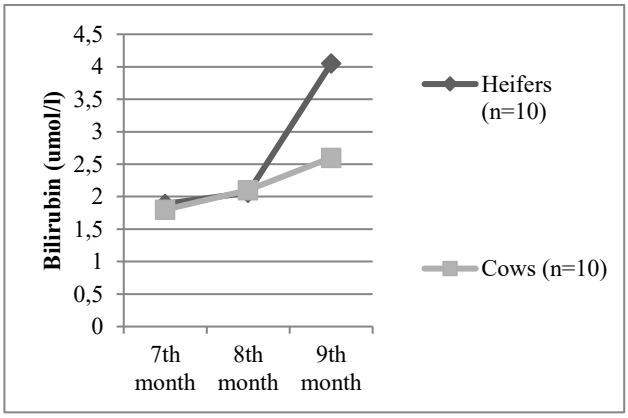

Fig. 7. Serum bilirubin level in heifers and cows during last trimester of pregnancy.

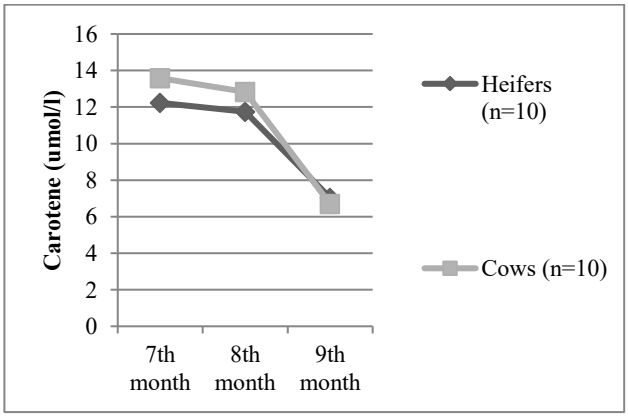

Fig. 8. Serum carotene level in heifers and cows during last trimester of pregnancy.

After thorough analysis of the received data there are evidences for high endogenous and exogenous load in pregnant animals. It is worth noticing that cows of 3rd lactation are in a worse condition when compared with heifers.

IV. Conclusion

After analyzing the received data, there was a revealed increase of exogenous and endogenous stress both in cows and in heifers. But it is worth noting that in cows (3rd lactation) damage was more severe. This was indicated by increase in serum enzyme activity, the serum bilirubin level, the serum creatinine level, as well as reduction of the serum carotene level in the course of pregnancy.

Thus it is essential not only to control health and wellbeing in pregnant cows, but also to improve environmental factors, feeding technique and veterinary work in order to reduce culling and early death in dairy herd. It is significant to introduce different feed supplements in order to reduce oxidative stress, manage excess loss of nutrients and prevent diseases [8].

Some of the most often reasons for dairy cattle culling in Russia are: liver failure, metabolic disorders and low dairy yield [7]. Liver disorder in dairy cattle is often asymptomatic and results not only in lower dairy yields, but also in low quality milk, lower offspring output and higher disease susceptibility.

One of these methods is mycotoxins eliminators application in cows during the last trimester of pregnancy [11]. Some researchers indicate that during the last trimester of pregnancy there is a great load in pregnant cow and mycotoxins of the feed has severe damaging impact for the fetus [18]. Moreover due to mycotoxins cumulative effects in body, the damaging effects may be more severe and prolonged. The oncoming lactation also will be less yielded and there will be possibility of mycotoxins excretion in milk and meat after mycotoxins infestation.

Mycotoxins are hard to detect in feed due to different specifics. For example, in order to destroy molecule of mycotoxin, the conditions have to be far more harsh and severe to destroy the mold or the fungus. So even if the feed underwent treatment or purification and there were no fungi or mold in the feed, the mycotoxins still may be there, and through milk and meat, get to the consumer (either human or calf) [18]. Also the assays to detect mycotoxins are very expensive – so it is impossible to check and detect all the mycotoxins in the feed. Moreover, some of the assays still cannot detect low levels of mycotoxins – the most dangerous doses, which trigger the cumulative effect [1].

It is important to monitor, prevent and when possible control not only mycotoxicosis in dairy cattle, but also metabolic distress, infectious diseases and high yield aftermaths in order to gain healthy and long-lasting highly yield dairy herd and reduce culling. Moreover, it is essential to monitor and regularly check feeds for mycotoxins, fungi and mold to avoid damage not only to health and wellbeing of the animals and offspring, but also to human's health.

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