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Plasma biochemical profile of dairy cows, with respect to diet and breed

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ABSTRACT

The aim of this study was to determine if total replacement of soybean meal (SBM) with a rapeseed by-product (RS-by) in the diets of Romanian Black Spotted (RBS) and Montbéliarde (MB) breeds will cause changes of the biochemical profile in lactating cows. Thirty-two healthy multiparous dairy cows, mid-lactation stage, were randomly assigned to four homogeneous groups (2 RBS and 2 MB) of 8 cows each for 42 days of experiment. Groups received the same basal diet (maize silage and alfalfa hay) and two types of compound feed: control (with 12.5% SBM) and experimental (with 12.5% RS-by). Individual blood samples were collected at the end of the trial from the mammary vein and plasma biochemical parameters were determined. The main effects of feed, breed and their interaction were statistically analyzed with SPSS software. Plasma energy parameters were not affected by the dietary treatments, but glucose was higher in MB breed. Total protein and urea were significantly higher (7.99 vs. 6.93 g/dL; 18.50 vs. 15.31 mg/dL) in control compared to the experimental group; urea was also influenced by the feed x breed interaction. Magnesium was significantly higher (1.98 vs.1.59 mg/dL) in the experimental group. Alanine aminotransferase and alkaline phosphatase were significantly higher (35.75 vs. 29.00 U/L; 43.62 vs. 36.40 U/L) in the control group. The mineral or enzimatic analytes were not affected by animal breed. The majority of the parameters ranged between the normal limits.

Keywords: Romanian Black Spotted, Montbéliarde, Plasma, Rapeseed by-product, Soybean meal

INTRODUCTION

Soybean meal (SBM) is one of the most expensive ingredients used in animal feed. Due to its high cost, especially in countries that need to import it,

there are continuously attempts to replace this major protein source with other economic alternatives. For example, rape (*Brassica spp.*) it is the 3rd most common oilseed crop grown in the European Union and rapeseed meal is a protein rich by-product that is widely used to feed all classes of livestock (USDA, 2016). The use of by-products with good protein and energy levels can reduce feed costs, decrease dependence on SBM and increase farmers profitability. But also the success (or failure) of replacing SBM rely on the advancement of the technological processes in order to improve the nutritional value of other alternative proteins or non-protein sources. Available research on this topic (Silva *et al.*, 2015; Yildiz *et al.*, 2015; Rinne *et al.*, 2015; Paula *et al.*, 2018) provide informations about the ruminants response (e.g. nutrient intake, diet digestibility, ruminal digestion, milk production and composition, body condition scores, efficiency of crude protein utilization, cost of milk) when diets with other protein sources [e.g. sunflower meal, rapeseed meal (canola type), rapeseed expeller, peanut cake, or dry distillers grain with solubles from maize] were fed compared to SBM. However, feeding regimen changes can be associated not only with animal performances but also with their health state. Therefore, the priority in high standards exploitation is to keep dairy cows healthy. So, appropriate analysis to evaluate the health condition of a herd are important in order to prevent metabolic or nutritional disorders in dairy farms. Related to this issue, blood parameters were studied in accordance with breeding stage in Japanese Black beef cows (Otomaru *et al.*, 2015), with production groups in Mexican Holstein cows (Garcia *et al.*, 2017), with early and mid-lactation stage in Simmental cows (Djoković *et al.*, 2016) or udder health (Qayyum *et al.*, 2016). Reference limits considering the effects of parity, stage of lactation, season and days relative to calving were determined for lactating (Cozzi *et al.*, 2011) or late-pregnant heifers and dry Holstein cows (Brscic *et al.*, 2015). Few available studies related to the plasma profile of the Montbeliarde breed were found.

In this context, the aim of this study was to evaluate how the biochemical profile of mid-lactating dairy cows will be affected by the total replacement of soybean meal (SBM) with a rapeseed by-product (RS-by), breed related.

MATERIALS AND METHODS

Ethical clearance for this research was approved by the Animal Care and Use Committee of the National Research-Development Institute for Biology and Animal Nutrition (IBNA), in accordance with Romanian Law no. 199/2018 and the Directive 2010/63/EU regarding the protection of animals used for scientific purposes.

The present study is part of a larger research.

