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Effects of Bentonite on Weight Gain, Feed Consumption, Blood Metabolites and Ruminal Protozoa in Dairy Calves

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ABSTRACT

Kirovski, D., Adamovic, M., Radivojevic, M., Samanc, H., Vujanac, I., Prodanovic, R. and Sladojevic, Z. 2015. The effects of bentonite on weight gain, feed consumption, blood metabolites and ruminal protozoa in dairy calves. Animal Nutrition and Feed Technology, 15: 11-20.

In order to ascertain the effects of addition of bentonite as a pelleting medium in the feed mixture for calves, fourteen 30d-old calves were randomly distributed into two equal groups (CON and EXP). From 30 to 120d of age the EXP group was fed a feed mixture containing 1.5% of natural bentonite while the CON group was fed the same pellets without added bentonite. Body weight was determined before and at the end of the trial. Feed intake was measured daily. Health status was monitored daily. Blood and rumen content samples were taken at 50 and 90d of experiment and analyzed for select parameters. The addition of bentonite had no effect ($P>0.05$) on average daily gain, feed intake and health. Blood pH, total number and motility of ruminal protozoa in the EXP group were significantly ($P<0.001$, $P<0.05$ and $P<0.05$; respectively) higher than in CON group at both the periods. At 120d of age the EXP calves had a significantly ($P<0.05$, $P<0.05$ and $P<0.01$; respectively) higher serum total protein, albumin and triglyceride concentrations and lower iron and IGF-I ($P<0.05$ and $P<0.01$, respectively) concentrations than respective controls values. It is concluded that the use of pelleted feed containing bentonite tended to improve growth of calves as well as the activity of protozoa in the rumen. Additionally, the results indicate a positive influence of the function of hepatocytes concomitant to a reduction in serum iron and IGF-I concentrations.

Key words: Bentonite, Blood metabolites, Calves, Ruminal protozoa, Weight gain.

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INTRODUCTION

The nutrition of calves is of primary importance for achieving the genetic potential, health, welfare and reproductive performance of cattle. Therefore, technological procedures that improve nutrition utilization in calfhood are gaining importance. One such procedure is pelleting of feed mixtures. Due to the effect of temperature during pelleting partial decomposition of carbohydrates occurs, resulting in increased digestibility, improved taste and increased metabolizable energy (Samarasinghe *et al.*, 2000). Also, under the influence of temperature, degradation of certain thermolabile anti-nutritive feed components (trypsin inhibitor, lectins, urease, peroxidase, lipoxygenase, myrosinase, glucosinolates, gossypol etc.) occurs, resulting in additional positive effects for achieving better production and health performance in animals (Raczyñski and Buraczewska, 1985). In addition, pelleted food improves the appetite, chewing and salivation and thus contributes to stabilizing the pH of the rumen. One of the effects of high temperature during pelleting is improved feed hygiene due to the reduction in the number of microorganisms present (Chukwuka *et al.*, 2010).

To improve the quality of pellets, especially their hardness, additives such as molasses, calcium lignosulfonate, sodium or calcium bentonite, raw fiber concentrate pulp, and other substances of organic and inorganic origins are used (Salari *et al.*, 2006; Pasha *et al.*, 2008).

