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Effect of glucogenic additive in transition dairy cow diets of varying ruminal starch degradability on blood metabolic profile

Wpływ dodatku preparatu glukogennego do dawek pokarmowych o różnej podatności skrobi na rozkład w żwaczu na profil metaboliczny krwi krów w okresie przejściowym

Summary. The objective of the study was to determine the effects of the supplemental feeding of glucogenic additive and increasing ruminal degradation of dietary starch on the blood biochemical parameters of high-yielding dairy cows during transition period. The study was carried out on 6 groups (10 animals each) of Polish Holstein-Friesian cows. Measurements of blood indices were carried out as a 2×3 experimental design of treatments with factors: glucogenic additive (GA; C – without *vs.* GA – with) and diet starch ruminal degradation rate (DR; M – maize *vs.* BW – barley and wheat *vs.* MBW – maize, barley and wheat). Blood samples were collected 2 weeks prior to calving and 1, 3 and 6 weeks lactation. Notable impact of the GA in the first weeks post-calving on β-hydroxybutyric acid (BHBA) and nonesterified fatty acids (NEFA) in the blood plasma was noted only in the treatments when the GA supplemented the barley-wheat or maize-barley-wheat based concentrates, whereas no effect was found when the GA enriched maize-based diets. Irrespective of grain species, the GA inclusion in the diet has clearly reduced the blood urea and total cholesterol levels, as well as elevated alkaline phosphatase activity.

Key words: cows, transition period, glucogenic additive, ruminal starch degradability, blood biochemical parameters

INTRODUCTION

The energy imbalance in the transition high-yielding dairy cows can lead to the occurrence of metabolic disorders related to nutrition and feed management problems, like ketosis. A direct reason inducing this periparturient disorder is a diminished level of

blood glucose generating the mobilization of body reserves (mainly fat) as an alternative metabolic fuel. At the scarcity of glucose, large amounts of body fats are mobilized faster than the liver can properly metabolize them. In this situation, ketone production exceeds ketone utilization by the cow and consequently, the level of many blood indices can be changed [Djoković *et al.* 2007]. A decreased blood glucose level is often associated not only with low feed intake noted during the periparturient period, caused by poor appetite [Nielsen and Ingvartsen 2004], but also with a diet composition (roughages and concentrate share) and high ruminal degradation of starch contained in wheat, triticale and barley (ca. 90 %), the most common grains used in animal feeding in Central Europe. From among yielded in the rumen by the fermentation volatile fatty acids (mainly acetic, propionic and butyric), only propionic acid is a glucogenic one [Bergman 1983]. The maize starch is degraded ruminally in much lesser extent (ca. 60%) which means that about 40% of the starch that escapes ruminal fermentation (bypass starch) is then digested in the small intestine and provides directly glucose blood pool [Orskov 1986]. On the other hand, too low capacity of starch for ruminal degradation may create shortage of energy essential for microbial protein synthesis in the rumen [Matras *et al.* 1991, Huntington 1997]. Besides, high bypass starch content in the diet can cause its diminished digestibility in the small intestine because of the compromised amylase activity [Huntington 1997]. Preventive measures against metabolic disorders involves dietary inclusion of various glucogenic preparations, like propionates or propylene glycol [Ballard *et al.* 2001, Patton *et al.* 2004, Klebaniuk *et al.* 2009]. The inclusion of glucogenic additives in ruminant diets may have different effects on blood parameters depending on the starch rumen degradation rate.

The aim of the present research was to evaluate the impact of glucogenic additive to transition dairy cow rations based on grains with varying starch degradability in the rumen on some blood biochemical parameters.