Design, animals, diets, and housing

The study population included 32 clinically healthy multiparous dairy cows, 3rd lactation, mid-stage (165±62.5, DIM±SD), used for 42 days in a 2x2 factorial design experiment. The animals were randomly assigned to four homogeneous groups of 8 cows each. An additive by-product based on rapeseed extraction meal and urea (RS-by, Wisan® 44), processed according to a customs procedure in HL Germany's special feed facilities was tested as an alternative protein source to SBM. The variation factors were: breed [Romanian Black Spotted (RBS) and Montbéliarde (MB)] and the compound feed (CF). Groups received the same basal diet (maize silage and alfalfa hay) fed ad libitum and two types of CFs fed at a rate of 8 kg/day on a fresh weight basis: control (with 12.5% SBM; 8 HF; 8 MB) and experimental (with 12.5% RS-by; 8 HF; 8 MB). Diets (basal plus CFs), formulated according to Burlacu et al. (1991, 2002), were isoenergetic and isonitrogenous and had the same nutritional characteristics as shown in Figure 1. The forage to CFs ratio was 63.5:36.5. The trial took place in IBNA experimental farm located in the South-East part of Romania. Cows were housed together in a stall barn and kept into four individual boxes. Diets were supplied daily at 0700 h and 1630 h and the access to water was permanent (constant level drinkers). Animals were milked in a parlor milking unit twice a day.

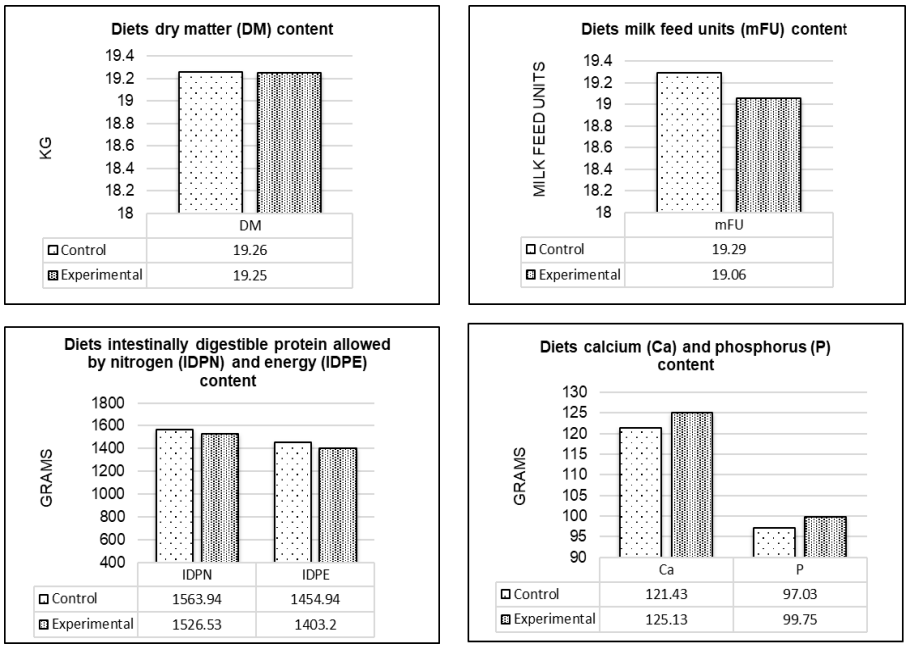


Figure 1. Dietary nutrient content of the daily rations

Sampling and biochemical analysis

At the end of the experiment, in the morning before feeding, individual blood samples were aseptically collected from the mammary vein of all animals into 6-mL vacuum sterile tubes containing 102 I.U. of lithium heparin (Vacutest, Arzergrande, Italy). All samples were then centrifuged at 3000 x g for 20 min at room temperature and the resulted plasma was stored at -80°C until proper analysis. Biochemical analytes concentration of energy [glucose, triglycerides, total cholesterol (T-Cho)], protein [total protein (TP), albumin (Alb), urea, creatinine (Cre), total bilirubin (T-Bil)], mineral [calcium (Ca), phosphorus (P), magnesium (Mg), iron (Fe)] and enzyme markers [alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), γ glutamyltransferase (GGT), creatinine kinase (CK)] were determined on an automatic BS-130 Chemistry analyzer (Bio-Medical Electronics Co., Ltd, China) using standardized kits ACCENT 200 supplied by PZ Cormay S.A. Poland.

