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Blood metabolic profile parameters of cows fed diet with glucogenic additive

RENATA KLEBANIUK, JAN MATRAS, EDYTA KOWALCZUK

Department of Nutrition, Institute of Animal Nutrition, Faculty of Biology and Animal Breeding, University of Life Sciences, Akademicka 13, 20-950 Lublin, Poland

Klebaniuk R., Matras J., Kowalczuk E.

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Summary

The objective of the study was to determine the effect of the dose and application period of a glucogenic additive (GA) to high-yielding dairy cows at the periparturient period and early lactation on the metabolic disorder incidence and some blood biochemical parameters. The study was carried out on 60 dairy cows which were at their dry-off period at the trial onset. They were divided into 5 groups – a control one (K) and four experimental ones, 12 animals each. In respect to the control group, the cows of the experimental groups received diets with GA (calcium propionate and propylene glycol mixture in loose form) at two levels (300 or 450 g/head/d) and during two different periods (4 or 7 weeks). Blood samples were collected 5 times to determine some blood biochemical parameters. The glucogenic supplement elevated the concentration of glucose and decreased beta-hydroxybutyric acid and free fatty acid accumulation in blood plasma. It also reduced aspartate aminotransferase and slightly elevated alkaline phosphatase activity. The best results were achieved when GA was used at the dose of 450 g/head/d for 7 weeks (1 week prior to calving and 6 weeks following it).

Keywords: dairy cows, periparturient period, propylene glycol, calcium propionate, blood biochemical parameter

High-yielding dairy cows require accurate balanced feed rations as well as an effective prophylactic nutritional management to prevent the incidence of various disorders, in particular at the periparturient period (5). At the onset of lactation the nutrient requirement increases faster than the increase in feed intake. It creates a negative energy balance (NEB) in the organism associated with alternations of the glucose level and some other metabolites and metabolic hormones in blood (24). The limited feed intake in the first stage of lactation may lead to a reduced blood glucose content. Its deficit in blood induces the mobilization of body fat reserves resulting in the usage of non-esterified fatty acids released from adipose tissue as an alternative metabolic fuel to be converted by the liver to ketone bodies. This process is accompanied by various changes in the contents of bovine blood biochemical indices and in a disease state, a dramatic lack of appetite that finally causes decreased milk yield and the animal's body weight loss (29).

A declined blood glucose content in high-producing cows in the initial lactation stage may be prevented to a large extent through the dietary provision of substances used by the animal organism in the gluconeogenesis process (4, 25). The present study aimed to evaluate the impact of a glucogenic additive (GA) – its dosage and the duration of application to periparturient cows – on the incidence of metabolic disorders and some blood biochemical parameters.

Material and methods

The investigations included a total of 180 dairy cows managed in an experimental cow barn with an average 7500 kg milk yield per year, the Lublin Province. The feed rations were formulated according to the real nutritional feed value in compliance with the INRA'88 feeding norms and nutritive values for ruminants (2). A basic feed ration for cows was composed of: maize silage (10 or 25 kg), grass haylage (3 or 5 kg) supplied at the dry-off and lactation, respectively; meadow hay was given *ad libitum.* Additionally, the cows received a concentrate mixture with a mineral-vitamin premix. The concentrate feeding ration was increased (0.5-3 kg) as the calving time approached, and then, after parturition, it was elevated (up to 6 kg) in accordance with the nutritional requirements.

