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Blood chemistry, thyroid hormones, and insulin serum content in bulls fed a ration limited in energy

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Abstract: The aim of this study was to determine blood biochemical profile and selected hormone concentrations in bulls fed a diet limited in energy content. Bulls in the experimental group (I) over the 63 days prior to slaughter were provided with ration energy limited to 80% of the maintenance requirement. Animals in the control group (II) received a balanced ration. Blood samples from all of the animals were collected 3 times (A, B, C). The decrease in the ration energy level did not have a significant effect on the concentrations of glucose, total protein, total cholesterol, high-density lipoproteins, triglycerides, and the activity of aminotransferase aspartate and aminotransferase alanine. Varied nutrition resulted in an increase in the content of albumins and urea ($P < 0.01$) in experimental group blood serum. The study did not show any significant changes in the insulin and thyroxine concentrations for the last blood collection date; however, a decrease in the triiodothyronine concentration was found ($P < 0.05$).

Key words: Bull, blood, biochemical profile, thyroid hormone, insulin, energy

1. Introduction

The growing consumer interest in the health-promoting properties of meat consumed observed in recent years has called for correction of the methods of cattle fattening and the development of methods for producing lean beef. The nutrient content in meat depends not only on the intensity of feeding and the kind of feed but also on the animal breed (1–3). A decrease in the meat fat content is possible by decreasing the energy level in the ration of fattened animals (4). One should consider, however, the threat of disturbing the inner balance of the body. Thanks to the role of blood in maintaining homeostasis as well as to easy blood sampling, blood tests can help us to determine whether the animal nutrition applied has been adequate. For such evaluation, the selection of adequate biochemical parameters defining the state of respective organs and organisms is of paramount importance.

Glucose blood concentration is considered to be a direct indicator of energy balance in the organism, while the concentration of triglycerides and cholesterol is also connected indirectly with energy metabolism and directly with fat transformations. Most blood lipid fractions occur in the form of protein-lipid bonds, namely lipoproteins. Depending on the current energy requirement, the fatty acids produced as a result of digestion processes are transformed into neutral fat or, having bonded with albumins and high-density lipoproteins (HDL), they are transported through the blood to the liver. It is there that in the process of beta-oxidation they are transformed into acetyl-CoA and then included into the Krebs cycle, thus constituting the source of energy. Following the transformation into triglycerides, the excess of fatty acids is released from the liver back into the blood (5). In ruminants, however, there is no hydrolysis of triglycerides in the liver, and so maintaining them in the organ, if the possibilities to remove them have been exceeded, is irrevocable (5). In providing a diagnosis of functional liver changes, determining the enzymatic activity of the liver by defining the level of aminotransferase aspartate (AST) and aminotransferase alanine (ALT) is justifiable. Animal nutrition also determines the level of cholesterol in serum. A variation in the concentration of that parameter affects all lipoproteins since insoluble cholesterol does not occur in serum in a free state but rather in soluble lipoproteins. The main lipoprotein fraction is made up by HDL. A strong correlation between changes in the HDL level and a variation in the other lipoproteins was observed (6). The levels of total protein, albumin, and urea are considered direct indicators of protein metabolism in the organism. Any deviations from the blood parameters can point to,

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for example, a disturbed protein–energy ratio in the ration (7,8), which, in turn, can result in the unwanted use of protein as a source of energy.

The role of hormones in maintaining body homeostasis by controlling metabolic transformations is vast and commonly known. The level of insulin in cattle serum is strongly correlated with the body weight increase rate (9–11), and limiting feed consumption by the animals results in a decrease in the content of insulin in blood serum (12). Limiting the amount of energy in feed also decreases the concentrations of triiodothyronine (T_3) and thyroxine (T_4) (9).

Since disturbed body homeostasis can occur due to a change in diet, we decided to compare the level of selected biochemical parameters and hormones in blood serum in bulls following a decrease in the ration energy level to 80% of the maintenance requirement.

2. Materials and methods

The study involved 60 bulls: 30 Black-and-White Polish Holstein–Friesians (PHFs) and 30 crossbreds obtained from Polish Holstein–Friesian dams sired by Limousins (LM × PHF). The animals were fattened indoors on individual stalls, on tethers, on shallow litter. They had access to a salt block and water and they were individually fed. The feed was administered twice every 24 h, with the same amount in the morning and in the evening. The ration was mostly made up of maize silage and grass hay silage. The ration was supplemented with concentrate containing bruised cereal grain, soy pellets and rapeseed meal, and a mineral–vitamin mixture. The rations for the animals were determined following the Ruminants Nutrition Norms in the French National Institute for Agricultural Research (INRA) system with the use of INRAtion software, version 2.x.x., drawing on the earlier chemical analysis of feeds and calculating their nutritive value. The total requirements for nutrients were determined based on the Ruminants Nutrition Norms in the system of IZ-INRA (13).