MATERIAL AND METHODS

The 2-factorial experiment was conducted on 60 multiparous Polish Holstein-Friesian transition cows allocated (by analogues) into 6 groups of 10 animals each. A glucogenic additive (GA) and type of starch regarding ruminal degradability rate (DR; M – maize *vs.* BW – barley and wheat *vs.* MBW – maize, barley and wheat) were the experimental factors. The glucogenic additive consisted of calcium propionate (99% purity, Pestell Minerals & Ingredients, New Hamburg, Canada) and loose propylene glycol (BASF) mixed 1 : 1. It was fed in a dose of 450 g per head/day. The feeding of particular groups was based on the same roughages (maize silage, haylage and meadow hay at 69 : 19 : 12 mix ratio, dry matter basis) but differed in the grain species – maize (M), barley (B) and wheat (W) – forming the concentrate and given in amounts to cover animal requirements determined by IZ-INRA [2009]. Therefore, the C-M and A-M groups were fed maize-based concentrate (low starch ruminal degradation; 1.16 UFL, 202 g PDIN and 184 g PDIE), C-BW and A-BW – barley and wheat-based (50 : 50, high degradation of their starch in the rumen; 1.11 UFL, 205 g PDIN and 179 g PDIE), while C-MBW and A-MBW groups – a concentrate based on maize, barley and wheat (50 : 25 : 25; 1.14 UFL, 204 g PDIN and 181 g PDIE). The full dose of a glucogenic additive (incorporated

as a mix into a concentrate, 2.5 kg per head/d, in the morning and evening meals) was given from 2 week before the expected date of calving until 6 week of lactation. Energy value of 1 kg GA was calculated at 2.0 UFL (Feed Unit for milk production), based on energy value (computed in NEL) by Miyoshi *et al.* [2001] for 1 kg propylene glycol and by Liu *et al.* [2010] for Ca-propionate. For more details on animal feeding in these trials refer to Matras *et al.* [2012].

Blood samples were collected 2 weeks prior to expected parturition and in 1, 3 and 6 week of lactation. They were taken from the jugular vein, right before the morning meal. In blood plasma, a content of glucose (GLU), total protein (TP), urea (UREA), as well as the enzyme activity i.e. aspartate aminotranspherase (AST) and alkaline aminotranspherase (ALT) and alkaline phosphatise (AP) and lipid indices: total cholesterol (CHOL), triacylglycerols (TG) and HDL cholesterol fraction was measured by colorimetric methods using Cormay monotests. The low density lipoprotein (LDL-chol) was calculated using Friedewald [1972] formula. A level of β-hydroxybutyric acid (BHBA) and nonesterified fatty acids (NEFA) in the blood plasma was determined by the enzymatic procedure with reagents by Randox.

The obtained results were subjected to 2-factorial analyses of variance using the Statistica 10.0 program [StatSoft 2011], according to the model:

$$Y_{ijk} = \mu + a_i + b_j + (ab)_{ij} + e_{ijk}$$

where: μ – overall mean; a_i – effect of the glucogenic additive, i = 2 (C – without *vs.* GA – with glucogenic additive); b_j – effect of diet starch ruminal degradability rate, j = 3 (M – maize, BW – barley-wheat, MBW – maize-barley-wheat); a × b – glucogenic additive × starch degradability ruminal rate interaction; e_{ijk} – random error.

RESULTS

A glucogenic additive increased ($p \leq 0.05$) the glucose level in blood plasma at all 4 terms (Table 1). The significant differences were noted 2 weeks prior to calving in the 3 and 6 week of lactation, they were reduced to only 0.05–0.06 mmol l^{-1} ($p \leq 0.05$). A starch sources did not have any impact on the blood glucose level.

The level of beta-hydroxybutyric acid (BHBA) in blood plasma was notably decreased when the GA enriched diets (Tab. 1), with the statistically confirmed differences during the first 6 lactation weeks. Its average level in the control groups in 1, 3 and 6 week of lactation reached 0.909, 0.909 and 0.967 mmol l^{-1} in comparison with 0.540, 0.697 and 0.738 mmol l^{-1} in the groups with glucogenic additive, respectively. Starch sources have also affected BHBA level during the 6-week study of lactation with the peak value (average 1.09 mmol l^{-1}) for blood plasma of cows receiving barley wheat grains mix (regardless of glucogenic additive) and the lowest (average 0.52 mmol l^{-1} from 1, 3 and 6 week of lactation) established in the blood of the cows fed the diets comprising of a maize-barley-wheat mix. The highest average content of BHBA (1.296 mmol l^{-1}) was recorded for the cows with barley-wheat concentrate, whereas the lowest (0.386 mmol l^{-1}) in the blood of cows given maize-barley-wheat concentrate supplemented with the GA.