Bentonite is a hydrated aluminum silicate of volcanic origin which contains the mineral montmorilonite (50-90%). It has thermoplastic characteristics which enable it to achieve good results during pelleting feedstuffs. It consists of exchangeable cations Na^{+}, K^{+}, Ca^{++}, and Mg^{++}. Bentonite has a high liquid binding (water and oil) capacity. Its cation exchange capacity (CEC) is 80-120 meq/100g. Due to its amphoteric features (releases and binds hydrogen ions) at the same time it is used as a means of maintaining the rumen pH. Bentonite binds aflatoxins (B_1, B_2, G_1 and G_2) present in the feed (Pasha *et al.*, 2007). Its ability to absorb zeralenone and ochratoxin is limited. Bentonite absorbs ammonia present in the rumen whenever its concentration is high and thereon it releases the absorbed when its concentration decreases (Britton *et al.*, 1978). This allows a more efficient use of ammonia nitrogen for microbial protein synthesis (Jacque *et al.*, 1986). In this way, it reduces the absorption of ammonia, liver load and energy consumption for urea synthesis. Due to its ability to bind water, bentonite increases its volume and thus the volume of the digested material in the digestive tract. The increased volume of the digesta has an effect on the reduction of the speed of passage through the digestive tract, thus enabling a prolonged action of digestive enzymes and subsequent increase of the digestibility of the nutrients (Pasha *et al.*, 2008). Studies on bentonite and its impact on the productive capacity of cattle were performed mainly in adult cows (Van den Hoek, 1980; Diaz *et al.*, 2004), while the number of publications relating to calves is limited.

The aim of this paper was to study the effects of pelleting medium based on natural bentonite (Bentopel®) on weight gain, feed consumption, blood metabolites and rumen protozoan activity in dairy calves.

MATERIALS AND METHODS

Bentonite composition

The pelleting mixture based on natural bentonite (Bentopel®) was manufactured with a special technological procedure at the Institute for the Technology of Nuclear and Other Raw Materials, Belgrade. The natural bentonite originated from the site Sipovo (Bosnia and Hercegovinia). The chemical composition of Bentopel® is shown in Table 2 and granulometric values are given in Table 3.

Calves and dietary treatments

The study was conducted on 14 Holstein calves. At the age of 30d the calves were divided into two equal groups of seven calves in each: the control (CON) and the experimental (EXP) groups. The CON group was fed a mixture of standard food pellets, while the EXP group received a pelleted mixture which included 1.5% pelleting medium based on natural bentonite (Table 1). The pellet diameter was 4 mm and the length measured 4-6 mm. In addition to the feed mixtures, both groups of calves were further fed milk replacer twice a day (the total daily consumption was 6L and up to the age of 70d, and then 3L up to the age of 80d). The substitute for milk powder was dissolved in water and diluted in a ratio of 1:8, at 38-42°C. Additionally all calves involved in the experiment received alfalfa hay *ad libitum*.

Table 1. Chemical composition of Bentopel®

Compound/Element	Content
SiO_2 (%)	48.37
Al_2O_3 (%)	22.39
Fe_2O_3 (%)	4.73
CaO (%)	5.86
MgO (%)	0.81
Na_2O %	0.07
K_2O (%)	0.40
TiO_2 (%)	0.34
Cd (ppm)	< 5
Pb (ppm)	< 30
Sb (ppm)	< 30
Cu (ppm)	< 30
Zn (ppm)	< 40
As (ppm)	< 20

BW gain, health status and feed intake

The BW of individual calves was measured at the start of the trial (at 30d of age) and at the end (at 120d of age). According to the differences in BW the daily weight gain was calculated. Health status of calves involved in experiment was monitored daily, especially the possible occurrence of diarrhoea. Feed intake was measured daily throughout the 90d duration of the trial.

Table 2. Granulometric composition of Bentopel®

Granulation size class (μm)	%
- 63 + 61	12.60
- 61 + 46	2.70
- 46 + 32	3.50
- 32 + 21	2.70
- 21 + 15	3.50
- 15 + 0	75.00

Rumen sample collection and analysis

Samples of rumen content were taken with the aid of a specifically designed probe from all the calves twice, at the age of 80 and 120d (Zwick and Klee, 1997). Rumen contents were taken 4 to 6h after feeding of the morning meal. Immediately after the samples were taken, the pH was recorded using a pH meter (WTW 330i), and a native slide was prepared for microscopic examination of the rumen protozoa and their motility. Their motility was numerically estimated (0- immobile, 1- poor motility, 2- optimal motility). Samples were mixed with an equal volume of 2% formalin in phosphate-buffered saline for microscopic counts. Protozoa were counted in whole rumen contents by light microscopy as per Dehority (1984). Counts of total protozoa were recorded; 20 fields of view were counted, and the highest and lowest counts were discarded.

