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Biochemical and Hormonal Characteristics of Peripheral Blood in Bulls in Relation to Genotype

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The study aimed at investigating differences in selected peripheral blood biochemical parameters and hormones in bulls of various genotype: Polish Holstein-Friesians (PHF; group I) and crossbreeds obtained from PHF dams sired by Limousin bulls (LMxPHF; group II). The blood for analysis was taken from the jugular vein twice. In blood serum the content of glucose, total protein, albumin, urea, total cholesterol, HDL, triglyceride , AST and ALT activity were determined. Insulin, triiodothyronine (T_3) and thyroxine (T_4) concentrations were also evaluated. The results showed that the insulin concentration and aspartate aminotransferase activity (AST) was significantly different ($P<0.05$) in both of the animal groups therefore it may be associated with bull genotype. Changes in triglyceride and urea levels were unclear thus long-term observation would be necessary to precisely interpret the relationships between blood serum parameters and bull genotype.

Key words: Bulls, blood chemistry, triiodothyronine, thyroxine, insulin, genotype.

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The phenotype of each animal is affected by two major groups of factors: genetic and environmental. Of all the genetic factors, the sex and the breed are most important. The same factors can also determine the quality of meat in animals allocated to consumption (BOYLSTON *et al.* 1995; ZEMBAYASHI & NISHIMURA 1996; LABORDE *et al.* 2001). The taste qualities, and mostly, the salutogenic qualities of meat, are defined not only by the total fat content, but mostly by the composition and the proportions of its fatty acids. Ruminant tissues show a high share of saturated fatty acids (SFAs) (about 50%), monounsaturated fatty acids (MUFAs) (about 45%) and polyunsaturated fatty acids (PUFAs) (about 5%) (MURPHY *et al.* 1995). Such an acid composition is unhealthy for humans. The conclusions of studies on the possibility of genetic improvement of dietary beef value by choosing appropriate fattened bull breeds differ significantly. DE SMET *et al*. (2004) suggest that the differences across breeds in particular types of fatty acids are low and they usually result from different feeding methods. However, an experiment presented by CHOROSZY *et al.* (2006) shows that Limousin and Simmental bulls are characterized by a higher content of *n-3* PUFA in the intramuscular fat of the *thoracis* muscle and a lower content of medium-chain SFA, as compared to Herefords. Compared to Simmentals, Hereford and Limousin bulls demonstrated a higher MUFA content and narrower and almost normal *n-6*/*n-3* PUFA ratio. ALBRECHT *et al.* (2006) found breed-dependent differences in the distribution, quantity and structure of marbling flecks in *longissimus* and *semitendinous* muscles. Breed-dependent genetic differences are also known to influence the disease resistance of animals, e.g. the risk for developing mastitis in Jersey cows is higher as compared to the Holstein breed (WASHBURN *et. al.* 2002; BERRY *et al.* 2007; BANNERMAN *et al.* 2008).

On cattle farms, especially those maintaining a high production level, irrespective of actions conditioning the best economic effects and considering consumer preferences, prophylactics and monitoring of animal health should be regularly conducted. Blood is a very sensitive indicator of metabolic changes in the body, both physiological and pathological. The transformations of all the compounds present and introduced into the body affect e.g. the level of biochemical parameters and

the concentration of hormones in blood serum. The results of lab tests make it possible to assess animal health, the functioning of individual organs and to identify any disorders. For this purpose the data recorded are compared with the reference values characteristic for a given animal group. The basic problem in interpreting the results is, in general, a very high range of physiological norms, which decreases their diagnostic value. Since the level of respective blood parameters is affected by various factors, including the animal genotype (KUPCZYŃSKI & CHUDOBA-DROZDOWSKA 2002; WINNICKA 2004), defining the values of the most essential parameters for respective cattle breeds seems justified.

The hypothesis of the present research was that differences in biochemical and hormonal characteristics of peripheral blood are connected with the genotype. Therefore, the experiment was arranged to determine and to compare the values of selected biochemical parameters and hormones in blood serum in Polish Holstein-Friesian bulls and crossbreeds with Limousin.

