Sel'skokhozyaistvennaya Biologiya [Agricultural Biology], 2011, № 6, p. 103-109

UDC 636.2:636.018:591.05

METABOLIC STATE AT THE END OF EARLY LACTATION IN HIGH- PRODUCING DAIRY COWS WITH DIFFERENT REPRODUCTIVE ABILITIES

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Received July 18, 2011

S u m m a r y

Biochemical blood indexes and metabolic relationships in high-producing dairy Black-and-White cows with different reproductive potential were investigated at the end of early lactation. A rise in the alanine aminotransferase activity in the blood of animals with the increased reproductive ability was found. This index was also negatively related to the length of the calving to conception interval and magnitude of the protein/glucose ratio in the cow blood. The role of integration of protein and carbohydrate metabolism during early lactation as one of the factors determining the reproductive potential of high-producing dairy cows is discussed.

Keywords: cows, metabolism, reproductive ability, glucose, aminotransferases.

Intense selection of dairy cattle today has significantly improved its milk productivity. At the same time, enhanced genetic potential of this trait in dairy cows has led to a number of undesirable side effects associated with disorders of various physiological processes including reproduction (1-3). These disorders are commonly related to complications of metabolic adaptation during a negative energy balance which arises in early lactation when the organism is not able to consume enough food (4, 5). The metabolism of high yielding cows is greatly modified – it is focused on milk production to the detriment of reproductive ability and energy reserves of the animal (5). The negative energy balance is mainly compensated by mobilization of the deposited body fat and, to much lesser degree, by protein reserves. Along with it, most of the endogenously synthesized glucose pool is spent on intense production of lactose, especially in early lactation. Such metabolic state is reflected by reduced blood level of glucose and increased levels of free fatty acids and ketone bodies (6). Both these phenomena of the negative energy balance are most pronounced in cows with reduced reproductive ability (7, 8). In this regard, the abovementioned biochemical blood indices are recommended for assessing the reproductive potential of high yielding dairy cows in early post-calving period (8).

Reduced reproductive ability of high-yielding cows is often caused by ovarian dysfunction associated with developmental disorders of the dominant follicle, that depends on basal concentration of luteinizing hormone (LH) or the absence of ovulation due to low preovulatory LH peak (9). Glucose is a nutritional factor important for reproductive function of cows. In hypothalamus, glucose modulates both basal LH secretion and the preovulatory release of this hormone (10), along with being the primary energy source for cells of the ovary (11). It has been shown in high-yielding cows that concentration of glucose in follicular fluid increases with growth of follicles, and during the post-calving period glucose content in the dominant follicles changes similar to that in blood (12, 13). This fact indicates that quality of sexual and somatic cells of antral follicles is directly determined by glucose blood level which, in turn, depends on total energy balance in the organism. In addition, the effect of glucose on the ovary can be mediated. Thus, blood level of this metabolite in cattle with a negative energy balance is positively correlated with contents of insulin and insulin-like growth factor I produced in the liver (7, 14). These hormones are the important modulators of viability and functional activity of ovarian follicles at all developmental stages including pre-antral follicles (9, 10). Thus, the decrease in blood level of glucose in high yielding cows with reduced reproductive ability can be accompanied with ovarian dysfunction caused by atresia of immature follicles.

Adaptation of high yielding cows to metabolic changes during a lactation depends on extent and duration of the negative energy balance. It has been reported that energy balance of cows attains equilibrium after 10-14 weeks post parturition, that is, at the end of early lactation (from the start of milking cow after calving till this period farmers perform activities aimed at increasing milk yield - *razdoi*) (4, 15). Prolongation of the negative energy balance reduces the chance of successful metabolic adaptation of cows and adversely affects their reproductive function (15).

The purpose of this research was biochemical study of the blood in high yielding dairy cows with different reproductive potential, as well as detection and comparative analysis of metabolic regularities in these animals.