The study was carried out on 60 cows selected from all the animals in the barn at the dry-off period at the research onset. The selection of animals to the treatment groups, 12 cows each, was based on the analog method, concerning cow age, annual milk yield from the last lactation, body weight and condition. The animals from the control group

Tab. 1. Experiment design

Group	GA* g/head/day	Duration (weeks) of GA* use (calving day – 0)
K	–	–
D1	300	–1 / +3
D2	450	–1 / +3
D3	300	–1 / +6
D4	450	–1 / +6

Explanations: * GA – glucogenic additive

(K) were fed the basic feed ration, whereas the cows from the experimental groups (D1-D4) received diets supplemented with GA (loose mixture of calcium propionate and propylene glycol). It was supplied to cows at two levels (300 and 450 kg) for different periods of time – 4 or 7 weeks (tab. 1). GA was introduced to the diet 3 weeks prior to the expected calving and its dose was gradually elevated. The full dose of this preparation was given from one week before the expected parturition until the 3rd or 6th week of lactation. It was supplied together with a concentrate for the morning meal.

The feedstuff samples were examined for the basic nutrient content, i.e. dry matter, crude protein, crude fiber, ether extract, crude ash in conformity with the standards (3) and starch by the polarimetric procedure in accordance with the Polish Directive of the Ministry of Agriculture and Rural Development, Journal of Law No 271, item 2688.

Tab. 2. Average intake of particular feeds and nutritive value of the cows' whole diets

Item	Observation week (calving related)				
	–2	1	3	6	10
Intake of dry matter of feeds, kg/head/day					
Maize silage	3.58	7.15	8.50	9.00	8.90
Haylage	1.15	1.15	1.85	1.92	1.90
Meadow hay	4.30	4.75	5.00	5.00	4.80
Concentrate mixture	1.20	3.00	4.40	5.50	5.00
Nutritive value of the diet					
Dry matter	10.2	16.1	19.8	21.4	20.6
LFU*	10.1	14.5	17.1	17.7	17.3
UFL**	8.44	14.04	17.58	19.33	18.52
PDIN***, g	728	1252	1634	1861	1753
PDIE****, g	790	1351	1730	1943	1843
Crude protein, g	1082	1879	2464	2812	2647
Crude fiber, g	2389	3347	4014	4234	4101
Calcium, g	63.7	102.9	132.7	148.7	140.9
Phosphorus, g	36.8	63.7	83.0	94.8	89.1

Explanations: * LFU – Fill Units for Cows; ** UFL – Unite Fourragere Lait (Feed Unit for Milk Production); *** PDIN – True protein truly digestible in the small intestine when N limits microbial protein synthesis; **** PDIE – True protein truly digestible in the small intestine when energy limits microbial protein synthesis

Cow blood samples were taken two weeks prior to calving and in the 1st, 3rd, 6th and 10th lactation weeks. Blood was collected from the external jugular vein early in the morning after milking but before the morning meal. Blood plasma was analyzed by means of Cormay monotests and the Cary 50 spectrophotometer to measure the amounts of glucose, crude protein, and urea, as well as lipid indices: total cholesterol (CHOL), triacyloglyceroles (TG) and HDL-cholesterol fraction (by colorimetric methods), as well as enzyme activity, i.e. alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (AP) (by a kinetic method). The content of beta-hydroxybutyric (BHB) acid and free fatty acids (FFA) was determined in blood plasma using the enzymatic procedure with reagents by Randox.

The feeds and blood plasma were studied to evaluate the level of calcium (atomic absorption spectrophotometry – AAS) and phosphorus (the Fiske-Subbarow method) (8).

The obtained results were analyzed statistically using Statistica 5.1.G (StatSoft, Inc. (1997) program for Windows, No lic: sp8 118638004g5) with the application of analysis of variance. The significance of differences between the means were determined by Duncan's multiple confidence interval at significance levels 0.05 and 0.01.

Results and discussion

A glucogenic additive did not have a significant influence on the feed intake or behaviour of cows. The average intake of dry matter, fill units, energy (UFL) and protein (crude protein, PDIN, PDIE) contained in the rations increased gradually with progressing lactation, until week 6 after calving (tab. 2).

