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Metabolic profile of the blood of Simmental cows during a production cycle

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ABSTRACT

The aim of this paper was to examine the metabolic profile of the blood of cows of the Simmental breed during a production cycle, from two aspects: by season (spring, summer, autumn and winter), and by stages of production (1/3, 2/3, and 3/3 of lactation and the dry period). The cows were kept in an intensive system in tie-stall housing, and fed with complete diets, which differed in composition throughout the seasons and stages of production. Blood samples of 10 cows were taken four times during the production cycle (winter, spring, summer, and autumn) in the corresponding production stages (1/3, 2/3, 3/3 of lactation and dry period). They weretaken by puncture of the tail vein. The content of Ca, P, Mg, glucose, urea, total proteins, total cholesterol, AST, bilirubin, and the hormones thyroxine (T4), triiodothyronine (T3) and cortisol were determined in blood serum of cows. The results obtained from the F - test indicated a statistically highly significant difference ($P<0.01$) in the contents of P, Mg, glucose, urea, cholesterol and thyroxine, depending on the season. Also, a statistically significant difference ($P<0.05$) was found in the content of AST and total proteins. The analysed parameters of the metabolic blood profile, tested with the F - test, indicated that there was a statistically significant difference ($P<0.05$) in Mg content depending on production stages.

Key words: Simmental breed, nutrition, metabolic profile, season, production stage

Introduction

There is a long tradition of keeping the Simmental breed of cattle in the northern part of Bosnia and Herzegovina. This area is characteristically flatlands with intensive agricultural production. Farmers in these conditions are able to prepare the necessary amount of feed and organize intensive cattle production. The Simmental breed is able to tolerate both extensive and intense systems of management. Moreover, it can achieve satisfactory production of milk and meat with good nutrition and care.

Today in Europe, and in our country too, efforts are being made to preserve a combined type of Simmental breed for the production of meat and milk, with harmonized production characteristics. Due to their ever-increasing genetic potential, the nutritional requirements of these cows are increasing and a more sophisticated feeding strategy is necessary, with improved feed quality (BRYDL et al., 2007). Cows kept in an intensive farming system through one production year, and fed with

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different diets, depending on their production needs. Winter meal is based on fodder (silage, haylage, hay) with the addition of an appropriate amount of concentrate, according to the production stage. In other seasons, the roughage part of the meal includes a green mass, with the addition of the required amount of concentrate. Cows in lactation are exposed to numerous changes in blood and biochemical parameters, which are particularly expressed in late gravidity and early lactation (JOKSIMOVIĆ-TODOROVIĆ and DAVIDOVIĆ, 2012). The most common diseases of lactating cows are: fatty liver, ketosis, oxidative stress, laminitis, mastitis, milk fever, retention of placenta, metritis and infertility (OETZEL, 2004; JOZWIK et al., 2012). Cows are affected by differing dietary systems and the influence of external factors during their production cycle. This may be extreme at some points. Changes taking place in the animal's body in response to external factors such as nutrition, housing system and microclimate may also affect the level of different blood parameters (WÓJCIK et al., 2004).

In one study, reduced nutrient intake accounted for just 35% of the HS (heat stress) induced decrease in milk production (RHOADS et al., 2009), and modest changes in the somatotropic axis may have contributed to a portion of the remainder. There is a decrease in glucose concentration and an increase in the concentration of non-esterified fatty acids (NEFA) in the blood during the negative energy balance in the period after calving (CINCOVIĆ et al., 2011). This is not the case in heat stress.