Table 1. Effect of glucogenic additive and grain species on plasma metabolites of dairy cows in transition period
Tabela 1. Wpływ preparatu glukogennego oraz gatunków zbóż na wskaźniki metaboliczne krwi krów w okresie przejściowym

Item Wskaźnik	Unit Jednostka	Week Tydzień	Treatment Grupa							Statistical significance Istotność statystyczna		
										factor czynnik		interaction interakcja
			C-M	C-BW	C-MBW	GA-M	GA-BW	GA-MBW	SEM	GA	DR	GA × DR
Glucose Glukoza	mmol l^{-1}	-2	3.09^{c}	3.67^{b}	3.82^{ab}	3.94^{a}	4.03^{a}	3.71^{b}	0.13	*	ns	ns
		1	2.48^{c}	3.66^{ab}	3.77^{ab}	3.81^{a}	3.80^{a}	3.50^{b}	0.21	*	ns	ns
		3	3.48^{b}	3.99^{a}	3.81^{ab}	3.72^{ab}	3.94^{a}	3.79^{ab}	0.17	*	ns	ns
		6	3.73^{b}	3.84^{ab}	3.73^{b}	3.76^{b}	3.91^{a}	3.79^{ab}	0.19	*	ns	ns
BHBA	mmol l^{-1}	-2	0.550^{b}	0.807^{a}	0.604^{b}	0.547^{b}	0.642^{b}	0.570^{b}	0.19	ns	ns	ns
		1	0.705^{b}	1.408^{a}	0.613^{b}	0.600^{b}	0.635^{b}	0.386^{c}	0.08	**	*	*
		3	0.636^{c}	1.417^{a}	0.673^{c}	0.847^{c}	1.033^{b}	0.210^{d}	0.09	**	**	**
		6	0.968^{a}	1.064^{a}	0.868^{b}	0.860^{b}	0.970^{a}	0.385^{c}	0.17	*	*	*
NEFA	mmol l^{-1}	-2	0.363^{a}	0.299^{ab}	0.263^{b}	0.260^{b}	0.230^{bc}	0.201^{c}	0.07	*	*	*
		1	0.341^{ab}	0.388^{a}	0.330^{ab}	0.305^{b}	0.313^{b}	0.259^{c}	0.08	*	*	*
		3	0.346^{bc}	0.510^{a}	0.406^{b}	0.310^{bc}	0.372^{b}	0.279^{c}	0.09	**	*	*
		6	0.333^{a}	0.344^{a}	0.337^{a}	0.275^{b}	0.275^{b}	0.254^{b}	0.04	**	ns	ns
Total protein Białko całkowite	g l^{-1}	-2	53.8^{ab}	55.4^{a}	53.0^{ab}	55.0^{a}	52.5^{b}	56.0^{a}	3.18	ns	ns	ns
		1	51.8^{ab}	49.4^{b}	51.4^{ab}	53.0^{a}	52.7^{ab}	51.8^{ab}	2.43	*	ns	ns
		3	55.0^{ab}	52.0^{b}	58.0^{a}	56.2^{ab}	57.0^{a}	60.0^{a}	4.39	ns	ns	ns
		6	64.3^{b}	58.7^{c}	63.3^{b}	62.5^{bc}	66.4^{ab}	68.1^{a}	3.90	*	ns	ns
Urea Mocznik	mmol l^{-1}	-2	5.21^{ab}	5.63^{a}	4.89^{ab}	3.41^{b}	4.17^{b}	3.80^{b}	1.34	*	ns	ns
		1	5.32^{a}	4.78^{a}	4.20^{ab}	3.16^{b}	3.58^{b}	3.41^{b}	1.81	**	ns	ns
		3	5.42^{a}	4.77^{ab}	4.93^{ab}	4.23^{b}	4.03^{b}	4.29^{b}	0.76	**	ns	ns
		6	6.20^{a}	5.16^{ab}	6.15^{a}	4.15^{b}	4.47^{b}	5.51^{ab}	0.92	**	ns	ns

