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The Influence of Keton Blend Preparation on Milk Yield and Energy Parameters of Cows in Early Lactation

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Abstract: The aim of undertaken studies was to determine an impact of Keton Blend SP-1 supplement (containing both propylene glicol and choline chloride) on milk yield and energy parameters of Holstein-Friesian cows in early lactation. Research was undertaken in 2013 in one of the agro-breeding farms in the Sztum district, Pomeranian voivodeship. Research material consisted of Holstein-Friesian race cows in 2nd lactation. Keton Blend SP-1 supplementation applied to the diet of cows from research group (20 pieces) was used in research. Application of the supplement started on the 15th day of lactation and continued for the next 30 days. During the experiment blood samples were collected 3 times from the jugular vein of each cow, i.e., on 14th day of lactation, after 30 days of supplementation and 30 days after discontinuation. During the experiment condition of cows was assessed by BCS (Body Condition Score) method using the 5-grade scale. Daily milk yield (kg) and content (%) of basic milk components as well as dry weight (%) and urea (%) in the studied periods were also determined. Basing on the undertaken experiment to apply Keton Blend SP-1 as nutritional supplement it is not possible to clearly define its positive impact on the energy balance of dairy cows in an early lactation. Research showed only a slight increase in daily milk yield of supplemented dairy cows with a slight decline in their condition.

Key words: Cows, milk yield, propylene glycol, choline, biochemical parameters

INTRODUCTION

The intensive genetic selection conducted for the last decades along with the introduction of new feeding and maintenance systems of cattle lead to a significant increase in performance of dairy cows, especially the H-F race. Unfortunately, genetically determined performance increase is only partially correlated with the possibility for high-efficiency cows to absorb a higher amount of feed. A phenomenon which very often accompanies the high productivity is a negative energy balance appearing in the 1st days after calving. At the time of calving an organism's hormonal profile change is happening consisting of reduction of insulin and thyroxine activity and growth of glucagon, growth hormone and cortisone levels which are stimulating an activation of gluconeogenic processes and mobilisation of fats and proteins from tissues. Diseases of the transitional period are a growing problem in a modern dairy farming. The most recent data indicate their higher prevalence and the most common disorders are: clinical mastitis-16.5% of animals, inflammation of hooves-14.0%, placenta retention-7.8%, perinatal retention-4.9%, inflammation of uterus-4.6% and the left-sided displacement of the abomasum-3.5% (Van Saun, 2005). Occurrence of clinical form of ketosis ranges from 7-15% and depends on the type of herd and method of diagnosis. Presently, greater attention is paid to the occurrence of diseases in sub-clinical form, e.g., sub-clinical hypocalcaemia ($Ca < 2$ mmol L^{-1}) occurs in 40-54% of cows in second and higher lactations and in 25% in first lactation (Reinhardt *et al*., 2011). Sub-clinical ketosis is observed in 15-43% of cows (Sobiech *et al*., 2008). In majority of cows (>50%) there is more than one perinatal period disorder (Oetzel, 2004).

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One of the most recommended ways of preventing the energy shortages of dairy cows, appearing after calving is using the glucoplastic compounds which include, among others, propylene glycol. It is used successfully since the 1950's to this day. It causes reduction of the negative energy balance occurring in high-efficiency dairy cows after calving and in the peak of lactation as well as reduction of ketosis and hepatic steatosis risk. Propylene glycol can affect the gluconeogenesis in different ways. A part of delivered preparation is metabolised to lactic and propionic acids in rumen which is then converted to glucosis in hepatocytes. A certain amount of glycogen which is not fermented in rumen is directly absorbed by a mucous membrane of this forestomach and then glucosis is produced in liver (Cozzi *et al*., 1996). Christensen *et al*. (1997), Butler *et al*. (2006) and Mikula *et al*. (2008) observed a beneficial impact of propylene glycol in different phases of lactation cycle in dairy cows.

The other substance being used increasingly for reduction of energy shortages disorders occuring in highly efficient dairy cows is choline. This compound, classified as one of the vitamins of the B group by Pinotti *et al*. (2002) plays a significant role in the organism as a precursor in synthesis of neurotransmitters in the transmission of signals across the cell membrane in transport of lipids or metabolism of methyl groups (Zeisel and da Costa, 2009). In ruminants, especially dairy cows, one of the most important choline functions is participation in the synthesis of Very Low Density Lipoproteins (VLDL) which facilitate transport of triglycerides from liver to blood thereby prevent steatosis of liver (Gruffat *et al*., 1996). It is proven that choline supplementation in cow's forage increased milk yield (Piepenbrink and Overton, 2003), methyl groups metabolism (Baldi and Pinotti, 2006), its excretion into milk and increased vitamin E concentration in the organism (Baldi *et al*., 2004).