The mean concentration of glucose (3.62 mmol l^{-1}) in the blood plasma of the analyzed cows (tab. 3) was similar to that reported in literature (18, 34). An extremely low plasma glucose content (2.4 mmol l^{-1}) was detected in blood taken from the control cows one week postpartum, whereas at the same time the cows with a GA-supplemented diet showed a blood plasma glucose level higher by approximately 50%. These differences diminished with the passage of time, and 3 weeks postpartum they amounted to only 7-15%. In the 6th week of lactation the glucose content in plasma was almost similar in all the treatments.

The average concentration of beta-hydroxybutyric acid in blood plasma (tab. 3) in all the cows showed a nearly threefold increase in the first three weeks postpartum compared to the dry-off period. From the 6th week of lactation, there was a significant decrease of the BHB acid level. A significantly ($p \leq 0.05$) higher average concentration of this acid in blood plasma during the whole period of the experiment was found in the control cows (K) in relation to its level in cows fed a diet with GA at the rate of 450 g/head/day (D2, D4) – by 32% and 53%, respectively.

The mean content of free fatty acids (FFA) in blood plasma of the cows under study (tab. 3) varied between the groups and the sampling dates. The FFA concentration in blood plasma during the first 6 weeks of lactation appeared to be almost twofold higher as com-

pared to the dry-off period. In the subsequent lactation weeks, the level decreased significantly. The higher GA dose (450 g/head/day) significantly ($p < 0.05$) decreased the FFA content in relation to the control treatment, whereas the lower dose of this preparation did not influence the FFA level.

The mean content of total protein (61.5 g l^{-1}) and urea (5.46 mmol l^{-1}) in the blood plasma of cows under investigations (tab. 3) was within the reference value range (18, 34). However, a tendency for a persistently lower level of total protein and urea in blood plasma was noted until week 3 of lactation as compared to the other lactation weeks. The duration of GA supply affected the level of these components, in particular the amount of total crude protein in bovine blood plasma.

Tab. 3. Some biochemical indices in the cows' blood plasma

Indice	Group	Observation week (calving related)					Mean
		–2	1	3	6	10	
Glucose, mmol l^{-1}	K	3.02	2.41	3.41	3.66	3.53	3.21^{a}
	D1	3.60	3.59	3.92	3.77	3.56	3.69^{b}
	D2	3.75	3.70	3.74	3.66	3.55	3.68^{b}
	D3	3.87	3.74	3.65	3.69	3.59	3.71^{b}
	D4	3.96	3.73	3.87	3.84	3.68	3.82^{b}
BHB, mmol l^{-1}	K	0.352	1.200	1.209	0.856	0.775	0.878^{a}
	D1	0.340	0.997	1.035	0.849	0.773	0.799^{ab}
	D2	0.396	0.825	0.772	0.723	0.618	0.667^{b}
	D3	0.285	1.006	0.979	0.845	0.592	0.741^{ab}
	D4	0.385	0.760	0.645	0.577	0.493	0.572^{c}
FFA, mmol l^{-1}	K	0.258	0.375	0.627	0.561	0.330	0.430^{a}
	D1	0.262	0.550	0.596	0.560	0.415	0.477^{a}
	D2	0.204	0.340	0.310	0.324	0.281	0.292^{b}
	D3	0.267	0.526	0.575	0.418	0.290	0.415^{a}
	D4	0.222	0.373	0.371	0.358	0.327	0.330^{b}
Total protein, g l^{-1}	K	57.4	52.8	50.9	57.7	60.5	55.8^{a}
	D1	59.4	57.3	55.8	59.6	58.5	58.1^{a}
	D2	56.4	57.3	60.9	62.6	70.4	61.5^{ab}
	D3	62.7	61.6	66.7	71.2	72.5	67.0^{b}
	D4	60.1	58.6	64.8	70.8	71.3	65.1^{b}
Urea, mmol l^{-1}	K	4.28	5.78	5.99	6.62	6.40	5.82^{a}
	D1	4.41	4.52	4.64	5.26	7.36	5.24^{ab}
	D2	4.18	6.02	5.49	6.92	7.18	5.96^{a}
	D3	4.69	5.19	5.26	4.88	4.96	5.00^{b}
	D4	5.12	5.08	5.52	5.59	5.04	5.27^{ab}