Statistical analysis

Data of plasma traits were statistically analyzed using the General Linear Model (GLM) of SPSS 20.0 software. The main effects of feed, breed and the interaction between them were determined. The significance level was set at $P < 0.05$. For P between 0.05 and 0.10 level of significance, it was considered that the fixed factors tended to influence the animal response. A multiple comparison test of Tukey was performed when the effect of treatments was found to be significant. The results are given as means with a standard error of means (SEM).

RESULTS AND DISCUSSION

The metabolic profile analysis, when the SBM was totally substituted by an RS-by, breed related, generally indicated that the majority of the values were within the normal reference ranges for the particular species and physiological category of farm animals (Merck Veterinary Manual, 2010).

Plasma energy profile

Glucose, triglycerides and total cholesterol concentrations were not affected ($P > 0.05$) by any of the main factors tested, and their means are reported in Table 1. When cows received either SBM or RS-by the glucose values were similar between experimental feeds and close to other such reports (Imaizumi et al., 2015; Silva et al., 2015). On the other hand, the fixed effect breed tended to increase the glucose concentration with 17% in MB compared to RBS cows, but even so, it was comprised in the range of normal limits. Also, although not significant, when SBM was fed, MB breed had a lower triglyceride (34.70 mg/dL) and a higher T-Cho (115.77 mg/dL) concentration.

As can be observed, all the values for energy plasma parameters were found in the lower half of the reference ranges. This may be due to the fact that the trial was conducted in the summer season.

Table 1. Mean (±SEM) plasma energy analytes concentration of dairy cows with respect to diet and breed

Item			**Glucose**	**Triglycerides**[ǂ]	**T-Cho**
Reference ranges*			40-100	8-50	62-193
Measurement units			mg/dL	mg/dL	mg/dL
Treatments	***Feed***	***Breed***			
1	SBM	RBS	53.47	45.62	93.22
2	RS-by		50.75	40.47	107.95
3	SBM	MB	59.07	34.70	115.77
4	RS-by		62.60	42.22	110.02
SEM			4.33	3.77	11.51
		Main effects			
Feed					
SBM			56.27	40.16	104.50
RS-by			56.67	41.35	108.99
SEM			3.31	2.88	8.79
Breed					
RBS			52.11	43.05	100.59
MB			60.83	38.46	112.90
SEM			3.06	2.67	8.14
P-value					
Feeds effect			0.930	0.768	0.715
Breed effect			0.079[T]	0.267	0.326
Feed x Breed effect			0.502	0.135	0.411

Note: *Merck Veterinary Manual: Tenth Edition; ǂ Brscic et al. 2015; T: tendency to be influenced by the treatment

So, in response to high temperatures, cattle have a greater energy expense for thermoregulation and a parallel reduction of dry matter intake (Wheelock et al., 2010), which may lead to reduced glucose (Abeni et al., 2007) or cholesterol plasma concentration (Goswami et al., 2000; Bertoni & Trevisi, 2013). AL-Saeed et al. (2009) noted that the seasonal variations between summer and winter climatic conditions alter biochemical parameters of blood plasma. Moreover, energy status in the mid-lactation stage of dairy cows is associated with intensive oxidative events (Svetina et al., 2011) and low levels of serum triglycerides (Marcos et al., 1990).

Plasma protein profile

The outcome means of statistical plasma proteins values are reported in Table 2. Generally, their analysis is used as an indicator that reflects the

metabolic and nutritive status of animals in response to the quality of the diet and the feed intake.

Table 2. Mean (±SEM) plasma protein analytes concentration of dairy cows with respect to diet and breed

Item			**TP**	**Alb**	**Urea**	**Cre**	**T-Bil**
Reference ranges*			6.7-7.5	2.5-3.8	10-25	0.5-2.2	0-1.6
Measurement Units			g/dL	g/dL	mg/dL	mg/dL	mg/dL
Treatments	***Feed***	***Breed***					
1	SBM	RBS	7.66	3.98	20.77^{a}	1.19	0.13
2	RS-by		6.79^{T}	4.05	15.22^{b}	0.96	0.13
3	SBM	MB	8.32^{T}	3.85	16.23^{ab}	1.17	0.17
4	RS-by		7.06	3.85	15.40^{b}	1.12	0.18
SEM			0.35	0.13	1.00	0.07	0.02
			Main effects				
Feed							
SBM			7.99	3.92	18.50	1.18	0.15
RS-by			6.93	3.95	15.31	1.04	0.15
SEM			0.26	0.10	0.77	0.06	0.02
Breed							
RBS			7.22	4.02	18.00	1.08	0.13
MB			7.69	3.85	15.82	1.15	0.17
SEM			0.24	0.09	0.71	0.05	0.02
P-value							
Feeds effect			0.013	0.807	0.011	0.107	0.804
Breed effect			0.221	0.262	0.061^{T}	0.400	0.108
Feed x Breed effect			0.061^{T}	0.834	0.045	0.251	0.804