Seventy days prior to slaughter, the animals were divided into 2 groups. The following method of animal selection was used: the bulls were divided into pairs of analogues of 2 individuals of similar age and with a similar body weight within the same breed. The animals were then randomly allocated to the control and experimental groups. Groups I and II included the same number of bulls of respective genotypes: 15 PHF and 15 LM × PHF. The initial average body weight was 503.63 kg (±52.48) in the experimental group (I) and 506.43 kg (±49.83) in the control (II). The mean age of the bulls was 23.92 months (±3.21) and 23.55 months (±3.77), respectively. The statistical analysis did not show any significant differences between the age and the body weight in respective groups.

The experimental and control group of animals varied in their nutrition. Group I (experimental) had 30 bulls provided with a ration energy reduced to 80% of the maintenance requirement 63 days before slaughter. Group II (control) had 30 bulls fed until slaughter with a ration that provided their maintenance requirement.

The rations offered to group II were balanced in terms of the content of energy, protein, fiber, mineral compounds, and vitamins. As for group I, the level of energy was limited and, due to restricting the amount of bulky feed, the level of fiber decreased while the contents of protein, mineral compounds, and vitamins were balanced. A lower level of energy in group I (experimental) was achieved by limiting the amount of maize silage and a change in the type of concentrate feed: crushed cereal grain was cancelled, the share of high-protein feeds (expelled soy meal) was increased, and feed containing protein protected against decomposition in the rumen was introduced. A change in the ration and the introduction of new feeds were done gradually within 7 days of the animals being divided into groups (adaptation period).

Blood was collected from the jugular vein 3 times (always before morning feeding): on the day when the animals were broken into groups (A), again 7 days after the completion of the adaptation period (B), and for the third time on the slaughter day (C). After coagulation, blood samples were centrifuged at 3000 rpm for 10 min and serum was separated. The samples were stored in a freezer at –20 °C until analyses.

In the blood serum the concentrations of glucose, total protein, albumin, total cholesterol, HDL, triglyceride, urea, AST, and ALT were determined with the Epoll-20 photometer using original Alpha Diagnostic kits (Alpha Diagnostic Intl. Inc., USA). Insulin, T_3, and T_4 in blood serum were evaluated with the radioimmunoassay method with Diagnostic Systems Laboratories kits (USA) for T_3 and T_4 and with the Nordic BioSite kit (Sweden) for insulin.

The individual animals constituted the experimental unit for analysis of all the parameters. The results were analyzed statistically using the Statistica 8.0 PL software by one-way analysis of variance. Significance of differences between the experimental (I) and control (II) groups was identified using Duncan's test.

3. Results

The levels of biochemical blood parameters in bulls are given in Table 1. The first parameter, glucose content, was very similar in both the animal groups prior to the experiment (date A of blood collection). The differences appeared on the second blood collection date (B). In the bulls with a lowered amount of energy in the ration (group I), the content of glucose decreased to 3.94 mmol/L and the difference, as compared with group II (control), was significant ($P < 0.05$).

Table 1. Mean ± standard deviation of biochemical blood indices in bulls of different groups.

Index / Group	Blood collection		
	A	B	C
Glucose, mmol/L			
I	4.18 ± 0.49	3.94 ± 0.51 [a]	4.72 ± 1.11
II	4.08 ± 0.30	4.25 ± 0.49 [b]	4.86 ± 0.56
Total protein, g/L			
I	79.20 ± 8.70	78.80 ± 9.80	90.50 ± 9.10
II	81.00 ± 8.90	77.70 ± 8.90	86.40 ± 8.30
Albumin, g/L			
I	42.40 ± 4.80	33.00 ± 2.40	37.40 ± 2.50 [A]
II	43.00 ± 4.60	32.70 ± 1.70	34.70 ± 2.50 [B]
Total cholesterol, mmol/L			
I	4.33 ± 1.07	2.01 ± 0.49 [a]	2.72 ± 0.50
II	4.17 ± 0.85	1.71 ± 0.54 [b]	2.68 ± 0.59
HDL, mmol/L			
I	1.41 ± 0.25	1.45 ± 0.26	1.69 ± 0.44
II	1.37 ± 0.23	1.38 ± 0.19	1.47 ± 0.55
Triglyceride, mmol/L			
I	0.25 ± 0.07	0.29 ± 0.09	0.19 ± 0.04
II	0.24 ± 0.05	0.30 ± 0.10	0.19 ± 0.04
Urea, mmol/L			
I	2.65 ± 1.08	4.37 ± 1.88 [A]	4.58 ± 1.01 [A]
II	2.58 ± 1.06	1.72 ± 0.69 [B]	2.34 ± 0.88 [B]

[a,b]: Values with different superscripts are significantly different at $P < 0.05$.
[A,B]: Values with different superscripts are significantly different at $P < 0.01$.