Item	Impact of *						
	factors			factors interaction			
	1	2	3	1 × 2	1 × 3	2 × 3	1 × 2 × 3
Glucose	***	***	**	***	ns	ns	***
BHB	***	***	***	***	**	**	***
FFA	***	ns	***	ns	ns	ns	**
Total protein	ns	***	**	**	ns	**	**
UREA	**	ns	***	ns	**	ns	**

Explanations: a, b, c – mean values in columns differ significantly at $p \leq 0.05$; * Determinations of statistical significance probability of factor impact: (1 – glucogenic additive dose; 2 – glucogenic additive supply period; 3 – sampling time) and interaction of factors: *** – $p \leq 0.05$; ** – $p \leq 0.01$; ns – > 0.05

The highest mean values of CHOL and TG (tab. 4) were established during the first week post calving. A somewhat higher but statistically insignificant CHOL level and a lower HDL fraction in the total cholesterol of the animals with the GA-supplemented diet in comparison with the control was observed. This additive provided at the dose of 450 g/head/day up to the 6th week of lactation has induced a significant decrease of TG concentration in blood plasma.

The highest AST activity value (tab. 5), that in the control group exceeded the reference range mentioned by Winnicka (34), was determined in the cows during the first week following calving. Both the glucogenic supplement and the duration of its use had a significant influence on the reduced AST activity in blood plasma.

The average ALT activity immediately postpartum was approximately 30% higher than in the dry-off period, and it increased slightly until week 6 of lactation. The glucogenic supplement did not exert any significant impact on the activity of this enzyme.

The activity of alkaline phosphatase (AP) in bovine blood plasma ranged within the limits reported in literature (18, 34) but differed significantly between the sampling dates (tab. 5). A significantly ($p \leq 0.05$) higher (on average by 43%) activity of this enzyme in cows fed the GA supplement was also noted.

The periparturient period of high yielding cows is associated with a high risk of metabolic disorder occurrence (16, 30), especially 1-2 weeks before calving and in the first 2-3 weeks following it (17). The reduction of the glucose level in the blood of these animals during the early lactation period may be effectively prevented to a large extent by providing carefully balanced feed rations as well as the implementation of substances used by animal organism at the gluconeogenesis (13). However, many concerns arise about the type and dosage of these substances as well as the duration of their application (11). An important management tool in assessing the health status and nutrition of the bovine organism proves to be the analysis of blood components (1) performed in

Tab. 4. Lipid indices in bovine blood plasma

Indice	Group	Observation week (calving related)					Mean
		–2	1	3	6	10	
Total cholesterol, mmol l^{-1}	K	3.91	4.69	3.54	3.61	3.63	3.88
	D1	3.61	4.68	4.40	4.51	3.83	4.21
	D2	3.71	4.90	4.83	4.37	3.38	4.24
	D3	3.59	4.96	4.72	4.15	3.81	4.25
	D4	3.96	4.72	4.69	4.13	3.75	4.25
TG, mmol l^{-1}	K	0.12	0.21	0.20	0.20	0.18	0.18^{ab}
	D1	0.22	0.27	0.27	0.20	0.13	0.22^{a}
	D2	0.16	0.24	0.21	0.12	0.15	0.18^{ab}
	D3	0.17	0.20	0.17	0.16	0.13	0.17^{ab}
	D4	0.14	0.14	0.12	0.13	0.14	0.14^{b}
HDL, %	K	57.4	60.7	82.4	70.0	73.9	68.9^{a}
	D1	62.4	66.4	65.4	59.4	62.4	63.2^{ab}
	D2	66.9	60.5	60.9	59.2	69.6	63.4^{ab}
	D3	60.8	57.1	56.3	65.8	60.8	60.2^{b}
	D4	55.2	62.2	61.6	66.5	67.5	62.6^{ab}