Technique. The object of study were Black-and-White cows aged 4-7 years and producing on average 7777±320 kg milk a year. Experiments were performed in the pedigree farm "Prinevskoe" (Vsevolozhsk region of Leningrad province) in 2008. The animals were kept in tied stalls; a diet corresponded to zootechnical standards. Biochemical blood tests were performed in 22 cows in the period from the 70th to the 90th days of lactation. Blood was collected once (jugular vein puncture 3-4 h after feeding). After the service period and subsequent calving, the cows were divided into two groups – cows with a service period of less than 150 days (short service period: 105±10 days, n = 11) and more than 150 days (long service period: 268±27 days, n = 11) (groups I and II, resp.). Duration of a service period was used as a characteristic of reproductive ability of cows.

Blood serum was tested to determine concentrations of protein and its fractions (albumin and globulin), glucose, urea, creatinine, cholesterol, bilirubin, minerals (calcium and phosphorus) and carotene, as well as activity of enzymes - aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) using commercial test kits by "DIALAB" (Austria) and "Randox" (UK). Laboratory studies were performed photometrically on the biochemical analyzer RX Daytona ("Randox Laboratories", UK).

The obtained biochemical data and characteristics of milk production were treated with univariate analysis of variance in the program SigmaStat. Reliability of differences of compared mean values was assessed by Tukey's test at a significance level $p<0,05$.

Correlations were analyzed using Pearson's correlations coefficient.

Results. Metabolic state of the cows was assessed at the end of early lactation w (Table 1). Milk production for 100 days of lactation (i.e. during the period of experiment) did not differ significantly in all investigated animals; in groups I and II it averaged to, respectively, 3015±265 and 2964±185 kg. However, milk production for 305 days of lactation was significantly higher in cows with a long service period (8466±336 vs. 7088±471 kg, $p < 0,05$), which, obviously, was the result of later occurrence of pregnancy in these animals (3 , 16).

1. Biochemical blood levels in Black-and-White cows with different duration of service period at the end of early lactation ($X \pm m$, pedigree farm "Prinevskoye", Vsevolzhsk region of Leningrad province, 2008).

Indicator	I group (n = 11)	II group (n = 11)	Reliability of differences, p
Glucose, mmol/l	2,18±0,18	1,82±0,15	NRD
Total protein, g/l	81,2±3,4	89,6±4,4	NRD
Protein/glucose	38,9±2,7	54,5±7,5	< 0,1
Albumin, g/l	32,0±1,9	33,7±2,3	NRD
Globulin, g/l	49,1±2,6	55,9±4,2	NRD
Urea, mmol/l	5,10±0,30	4,38±0,31	NRD
Creatinine, mmol/l	94,1±3,0	88,5±2,8	NRD
Cholesterol, mmol/l	6,81±0,79	7,55±0,79	NRD
Bilirubin, umol/l	2,24±0,35	2,05±0,29	NRD
Alkaline phosphatase (ALP), units/l	55,2±5,0	52,7±2,8	NRD
Aspartate aminotransferase (AST), units/l	91,7±3,0	94,8±8,5	NRD
Alanine aminotransferase (ALT), units/l	27,4±1,6	21,2±1,3	< 0,01
AST/ALT	3,46±0,22	4,78±0,65	< 0,1
Calcium, mmol/l	2,46±0,06	2,44±0,05	NRD
Phosphorus, mmol/l	2,00±0,08	2,13±0,04	NRD
Calcium/phosphorus	1,23±0,06	1,15±0,03	NRD
Carotene, mg %	0,236±0,031	0,273±0,020	NRD

Note. Groups I and II – cows with a service period of, respectively, less and more than 150 days; NRD – no reliable differences.

High yielding cows whose service period was less than 150 days showed higher values of ALT activity ($p < 0,01$) at the end of early lactation compared with cows having a longer service period (Table 1). This fact indicates the activation of glucose-alanine cycle and subsequent elevation of gluconeogenesis in cows with better reproductive ability. In these animals there was also revealed a trend ($p < 0,1$) to lower values of the ratios protein/glucose and AST/ALT, which suggests more intense use of glucogenic amino acids (at least, alanine) in the synthesis of glucose. These amino acids were probably obtained from food protein and deposited body protein (6). Indeed, the fact of using the muscle protein to supply the increased demand for glucose during the early lactation in high yielding cows has been reported by S. Kessel et al. (15). Another data represent the blood levels of total protein, globulin and cholesterol significantly exceeding the norm in cows with a long service period (17). High blood level of cholesterol can be the result of increased mobilization of fat depots in individuals with reduced reproductive ability (7, 8). These data are generally consistent with findings of other authors that the balance of adipogenic and glucogenic compounds in the organism of high yielding cows causes a great effect on their reproductive ability (6).