Due to the earlier mentioned effects of those substances the aim of undertaken research was to determine the effect of Keton Blend SP-1 (containing propylene glycol as well as choline chloride) on milk yield and energy transformations in Polish Holstein-Friesian cows in an early lactation.

MATERIALS AND METHODS

The research was undertaken in 2013 in one of the agro-breeding farms in the Sztum district, Pomeranian voivodeship. Research material consisted of Polish Holstein-Friesian cows in second lactation. Keton Blend SP-1 supplementation applied to the diet of cows was used in research (Table 1). Two groups of cows (the control and study group) of 20 pieces in each were made. Control group did not receive preparation and study group received Keton Blend SP-1. Cows were selected for each group by analogues in terms of daily milk yield and day of lactation. Supplementation was constant because cows received it with water using troughs. By DOSTEC pump preparation in the amount of 250 mL/pcs./day got into drinking water. Supplementation started on the 15th day of lactation and continued through the next 30 days. During the experiment blood samples were collected 3 times from the jugular vein of each cow, i.e., on 14th day of lactation after 30 days of supplementation and 30 days after discontinuation.

During the experiment condition of cows was assessed by BCS (Body Condition Score) Method using the 5-grade scale (Wildman *et al*., 1982) taking into account the level of subcutaneous adipose tissue development in the visual and tactile evaluation. The following grading was adopted: emaciated cow in a very bad condition-1 point, cow in mediocre condition-2 points, cow in an average condition-3 points, very good condition-4 points, very fattened cow-5 points. The evaluation used a clearance of 0.25 points. The condition of cows was evaluated 3 times during collection of blood for biochemical studies (Table 2).

Blood for biochemical studies was collected to tubes with clotting activator. To obtain serum samples were centrifuged for 10 min at 2000 rpm min^{-1}. In terms of biochemical tests glucose, triglycerides, cholesterol and total bilirubin concentration were identified in serum. These parameters were determined by a biochemical analyzer ACCENT 200 by Cormay (Table 3).

Additionally, concentration of free fatty acids was also determined by ACS-ACOD Method, Wako NEFA-HR test and concentration of Beta-Hydroxybutyric acid (BHB) by Wako Autokit 3-HB. In terms of enzymatic analysis Aspartate Transaminase (AST) and Gamma-Glutamyl Transpeptidase (GGTP) activity in serum were determined by a biochemical analyzer ACCENT 200 by Cormay. In the next part of research daily milk yield (kg) and concentration (%) of basic milk components as well as dry

Table 1: Components of Keton Blend SP-1

Ingredients contained in 1 kg	Values (units)
Choline chloride	175.0 g kg^{-1}
Magnesium (Mg)	16.2 g kg^{-1}
Zinc (Zn)	1.8 g kg^{-1}
Manganese (Mg)	1.8 g kg^{-1}
Copper (Cu)	0.5 g kg^{-1}
Iodine (J)	40.0 mg kg^{-1}
Selenium (Se)	18.0 mg kg^{-1}
Cobalt (Ko)	18.0 mg kg^{-1}
Monopropylene glycol	729.0 g kg^{-1}

weight and urea (%) were determined: before beginning of the study (14th lactation day) after 30 days of supplementation (45th lactation day) and 30 days after discontinuation (75th lactation day).

Obtained results were statistically developed using one-way analysis of variance in the non-orthogonal system. The mean (LSM) and Standard Deviation (SD) were determined. The significance of differences was verified by a Fisher LSD test. Results were developed statistically using the Statistica Ver. 10 computer program (StatSoft, 2011).

Table 2: Ration for a cow of an average daily capacity of 30 kg

Type of feeds	Dry weight (%)	Feed (kg) for a daily efficiency level = 30 kg
Corn silage (300 g of starch)	35.0	22.00
3rd swath of grass silage	30.0	12.00
Pickled beet pulp, dried	91.0	8.00
Wheat straw	86.0	1.00
Corn DDGS-granules	80.0	1.50
Soybean meal (46% of total protein)	88.0	0.50
Rapeseed meal “00”	89.0	1.00
Own concentrated feed 12×12	87.0	3.00
Mlekomix chemirol	88.0	1.00
Winter barley	89.0	2.50
KM TMR chemirol	86.0	0.30
Sodium bicarbonate	99.9	0.15
Fodder chalk	99.9	0.10
Magnesium oxide 56	98.0	0.05
Salt (sodium chloride)	99.0	0.05

