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Original article

The influence of administration of biotin and zinc chelate (Zn-methionine) to cows in the first and second trimester of lactation on their health and productivity*

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Abstract

In cattle, the prevention of diseases might be possible using dietary methods, with nutritional optimization of feed rations. For years significant influence has been associated with the addition of biotin, methionine and zinc (each given individually, or as chelate – Zn-methionine). The aim of this study was to evaluate the influence of biotin or biotin + Zn-methionine additives on the health and performance of cows, in the first and second trimester of lactation.

This study was carried out in 3 groups of cows, 30 animals in each group. In the first group cows were fed the usual fodder used on the farm. In the second group biotin at 10 mg/day/cow was added. In the third group both biotin at 10 mg/day/cow and Zn-methionine at 5 g/day/cow were added. The administration of biotin increased the milk yield and reduced the occurrence of retained placenta, as well as *endometritis puerperalis*. However, the high doses of biotin suppressed ovulation and oestrus symptoms in the investigated cows (low progesterone levels on days 15, 21 and 45 after calving). Zn-methionine in comparison to biotin had a lower effect on the milk yield in the cows. Moreover, its supplementation improved the milk content, as well as some of the blood parameters; it also decreased the number of somatic cells in milk. The concurrent administration of biotin and zinc-methionine seemed to be a good method for the prophylaxis of subacute mastitis and for the improvement of the high yielding dairy cows' productivity.

Key words: dairy cow, biotin, Zn-methionine, health, productivity

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Introduction

Many experiments have shown that in the treatment and the prevention of limb disorders in cows, biotin and zinc-methionine play an important role. However, the influence of these additives on the health and production of dairy cows has rarely been investigated (Kinal et al. 2008).

In cattle, the prevention of diseases might be possible using dietary methods, with the nutritional optimization of feed rations and the addition of indispensable nutrients, i.e. amino acids – lysine, cysteine, methionine and histidine, and vitamins. One of the important elements is thought to be biotin, though its synthesis in the rumen is very low (Santschi et al. 2005). Biotin is an essential coenzyme for mammals' four carboxylases, two of them, pyruvate carboxylase and propionyl-coenzyme A carboxylase, are very important enzymes in gluconeogenesis (Rosendo et al. 2004, Ferreira and Weiss 2007). The effect of biotin supplementation on the milk yield is hard to determine. Several studies (Zimmerly and Weiss 2001, Majee et al. 2003, Ferreira et al. 2007) have demonstrated that administration of biotin increased the milk production, but in another study (Rosedno et al. 2004) there was no influence of biotin on the milk yield. The method of biotin action on milk synthesis in the mammary gland is still unknown. Ferreira and Weiss (2007) suggested that biotin may increase gluconeogenesis, presumably in the liver. It was confirmed that biotin administration in cows significantly improves the fertility and health (Zimmerly and Weiss 2001).

In particular conditions, organic Zn sources may improve the production, health and fertility of animals; nevertheless the underlying processes remain unknown (Spears, 1996). In ruminants the absorption of both organic and inorganic zinc is similar (Spears 1996). However, some researchers suggest that the metabolism of organic trace minerals may differ significantly from the inorganic forms (Spears 1996). Smith et al. (1999) observed that the mammary gland epithelium, after the application of Zn proteinates or Zn compounded with amino acids, had a greater ability to regenerate.

Engelhard and Helm (1998) showed that the addition of protected DL-methionine to the TMR increased the milk protein content and reduced the number of somatic cells, and also decreased BHBA concentration in blood. The number of ketosis cases was also reduced after this treatment. Another study (Cope et al. 2009) demonstrated that the use of organic Zn chelates in dairy cows did not affect the BCS or body weight. In the investigated animals blood plasma Zn, Cu, Mo and Fe levels and hematology were unchanged, and the supplementation of organic zinc increased the milk yield. The substrate and donor of methyl groups in the synthesis of phosphatidylcholine – an element of VLDL – is methionine. Buchart et al. (1998) (cited by Grunner 2008) conducted a study which included the application of a protected methionine, or both methionine and lysine. A significant reduction in the concentration of triglycerides in the liver of the investigated cows was observed.