Index	Impact of *						
	factors			factors interaction			
	1	2	3	1 × 2	1 × 3	2 × 3	1 × 2 × 3
Total Chol.	ns	ns	**	ns	ns	ns	ns
TG	ns	**	**	**	ns	ns	ns
HDL, %	**	**	**	**	ns	ns	**

Explanations: as in tab. 3.

the light of nutrition management evaluation (6, 9, 24). The most critical blood parameters related to the organism's energy balance are the contents of glucose, free fatty acids (FFA) and beta-hydroxybutyric acid as well as metabolic hormones – among others insulin, IGF-1, cortisol and leptin (12, 15, 27). The glucogenic additive discussed in the present study had a significant impact on the plasma glucose concentration. Its level was the highest in the blood plasma of the cows fed the GA-supplemented diet at the amount of 450g/head/day for seven weeks. At the same time, the cows from this group showed the lowest beta-hydroxybutyrate acid content and a low free fatty acid level. That may give evidence for the dietary provision of an optimal energy level to these cows as the BHB acid content in blood plasma reflects the short-term energy status (15, 31, 33), whereas the free fatty acid (FFA) level – distant energy processes. An elevated FFA level in blood plasma represents the magnitude of body fat mobilization from adipose stores in the organism (31). McNamara et al. (22) imply that the FFA content should be < 0.7 mmol l^{-1} in early lactation and < 0.4 mmol l^{-1} in late pregnancy. A raised FFA content in blood shows a significant correlation with the incidence of metabolic diseases and reproductive disorders (21, 26).

The total protein content in blood shows the long-term protein supply (15, 31, 33), while the blood urea level reflects mainly protein metabolism in the rumen. It informs about the animal's short-term protein and energy supply (33). An increased total protein concentration in blood plasma, from a nutritional standpoint, may indicate an energy deficit (33). A physiological reduction of protein level occurs in late pregnancy and the first weeks of lactation. In the present investigation, the greatest decrease in the total protein content in the cows' blood plasma postpartum was found in the control cows. An elevated content of blood urea nitrogen with concomitant total crude protein deficiency may point to an insufficient amount of protein digested in the intestine (31, 33) or an improper ratio of protein to energy undergoing fermentation in the rumen. A raised energy content in a feed ration, especially the energy produced during ruminal fermentation, seems absolutely indispensable.

Lipid metabolic profile changes are critical for assessing the course of the metabolic process in the organism. The present research highlights the fact that the mean concentration of total cholesterol varied slightly and some increase of it in the blood plasma of the cows fed the diet with the glucogenic additive can be considered beneficial. In ruminants, a decrease of cholesterol, which is the precursor to all steroid hormones, may be a challenge (28, 32). Its concentration in blood and steroid hormone synthesis are positively correlated with feedstuff intake and animal health status, while a low content of cholesterol and glucose contributes to a delayed subsequent conception (28). The present investigation revealed the effect of the sampling date on the blood plasma total cholesterol level and its fractions. Its concentration, the highest in week 1 postpartum, decreased gradually with progressing lactation. An analogical relationship, with exception of group D4, was observed for the level of TG, which constitute the major form of fat reserve stored in the organism and released to the circulation when needed (14). A triglyceride content in blood plasma of cows with dietary GA at the dose of 450 g/head/day got reduced within the whole trial period and was closest to the reference value means (34).