The highest levels of total protein were recorded on the third blood collection date (C) in group I, namely in the animals provided with a lower energy level and increased share of high-protein feeds. However, the results did not differ significantly. The difference in the level of albumins occurred on the last date of blood collection (C). In the bulls from group I there was a significant increase in the content of albumins ($P < 0.01$).

In the blood collected from the bulls prior to the change in the ration energy value (date A of blood collection), there were no recorded differences in urea concentration between the animals. The results of the second blood collection date (B) clearly show the effect of experimental nutrition on the concentration of urea in blood. A significant increase in the level of that parameter ($P < 0.01$) was reported in the bulls provided with a limited energy. Exactly the same direction of changes as above persisted until the end of the experiment, which was confirmed by the third and last blood collection (C).

In the present experiment the total cholesterol content of the second blood collection date (B) increased in the bulls with a lowered amount of energy in the ration. At the end of experiment (C), there was no observed effect of the ration energy level on the content of total cholesterol in blood serum. In the present results, there were also no observed differences in the content of HDL, having introduced the animals to the ration with a limited energy level and increased protein amount. The last parameter researched, connected directly with fat metabolism and indirectly with the body energy transformations, was the level of triglycerides (Table 1). In that case, throughout the experiment, there were no significant differences across the bulls fed with rations of a varied energy level.

Table 2 presents changes in the level of selected enzymes and hormones in blood serum. One can observe a clear growing tendency for the AST activity with time in both the animal groups, but no significant differences occurred. In the case of ALT, a significant difference on day B of blood collection was recorded.

There was no observed effect of energy level in the ration on the content of insulin in blood serum.

The content of thyroid hormones in the bulls' blood changed significantly. The highest level of T_3 was reported in group II (date B of blood collection), and it differed significantly ($P < 0.01$) as compared with the animals provided with a lowered energy level in the ration (group I). The tendency for such variation in the level of T_3 persisted until the last (C) blood collection ($P < 0.05$). As

Table 2. Mean ± standard deviation of blood enzymes, insulin, and thyroid hormone concentration in bulls of different groups.

Index / Group	Blood collection		
	A	B	C
AST, U/L			
I	46.23 ± 13.87	58.53 ± 12.36	63.77 ± 12.32
II	43.13 ± 11.41	52.07 ± 10.61	70.03 ± 21.63
ALT, U/L			
I	17.57 ± 4.66	13.53 ± 4.71 [a]	17.03 ± 5.86
II	17.43 ± 4.38	17.17 ± 8.01 [b]	16.87 ± 4.38
Insulin, pmol/L			
I	100.08 ± 24.65	116.54 ± 28.34	101.67 ± 33.27
II	92.44 ± 24.59	106.19 ± 25.84	98.06 ± 30.84
T_3, nmol/L			
I	2.01 ± 0.36	1.48 ± 0.46 [A]	1.88 ± 0.35 [a]
II	1.97 ± 0.35	2.43 ± 0.35 [B]	2.13 ± 0.39 [b]
T_4, nmol/L			
I	86.24 ± 9.40	82.74 ± 9.93 [A]	61.65 ± 5.48
II	84.54 ± 16.20	73.18 ± 11.66 [B]	63.09 ± 6.02

[a,b]: Values with different superscripts are significantly different at $P < 0.05$.
[A,B]: Values with different superscripts are significantly different at $P < 0.01$.

for T_4, a decrease in the ration energy level resulted in an increase in the content of the parameter, but only for the second (B) blood collection ($P < 0.01$).

4. Discussion

The results for glucose level show that, with time, the bodies of the bulls were adapting to the new nutrition. There was a very similar glucose content in blood collection C despite a significant difference in collection B. It is common knowledge that with the insufficient supply of energy in the feed, the animal body triggers the process of gluconeogenesis (mostly in the liver and kidneys), in which the substrate is made up of glycerol released from fat tissue during triglyceride hydrolysis. Rule et al. (14) recorded a significant decrease in the level of glucose (from 4.55 to 3.31 mmol/L) after 2 days of complete starving in steers; however, during the 3 successive days, the content of that parameter increased to 3.82 mmol/L. The increase persisted to the end of the experiment, namely to the last day prior to slaughter (the eighth starving day).