Since more and more frequently in veterinary prophylaxis the administration of large doses of biotin and zinc methionine is recommended, in our study we decided to investigate the influence of biotin and zinc methionine on the health and production of dairy cows.

Materials and Methods

The study protocol was approved by the Local Ethics Commission.

The farm, where the study was carried out, consisted of 500 black-and-white dairy cows with a large share of hf genes (70-90%), with an average milk yield about 7200 kg. The cows were housed in shallow bedding tying stalls. Samples of fodder were taken and analysed to determine the primary nutrients (dry matter, protein, fat, fiber) in accordance with the accepted official methods; minerals – Ca, Mg, Na, K – atomic spectrophotometry absorption, EN-ISO 6869:2002; P – photometric method according to ISO-6491 2000; S – by PN-93/A-7485.15 and Cl by PN-81/R-64780. This allowed the calculation of the nutritive value of feed doses according to German norms (DLG) and mineral content according to American standards (NRC), and the calculation of cationic-anionic balance (DCAD). The feed ration for the cows consisted of: maize silage 35.0 kg, grass silage 10.0 kg, wheat bran 3.0 kg, barley meal 2.8 kg, malt sprouts 1.0 kg, mineral-vitamin mixture 0.1 kg. This ration provided: 18 kg of dry matter, 135 MJ NEL, 2873 g metabolizable protein, 120 g Ca, 100 g P, 41,4 g Mg, 151 g K, 31 g Na, 41 g S, 50 g Cl. The dietary cation-anion difference (DCAD) ratio amounted to 185 meq/kg DM.

The study was carried out on 90 dairy cows, divided into 3 groups, 30 animals per group, based on the analogue method, taking into account productivity and lactation (second or third). At the beginning of the study, the average production of each investigated cow was checked, and it was similar in all the chosen cows, confirming the appropriate selection of the animals. The study was conducted in the first seven months of lactation.

In the first (I, control) group cows were fed the usual fodder used on the farm. In the second (II, experimental) group biotin at 10 mg/day/cow was added. In the third (III, experimental) group both biotin at 10 mg/day/cow and Zn-methionine at 5 g/day/cow were added. Additives were given in a mineral-vitamin mixture.

At the start and the end of the study, jugular vein blood samples were collected from 10 cows in each group, and acid-base balance indicators were marked (following the Astrup method, using Coring 286 apparatus). The content of K, Na and Ca (using AAS) and blood cell count (Animal Blood Counter – Horiba) was also determined. At that time, the zinc content in blood and acute phase protein – haptoglobin were determined (Gayacol method).

Furthermore, on days 15, 21 and 45 of lactation blood samples were collected and the level of progesterone was measured in serum by the immunoensymatic method (Biokom EXL 800).

At the start, halfway, and end of the study milk samples from all the investigated cows were collected (authorized collection), and milk fat, protein, lactose and dry matter contents were determined (MilcoScan 133, Foss Electric Company). The level of urea (AA analyzer II, Lubbe) and the somatic cell content was also measured using flow cytometry (Bactocount Bentley 700).

Results

At the beginning and at the end of the study blood pH value remained similar (Table 1) and ranged within reference values. The BE index in blood serum at the beginning of the study was significantly higher than at the end, indicating a slight alkalosis in the group II and III (Table 1). The level of HCO_3^-, especially at the beginning of the experiment, was rather high, which rather excluded the possibility of subclinical acidosis. However, at the end of the study HCO_3^- values ranged from 27.5 to 28.2 mmol/l and were slightly higher than the reference values: 25 mmol/l. The sodium and potassium levels in serum remained the same throughout the study and were within the standard range (Table 1). The content of calcium at the beginning and at the end of the experiment was within the reference ranges (2.25-3.03 mmol/l). Low zinc levels in the blood serum might indicate an insufficient supply of zinc in the investigated cows (Table 1). Kendall and Bone (2006) estimated that the lower range of the normal limit is 10 µmol/l. These values for zinc concentration in blood serum were not statistically significant.