Note: *Merck Veterinary Manual: Tenth Edition; Different superscripts letters within the same column: significant differences between groups (P <0.05); T: tendency to be influenced by the treatment

In the present study, total protein and urea concentrations were significantly higher in SBM group compared to RS-by (with 15% and 21% respectively); breed factor tended to influence urea; feed x breed interaction tended to influence the total protein and significantly affected urea. Albumin, creatinine and total bilirubin concentrations were not altered by any of the main factors tested or by their interaction. Increased total protein value in plasma of the SBM fed cows might suggest a more efficient protein utilization of the diet containing this ingredient. Although the values for this parameter are slightly above the upper limit, they are in the normal reference range found in other research studies (Otomaru et al., 2015; Bezerra et al., 2013) and close to those obtained by Neto et al. (2015), Radkowska & Herbut (2014), Rutkowska et al. (2015) when similar experimental designs were used. The urea concentration is one of the measurements of the available digestible crude protein and indicates how dietary protein is used by rumen

microflora. Thus, the level of urea from blood is closely linked to the level of urea in the rumen and to the degree of protein degradation to ammonia (Brzóska, 2006; Davidson et al., 2003). Generally, feed sources with high undegradable protein content could reduce the level of ammonia nitrogen in the rumen and so the level of urea in blood plasma. Furthermore, ruminal ammonia depends on the metabolic activity of ruminal microorganisms that transform the ammonia into microbial protein, a process that requires energy (Neto et al., 2015), and which produce changes in plasma urea concentration as well. Although the RS-by contained urea it is well known that SBM delivers a high amount (over 60%) of rumen degradable protein which may result in a greater amount of urea synthesized in the liver that can exceed the microbial growth rate. In the present study, the results were in line with the above. The lowest value of urea was found in the plasma of RS-by dietary regimen animals (15.31 mg/dL). However, our values were in normal literature reference limit.

Plasma mineral profile

It is generally known that minerals are essential nutrients in animals' diets, in order to maintain the normal function of biochemical processes. In dairy cows, plasma levels of calcium, phosphorous, magnesium and iron reflect the supply of these macros- and micronutrients through the feed, their metabolism and their utilization by peripheral tissues, particularly by the mammary gland (Djokovic et al., 2014). Any changes beyond their physiological limits (especially deficiencies) may suggest an imbalance (for example an unfavorable Ca:P ratio in the diet), insufficient intake, poor bioavailability (for example of magnesium in the digestive tract) or other animal health problems (mostly hypocalcemia). In the present study, among plasma electrolytes (Table 3), magnesium content was significantly higher in cows fed RS-by; a tendency (P =0.057) toward an increase of iron in the SBM (169.55 μg/dL) group compared to the RS-by group (154.75 μg/dL) by 1.09 times was found; phosphorus content tended to be influenced by the fixed factor breed; likewise, the interaction of the main factors tended to influence the magnesium and phosphorus levels. Because there are only limited readily accessible body reserves of magnesium, thus plasma concentrations are indicative of current dietary supply and intake of this electrolyte. Also, ruminants can only absorb approximately 20% of the dietary magnesium via the ruminoreticulum, and this dietary absorption is affected by high concentrations of potassium and low sodium concentrations in the diet, or high rumen pH which reduces magnesium absorption (Macrae, 2017; Kurćubić et al., 2010). Our results indicated a plus of 24% statistically difference of RS-by diet over SBM diet, and although not significant, a slightly high content of Mg was found in MB breed compared to RBS cows which may

indicate, in both cases, a better availability or, on the contrary, a higher degree of Mg utilization by SBM group or RBS cows.