[a, b, c] Values in the rows with different letters differ significantly ($p < 0.05$).
Determinations of statistical significance probability of factor impact (C – control, GA – glucogenic additive, DR – starch ruminal degradability rates: M – maize, B – barley, W – wheat) and interaction of factors: *$p < 0.01$; **$p < 0,05$; ns – $p > 0,05$.
[a, b, c] Wartości w wierszach różnią się istotnie pomiędzy grupami ($p < 0.05$).
Oznaczenia prawdopodobieństwa istotności statystycznej wpływu czynnika (C – kontrola, GA – dodatek glukogenny, DR – skrobia o różnej podatności na rozkład w żwaczu: M – kukurydza, B – jęczmień, W – pszenica) i interakcji czynników : *$p < 0.01$; **$p < 0,05$; ns – $p > 0,05$.

Both, the GA as well as grain species were shown to significantly influence a non-esterified fatty acid level (NEFA) in the blood plasma of the cows throughout the examined period (Tab. 1). The average NEFA level in the cows from the control groups were 0.308, 0.353, 0.421 and 0.338 mmol l^{-1}, respectively in comparison with 0.230, 0.292, 0.320 and 0.268 mmol l^{-1} in the cows fed the GA supplemented diet. The differences have been statistically significant. Effect of starch ruminal degradability rates on the NEFA content in blood was also seen; the generally highest level ($p \leq 0.05$) was recorded in 1 and 3 wk in the group fed a barley-wheat concentrate. A significant glucogenic additive × starch degradability rates interaction on plasma BHBA and NEFA level was observed. The most significant interaction ($p \leq 0.01$) on decreasing blood plasma BHBA content was noted especially in the GA-BW and GA-MBW groups comparing to control groups (C-BW and C-MBW). This tendency was not observed in the group of cows fed the diet based on grain of low starch rumen degradability rate (maize) as the only grain. The increased concentration of BHBA in 3 and 6 lactation weeks in GA-M was noted, as compared to C-M group. Similarly, a beneficial interaction on the plasma NEFA concentration of both experimental factors GA × DR was stated ($p \leq 0.01$). That notable effect carried through the whole 6-week observation period after parturition.

A content of total protein in the blood plasma was slightly increased ($p \leq 0.05$) in 1 and 6 lactation week in the groups fed the GA-supplemented diets, whereas grain species had no influence on this parameter.

A glucogenic additive significantly decreased cows blood urea level. Besides, grain species was found to affect a blood plasma urea content inducing a slightly lower level of it in the cows during lactation when fed a barley-wheat concentrate. A significant GA × grain species interaction shows the biggest fall (by about 32% during lactation) of this parameter resulting from the GA inclusion in the cow concentrate incorporating maize as the only grain and the lowest (decrease by about 14%) in the treatments with a maize-barley-wheat concentrate mixture.

Neither GA nor starch degradability had any clear influence on the triacylglicerols content in the blood plasma, whereas the GA have evidently raised ($p \leq 0.05$) the total cholesterol level before calving as well as in lactation by about 12% (Tab. 2). The most pronounced difference (over 19%) as against the control cows was observed in 3 lactation week. Similar impact of the GA was noted on HDL cholesterol fraction. Also grain species influenced both fractions of cholesterol with the lowest average HDL levels in 3 and 6 week of lactation in the maize-based diet groups, whereas the lowest LDL concentrations were established in the blood of the cows fed maize-barley-wheat concentrate. A statistically confirmed GA x grain species interaction regarding the HDL level in cow blood in 3 wk of lactation points to the lowest (2.72 mmol l^{-1}) HDL content in C-M group and the highest content (4.22 mmol l^{-1}) in A-MBW group.