Further analysis revealed the negative correlation between a duration of service period and glucose content ($p < 0,05$), as well as ALT activity ($p < 0,01$) in the blood of high yielding cows (Table 2 .)

Previously, M. Reist et al. (18) have established a similar relationship between the blood level of glucose and interval from calving to fertilization during the first half of lactation in high yielding cows; in this study, such dependence for the first time was established in respect to ALT activity. At the same time the ratio protein/glucose and AST/ALT were found to be positively correlated with duration of a service period (at least at $p < 0,05$). Thus, results of correlation analysis confirm the earlier assumption that high yielding cows with better reproductive ability have more intense utilization of amino acids as a substrate of gluconeogenesis.

2. Correlations between biochemical blood indices, enzyme activity and productivity in Black-and-White cows at the end of early lactation ($X \pm \bar{m}$, pedigree farm "Prinevskoye", Vsevolzhsk region of Leningrad province, 2008).

Pairs of compared indices	Correlation coefficient r (n = 22)	Reliability p
Service period —Glucose content	–0,467	< 0,05
Service period —Protein/glucose	0,478	< 0,05
Service period —ALT activity	–0,533	< 0,01
Service period —AST/ALT	0,616	< 0,01
Protein content —Globulin content	0,858	< 0,001
Protein content —Albumin content	0,476	< 0,05
Protein content —ALT activity	–0,646	< 0,001
Protein content —AST/ALT	0,656	< 0,001
Protein content —ALP activity	–0,430	< 0,05
Protein content —Creatinine content	–0,672	< 0,001
Glucose content —Albumin content	0,439	< 0,05
Glucose content —AST/ALT	–0,437	< 0,05
Glucose content —Cholesterol content	0,506	< 0,05
Protein/glucose —ALT activity	–0,646	< 0,001
Protein/glucose —AST/ALT	0,794	< 0,001
Protein/glucose —Creatinine content	–0,435	< 0,05
Globulin content —ALT activity	–0,517	< 0,05
Globulin content —AST/ALT	0,548	< 0,01
Globulin content —ALP/ALT	0,420	< 0,05
Globulin content — Creatinine content	–0,519	< 0,05
Albumin content — ALP activity	–0,502	< 0,05
Albumin content — Urea content	–0,552	< 0,01
Albumin content — Creatinine content	–0,467	< 0,05
Albumin content — Cholesterol content	0,780	< 0,001
Albumin content —Calcium content	0,495	< 0,05
Urea content — Creatinine content	0,430	< 0,05

Urea content — Cholesterol content	–0,472	< 0,05
Urea content —Phosphorus content	–0,533	< 0,01
Creatinine content — ALT activity	0,580	< 0,01
Creatinine content —AST/ALT	–0,479	< 0,05
Creatinine content — ALP activity	0,545	< 0,01
Cholesterol content — AST activity	0,423	≤ 0,05
Cholesterol content —Calcium content	0,469	< 0,05

Note. See Table 1.

Numerous correlations between biochemical blood indices were revealed as the evidence of faithful metabolic interrelations in the organism of high yielding dairy cows (Table 2). Negative correlations between ALT activity and total protein content were found, as well as between ALT activity and the ratio protein/glucose ($p < 0,001$), between glucose content and the ratio AST/ALT ($p < 0,05$). These relationships show the important role of ALT in integration of protein and carbohydrate metabolism of high yielding cows at the end of early lactation. The established positive correlation between blood levels of glucose and cholesterol ($p < 0,05$) is consistent with findings of other researchers who observed the correlation between a short service period and high blood levels of glucose and cholesterol (18). Unidirectional change in glucose and cholesterol blood levels in high yielding cows can be explained by the fact that using acetyl-CoA in cholesterol synthesis results in producing free fatty acids and ketone bodies, the contents of which, in turn, are negatively correlated with concentration of glucose (7, 8).