The number of WBC's in the blood at the beginning of the study, was higher in the cows in the experimental groups, than in the control (Table 2). However, it was not statistically significant. At the end of the study the number of WBC increased significantly (Table 2). Similar changes were observed for RBC. The HGB level was high and the standard deviation was low. At the beginning of the experiment Ht was within the lower range of normal values and was slightly lower ($P \leq 0.05$) in group III. At the end of the study Ht values were significantly higher and similar in all investigated groups. The number of platelets at the beginning of the study differed significantly among cows, but at the end of the study its values were within the same range in all the groups. MCV

Table 1. Indexes of acid-base balance and level of some minerals in blood serum of cows – start and end of study.

Parameter	Mean/Standard deviation	Group					
		I		II		III	
		start	end	start	end	start	end
pH	x sd ±	7.39 ± 0.05	7.44 ± 0.05	7.49 ± 0.14	7.45 ± 0.05	7.41 ± 0.03	7.41 ± 0.03
pCO_2 mmHg	x sd ±	54.57 ± 7.19	42.00 ± 5.60	49.47 ± 8.68	42.00 ± 5.61	52.70 ± 3.50	52.70 ± 3.50
HCO_3^- mmol/l	x sd ±	31.77 ± 1.08	27.50 ± 1.06	31.37 ± 3.63	27.78 ± 0.82	32.63 ± 1.91	32.63 ± 1.91
BE mmol/l	x sd ±	5.20 ± 1.46	3.03 ± 1.56	5.73 ± 2.42	3.48 ± 0.94	6.43 ± 2.05	6.43 ± 2.05
Na^+ mmol/l	x sd ±	133.00 ± 1.26	137.25 ± 3.10	132.67 ± 1.15	137.20 ± 1.10	133.86 ± 1.21	133.86 ± 1.21
K^+ mmol/l	x sd ±	4.31 ± 0.21	4.64^{A} ± 0.17	4.44 ± 0.26	4.46^{AB} ± 0.28	4.18 ± 0.52	4.18 ± 0.52
Ca^{++} mmol/l	x sd ±	1.13 ± 0.05	2.23 ± 0.15	1.12 ± 0.05	2.12 ± 0.11	1.13 ± 0.10	1.13 ± 0.10
Zn µmol/l	x sd ±	5.70 1.31	6.27 1.48	6.04 1.62	6.05 1.63	5. 67 0.98	5.88 1.70

Table 2. Haematology – start and end of study.

Parameters	Mean/Standard deviation	Group						Reference values
		I		II		III		
		start	end	start	end	start	end	
WBC	x	6.82	9.64	7.32	9.94	7.73	9.23	6.2 – 9.5
10^9/l	sd ±	1.28	3.83	1.47	3.18	1.74	0.99	
RBC	x	6.02	7.02	5.91	6.39	5.86	6.33	5 – 7
10^{12}/l	sd ±	0.55	0.79	0.53	1.28	0.52	0.48	
HGB	x	7.04	7.14	6.86	6.68	6.60	6.59	4.9 – 8.7
mmol/l	sd ±	0.55	0.78	0.52	1.04	0.62	0.47	
HCT	x	0.25^{a}	0.32	0.23^{ab}	0.30	0.22^{b}	0.30	0.24 – 0.46
l/l	sd ±	0.02	0.04	0.02	0.06	0.02	0.02	
PLT	x	785	438.00	615	435.00	267	437.38	200 – 800
10^9/l	sd ±	419	48.23	204	176.33	267	144.70	
MCV	x	40.99	46.14	38.93	47.75	38.50	47.38	40 – 60
Fl	sd ±	2.67	6.47	3.31	2.49	1.98	3.54	
MCH	x	1.17	1.03	1.16	1.05	1.12	1.05	0.9 – 1.5
Fmol	sd ±	0.07	0.12	0.08	0.07	0.06	0.09	
MCHC	x	28.61^{aA}	22.27	29.97^{bB}	22.10	29.30^{AB}	22.05	19 – 23
mmol/l	sd ±	1.06	0.61	0.73	0.74	1.22	0.49	18.6 – 22.3

Table 3. Progesterone (ng / ml) in blood serum of cows.