Table 3. Mean (±SEM) plasma mineral analytes concentration of dairy cows with respect to diet and breed

Item			**Ca**	**P**	**Mg**	**Fe**[ǂ]
Reference ranges*			8-11.4	5.6-8	1.5-2.9	57-162
Measurement Units			mg/dL	mg/dL	mg/dL	µg/dL
Treatments	***Feed***	***Breed***				
1	SBM	RBS	9.93	4.99[T]	1.53[T]	173.30
2	RS-by		10.08	5.42	1.90	151.47
3	SBM	MB	10.28	6.13[T]	1.65	165.80
4	RS-by		10.07	5.35	2.07[T]	158.02
SEM			0.34	0.28	0.13	6.69
		Main effects				
Feed						
SBM			10.10	5.56	1.59	169.55
RS-by			10.07	5.39	1.98	154.75
SEM			0.24	0.20	0.09	4.73
Breed						
RBS			10.00	5.21	1.71	162.39
MB			10.17	5.74	1.86	161.91
SEM			0.26	0.21	0.10	5.11
P-value						
Feeds effect			0.928	0.556	0.012	0.057[T]
Breed effect			0.639	0.094[T]	0.295	0.947
Feed x Breed effect			0.619	0.062[T]	0.060[T]	0.335

Note: *Merck Veterinary Manual: Tenth Edition; ǂ Mordak, 2008; T: tendency to be influenced by the treatment

The mean values of plasma Mg in the present study were in close agreement with those obtained by Rutkowska et al. (2015), Yokus & Cakir (2006), and much lower than the values given by Ambica & Rao (2012), Shrikhande et al. (2008), Piao et al. (2015), Djokovic et al. (2014) or Nehra (2016).

Conventional dairy cows diets mostly contain sufficient amounts of iron and deficiencies rarely occurs, the situation also observed in our study, suggesting an adequate feed supply of this micronutrient. Yet, the total substitution of SBM by RS-by tended to decrease the plasma level of iron with 9%. However, our results were within the physiological limits, close to those found by Rutkowska et al. (2015), higher than values of Yokus & Cakir (2006) and lower than in the study reported by Djokovic et al. (2014). Among breeds, no differences were observed.

Calcium and phosphorus are the most important macronutrients in dairy cows because of the homeostasis need, the physiological status, and animal's

productivity. Due to their low variation in blood, and high variation in feeds, Ca and P have high diagnostic meaning in determining the nutritional status of animals. Our results were within normal ranges for mid-lactation stage and similar with the findings reported in other studies (Brscic et al., 2015; Rutkowska et al., 2015; Piao et al., 2015; Djokovic et al., 2014). Thus, the values obtained in this study reinforces the fact that Ca varies mostly with the cow's physiological state while P varies more with the nature of the feed or with season, with lower levels in the summer period (Yokus & Cakir, 2006), conversely to other research (Shrikhande et al., 2008) who found no variation in season. Although the P values were just slightly below the lower limit, it must be remembered, however, that hypophosphatemia indirectly stimulates secretion of parathyroid hormone (PTH), thus promoting intestinal Ca absorption. The effect of PTH also increases urinary P elimination. The same mechanism occurs for magnesium, as well (hypomagnesemia). Nevertheless, the plasma levels of P in our study were higher than those reported by Yokus & Cakir (2006), Shrikhande et al. (2008), Piccione et al. (2012) and Naser et al. (2014).

Plasma enzymatic profile

Evaluation of plasma enzymes is an important tool in detecting and correcting metabolic and/or nutritional disturbances. In order to asses' liver functioning in the given experimental design, catabolic enzymes profile was determined (Table 4). The SBM diet significantly increased ALT and ALP concentrations, with 23% and 20% respectively, and tended to increase AST; breed had no effect on the studied markers, while the feed x breed interaction also, just tended to influence the animal's response. Although there were differences between groups for two of the parameters mentioned above, they had values within the normal range. The exception makes GGT concentrations whose values have exceeded (according to the reference used) the upper limit for the studied category of animals and for all factors involved. However, the GGT values resulted in the present study were in line with other accepted reference intervals (Winnicka, 2004; Meyer & Harley, 1998) fell within 22-64 U/L and 20-48 U/L respectively. More, Svetina et al. (2011) concluded that in lactating cows, because of the high energy demands for milk production there is an increase in fatty acid mobilization from adipose tissue resulting in lipid accumulation in the liver and impaired liver function. So, high GGT activity might point out increased hepatic efforts during lactation. A study carried by Jozwik et al. (2012) in Polish Holstein-Friesian Black and White dairy cows revealed highly significant GGT level in mid-lactation (46.18 U/L) than early lactation (38.23 U/L), a value much higher than those obtained in the present study. The same authors revealed a positive significant correlation between GGT level and cholesterol content in blood serum. Also, the higher GGT activity might be a long-term effect of the hepatic stress induced by the preceding

lactation (Brscic, 2015) or, generally, the increased liver enzymes activities in animals could be due to exposure to any stress like nutritional stress, environmental stress, and physiological stress (pregnancy and lactation) (Nessim, 2010).