In heat stress, the negative energy balance implies a decrease in the glucose concentration, but also a reduction in the NEFA concentration in the blood (WHEELOCK et al., 2010; CINCOVIĆ et al., 2010). The hormonal status of dairy cows can be seen by the content of the following: thyroxine, triiodothyronine and cortisol. There may be less triiodothyronine (T3) than thyroxine (T4) in the blood, but the physiological activity of T3 in the activation of the metabolism is about 4 times greater. Under the conditions of negative energy balance and high lipid mobilisation, the concentrations of thyroid hormones were reduced in some studies in the blood of dairy cows in the transitional period, with markedly less triiodothyronine in the blood shortly before and after calving (REIST et al., 2002; PEZZY et al., 2003). The metabolic system is closely connected with the hypothalamus-pituitary-adrenocortical axis (CHROUSOS, 2000), the neuroendocrine system that orchestrates responses of the body to many different types of challenges (BEERDA et al., 2004). The hypothalamus-pituitary-adrenocortical axis effectuates allostasis, *i.e.*, stability through change, and its normal functioning is important for successful adaptation. The external temperature has a major influence on the secretion of the thyroid gland hormone, while the secretion of adrenal gland hormones (cortisol and adrenaline) is affected by various external stresses. Economic cattle production and good management requires healthy animals, high milk production, adequate nutrition and animal welfare. In recent years there has been growing concern for animal welfare, with consumers paying increasing attention to farm management conditions and procedures, especially those that may inflict pain and suffering on animals (GODYŃ et al., 2013). One of the most important challenges in modern herds is to maintain a proper in-door climate, and the interrelation between air temperature and humidity is of prime importance for animal welfare and production profitability (HERBUT and ANGRECKA, 2012).

The aim of this research was to examine the blood metabolic profile of Simmental breed cows during a production cycle, from two aspects: by season (spring, summer, autumn and winter) and by stages of production (1/3, 2/3, 3 /3 of lactation and the dry period).

Materials and methods

The experiment was conducted on a dairy farm with Simmental breed cows. The cows were kept in an intensive production system, in tie-stall housing, and fed with complete diets, depending on the season. The animals were fed with silage and high quality hay, with the addition of concentrate in the winter period, in accordance with the stage of production. Spring meal was based on fodder as was the winter feed. However, before the end of the spring the cows received a certain amount of green mass as the roughage part of the meal. Also, they received appropriate amounts of concentrate in accordance with production achieved and the production phase. The summer meal was based on silage and green mass, with the addition of the necessary concentrates. The meal for the cows in the autumn season consisted of silage and green mass, which was replaced by good quality hay before the end of the autumn. Additionally, appropriate amounts of concentrates were added, according to their needs. Furthermore, the cows did not have any clinical signs of disease.

Blood samples were taken from 10 cows four times during one production cycle, *i.e.* in the winter, spring, summer and autumn, and in the corresponding production stages (1/3, 2/3, 3/3 of lactation and the dry period). The samples were taken by puncture of the tail vein, in sterile vacutainers that did not contain anticoagulants.

After spontaneous coagulation (30 minutes), the samples were centrifuged at 3000 rotation/minute for 10 minutes. The serum was stored at -20 °C until analysis.

The content of the following parameters: Ca, P, Mg, glucose, urea, total proteins, total cholesterol, AST, bilirubin, and the hormones: thyroxine (T4), triiodothyronine (T3) and cortisol, were determined in the cows' blood serum. The concentrations of the biochemical components in the blood serum was determined using an automatic veterinary biochemical analyser (VetEvolution, Italy), while the hormone concentration was determined by the radioimmune method (RIA), using commercial kits.

The results obtained were processed by simple analysis of variance using the statistical program SPSS (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.). The differences between the LSmeans of these parameters, according to the season and stage of production, were tested using the Duncan test. A significance value of 0.05 and 0.01 was used.

Results and discussion

The metabolic blood profile represents the state of an organism in a given time period, if the parameters for analysis are properly selected. According to SCAMELL (2006), biochemical tests evaluate the body's internal condition, the function of different organs (including the kidneys and liver), and the course of metabolic changes in the body.

The metabolic status of macroelements is determined by the content of Ca, P and Mg, and energy metabolism through glucose content, while protein metabolism is based on the content of total proteins and urea. Liver status is determined by AST activity, and the hormonal state through the concentrations of thyroxine, triiodothyronine and cortisol in the blood.

The contents of P, Mg, glucose, urea, cholesterol and thyroxine differ highly significantly ($P<0.01$) according to season. The activity of AST and total proteins in the blood samples of the cows differed significantly ($P<0.05$) between the seasons (Table 1).