The activity of AST and ALT (Tab. 2) in the blood plasma of all the groups ranged within the limits reported in the literature [Winnicka 2015]. The highest activity of AST (91.1 U l^{-1}) was established in 1 week postcalving in the groups receiving the GA and also in 3wk in the blood plasma of the control group cows (92.3 U l^{-1}). Neither GA nor grain species affected this enzyme activity evidently. The average ALT activity was found in the blood of the cows during the first 6 weeks of lactation ca. 36% higher in comparison with the activity in 2 wk precalving. The GA supplementation of the diets increased ($p \leq 0.05$) its activity in 3 and 6 wk of lactation. Grain species did not influence

Table 2. Effect of glucogenic additive and grain species on plasma lipid indices of dairy cows in transition period
Tabela 2. Wpływ preparatu glukogennego oraz gatunków zbóż na wskaźniki lipidowe krwi krów w okresie przejściowym

Item Wskaźnik	Unit Jednostka	Week Tydzień	Treatment Grupa							Statistical significance Istotność statystyczna		
										factor czynnik		interaction interakcja
			C-M	C-BW	C-MBW	GA-M	GA-BW	GA-MBW	*SEM*	GA	DR	GA × DR
Total cholesterol Cholesterol całkowity	mmol l^{-1}	-2	3.67^{ab}	3.37^{b}	3.47^{b}	3.73^{ab}	4.10^{a}	3.89^{a}	0.71	*	ns	ns
		1	4.45^{b}	4.44^{b}	4.66^{ab}	5.10^{a}	4.86^{a}	4.93^{a}	0.32	**	ns	ns
		3	3.30^{b}	4.16^{ab}	4.59^{a}	4.86^{a}	4.83^{a}	4.58^{a}	0.48	*	ns	ns
		6	3.37^{b}	4.27^{a}	4.13^{a}	4.29^{a}	4.27^{a}	4.29^{a}	0.56	*	ns	ns
TG	mmol l^{-1}	-2	0.21^{ab}	0.19^{ab}	0.17^{b}	0.21^{ab}	0.18^{b}	0.23^{a}	0.09	ns	ns	ns
		1	0.23^{a}	0.21^{ab}	0.23^{a}	0.21^{ab}	0.20^{ab}	0.17^{b}	0.06	ns	ns	ns
		3	0.25^{a}	0.22^{ab}	0.17^{b}	0.21^{ab}	0.17^{b}	0.25^{a}	0.10	ns	ns	ns
		6	0.15^{ab}	0.17^{a}	0.11^{b}	0.17^{a}	0.16^{ab}	0.13^{b}	0.08	ns	ns	ns
HDL	mmol l^{-1}	-2	3.18^{ab}	3.04^{b}	3.12^{ab}	3.14^{ab}	3.29^{a}	3.26^{a}	0.21	*	ns	ns
		1	3.35^{b}	3.50^{ab}	3.65^{ab}	3.75^{ab}	3.74^{ab}	3.92^{a}	0.19	*	ns	ns
		3	2.72^{c}	3.45^{b}	3.63^{b}	3.45^{b}	3.69^{b}	4.22^{a}	0.33	**	*	*
		6	2.47^{b}	3.52^{a}	3.29^{ab}	3.46^{a}	3.54^{a}	3.73^{a}	0.29	**	*	ns
LDL	mmol l^{-1}	-2	0.48^{b}	0.32^{b}	0.34^{b}	0.58^{ab}	0.80^{a}	0.62^{ab}	0.12	**	ns	ns
		1	1.09^{ab}	0.93^{b}	1.00^{ab}	1.34^{a}	1.11^{ab}	1.00^{ab}	0.09	**	ns	ns
		3	0.57^{bc}	0.70^{b}	0.95^{ab}	1.40^{a}	1.13^{a}	0.35^{c}	0.24	**	*	*
		6	0.89^{a}	0.74^{ab}	0.84^{a}	0.82^{a}	0.72^{ab}	0.55^{c}	0.31	**	*	*
AST	U l^{-1}	-2	73.88^{b}	81.42^{a}	78.28^{ab}	77.38^{ab}	75.48^{ab}	77.28^{ab}	3.65	ns	ns	ns
		1	84.12^{b}	92.58^{a}	88.78^{ab}	90.88^{ab}	88.08^{ab}	94.38^{a}	4.21	ns	ns	ns
		3	91.12^{ab}	96.28^{a}	89.58^{ab}	89.88^{ab}	84.38^{b}	90.18^{ab}	3.96	ns	ns	ns
		6	66.12^{d}	93.18^{a}	69.08^{d}	84.78^{b}	77.38^{c}	78.08^{c}	3.72	ns	ns	ns
ALT	U l^{-1}	-2	47.41^{ab}	49.01^{a}	43.11^{b}	46.21^{ab}	46.21^{ab}	45.21^{ab}	2.84	ns	ns	ns
		1	58.62^{b}	66.02^{a}	61.22^{ab}	57.62^{b}	60.02^{ab}	56.72^{b}	2.91	ns	ns	ns
		3	65.02^{a}	67.62^{a}	63.32^{ab}	62.42^{b}	64.02^{ab}	61.82^{b}	3.15	*	ns	ns
		6	67.02^{a}	66.42^{a}	64.02^{ab}	64.92^{ab}	65.02^{ab}	62.42^{b}	2.36	*	ns	ns
AP	U l^{-1}	-2	71.64^{b}	73.10^{b}	68.52^{b}	83.00^{ab}	99.16^{a}	97.70^{a}	4.87	**	ns	ns
		1	72.18^{b}	72.64^{b}	73.22^{b}	88.34^{a}	87.67^{a}	91.49^{a}	5.16	**	ns	ns
		3	68.31^{b}	71.15^{b}	70.92^{b}	85.66^{a}	90.41^{a}	81.66^{a}	4.92	**	ns	ns
		6	64.04^{b}	61.68^{b}	64.69^{b}	69.34^{ab}	80.28^{a}	75.49^{a}	4.36	**	ns	ns