Metabolic relationships in cows with different productive potential were the essential issue of this research (Table 3). A comparative analysis of biochemical indices revealed the dependence in 24 cases; in 14 of them correlation coefficients calculated for two groups of cows differed more than 2-fold, which reflected the unequal metabolic status of these individuals. The direction of correlations between glucose and enzyme activity of ALT and AST in the blood was determined by reproductive potential of cows. Thus, in cows with a long service period, this trait was positively correlated with AST activity ($p < 0,05$) although this wasn't peculiar to cows with a short service period.

3. Coefficients of correlation (r) between biochemical blood indices and enzyme activity in Black-and-White cows with different duration of service period at the end of early lactation ($X \pm m$, pedigree farm "Prinevskoye", Vsevolzhsk region of Leningrad province, 2008).

Pairs of compared parameters	I group (n = 11)	II group (n = 11)
Service period —AST activity	–0,09	0,60*
Service period — AST/ALT	–0,31	0,64*
Protein content — ALT activity	–0,59	–0,64*
Protein content — AST/ALT	0,66*	0,64*
Protein content — ALP activity	–0,66*	–0,18
Protein content — Globulin content	0,83**	0,86***
Protein content — Albumin content	0,62*	0,37
Protein content — Creatinine content	–0,46	–0,80**
Protein content — Calcium content	0,67*	–0,04
Glucose content — AST activity	0,67*	–0,54
Glucose content — ALT activity	–0,14	0,62*
Glucose content — AST/ALT	0,42	–0,75**
Glucose content — Globulin content	0,11	–0,60*
Glucose content — Albumin content	0,68*	0,37
Glucose content — Cholesterol content	0,82**	0,33
Protein/glucose — AST activity	–0,63*	0,47
Protein/glucose — ALT activity	–0,31	–0,82**
Protein/glucose — AST/ALT	0,02	0,84**
Protein/glucose — Globulin content	0,35	0,82**
Globulin content — Creatinine content	–0,22	–0,66*
Albumin content — Urea content	–0,37	–0,69*
Albumin content — Cholesterol content	0,79**	0,77**
Albumin content — Calcium content	0,62*	0,42
Albumin content — Calcium content	0,39	0,63*

Note. See Table 1. *, ** and *** respectively $p < 0,05$; $p < 0,01$ and $p < 0,001$.

Along with it, in animals with high reproductive ability the blood level of glucose was positively correlated with AST activity ($p < 0,05$). On the contrary, cows with reduced reproductive potential showed a positive correlation of glucose content and ALT activity ($p < 0,05$), as well as the trend to a negative correlation between glucose level and AST activity ($p < 0,1$).

The obtained data can be interpreted in a view of acute glucose deficit in the organism of high yielding cows during an early lactation (6) and unequal activity of ALT in the blood of animals with different reproductive potential (Table 1). Increased ALT activity in cows with a short service period possibly contributed to the activation of glucose-alanine cycle conjugated in the liver with ornithine cycle, which requires aspartate participating nitrogen elimination. Elevation of AST activity apparently stimulated aspartate synthesis, which then resulted in a positive correlation between AST activity and glucose content. At the same time, when the intensity of using amino acids in gluconeogenesis exceeds a certain threshold, it most likely will suppress reproductive potential of animals. That's why the group of cows with a short service period didn't manifest the regularities found in another group - negatively correlated duration of a service period and ALT activity along with positively correlated ALT activity and glucose content. On the contrary, in animals with reduced reproductive ability and low ALT activity, the increase in AST activity aimed at aspartate production can inhibit the synthesis of oxaloacetate (substrate of gluconeogenesis), which leads to a negative correlation between the blood level of glucose and AST activity.

So, high yielding cows manifest different adaptability at the end of early lactation even under similar conditions of feeding and keeping. Animals with better reproductive ability show higher activity of alanine aminotransferase, which value is negatively correlated with duration of a service period and the ratio protein/glucose in blood. The direction of interrelations between metabolism of proteins and carbohydrates at the end of early lactation is one of the factors determining reproductive potential of high yielding cows.

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