Day of lactation	Group					
	I		II		III	
	x	sd	x	sd	x	sd
15-21	0.161	± 0.112	0.268	± 0.324	1.077	± 1.762
45	2.745^{A}	± 1.156	0.367^{B}	± 0.423	1.556^{C}	± 0.745

$^{A, B}$ values in the rows marked with different letters differ significantly at $p \leq 0.01$.

Table 4. Level of haptoglobin in serum of cows.

No.	Group			Group		
	I	II	III	I	II	III
	HP g/L start of experiment			HP g/L end of experiment		
1	0	0	0	0	0	0.29
2	0	0.275	0	0	0.28	0
3	0	0	0	0	0	0
4	0	0	1.08	0	0	1.08
5	0	0.16	0.16	0	0.16	0.165
6	0	1.075	0.51	0	1.08	0.51
7	0	0	0	0	0	0
8	0	0	0	0	0	0
9	1.065	0	0.025	0.999	0	0.03
10	0	0>	0	0	0	0

Table 5. Level of haptoglobin in serum of cows.

Time of examination	Hp G/l level	Group					
		I-control		II		III	
		Number of animals					
		No.	%	No.	%	No.	%
Start of experiment	0-0.1	9	90	7	70	7	70
	0.1-0.4	0	0	2	20	1	10
	0.4-1	0	0	0	0	1	10
	>1	1	10	1	10	1	10
End of experiment	0-0.1	9	90	7	70	6	60
	0.1-0.4	0	0	2	20	2	20
	0.4-1	1	10	0	0	1	10
	>1	0	0	1	10	1	10

Table 6. Productivity and composition of milk – start, halfway point and end of study.

Specification	Start						Halfway						End					
	Group						Group						Group					
	I		II		III		I		II		III		I		II		III	
	x	sd	x	sd	x	sd	x	sd	x	sd	x	sd	x	sd	x	sd	x	sd
Milk yield (kg)	25.4	5.1	26.6	6.2	25.1	5.8	25. 9	6.3	27.2	9.5	23.9	8.4	24.7 a	5.4	31.3b	6.76	28.8^{ab}	7.47
Milk composition																		
Dry meter (%)	13.7^{A}	1.1	13.1^{B}	0.9	13.8^{A}	0.9	13.5	0.7	13.1	1.13	13.3	0.86	13.2	0.86	13.0	0.91	13.2	1.31
Fat (%)	4.56^{a}	0.77	4.09^{b}	0.63	4.51^{a}	0.78	4.57	0.68	4.21	0.97	4.28	0.69	4.02	0.69	4.15	0.80	4.36	0.75
Lactose (%)	4.89	0.15	4.84	0.23	4.89	0.24	4.80	0.16	4.88	0.19	4.81	0.19	4.80	0.19	4.80	0.09	4.77	0.30
Protein (%)	3.74^{a}	0.42	3.54^{b}	0.43	3.62^{ab}	0.27	3.48	0.36	3.38	0.51	3.56	0.37	3.50	0.37	3.33	0.45	3.42	0.49
Urea (mg/l)	354^{ABa}	50	358^{Aa}	64	317^{Bb}	34	156	73	179	83	166	59	140	59	143	39	110	61
Somatic cell count 1000	257	154	361	226	269	181	396	150	461	167	650	186	655	186	404	242	466	155

$^{a,\,b}$ values in the rows marked with different letters differ significantly at $p \le 0.05$; $^{A,\,B}$ values in the rows marked with different letters differ significantly at $p \le 0.01$.

remained within normal range and did not differ among the groups, while the MCHC at the beginning of the study were high and differed significantly among the groups ($P \le 0.01$). In all groups, at the end of the study, MCV and MCHC values were remarkably lower and close to the normal range. It is clearly visible that at the end of the experiment most of the hematology parameters improved, very probably as a result of the food additives used. Most of the investigated hematology parameters (except HGB and MCHC, which were higher) were similar to a results of the study conducted in the UK (Cope et al. 2009).