Table 3: The nutritional value of fixed dose for one average cow in the herd with a daily capacity of 30 kg

Nutrients	The nutritional value of dose for the daily performance level = 30 kg
Dry Weight (DW)	21011 g
The share of roughage in dry weight	64%
The share of roughage in dry weight	13478 g
Dry Weight (DW)	44.51%
The share of total protein in dry weight	14.95%
NEL energy in dry weight	6.55 MJ
The share of structural fibres	2658 g
The share of crude fibres in dry weight	18.3%
Fat content in dry weight	3.37%
The share of DJ starch	1159 g
The share of starch+sugar in dry weight	26.46%
DŻ starch+sugars	20.94%
Sugar content in dry weight	2.42%
ADF in dry weight	23.23%
NDF in dry weight	40.13%
NFC in dry weight	38.94%
Milk yield of NEL	30.42 kg
Milk yield of protein	31.07 kg
Milk yield of nXP	32.43 kg
BNŻ	-3.20 g
UDP	30.66%
Calcium (Ca)	120.52 g
Phosphorus (P)	93.22 g
Sodium (Na)	86.26 g
Magnesium (Mg)	80.66 g

RESULTS AND DISCUSSION

The experiment was conducted on the farm specialized in milk production with Holstein-Friesian cattle (HF). On the beginning of the study the average daily milk yield of cows from the control group was 27.71 kg of milk with 4.01% of fat, 3.21% of protein with 261 mg L^{-1} urea content (Table 4). The ratio of fat to protein is thus, 1.24. In contrast, among cows from the study group the average performance numbers were: 27.44 kg of milk, 4.08% of fat, 3.2% of protein with a similar urea content and with a similar fat to protein ratio (1.28). Condition of cows in the 14th day of lactation was 3.63 pts. BCS in the control group and 3.7 pts. in the study group.

After a 1 month Keton Blend SP-1 supplementation the daily milk yield was 30.27 kg of milk, exceeding yield in the control group by 1.5 kg. The difference was statistically significant with $p \leq 0.05$. In this period condition of cows reduced slightly. In research of Chung *et al*. (2009) cows which were fed using the TMR system and received propylene glycol were characterised by a daily milk yield exceeding 5 kg of milk in comparison to cows that did not receive it. Rukkwamsuk and Panneum (2010) lasting for the first 4 weeks of lactation, propylene glycol supplementation resulted in a yield growth for 3 weeks since calving in comparison to the control group (without supplementation).

Table 4: Daily yield, milk composition and condition of cows

		Research groups of cows			
		Control		Study	
Features	Term of designations (dl)	LSM	SD	LSM	SD
Milk (kg)	14	27.71	8.21	27.44	6.96
	45	28.79	6.50	30.27^x	7.50
	75	27.17	7.18	29.07^x	7.12
Fat (%)	14	4.01	0.89	4.08	1.15
	45	3.84^x	0.84	3.78	0.67
	75	3.91	0.69	4.09^x	0.70
Protein (%)	14	3.21	0.33	3.20	0.43
	45	3.18	0.30	3.11	0.27
	75	3.22^x	0.31	3.23	0.28
Lactose (%)	14	4.82	0.20	4.80	0.17
	45	4.73	0.20	4.77	0.17
	75	4.77	0.18	4.75	0.18
Dry weight (%)	14	12.75	1.12	12.78	1.45
	45	12.50	0.88	12.36	0.86
	75	12.62	0.91	12.77	0.78
Urea (mg dL^{-1})	14	261.00^x	77.46	252.00	78.71
	45	186.00^{xx}	61.49	201.00	78.58
	75	262.00^{xx}	44.88	239.00	52.57
T/B ratio	14	1.24	0.20	1.29	0.15
	45	1.17	0.20	1.21^x	0.18
	75	1.19	0.12	1.27^x	0.28
BCS (pts)	14	3.63	0.37	3.70	0.36
	45	3.60	0.38	3.65	0.30
	75	3.60^x	0.25	3.40	0.32

xx-$p \leq 0.01$; x-$p \leq 0.05$; 14 dl: 14th day of lactation since calving; 45 dl: 45th day of lactation since calving; 75 dl: 75th day of lactation since calving

In the 75th day of lactation the efficiency of cows in both groups lowered to 27.17 kg of milk in the control group and 29.07 kg in the study group while maintaining statistical differences between groups on the $p \leq 0.05$ level. A higher level of cows efficiency from the study group affected the reduction of these cows condition to 3.4 pts. BCS while condition in control group maintained on 3.6 pts. BCS level.