Proteins circulating in the body with blood undergo a continuous exchange with the proteins of organs and tissues of the organism, and so the level of total protein in serum is a reflection of the processes that occur in the body. A lack of differences across the groups in the present experiment can suggest a sufficient supply of protein in the body, with this protein not being used by bulls as the source of energy. The content of total protein in both groups, irrespective of the energy level of the ration, did not differ significantly (Table 1). A similar relationship in calves receiving the ration with a high content of protein was reported by Park (8). This could be a reflection of compositional changes of apoprotein in circulating lipoproteins in blood serum (15,16). The content of urea in serum, next to the level of total proteins and albumins, is yet another direct parameter of protein metabolism in the organism. The increase in the level of urea was due to a changed protein-to-energy ratio of the ration and an incomplete use of ammonia produced in the process of deamination, which transferred to the liver for the synthesis of urea by rumen bacteria. The ammonia production intensity in the rumen, however, depends on the amount of protein in the feed, which coincides with the earlier reports by Fenderson and Bergen (17), Park (8), and Rule et al. (14).

The results for total cholesterol differ from those reported earlier by other authors. The connection between the dietary protein level and plasma cholesterol content has been observed in several experiments with ruminants (8,18,19). The authors suggest that the level of dietary protein has an influence on cholesterol concentration by affecting the rate of cholesterol synthesis. Cholesterol circulated in the blood serum may provoke changes in the concentration or metabolism of cholesterol in other tissues and organs. Park (8), in an experiment that involved calves, observed that the level of dietary protein did not affect blood serum cholesterol, but when HDL was presented as a percentage of total cholesterol, a significant increase in the type of lipoprotein-bound cholesterol was noticed for the group receiving high dietary protein. Triglycerides

are products of the decomposition of reserve fat, and their level in the present experiment did not increase. There was no increase in the intensity of fat tissue lipolysis, which occurs for an excessively low energy value of the ration (5).

AST and ALT are enzymes associated with liver parenchymal cells, facilitating the conversion of aspartate and alpha-ketoglutarate to oxaloacetate and glutamate. When body tissue or an organ, such as the liver, is diseased or damaged, AST is released into the blood; thus, the amount of that enzyme in the blood is related to the extent of damage.

Rule et al. (14) reported an 82% decrease in insulin content after 2 days of complete starving in steers. Such a low level of the hormone persisted until the eighth starving day, upon which slaughter took place. However, in this experiment, the animals were completely and rapidly deprived of feed with no adaptation period. Hayden et al. (10) reported that in steers, decreased plasma concentration of glucose corresponded with a decreased concentration of insulin, which at low concentrations is associated with lipolysis. Steer rate of gain is correlated with plasma concentrations of insulin and insulin-like growth factor 1 (IGF-1) (9), and nutritional restriction decreases plasma concentrations of insulin (12) and IGF-1 (9,20,21). Eisemann et al. (21) examined insulin responsiveness and sensitivity in beef steers of different ages and body weights and determined that the metabolism of glucose by the hindquarters decreased in sensitivity and responsiveness to insulin at a heavier body weight. The results would indicate insulin resistance by peripheral tissues of beef steers as body weight, age, and body fat content increases.

A similar tendency to that of the present study for thyroid hormone concentrations was reported by Murphy and Loerch (22), investigating the effect of limited feed intake on the level of selected parameters in the blood of young steers. This research demonstrated a 10% and 20% decrease in the T_3 level in the animals provided with a limited amount of the feed uptake, as compared with the uptake ad libitum. Ellenberger et al. (9) and Yambayamba et al. (20) also reported that nutritional restriction decreases plasma concentrations of T_3 and T_4. Hayden et al. (10) stated that T_3 concentrations are indicative of energy balance and T_4 appears to be positively associated with energy consumption.

In conclusion, the present study shows that a decrease in the energy level in the ration against the recommended norms at the final period of the bulls' fattening changed the protein-to-energy ratio which, in turn, resulted in a significant increase in the level of albumins and urea in blood serum. The changes in the activity of AST and ALT enzymes observed confirmed the adequacy of the functioning of the liver, which was not burdened with a new composition of the ration. There was no observed effect of energy level in the ration on the content of insulin in blood serum. Limiting the energy in the animal feed to 80% of the maintenance requirement resulted in a temporary disturbance of homeostasis of the organism by an increase in the level of T_4 and a decrease in the content of T_3 in blood serum. The present study may be helpful in interpreting the changes in health condition and in metabolic profiles in bulls fed a ration limited in energy at the final period of fattening.

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