On days 15 and 21 postpartum progesterone level in the blood serum ranged from 0.161 – 1.077 ng/ml (Table 3). These results, due to the large variability and the high standard deviation, were not confirmed statistically. The highest level of this hormone was measured in cows receiving both biotin and zinc-methionine (group III), while the progesterone level in cows receiving only biotin was 0.268 ng/ml (group II). The progesterone level significantly increased on day 45 postpartum in cows from the control group and was significantly higher ($P \le 0.01$) than in the experimenta1 groups (groups II and III). In cows fed biotin and zinc methionine (group III) progesterone levels were significantly ($P \le 0.01$) higher than in cows fed biotin alone (Table 3). The progesterone level in the control group (2.745 ng/ml) may indicate the early return of the ovarian function. It is possible that after a very rapid and complete involution of the uterus, an ovulation of a mature ovarian follicle occurred and the corpus luteum developed, which subsequently produced progesterone. The administration of biotin might suppress the function of the ovaries.

The haptoglobin level (Tables 4 and 5) showed that in each group, at the start and at the end of the

Table 7. Occurrence of some disorders.

Disorder	Group					
	I-control		II		III	
	A	B	A	B	A	B
Retained placenta *Retentio placentae*	2	3	3	1	2	1
Endometritis puerperalis	3	4	4	2	3	2
Subacute mastitis *Mastitis subclinica*	14	13	11	10	14	8

A – previous lactation, B – experimental lactation

study, at least one cow suffered from an acute inflammation (>1.0 gL). In the rest of the investigated cows, the levels of haptoglobin indicated only a soft to mild disorder, and in all cases the problem remained at the same level at the end of the study. The exception was one cow in group II receiving biotin, which improved at the end of the investigation. Based on the acute phase protein level, the overall condition of the cows may be evaluated as satisfactory (Stefaniak 2000, Cope at al. 2009).

At the beginning of the study milk production in all groups was similar (Table 6). However, the composition of milk varied greatly. The content of dry matter (DM) was significantly ($P \leq 0.01$) higher in milk of cows from groups I and III, in comparison with DM content in milk of cows from group II, which also showed lower ($P \leq 0.05$) fat and protein content. A slightly higher somatic cell count (SCC) was found in the milk of cows from group II in comparison to SCC in the milk of cows from other groups. The difference was not statistically significant, due to individual variability, confirmed by the standard deviation. The content of urea in the milk was high in all the groups (Table 6). In the third month of lactation (halfway point of the study) the milk yield in the control group and in cows treated with biotin (group II) increased by 0.5 kg and 0.6 kg respectively, and in cows treated with both biotin and zinc – methionine (group III) decreased by 1.2 kg (Table 6). There were no significant differences in the composition of the milk, although the fat and the protein content in the milk of group II were the lowest (Table 6). The level of urea ranged from 156 to 179 mg/l and was at the lower range of the recommended values. The SCC, especially in group III receiving both biotin and Zn-methionine, was higher in comparison with SCC from the other groups. The difference might be due to the high individual variability indicated by the standard deviation, and was not statistically confirmed. At the end of the study, in month 7 of lactation, the milk yield increased ($P \leq 0.05$) by 6.6 for the cows treated with biotin (group II) and by 4.1 kg in the cows treated with both biotin and Zn-methionine, in comparison with the control group (Table 6). The composition of milk showed no significant differences; however urea content decreased significantly comparing to the values obtained in months 1 and 4 of lactation. The values were low (110-143 mg/l – below the accepted standards). This might indicate a minor shortage of protein content in the food rations for the cows. The SCC in the milk was high – the highest SCC was measured in the milk of cows from the control group – 665 000, and SCC was significantly lower in the milk of the cows receiving biotin or biotin and Zn-methionine: 404 000 and 466 000, respectively.

The supplementation of biotin or biotin with Zn-metihionine reduced the occurrence of various disorders (Table 7). The frequency of problems in groups II and III showed that biotin had significantly decreased retained placenta and *endometritis puerperalis* cases. Zn-methionin seemed to be more effective in prophylaxis of subacute mastitis.