In both groups during the experiment fat to protein ratio did not change significantly and ranged from 1.17-1.24 in the control group and 1.21-1.28 in the study group (Table 4).

Results of performance efficiency assessment of dairy cattle conducted in the analysed farm in 2013 indicate a high performance of cows. The average annual productivity of a cow was then 8867 kg of milk with 4.20% of fat and 3.40% of protein content. Polish Federation of Cattle Breeders and Dairy Farmers provides that the annual performance of Holstein-Friesian cows in 2013 in Poland was as follows: 7588 kg of milk with 4.15% of fat and 3.35% of protein content.

Concentration of β-hydroxybutyric acid among cows from control and study groups in the first sampling was similar and was 1.39 and 1.32 mmol L^{-1}, respectively. In the next studies it underwent a gradual reduction in all animals but these changes were not statistically significant (Table 5). It should be noted that the BHB values obtained in the first sampling were quite high reaching the border regarded by Duffield *et al*. (1998) as indicative of a subclinical ketosis (>1.4 mmol L^{-1}). Results obtained in own research do not indicate a reduction of BHB concentration after Keton Blend supplementation. Research of other researchers (Kristensen and Raun, 2007) showed that the use of propylene glycol can lower BHB concentration in serum by reducing level of fat mobilisation or increasing the affinity of tissues for ketone bodies and their faster metabolism. However, these researchers used a higher dose of propylene glycol than used in own research.

Glucose concentration in both groups was equal during the experiment and remained on a fairly low level comprised within reference limits for cattle (Table 5). Obtained glucose values in examined cows might indicate a small energy shortage associated with an increased lactation. Cozzi *et al*. (2011) research indicate a reduction of glucose level in cows in the first period of lactation and the subsequent growth. On the other hand, Grunwaldt *et al*. (2005) point to the fact that the glucose concentration is not a specific indicator of cattle's energy status due to its homeostatic regulation in the organism. In own research the impact of used preparation on glucose concentration change in cows from the experimental group was not proven. Obtained results are contradictory to Grummer *et al*. (1994) who observed statistically significant increase of glucose concentration during propylene glycol supplementation. Butler *et al*. (2006) have stated that propylene glycol is effective in increasing of glucose concentration among cows being only in negative energy balance state that is most often after parturition. Mikula *et al*. (2008) studies as well as own results did not confirm an impact of propylene glycol supplementation in cows on glucose concentration increasing.

Table 5: Blood biochemical parameters of cows under experiment

Features	Term of designations (dL)	Research groups of cows: Control LSM	Control SD	Study LSM	Study SD
BHB (mmol L^{-1})	14	1.320	0.36	1.390	1.68
	45	1.020	0.75	1.180	1.23
	75	1.160	0.82	1.110	0.94
GLUC (mmol L^{-1})	14	2.190	6.48	2.150	9.93
	45	2.180	8.76	2.110	7.89
	75	1.950	5.94	2.090	9.36
CHOL (mmol L^{-1})	14	5.560	45.03	5.040	61.35
	45	5.080	52.45	5.350	39.26
	75	5.310	44.32	4.570	40.67
TGL (mmol L^{-1})	14	0.160	3.16	0.150	2.51
	45	0.210	3.72	0.210	2.29
	75	0.320	2.55	0.320	3.98
BILIR (μmol L^{-1})	14	1.670	0.03	2.890	0.03
	45	3.550	0.04	3.830	0.10
	75	2.310	0.02	2.580	3.76
FFA (mmol L^{-1})	14	0.325	0.03	0.432	3.76
	45	0.353	0.06	0.382	0.16
	75	0.326	0.05	0.562	3.32
AST (U L^{-1})	14	98.310^{xx}	18.93	91.650^{xx}	29.02
	45	74.300	19.07	72.500	21.22
	75	71.350	19.57	75.400	17.55
GGTP (U L^{-1})	14	35.820	12.86	35.550	9.89
	45	30.900	14.46	30.850	37.54
	75	32.730	12.87	33.550	20.94

xx-$p \leq 0.01$; x-$p \leq 0.05$; 14 dl: 14th day of lactation since calving; 45 dl: 45th day of lactation since calving; 75 dl: 75th day of lactation since calving

Cholesterol concentration in control group cows was slightly higher compared to experimental group, especially in the last sampling (Table 5). Obtained values of this parameter, however, housed within the reference standards for cows. Rise in a cholesterol level in dairy cows after calving is a physiological and confirmed by Pysera and Opalka (2000) and Cozzi *et al*. (2011) process. In own research, statistically significant differences between a concentration of cholesterol in serum in cows from control and experimental groups were not observed indicating a lack of impact of used supplementation with propylene glycol and choline chloride on the level of this parameter. Similar results were acquired by Rukkwasmsuk *et al*. (2005) when testing the effects of propylene glycol use only.