Explanation as in Table 1/ Objaśnienia jak w tabeli 1.

this index. Both, GA and grain species showed statistically confirmed impact on the alkaline phosphatase (AP) activity. The glucogenic supplement enhanced ($p \leq 0.01$) the activity of this enzyme by ca. 30 and 20%, respectively in the blood of the cows before parturition and during the examined lactation period.

DISCUSSION

Glucose, nonesterified fatty acid and β-hydroxybutyric acid levels in blood plasma are commonly used to assess the energy status of transition dairy cow [Klebaniuk *et al.* 2009]. In the present study, the average levels of blood glucose in cows experimental exceeded 3.0 mmol l^{-1} – the level recognized as minimum by Whitaker *et al.* [1997]. The glucogenic additive elevated glucose content markedly ($p \leq 0.01$) during the whole experimental period. The results of other studies exploring the impact of GA on blood glucose level are controversial. Some investigations highlighted significant growth of this parameter [Grummer *et al.* 1994, Patton *et al.* 2004, Liu *et al.* 2010], whereas in the others [Ballard *et al.* 2001, DeFrain *et al.* 2005, Moallem *et al.* 2007], no effect of the GA was reported. The time of blood sampling in relation to GA allocation method and its dose, is likely to affect this discrepancy [Nielsen and Ingvartsen 2004]. According to Brockman and Laarveld [1986], the explanation of its only slight impact on the glucose level, although significant in some other trials, lies in a large rise of insulin that maintains plasma glucose homeostasis. The action of this hormone keeping up the glucose level in blood, might also mask possible influence of grain species used in the studies and presented in this paper. Importantly, they differ in ruminal degradation of their starch and this attribute specific for particular grains species conditions the quantity of ruminal bypass starch degraded in the small intestine and absorbed to the blood in glucose form [Orskov 1986].

Whitaker [1997] implied that NEFA and BHBA of blood plasma are much more sensitive than glucose to any change in the energy status of dairy cow. NEFA correlate negatively with energy balance and its increased level indicates the higher mobilization of body fat. The desired plasma concentration for NEFA is according to Whitaker *et al.* [1997] below 0.4 mmol l^{-1} in late pregnancy and it should not exceed 0.7 mmol l^{-1} in early lactation. In our study, the average level of NEFA was not high. Regardless of a grain species, the GA reduced NEFA content by 25% in late pregnancy and up to 24% in early lactation. A similar beneficial response of blood NEFA level in transition dairy cow to a glucogenic additive was highlighted in other studies [Hoedemaker *et al.* 2004, Patton *et al.* 2004]. Besides, in the cows in late pregnancy and in 1 and 3 lactation week the influence of a grain species on NEFA levels was found ($p \leq 0.05$), with the highest level recorded in the cows fed a concentrate based on barley and wheat, that is the grains containing high ruminally degraded starch. The GA × starch degradation rate interaction ($p \leq 0.05$) noted in the late lactation and in 1 and 3 wk of lactation revealed the strongest effect on NEFA concentration in the blood of cows receiving the maize-barley-wheat-based concentrate.