The highest content of P in the blood of Simmental cows was determined during the summer, and the smallest during the spring season. A statistically highly significant difference ($P<0.01$) in P content in the blood was determined between the spring and summer seasons. Furthermore, the difference in the content of phosphorus in the blood sampled during the spring and winter seasons, compared to the autumn and summer seasons, was statistically significant ($P<0.05$).

The highest Mg content was found in the blood of cows during the spring, and the smallest during the summer. The difference found in Mg content in the cows' blood was highly statistically significant ($P<0.01$) between the summer and spring, as well as the autumn and spring seasons. A statistically significant difference ($P<0.05$) in Mg content was recorded in the cows' blood during the summer and winter, as well as the autumn and winter seasons.

The highest blood glucose concentration was recorded during autumn and the smallest during the summer. Statistically highly significant differences ($P<0.01$) in blood glucose content were recorded between the following seasons: 1) summer-autumn, 2) summer-winter, 3) spring-autumn and 4) spring-winter. A statistically significant difference ($P<0.05$) in blood glucose content was recorded between the summer and spring seasons.

The content of glucose in the blood of the cows in this research is in line with the research by BELIĆ et al. (2011). These authors found a

Table 1. LSmeans of the metabolic profiles of cows' blood according to season

	Winter	Spring	Summer	Autumn	P
No. of cows (n)	10	10	10	10	
Ca (mmol/L)	2.55	2.33	2.35	2.33	NS
P (mmol/L)	2.09[Abab]	1.78[Bb]	2.36[Aa]	2.00[ABb]	<0.01
Mg (mmol/L)	1.39[Aba]	1.52[Aa]	0.98[Bb]	1.02[Bb]	<0.01
Glucose (mmol/L)	3.50[Aa]	2.37[Bb]	1.73[Bc]	3.97[Aa]	<0.01
Urea (mmol/L)	4.53[Bb]	4.20[Bb]	9.85[Aa]	4.98[Bb]	<0.01
Total proteins (g/L)	81.82[b]	81.75[b]	76.08[b]	99.84[a]	<0,05
Cholesterol (mmol/L)	3.71[Aa]	2.47[ABb]	3.61[Aa]	2.25[Bb]	<0.01
AST (IU/L)	65.27[b]	101.33[a]	82.78[ab]	105.72[a]	<0.05
Bilirubin (µmol/L)	7.90	4.54	7.64	9.89	NS
T_4 (mmol/L)	73.10[BCb]	51.50[Cc]	85.00[ABab]	101.30[Aa]	<0,01
T_3 (mmol/L)	1.99	1.92	2.46	1.67	NS
Cortisol (nmol/L)	3.47	7.19	7.34	7.46	NS

NS - non-significant; values by letters ([a,b,c]) in one row describe significant differences; values marked by a lower case letter differ significantly (P<0.05); values marked by a capital letter differ highly significantly (P<0.01)

decrease in blood glucose concentration during heat stress. Glucose is a better source of energy than fat during heat stress, because the combustion of glucose produces a smaller amount of heat energy compared to the burning of the same amount of fat (POPOVIĆ, 2008), which reduces the thermal load of the organism.

The highest concentration of urea in the cows' blood was determined during the summer season, and the smallest during the spring season. The content of urea in the blood sampled during the summer season was highly significantly different (P<0.01) compared to the urea content in other seasons. There were no statistically significant differences in the content of the same blood parameter between the other seasons. This may be due to diet and the presence of increased outdoor temperatures during the summer season.

RADKOWSKA and HERBUT (2014) found higher urea concentrations in a group of dairy cows that were grazing outside in relation to groups of cows that were in a barn and fed with a complete meal. The slightly higher blood urea concentration in the pasture fed cows compared to the other groups may be due to the fact that pasture vegetation, especially legumes, is rich in protein and poor in energy, which may increase the amount of nitrogen in the diet, and thus in blood, which initiates an intense ureopoietic cycle in the liver, and increases urea concentration in the blood.

The other reason is the higher outside temperature (heat stress) during the summer season.