Discussion

The administering of biotin or biotin with Zn-methionine to the cows in the first and the second trimester of lactation had no effect on the blood pH, which ranged within normal values. The BE index in blood serum indicated a slight alkalosis. In contrast, the bicarbonate (HCO_3^-) levels were above the reference standards – 25 mmol/l, which excluded subclinical acidosis. The administration of the investigated supplements improved some parameters in blood serum and had a positive influence on the cows' health. The beneficial effect of biotin were also indicated in the studies of Zimmerly and Weiss (2001) and Cope et al. (2009). Burgsten et al. (2003) emphasized the role of biotin as an important factor in the process of gluconeogenesis, lipogenesis and protein synthesis. The use of biotin and biotin with Zn-methionine had a positive effect on blood cells. A low level of zinc in the blood serum usually indicates insufficient Zn supplementation.

The level of haptoglobin in the blood serum of cows in the experiment showed that the additives used had no significant effect on acute phase protein. Haptoglobin is a dominant protein in acute phase inflammation in the cows. Moreover, it is one of the most useful parameters for monitoring the development and severity of inflammation, as well as for controling the health of cattle (Kostro et al. 2001). In clinically healthy animals, except in the early perinatal period, haptoglobin should be below the detectable leve1 (Stefaniak 2000).

The milk yield and its composition clearly showed that the administration of biotin and biotin with Zn-methionine had a positive influence, in particular in the cows treated with biotin at a dose of 10 mg/day/cow. Long-term studies (Bergsten et al. 2003) indicated that the addition of biotin increased the milk yield. Majee et al. (2003) supplemented cows with biotin, which resulted in an increase in the daily milk production, as well as in higher protein and lactose content. In the studies of Rosendo et al. (2004) the biotin addition caused a slight increase in protein concentration in milk and a decrease in the NFE in blood plasma. The average concentration of glucose in blood plasma increased, whereas the BHBA level was stable. Milk fat was not affected by biotin. Ferreira et al. (2007) in their studies showed that the administration of biotin favorably affected the yield and composition of milk only in cows with higher milk yields. Combined administration of biotin and zinc-methionine had no effect on the milk yield, but affected the fat and the protein content, which increased; and urea and the number of somatic cells, which decreased. Gaynor et al. (1988) reported that the administration of large amounts of inorganic zinc (130 ppm) did not affect the milk yield in dairy cows. Significantly better results were obtained by Kinal et al. (2005b, 2007) with the use of organic forms of zinc – chelates. In this study an increase in milk production was observed. Moreover, a clear (20-30%) reduction in the SCC was noted. In contrast, in the study of Cope et al. (2009) it was noted that the organic form of zinc had no effect on milk composition and SCC, whereas milk yield was improved. According to Kowalski et al. (2003) an addition of protected methionine did not affect the milk yield of the cows, but increased the protein content in the milk and decreased SCC. The supplementation of an organic form of zinc is successful only when the somatic cell count is high (Kinal et al. 2005a).

Summary

The results obtained concerning the administration of biotin to dairy cows in the first and the second trimester of lactation, indicated that it was profitable to use biotin at 10 mg/day/cow. Such high doses of biotin (nutritional standards are usually in micrograms) are required by the low synthesis of biotin in the rumen, especially when high doses of concentrates are fed, and from a wide (50%) breaking down of this vitamin by rumen bacteria. The administration of biotin increased the milk yield and reduced the occurrence of retained placenta, as well as *endometritis puerperalis*. High doses of biotin suppressed the ovulation and oestrus symptoms in cows (low level of progesterone on days 15, 21 and 45 after calving). The administration of Zn-methionine (5 g/day/cow) affected the milk yield in cows. However, its supplementation improved the milk composition and some of the blood parameters, and decreased the number of somatic cells in the milk. Simultaneous administering of biotin and Zn-methionine might be a good method for the prophylaxis of subacute mastitis and the improvement of productivity in high yielding dairy cows.

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