BHBA serum concentration characterize a short-time energy status of cow organism and according to Duffield [2000] and Herdt [2000], because of its stability its measurement in the blood is the gold standard diagnostic test for subclinical ketosis. Filar [1997]

recognized the blood BHBA concentrations lower than 1000 µmol l^{-1} to be normal while the ranges from 1000 to 3000 µmol l^{-1} of blood BHBA were proposed to indicate subclinical ketosis. Whereas, Duffield [2000] considered the value of 1400 µmol l^{-1} as the threshold between the normal and abnormal BHBA levels. In the present study, the average BHBA content in the groups, fed without glucogenic additive regardless of grain species, was below 1000 µmol l^{-1}. However, in the group receiving barley-wheat-based concentrate it exceeded 1400 µmol l^{-1} in the 1 and 3 lactation week, that is the threshold which classify cows as experiencing subclinical ketosis. The employed GA significantly decreased the BHBA level in the blood plasma of the lactating cows. Irrespectively of a grain species, an average decline of the blood BHBA level caused by the glucogenic supplement was over 40% in 1 wk and about 23–24% in 3 and 6 wk postcalving. A similar fall in the BHBA content until 7 wk postpartum was observed by Hoedemaker *et al.* [2004] as a response to propylene glycol (300 ml/head/d) addition. Alike, as reported by Liu *et al.* [2010], the supplementation of dairy cow diets in early lactation with calcium propionate doses up to 300 g per cow/day lowered significantly the BHBA but only by 3.4%. In the Patton *et al.* [2004] studies where the dairy cow diets contained a mix (409 g/head/d) of calcium propionate, propylene glycol and calcium salts of fatty acids, the determined BHBA level was reduced by 20% on 7 d postpartum in comparison with control. However, the difference was not confirmed statistically. In other studies [DeFrain *et al.* 2005, Moallem *et al.* 2007] no influence of GA was stated. Lack of glucogenic additive effect on blood plasma BHBA level determined in many researches might be due to a grain species incorporated in a concentrate. In the majority of the experiments, the concentrate was based on maize as the main grain. Also in the present trial, no considerable differences were recorded in BHBA blood plasma level of the cows fed the diets enriched with GA in comparison with control (except lower by 11% in 6 wk, $p \leq 0.05$) when the concentrate comprised maize grain. On average (the entire examined lactation period), the decrease of this parameter resulting from the GA supplementation reached 32 and 54% in the groups with the barley-wheat and maize-barley-wheat concentrate, respectively. The BHBA content in C-M group was substantially lower than in the C-BW group. The greatest differences (nearly 2-fold) were stated in 1 and 3 wk of lactation. These research results correspond to Strzetelski *et al.* [2008] observations that indicate a tendency for lower BHBA level in the blood of the cows supplied with maize-based concentrate as against those receiving the concentrate with barley (0.92 *vs.* 1.19 mmol l^{-1}).

Glucogenic additive has significantly declined the blood plasma urea level in both, late pregnancy and early lactation, on average by over 20%. Its employment in the diet might save glucogenic amino acids used in gluconeogenesis process, which may provide significant amount of glucogenic material [Bergman 1983], especially in the case of negative energy balance occurring in the transition period of cows. These findings correspond closely to Klebaniuk *et al.* [2009] study, who used a glucogenic additive (calcium propionate and propylene glycol mix) at 2 levels (300 or 450 g/head/d). Grain species did not have any clear impact upon plasma urea content. A lack of direct influence of grain source on blood urea level stated in the present research agrees with the results presented by Strzetelski *et al.* [2008], who compared impact of barley *vs.* maize on this blood parameter, whereas in similar study carried out by Grings *et al.* [1992] a considerably lower urea level was found in blood of cows fed the maize-based diet.