Due to the catabolism of proteins and excess ammonia from the rumen, urea in the liver is created in larger quantities during heat stress (CINCOVIĆ et al., 2010). The urea in the bloodstream is derived from the rumen or muscle tissue. It is produced from ammonia in the liver, which is created by the degradation of proteins in the rumen, or the creation of amino acids in the process of gluconeogenesis. It is a characteristic of the metabolic process during heat stress (JENKINS and McGUIRE, 2006).

The total protein content in the blood sampled during the summer season was the smallest in comparison with other seasons during the year. It is evident that there was a change in the metabolism of proteins under the influence of heat stress. The blood cholesterol content in this study was the lowest during the spring and autumn, when cows received a higher amount of green matter in the diet. This is in agreement with the studies of EL-MASRY (1991) and GOSWAMI et al. (2000), who found the lowest concentrations of cholesterol in the blood of cows on a summer diet and when cows were kept on pasture. RADKOWSKA and HERBUT (2014) also found a decrease in blood cholesterol levels in dairy cows on pasture.

In this study, the highest content of cholesterol in the blood of the Simmental cows was recorded in the winter and summer seasons.

In contrast to the content of cholesterol, the highest AST activity in the blood was recorded in the autumn and spring seasons, while the least activity was recorded during the summer and winter seasons.

A statistically significant difference ($P<0.05$) was determined for AST activity in the blood of the cows between the winter and the autumn and spring. RADKOWSKA and HERBUT (2014) also found an increase in blood AST activity during grazing. The explanation for this may be found in the content of the diet. These cows take in a higher amount of green matter with relatively higher protein content, which is rapidly decomposed to ammonia in the rumen.

The higher content of nitrogenous substances in feed certainly affects the metabolism of nitrogen in the liver, and increases blood AST activity. In addition to AST activity, the higher concentration of bilirubin in the blood during the autumn season is a sign of the disruption of the hepatocyte structure.

The smallest concentration of thyroxine in the blood was determined during the spring season, which leads us to conclude that the cows were in a negative energy status during this period. The difference in the content of thyroxine in the blood during the spring season was statistically highly significant ($P<0.01$) in relation to the blood of cows taken during the autumn season.

The difference in the content of this hormone was statistically significant ($P<0.05$) between the spring and winter seasons, as well as between the winter and autumn seasons. Thyroid hormones specifically affect the metabolism of certain nutrients, by increasing the intake of glucose from the gut and stimulating its decomposition in the muscle. In addition, these hormones prevent the synthesis of fatty acids from carbohydrates, stimulate the breakdown of fat, and do not allow it to be deposited in the liver. They also reduce blood cholesterol levels.

Based on the F-test, it may be concluded that there was no statistically significant difference between the blood parameters determined in relation to the production stages of the Simmental cows, with the exception of the Mg content (Table 2).

Table 2. LSmeans of the metabolic profile of cows' blood according to the stage of production

	1/3	2/3	3/3	Dry period	P
No of cows	10	10	10	10	
Ca (mmol/L)	2.53	2.19	2.44	2.40	NS
P (mmol/L)	1.98	2.12	2.20	1.98	NS
Mg (mmol/L)	1.14^{b}	1.19^{b}	1.65^{a}	1.65^{a}	<0.05
Glucose (mmol/L)	2.54	2.48	3.20	3.43	NS
Urea (mmol/L)	6.20	6.31	6.34	4.71	NS
Total protein (g/L)	85.97	80.51	91.70	85.96	NS
Cholesterol (mmol/L)	2.99	2.59	3.57	2.77	NS
AST (IU/L)	92.46	86.43	89.11	88.06	NS
Bilirubin (μmol/L)	5.82	6.31	9.84	6.96	NS
T_4 (mmol/L)	64.10	77.50	83.60	75.50	NS
T_3 (mmol/L)	2.32	1.97	1.99	1.89	NS
Cortisol (nmol/L)	5.69	6.48	6.91	6.90	NS

NS - non-significant; values by letters (^{a,b,c}) in one row describe significant differences; values marked by a lower-case letter differ significantly ($P<0.05$); values marked by capital letter differ highly significantly ($P<0.01$)

The highest concentration of Mg in the cows' blood (P<0.05) was recorded in the dry period and 3/3 of lactation, in relation to 1/3 and 2/3 lactation. The difference in the content of Mg between 1/3 and 2/3 lactation was not statistically significant.