Table 3. Physical and chemical composition of feed pellet

Attributes	Dietary group	
	CON	EXP
Physical composition (%)		
Corn, wholemeal	34.30	34.30
Barley, wholemeal	10.00	10.00
Soybean meal, full fat	22.50	22.50
Sunflower meal, 33% CP	10.50	10.50
Wheat flour	16.50	15.00
Alfalfa meal	3.00	3.00
Calcium carbonate	1.20	1.20
Dicalcium phosphate	0.40	0.40
Salt	0.60	0.60
Vitamin and mineral supplement	1.00	1.00
Bentopel®	0.00	1.50
Chemical composition (% as feed)		
Protein	18.51	18.33
Fat	5.42	5.36
Crude fiber	6.48	6.39
ADF	7.35	7.29
NDF	15.11	14.95
Ash	3.96	5.13
Calcium	0.72	0.74
Phosphorous	0.60	0.58
NE_L, MJ/kg	6.86	6.78

Blood sample collection and analytical methods

Blood samples were taken from calves at 50 and 90d of experimental feeding by jugular venipuncture 30 min before rumen content sampling to eliminate the effects of stress on the results. Blood samples were taken into test tubes with no added anticoagulant; samples taken for glucose determination were collected with the anticoagulant Na-fluoride. After 15 min at room temperature, all samples were centrifuged at 1,000 x *g* for 20 min and the blood serum and blood plasma separated and stored at -18°C until analyses. Blood samples were tested by photometry for Ca and P concentration, total protein, albumin, triglycerides, urea, iron, and glucose with commercial test kits (BioMedica, Serbia). The concentrations of insulin like growth factor-I (IGF-I) and insulin were determined by RIA method using commercial kits (INEP, Zemun). The homeostasis model assessment of insulin resistance (HOMA-IR; an indicator of the susceptibility to insulin resistance) was calculated based upon the values measured for blood glucose and insulin concentrations according to the formula: HOMA-IR= glucose (mmol/L) x insulin (mIU/L) / 22.5.

Statistical analysis

The results obtained were analyzed statistically using Statistica v. 6. (StatSoft, Inc., Tulsa, OK, USA). Experimental data are presented as means±SE. For testing and determination of statistically significant differences two tests were applied. The first test was the repeatet-measure ANOVA with 3 variables (calf, treatment and time). When "F" for treatment, time, or interaction showed statistical significance ($P<0.05$), the second (t) test, for evaluation of differences between mean values ($n=7$), was applied. Differences were considered statistically significant as $P<0.05$, $P<0.01$ and $P<0.001$.

RESULTS AND DISCUSSION

Body weight gain

At the start of the trial the mean BW did not differ significantly between the experimental and control group (Table 4). At the end of the experiment the EXP group had on average 9.55 kg higher BW compared to CON calves. When calculated in terms of average daily gain (ADG) the EXP calves attained 112g more body weight than the CON calves. However, these differences were not significant; thus, it can be concluded that bentonite had no effect on growth of dairy calves.

Feed intake

The results have shown that on an average, the EXP calves consumed 4.42% more feed and had a 6.45% higher efficiency compared to the CON calves (Table 4). Similar effects on weight gain of Bentonite-based mixtures have been described in lambs by Khadem *et al*. (2007) and piglets by Thieu *et al*. (2008). There is no literature available related to feed intake in calves fed bentonite added diets. Possible explanation of increased weight gain and feed intake in calves fed Bentonite-based diets could be increased ruminal pH, total number and motility of protozoa in those animals (Diaz *et al*., 2004), as have been observed in present study (Table 5). There was no occurrence of diarrhoea.