Supplementation of the diets with GA has significantly raised (average by ca. 12%) total cholesterol level. Similar effect (by about 20%) was noted in Ballard *et al.* [2001] investigations performed to explore the effect of an energy supplement containing 150 or 300 g propylene glycol and calcium propionate mix. An elevated level of cholesterol – a precursor of steroid hormones can be considered beneficial in farm animals [Rabiee 2000]. Kappel *et al.* [1984] revealed that higher cholesterol and glucose concentrations after calving were associated with lower number of days from calving to conception. The raised concentration of blood cholesterol determined in the present research may indicate its positive influence on the reproductive parameters as reported in the paper by Matras *et al.* [2012].

The activities of the all three enzymes determined aspartate aminotranspherase (AST), alanine aminotranspherase (ALT) and alkaline phosphatase (AP) were found within the reference limits [Winnicka 2015]. There was not stated influence of GA or grain species on AST or ALT activity in cows blood in late pregnancy or in the first week postcalving. Ballard *et al.* [2001] and Hoedemaker *et al.* [2004] did not find any significant effect of GA on the activity of AST in blood, whereas Klebaniuk *et al.* [2009] highlighted significantly lower activity of this enzyme in the cows whose diets were enriched with glucogenic additive. According to Mandebvu *et al.* [2003], the enhanced activity of these enzymes in the blood serum of cows postpartum are attributed to an energy deficit and improper protein to energy ratio in a feed ration. The grain species employed in the present study did not affect AST or ALT activities that conforms to Strzetelski *et al.* [2008] observations. Heightened AP activity is usually observed when the increased activity of liver is exhibited, *e.g.* in the intensified gluconeogenesis. The research findings of the present study seem to confirm it. The GA consisted of propylene glycol and propionate, the substances undergoing gluconeogenesis, have increased markedly (by nearly 22%) this enzyme activity, irrespective of a grain species. These results correspond to those presented by Klebaniuk *et al.* [2009].

CONCLUSIONS

The results obtained in the present study have revealed the significant interaction of a glucogenic additive and grain species of varying ruminal starch degradability and its effect on some biochemical (mainly energy) blood indices. Notable impact of the GA on blood plasma BHBA and NEFA content was noted only in the treatments when the GA supplemented the barley-wheat mix based concentrates (BW and MBW groups). Whereas, inclusion of GA in diets based only on maize (low starch ruminal degradability) had not improved significantly the imbalanced energy status of lactating cows. Irrespective of grain species, the GA inclusion in the diet has clearly reduced the blood urea and total cholesterol levels, as well as elevated alkaline phosphatase activity.

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Streszczenie. Celem badań była ocena wpływu preparatu glukogennego (GA) oraz gatunków zbóż o różnej podatności skrobi na rozkład w żwaczu, podawanych krowom mlecznym w okresie przejściowym, na profil metaboliczny krwi. Doświadczenie przeprowadzono na 60 krowach podzielonych na 6 grup: 3 kontrolne (C) i 3 eksperymentalne (A) – otrzymujące preparat glukogenny. Zwierzęta otrzymywały 3 analogiczne dawki różniące się komponentami mieszanki treściwej [kukurydza (grupy C-M i GA-M), jęczmień i pszenica (C-BW and GA-BW) oraz kukurydza, jęczmień, pszenica (C-MBW i GA-MBW)]. W badaniach wykazano, że dodatek glukogenny (GA) w sposób istotny obniżył zawartość kwasu β-hydroksymasłowego oraz niezestryfikowanych kwasów tłuszczowych w osoczu krwi krów z grup GA-BW i GA-MBW w pierwszych tygodniach po wycieleniu. Takiej zależności nie odnotowano natomiast w grupie krów żywionych mieszanką bazującą wyłącznie na kukurydzy. Niezależnie od gatunku zboża suplementacja dawki dodatkiem glukogennym wyraźnie wpływała na obniżenie poziomu mocznika i cholesterolu całkowitego we krwi, a także na wzrost aktywności fosfatazy zasadowej.

Słowa kluczowe: krowy, okres przejściowy, dodatek glukogenny, rozkład skrobi, wskaźniki biochemiczne krwi