Free Fatty Acids (FFA) concentration was similar in both animal groups in all samplings, only in the experimental group its level was insignificantly higher in the last sampling (Table 5). Thus, there was no significant effect of applied supplementation to changes in this parameter. Similar results were obtained by Chung *et al.* (2009) with concomitant application of propylene glycol and choline chloride in dairy cows in an early lactation. Vazquez-Anon *et al.* (1994) and Nielsen and Ingvartsen (2004) proved the beneficial effect of propylene glycol on reduction of FFA concentration in cows during intensive fat mobilisation after parturition and in negative energy balance conditions. In own research, used cows were in the 14th lactation day at the beginning of the experiment, therefore their degree of fat tissue lipolysis was not as high as right after parturition. Stephenson *et al.* (1997) studies showed that the FFA level in cows in calf increases gradually in the last period of pregnancy reaching its peak at the moment of labour and then gradually decreases during lactation. Observed increase in FFA content in the perinatal period may be a result of hormonal changes and appearance of trauma connected with parturition and the consequent reduction in feed intake. According to Whitaker (1997), determination of FFA in serum is a more sensitive indicator in the diagnosis of a negative energy balance than determining the concentration of glucose.

Triglycerides levels in serum in both groups were similar throughout the whole experiment while increasing not significantly from the first sampling (Table 5). The impact of Keton Blend SP-1 supplementation on this parameter was not proven. Similar results were achieved by Mikula *et al.* (2008) and Rukkwamsuk and Panneum (2010) in research on use of propylene glycol in cows in early lactation. Data published by Basoglu *et al.* (1998) show a gradual decrease in serum triglycerides concentration in the 1st day of lactation connected with the transition state of hepatic steatosis and triglyceride accumulation in this organ. In own research, a small increase in levels of triglycerides in this organ was observed from 14-75th day of lactation, indicating a normalisation of lipid metabolism and no evidence of steatosis.

Total bilirubin concentration did not show statistically significant differences between individual samplings as well as groups of animals used in the experiment, indicating lack of impact of supplementation on this indicator. Kabu and Civelek (2012) research show a similar reaction of serum total bilirubin in the case of both propylene glycol and methionine (a lipotropic compound, as choline) addition in cows in early lactation. Van den Top *et al.* (1995) and Bionaz *et al.* (2007) emphasize that the serum concentration of total bilirubin increases in dairy cows on the day of calving and maintains on the high level during the 1st week after it.

Concentration of this parameter corresponds with the intensity of hepatic metabolism associated with the parturition trauma.

AST activity in both groups was the highest in the first sampling (14th day of lactation) and then was decreasing statistically significantly while there was no difference between the groups (Table 5). Many researchers indicate a positive correlation between the activity of this enzyme (Steen, 2001; Kabu and Civelek, 2012) lipolysis of fat tissue and the level of hepatic steatosis. Seifi *et al.* (2007) emphasize the essential positive correlation between AST activity and BHB, FFA and cholesterol concentration. Significantly higher AST activity in the 14th day of lactation, observed in own research is probably connected with the intensification of metabolic changes in liver at the beginning of lactation and its later decrease indicates liver adapting to these changes. Obtained results indicate no impact of supplementation on hepatic functions. GGTP activity maintained on a similar level in all animals throughout the experiment. This enzyme, like AST, is regarded as an indicator of the health status of the liver. Vergara *et al.* (2014) suggest that determination of GGTP activity is a valuable diagnostic test in the case of damage of liver cells and its increase appears in cases of energy shortages. In own research, the impact of Keton Blend supplementation on GGTP activity changes was not confirmed, similar results were obtained by Mikula *et al.* (2008) using the propylene glycol.

CONCLUSION

Basing on the undertaken experiment involving the introduction of Keton Blend SP-1 as a nutritional supplement it is not possible to clearly determine its positive impact on energy balance of dairy cows in an early lactation. Research have shown only a small increase in a daily milk yield among supplemented cows with a slight decline in their condition. Analysing the obtained biochemical results the essential impact of supplementation on energy and fat administration was not stated.

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