Table 4. The effect of bentonite treatment on body weight changes and feed intake of dairy calves

Parameters	Dietary group	
	CON	EXP
Initial BW, kg	54.55±1.56	54.05±1.16
Final BW, kg	142.06±3.69	151.61±5.29
Net BW gain, kg	87.51±3.88	97.56±4.81
Average daily gain, kg	0.972±0.04	1.084±0.05
Mixture intake*, kg/d	1.81±0.01	1.89±0.02
Mixture utilization*, kg/kg BW gain	1.86±0.08	1.74±0.09

*Significant difference between groups at $P<0.05$

Table 5. The effect of bentonite treatment on select blood and rumen parameters of dairy calves

Attributes	Period	Dietary groups	
		CON	EXP
Blood parameters			
pH	50d	7.40±0.01***	7.45±0.0[a]
	90d	7.39±0.01***	7.49±0.01
Calcium (mmol/L)	50d	2.20±0.04	2.12±0.03
	90d	2.20±0.04	2.24±0.03
Phosphorus (mmol/L)	50d	2.98±0.13	2.71±0.12
	90d	3.05±0.08	2.83±0.12
Total protein (g/L)	50d	61.40±2.12	63.60±2.11
	90d	75.04±0.94*	79.56±1.77
Albumin (g/L)	50d	23.36±0.92[aaa]	21.58±0.74[aa]
	90d	58.40±1.54*	63.84±1.11
Urea (mmol/L)	50d	3.60±0.13[aaa]	3.26±0.19[aaa]
	90d	5.51±0.34	6.15±0.18
Total bilirubin (mmol/L)	50d	11.04±1.33[aaa]	11.08±1.27
	90d	4.46±0.38	5.36±0.28
Triglycerides (mmol/L)	50d	0.45±0.03[aaa]	0.53±0.27[aaa]
	90d	0.13±0.04**	0.31±0.02
Cholesterol (mmol/L)	50d	2.40±0.07[aaa]	2.60±0.06[aa]
	90d	1.37±0.06	1.56±0.08
Fe (μmol/L)	50d	38.36±4.38[aaa]	33.48±1.89
	90d	38.01±0.39*	29.84±3.45
IGF-I (nmol/L)	50d	18.16±1.49[aaa]	19.66±1.88[aaa]
	90d	32.01±2.69**	22.32±1.15
Insulin (mIU/L)	50d	22.16±2.70	25.83±2.78
	90d	32.58±3.59	37.66±3.74
Glucose (mmol/L)	50d	4.74±0.18[a]	4.50±0.06
	90d	4.42±0.13	4.36±0.12
HOMA[†]	50d	4.62±0.52[a]	5.19±0.61[a]
	90d	6.51±0.84	7.35±0.80
Rumen parameters			
pH	50d	6,28±0.09	6,44±0.08[a]
	90d	6,14±0.04	6,25±0.11
Total protozoa (x 10^5)	50d	3.28±0.61*[a]	5.00±0.44[a]
	90d	3.86±0.63**	6.43±0.43
Protozoa motility	50d	1.00±0.22*	1.71±0.18
	90d	1.14±0.26*	1.86±0.14

*Significant difference between groups at $P<0.05$; **$P<0.01$; ***$P<0.001$

[a]Significant difference within group at $P<0.05$; [aa]$P<0.01$; [aaa]$P<0.001$

[†]Homeostasis model assessment of insulin resistance

Blood parameters

Analysis of blood biochemical parameters in both groups of calves at the age of 80 days, which corresponded to the 50th day of the experiment, did not differ significantly ($P > 0.05$) with the exception of the pH which was significantly ($P < 0.001$) higher in the EXP group (Table 5). The difference in blood pH between the two groups at the age of 80 and 120 days can be accounted by the differences in ruminal pH. When analyzing the parameters whose concentrations differed between the groups it could be assumed that the added pelleting agent possibly had a positive effect on the activity of hepatocytes. Namely, the concentration of albumin, which is primarily synthesized by the liver and total protein were significantly ($P < 0.05$) higher in the EXP group of calves. In addition, significantly ($P < 0.01$) higher triglyceride concentrations in the EXP group may confirm the assumption of an improved synthetic activity of hepatocytes in experimental calves. The numerically higher bilirubin concentration (although not significantly) in the EXP calves may indicate the increased activity of liver tissue in the experimental groups. These results are consistent with the available data in the literature (Dunn *et al.*, 1979; Mckenzie, 1991). Given the fact that preparations of bentonite absorbed toxic substances within the small intestine, it is possible that this relieves the liver, thus reducing its detoxifying activity and in compensation leaves space for an increased synthetic activity.