Material and Methods

The study was conducted under a research protocol approved by the Local Ethical Committee in Bydgoszcz (No. 12/2006 from the 6 April 2006).

The experiment involved 30 bulls: 15 Black-and-White Polish Holstein-Friesian bulls (PHF)- group I (23.97 ± 0.73 months of age) and 15 crossbreeds obtained from Polish Holstein-Friesian dams sired by Limousin bulls (LMxPHF)- group II (23.14 ± 1.19 months of age). Throughout the fattening period the animals stayed indoors and had access to fresh water. They were individually fed twice every 24 hours; the same amount in the morning and in the evening. The ration for both experimental groups was the same and was mostly made up of maize silage and grass hay silage. The ration was supplemented with concentrate mixture containing bruised cereal grain, soy pellets and rapeseed meal and a mineral-vitamin premix. The diets were formulated according to IZ-INRA (2001) feeding standards drawing on the earlier chemical analysis of feeds and calculating their nutritive value. The total requirements for nutrients were determined based on the IZ-INRA system. The initial average body weight in the first experimental group (I) was 512.73 kg (±14.76) and 505.40 kg (±11.20) in the second group (II). The final average body weight was respectively: 555.40 kg (± 16.14) and 543.47 kg (±13.05).

Blood from all the animals was collected from the jugular vein two times: 56 days before slaughter (blood collection A) and on the day of slaughter (blood collection B). The blood was sampled before morning feeding. After coagulation, the blood was centrifuged at 3000 rpm for 10 min and the serum was separated. The samples were stored in a freezer at -20^{o}C until the analyses.

In the blood serum the content of glucose, total protein, albumin, urea, total cholesterol, high density lipoproteins (HDL), triglyceride, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined with the EPOLL-20 photometer using original Alpha Diagnostic kits (Alpha Diagnostic Intl. Inc., US). Insulin, triiodothyronine (T_3) and thyroxine (T_4) in blood serum were evaluated by applying the radioimmunoassay method (RIA) with DSL kits (Diagnostic Systems Laboratories, Inc., US) for T_3 and T_4 and the Nordic BioSite kit for insulin.

All the results are given in tables as means ($\bar{x}$) with standard error (SE) and were subjected to one way analysis of variance to evaluate the influence of genotype on blood parameters. The *post hoc* Duncan test was applied and the probability of $P<0.05$ was accepted as significant. Data were analysed using Statistica 8.0 PL Software (STATSOFT, INC. 2008).

Results and Discussion

Table 1 presents the results of selected biochemical parameters in bull blood serum in relation to genotype.

In ruminants blood glucose content can be supplied through rumen escape or bypass, however, the balance must be maintained by gluconeogenesis from other absorbed products. The main source of glucose in gluconeogenesis is volatile fatty acids (VFAs) absorbed from the rumen after bacterial fermentation. In general, among all the VFAs, acetate and butyrate acids are the major energy sources (for oxidation) and propionate is reserved for gluconeogenesis (VAN SOEST 1994). The concentration of blood serum glucose in the present study was very similar prior to the experiment (A) as well as at the end of the study (B) in both animal groups. The glucose level during the first blood collection (A) fell into the range of physiological values defined for adult cattle by MEYER & HARLEY (1998) as well as WINNICKA (2004). However, at the end of the experiment (B) the concentration was higher than reference values (2.2-4.5 mmol/l) in both animal groups. We did not find significant breed-dependent differences in the blood glucose level during the first nor the second blood collection time. Earlier studies carried out on cows and heifers did not show any significant changes in the glucose content across six cattle breeds (KUCERA & CHLADEK 2004).