Of the total number of cows in 1/3 of lactation, 20% of them had a lower glucose value of 2.5 mmol / L, and 50% of cows had glucose below this value in 2/3 of lactation. Similar results may be found in the papers by SEVINC et al. (2003); DJOKOVIĆ et al. (2011); PICCIONE et al. (2011, 2012, 2012a); CINCOVIĆ et al. (2012), who suggest that fatty liver infiltration may affect the concentrations of several blood components, such as glucose, total proteins, albumin and urea. The highest average AST activity was recorded in 1/3 of lactation compared to other stages of production.

In this production phase, 50% of cows had higher AST activity of 100 U/L. This leads to the conclusion that there were cows with liver lesions among the examined cows. The result obtained is in agreement with the result of DJOKOVIĆ et al. (2013). The lowest concentration of thyroxine in the blood of the cows was determined in 1/3 of lactation. This was not the case for triiodothyronines, whose lowest concentration was determined during the dry period, and highest in 1/3 of lactation.

The content of thyroxine and triiodothyronine in blood decreases in cows that are in a negative energy balance. In this research, this was not the case with the content of triiodothyronine. The reason for this may be found in the time of taking blood samples. The characteristic of triiodothyronine is that its blood concentration rapidly decreases before and immediately after calving.

In addition, since the metabolic profile was tracked through an entire production cycle, it may be expected that the temperature of the external environment affected the secretion of thyroid hormones.

Conclusion

The examined parameters of the metabolic blood profile of the cows indicate that there were statistically significant differences depending on the season (due to different diets, temperatures and other paragenetic factors).

The results obtained from the F - test indicate a high level of statistically significance differences (P<0.01) in the contents of P, Mg, glucose, urea, cholesterol and thyroxine, depending on the season. Also, a statistically significance difference (P<0.05) due to season was found in the content of AST and total proteins. The metabolic blood profile parameters analysed, tested using the F - test, indicate that there was a statistically significant difference (P<0.05) in Mg content depending on production stage.

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SAŽETAK

Cilj ovoga rada bio je praćenje metaboličkog profila krvi krava simentalske pasmine tijekom proizvodnog ciklusa s dva aspekta: po sezoni (proljeće, ljeto, jesen i zima) i po fazama proizvodnje (1/3, 2/3, 3/3 laktacije i suhostaj). Krave su držane intenzivno, na vezu, i opskrbljene su kompletnim obrocima koji su se razlikovali po sastavu ovisno o godišnjim dobima i stadijima proizvodnje. Uzorci krvi od 10 krava uzimani su punktiranjem repne vene, četiri puta tijekom proizvodnog ciklusa (zima, proljeće, ljeto i jesen) u odgovarajućim proizvodnim fazama (1/3, 2/3, 3/3 laktacije i suhostaj). U krvnom serumu krava određivan je sadržaj Ca, P, Mg, glukoze, ureje, ukupnih bjelančevina, ukupnog kolesterola, AST-a, bilirubina i hormona: tiroksina (T4), trijodotironina (T3) i kortizola. Rezultati F-testa upućuju na statistički visoko znakovitu razliku ($P<0,01$) u sadržaju P, Mg, glukoze, ureje, kolesterola i tiroksina, ovisno o sezoni. Također, statistički znakovita razlika ($P<0,05$) zbog utjecaja sezone utvrđena je i u sadržaju AST-a i ukupnih bjelančevina. Analizirani pokazatelji metaboličkog profila krvi, testirani F-testom, upućuju na postojanje statistički znakovite razlike ($P<0,05$) u sadržaju Mg, ovisno o fazi proizvodnje.

Ključne riječi: simentalska pasmina, hranidba, metabolički profil, sezona, proizvodna faza