The concentration of iron was significantly ($P < 0.05$) lower in the EXP group compared to the control group at 90d post-feeding. This finding can be considered as the result of supplementation with bentonite and it has been previously reported as a consequence of the use of mineral supplements in calf feeding. Literature indicates that low iron concentration in calves occurs concurrently with low IGF-I concentration (Blum and Hammon, 1999; Prodanovic *et al.,* 2014), the observed significantly ($P < 0.01$) lower concentration of IGF-I in the blood serum of the EXP calves is a confirmation of the above reports. Moreover, higher serum triglycerides concentrations in EXP group of calves likely to have contributed to the reduction of blood IGF-I concentration. An increase in serum triglycerides concentrations inhibits GRF-mediated GH secretion in calves, as demonstrated by Coxam *et al.* (1989). If the HOMA indices between the two groups are compared the fall in IGF-I concentration can be partially explained by the established relationship between insulin and glucose in the EXP group of calves. Although HOMA-IR is widely used in human medicine to determine the susceptibility to insulin resistance, it may give certain information in other species as well. Thus, if the HOMA-IR value is high then the predisposition to insulin resistance is increased and vice versa (Borai *et al.*, 2011). Although the concentration of insulin and glucose between the CON and EXP groups of calves did not differ ($P > 0.05$), the calculated HOMA index indicates that in the EXP group there was a tendency toward insulin resistance. From the available literature it is known that the tendency toward insulin resistance leads to lower IGF-I. Also, it is possible that the significantly higher triglycerides concentration at 90d post-feeding can partially

explain the tendency of these animals towards insulin resistance, as documented in children (Tresaco *et al.*, 2003).

Rumen parameters

Data presented in Table 5 showed that the pH value was higher, though not significantly, in the EXP group at 50 and 90d. However, it should be emphasized that throughout the experiment the rumen content pH value was within the physiological range (6.2-6.8). At the end of the trial ruminal pH in the CON group decreased below the optimal physiological value. This indicates that the tested pelleting mixture had a positive impact on ruminal pH and maintained the pH continuously within the physiological values. In such conditions, the electrochemical reaction of ruminal contents and blood do not change significantly compared to physiological values. It is the buffering activity of bentonite at different pH values, and its basic chemical properties that make it interesting for application in the feed industry and animal nutrition. While studying the influence of bentonite on fermentation in the rumen it was observed that bentonite slows fermentation in the rumen especially in terms of diet and concentrated feeds thus preventing a pronounced fall in rumen pH (Aitchison *et al.*, 1986). The data on the presence of protozoa in rumen have shown significant ($P<0.05$ and $P<0.01$; respectively) differences between the EXP and CON groups at both the tested periods. In the CON group there were a significantly ($P<0.05$ and $P<0.01$; respectively) reduced number of total protozoa which had lower ($P<0.05$) motility (Table 5). The beneficial impact of bentonite on protozoa activity has been reported earlier as well (Abudullah *et al.*, 1995). However, increased ruminal pH, higher total number and increased motility of protozoa in EXP calves consuming the feed mixture containing 1.5% of natural bentonite, had no adverse impact on animal health since all calves during experimental period did not show any visible sign of disease.

CONCLUSIONS

The use of pelleted feed containing bentonite tended to improve growth of calves and also the activity of protozoa in the rumen. Additionally, the results indicate a positive influence of the function of hepatocytes concomitant to a reduction in serum iron and IGF-I concentrations.

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