Table 1

Biochemical blood profile in bulls of various genotype (x ± SE)

Parameter / Group	Blood collection	
	A	B
Glucose, mmol l^{-1}		
I	4.07 ± 0.07	4.86 ± 0.15
II	4.09 ± 0.08	4.85 ± 0.15
Total protein, g l^{-1}		
I	82.90 ± 2.92	87.00 ± 2.75
II	79.10 ± 1.39	85.90 ± 1.38
Albumin, g l^{-1}		
I	42.90 ± 1.09	34.60 ± 0.67
II	43.00 ± 1.30	34.80 ± 0.62
Urea, mmol l^{-1}		
I	3.13 [a] ± 0.30	2.20 ± 0.14
II	2.04 [b] ± 0.14	2.49 ± 0.16
Total cholesterol, mmol l^{-1}		
I	4.42 ± 0.25	2.64 ± 0.17
II	3.92 ± 0.17	2.71 ± 0.14
HDL, mmol l^{-1}		
I	1.35 ± 0.07	1.43 ± 0.15
II	1.39 ± 0.04	1.51 ± 0.14
Triglyceride, mmol l^{-1}		
I	0.25 [a] ± 0.006	0.19 ± 0.01
II	0.22 [b] ± 0.014	0.19 ± 0.01

[a, b] values with different superscripts are significantly different at $P<0.05$.

The levels of total protein and albumin are considered direct indicators of protein metabolism in the organism. Plasma proteins synthesised by the liver play many important roles, e.g. by binding and transporting minerals and biologically active compounds, forming the immune system in the organism and maintaining plasma oncotic pressure. The level of albumin in the present experiment was within reference values for adult cattle (MEYER & HARLEY 1998; WINNICKA 2004). However, the total protein concentration in plasma was slightly higher than physiological values (51-77 gl^{-1}). The content of total protein and albumin in various genotypes in the present study did not differ significantly during A nor B blood collection. The content of urea in blood serum is yet another direct parameter of protein metabolism. The results of the present experiment showed that in blood collected from animals for the first time (A) a significantly higher ($P<0.05$) concentration of urea was found in purebred Holstein-Friesian bulls (group I), as compared with crossbreeds (group II). However, on the slaughter day (B) the level of this parameter in all animals was very similar and significant differences were not found. KUCERA and CHLADEK (2004) showed that cattle breed affected the blood plasma urea. Significantly lower values of this parameter were found in Aberdeen Angus, Charolais and Limousin, as compared to higher values in Beef Simmental, Blonde d'Aquitaine and Hereford.

The concentration of cholesterol and triglycerides in blood serum is connected with fat and carbohydrate transformation. In ruminants the intake of diets containing more than 5% fat can inhibit ruminal carbohydrate fermentation into volatile fatty acids (VFAs) (DODSON *et al.* 2010). These acids are shorter than 6 carbons in length and are the main energy source for these animals. However, the principal precursor for *de novo* fatty acid (FA) synthesis in ruminants is acetate acid (BERGEN & MERSMANN 2005). Cholesterol closely associated with fat metabolism does not occur in blood serum in a free state but in soluble lipoproteins. The main lipoprotein fraction is made up by HDL, the concentration of which is to much extent genetically determined, resulting in a strong correlation between changes in HDL level and variation in other lipoproteins, including the content of total cholesterol (COOPER *et al.* 1992). In the present study, we did not observe the effect of bull genotype on the content of total cholesterol and high density lipoproteins (HDL). Both the indices tested remained within the reference values (WINNICKA 2004). RAMZAN *et al.* (1988) reported that in goat kids, an increase in blood cholesterol level was greater in the Beetal than the Barbari breed. Genetically determined levels of physiological plasma cholesterol and triglyceride were noticed by OGUNSANMI *et al.* (2000) in N' Dama and Zebu cattle. The present study showed a significantly ($P<0.05$) higher content of triglycerides in purebred bulls (group I), as compared with crossbred animals (group II). Nevertheless, at the end of the study (blood collection B) we did not detect an effect of genotype on the level of triglycerides in blood.

Table 2 presents changes in the concentration of selected enzymes and hormones in blood serum.