Table 4. Mean (±SEM) plasma enzyme markers concentration of dairy cows with respect to diet and breed

Item			**ALT**	**AST**	**ALP**	**GGT**	**CK**
Reference ranges*			6.9-35	60-125	18-153	6-17.4	0-350
Measurement Units			U/L	U/L	U/L	U/L	U/L
Treatments	***Feed***	***Breed***					
1	SBM	RBS	38.70^{T}	68.87	44.90	34.10	92.85
2	RS-by		27.70^{T}	67.87	37.45	30.07	88.92
3	SBM	MB	32.80	82.40^{T}	42.33	25.63	100.03
4	RS-by		30.30	62.45^{T}	35.35	33.95	84.47
SEM			2.77	4.95	2.65	3.90	9.26
		Main effects					
Feed							
SBM			35.75	75.64	43.62	29.87	96.44
RS-by			29.00	65.16	36.40	32.01	86.70
SEM			1.95	3.50	1.88	2.76	6.55
Breed							
RBS			33.20	68.37	41.17	32.09	90.89
MB			31.55	72.42	38.84	29.79	92.25
SEM			2.11	3.78	2.03	2.98	7.07
P-value							
Feeds effect			0.039	0.067	0.024	0.608	0.334
Breed effect			0.579	0.448	0.416	0.583	0.890
Feed x Breed effect			0.084^{T}	0.093^{T}	0.934	0.157	0.559

Note: *Merck Veterinary Manual: Tenth Edition; T: tendency to be influenced by the treatment

However, our GGT results were close to those reported by Nehra (2016), higher than those obtained by Piao et al. (2015), Todorović & Davidović (2012), Otomaru et al. (2015) and lower than those determined by Górski & Saba (2012), Mohamed (2014) and Rutkowska et al. (2015). Considering the level of other enzymes reported in the present study, which was inside the lower and upper physiological limits, we can conclude that the liver function was not affected by any of the main factors. Anyway, we can notice that the SBM diet overstrained more the liver metabolism than RS-by diet and that RBS cows have shown greater sensitivity, from this point of view, compared to the MB breed. Jozwik et al. (2012) found a positive significant correlation between AST and ALT levels and daily milk yield and some milk traits, between ALT level and cholesterol content, and between AST and ALT blood levels. High alkaline phosphatase activity in the plasma of lactating cows may be a result of disturbed Ca and P metabolism (e.g. calcium and phosphorus deficiencies)

(Górski & Saba 2012) or increased placental ALP isoenzymes (Yokus & Cakir 2006). Increased transaminase activities were also observed as a result of microelement deficiency (Martyna et al., 2006), disorders in the energy metabolism of the body, stress (Krupczynski & Chudoba-Drozdowska, 2002; Darul & Kruczynska, 2005) or either as a consequence of increased permeability of the membrane of the hepatocyte (Todorović & Davidović, 2012). Stojevic et al. (2005) reported significant high ALT activity during mid-lactation than in early stage and dry period in Holstein cows. Similarly, Liu et al. (2012) also reported, in Holstein cows, a significant increase in ALT level in mid-lactation than other stages. The possible reason for this highest ALT activity in mid-lactation may be associated with the intensity of the milk production. According to Radkowska & Herbut (2014), the high feed content of nitrogen compounds may have a certain influence on hepatic nitrogen metabolism, which may be manifested by higher serum activity of AST and ALT enzymes. The concentration of these enzymes can be also influenced by ambient temperature. Marai et al. (1995, 1997) found aminotransferase activity to increase during the summer in response to high temperature.

Conclusion

According to the results of the present study, it can be concluded that total replacement of SBM with RS-by, breed related, had no effect on the biochemical profile of mid-lactating dairy cows and that the animals health was not affected. The study outcomes indicate once again the importance of a proper management and nutrition programme, along with animals health monitoring.

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