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are enzymes recognized as indices of protein reserve mobilization in the organism in the case of negative energy balance (27). However, different views are shared by the authors (20). Herdt et al. (15) think that higher AST parameters in blood plasma may indicate the occurrence of the Fat Cow

Tab. 5. Activity of some enzymes (U l^{-1}) in bovine blood plasma

Indice	Group	Observation week (calving related)					Mean
		–2	1	3	6	10	
AST	K	73.8	120.5	96.2	93.1	70.7	90.9^{a}
	D1	81.3	84.0	91.0	66.0	64.8	77.4^{b}
	D2	78.2	88.7	89.5	69.0	67.0	78.5^{b}
	D3	77.4	90.9	89.9	84.8	81.9	85.0^{ab}
	D4	75.5	88.1	84.4	77.4	75.0	80.1^{ab}
ALT	K	47.4	58.6	65.0	67.0	64.8	60.6
	D1	49.0	66.0	67.6	66.4	60.8	62.0
	D2	43.1	61.2	63.3	64.0	56.1	57.5
	D3	46.2	57.6	62.4	64.9	65.5	59.3
	D4	45.2	56.7	61.8	62.4	63.9	58.0
AP	K	64.2	55.8	53.5	44.5	46.2	52.8^{a}
	D1	69.8	102.2	86.8	55.3	51.0	73.0^{b}
	D2	61.4	87.6	90.0	65.0	52.0	71.2^{b}
	D3	78.4	81.5	80.3	80.9	67.0	77.6^{cb}
	D4	86.8	84.9	81.9	83.6	64.2	80.3^{c}

Index	Impact of *						
	factors			factors interaction			
	1	2	3	1 × 2	1 × 3	2 × 3	1 × 2 × 3
AST	**	***	**	**	ns	ns	**
ALT	ns	ns	***	ns	ns	ns	ns
AP	***	**	**	**	**	ns	**

Explanations: as in tab. 3.

Syndrome, though this parameter is not specific to such a clinical status. An elevated activity of this enzyme in blood serum is a marker of an extensive liver endothelial cell damage rather than the organ dysfunction (20). An elevated activities of AST and ALT or either of them in the blood serum of cows postpartum is attributed to an energy deficit and improper protein to energy ratio in the feed ration (19). A more intensive enzyme activity recorded during early lactation suggests the highest activity of the liver (7). In the present study the elevated AST and ALT activities in the first weeks of lactation and then their decline were shown to confirm the fact that these enzyme activities were induced by an energy deficit. The dietary GA implementation limited this activity, of AST in particular.

A physiological elevation of alkaline phosphatase activity occurs at the time of an increased liver activity, e.g. during pregnancy, enhanced gluconeogenesis processes (10, 23, 34). The study results apparently seem to confirm it. The average activity of this enzyme after an approximately 15% rise in week 1 of lactation as compared to the dry-off period, showed a significant decrease in the subsequent weeks. The glucogenic supplement increased the AP activity in bovine blood plasma, especially when employed at the amount of 450 g/head/day until week 6 of lactation. The obtained values of alkaline phosphatase activity were within the reference range (34), thus the changes in the enzyme activity are the sequel of an intensive but appropriate processes in the organism.

Currently, dairy cows with a high potential for performance need an optimal dietary provision of not only energy and protein but essential macro- and micro-elements as well, to meet their physiological and productive requirements (6). The levels of calcium and phosphorus established in this study were within the physiological reference value (34).

Conclusions

The dietary glucogenic additive (calcium propionate and propylene glycol mixture) affected the stability of biochemical changes in the organism.

– The additive dose enhanced the glucose synthesis in the liver and increased its blood plasma concentration, whereas it decreased the beta-hydroxybutyric acid level as well as the free fatty acid content in blood plasma.

– It contributed significantly to the reduction of aspartate aminotranferase activity and a slight rise of alkaline phosphatase activity at optimal phosphorus content.

– The efficiency of the supplement implementation was dependent on its dosage and duration of application. The best test results were obtained when the glucogenic supplement was added at the dose of 450 g/head/day for 7 weeks (1 week pre- and 6 weeks postpartum).

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Author's address: Dr. Renata Klebaniuk, Institute of Animal Nutrition of the University of Life Sciences, Akademicka 13, 20-950 Lublin, Poland; e-mail: rklebaniuk@poczta.fm