Increased AST activity in the blood plasma is an accurate marker of liver damage. When body tissue or an organ such as the liver is diseased or damaged, additional AST is released into the blood. Unlike AST, ruminant liver cells do not show high ALT activity, and the increased activity of this enzyme in the serum during liver damage is insignificant (STOJEVIC *et al.* 2005). The data recorded in our study showed an increase in AST activity during A and B blood collection in both experimental groups. At the end of the study the level in LMxPHF bulls (group II) was significantly higher ($P<0.05$) as compared to PHF (group I). Changes

Table 2

Serum enzyme, insulin and thyroid hormone profile in bulls of various genotype ($\bar{x} \pm SE$)

Parameter / Group	Blood collection	
	A	B
AST, U l^{-1}		
I	41.93 ± 2.91	61.40 [a] ± 2.20
II	44.33 ± 3.05	78.67 [b] ± 7.01
ALT, U l^{-1}		
I	19.13 [a] ± 0.99	16.80 ± 1.27
II	15.73 [b] ± 1.12	16.93 ± 1.03
Insulin, pmol l^{-1}		
I	100.42 ± 5.52	112.86 [a] ± 6.63
II	84.52 ± 6.62	83.34 [b] ± 7.48
T_3, nmol l^{-1}		
I	1.95 ± 0.11	2.20 ± 0.14
II	1.99 ± 0.07	2.07 ± 0.05
T_4, nmol l^{-1}		
I	79.81 ± 5.58	63.75 ± 2.06
II	89.27 ± 1.39	62.43 ± 0.83

[a, b] values with different superscripts are significantly different at $P<0.05$.

in ALT activity were different. A significantly higher level of ALT was found in Holstein-Friesians at the first date of blood collection (A), unlike at the end of the study (B) when no significant differences were noted. The experiment reported by MAPIYE *et al.* (2010) on Nguni cattle and local crossbreds showed genetically dependent serum aminotransferase activity. Crossbreds showed a higher alanine aminotransferase concentration and a lower aspartate aminotransferase level in blood serum than Nguni cattle. KUCERA and CHLADEK (2004), on the other hand, concluded that the cattle breed did not affect the blood plasma AST and ALT activity.

The next studied parameter was the content of insulin in the bull blood serum. Insulin is secreted from pancreas beta cells in response to various factors, however, glucose is the major factor. The correlation between plasma insulin and plasma glucose levels (MATSUZAKI *et al.* 1997; SASAKI *et al.* 2003; FIEDOROWICZ *et al.* 2008) well complies with the accepted role for insulin in the regulation of glucose metabolism. At the end of the present study (B blood collection) the level of insulin in purebred animals (group I) was significantly higher than in crossbreds (II). There was no relationship between this variation and serum glucose concentration. As mentioned above, the content of glucose reached almost the same values in both animal groups. Previous experiments on cattle revealed a breed-dependent plasma insulin concentration. GRIGSBY and TRANKLE (1986) observed higher serum insulin content in small Angus steers than in larger Limousin or Simmentals. Japanese Black steers demonstrated higher insulin levels over the fattening period than Japanese Brown or Holstein steers (MATSUZAKI *et al.* 1997). Differences in plasma concentration of insulin occurred in Charolais (beef) and German Holstein (dairy) cattle (BELLMANN *et al*. 2004).

The levels of triiodothyronine (T_3) and thyroxine (T_4) in blood serum were the final studied parameters. Hormones in cattle influence many economically important traits, such as the rate of growth, carcass characteristics, meat quality and milk production (VAN SOEST 1994; MATSUZAKI *et al.* 1997; BELLMANN *et al.* 2004). Thyroid hormones influence the energy demand by boosting energy metabolism (oxygen consumption) in mitochondria. Many environmental factors such as climate, season and nutrition can affect thyroid activity and hormone concentrations in blood (TODINI 2007). The breed is one of the endogenous factors affecting the determination of the triiodothyronine and thyroxine content in blood serum. ROY *et al*. (1983) indicated lower concentrations of T_3 and T_4 in purebred Friesian than in beef type x Friesian crosses. TONG *et al.* (1986) reported that Charolais and Limousin crosses had a lower level of T_3 than Hereford x Angus. Our results did not reveal significant breed-dependent differences in blood triiodothyronine nor in thyroxine content.

The present experiment provided some significant data although some changes observed remain unclear. It can be concluded that long-term observations with larger samples of bulls would be necessary to provide a precise interpretation of the relationship between blood serum parameters and bull genotype.

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