




Effects of dietary supplementation of bentonite and yeast cell wall on serum blood urea nitrogen, triglyceride, alkaline phosphatase, and calcium in high-producing dairy cattle during the transition period

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Abstract

This study was conducted to evaluate the effects of dietary supplementation of endotoxins binders (*Saccharomyces cerevisiae* cell wall (SCW) and bentonite (BEN)) on blood urea nitrogen (BUN), triglyceride, alkaline phosphates (ALP), and calcium (Ca) in high-producing dairy cows during the transition period. Twenty-four Holstein dairy cows ($n = 6$, average milk production = 35.8 ± 1.6 kg/day, body weight (BW) = 567.5 ± 40.3 kg, body condition score (BCS) = 3.5 ± 0.26 out of 5) were assigned in a completely randomized design, to the following treatments: (1) basal diet (BD), (2) BD + SCW, (3) BD + BEN, and (4) BD + SCW + BEN. Blood samples were taken 4, 3, and 1 weeks before and 1 and 3 weeks after parturition. Serum concentrations of BUN, triglyceride, ALP, and Ca were measured. It was observed that, BUN concentration was lower in SCW + BEN group, probably because of the better utilization of NH₃-N and better stimulation for ruminal microbial protein synthesis. Triglyceride plasma concentration was lower in the control group, which might be due to decreased lipolysis and a lower pH. The treatment groups showed no effect for Ca at any time point. Serum ALP concentration was within references range. In conclusion, lower BUN concentration could be a result of better stimulation of ruminal microbial protein synthesis in the treatment groups and also due to lower triglyceride in control group, which might be result of lower pH in the rumen, we suggest that endotoxins binders (especially combined feeding of endotoxin binders) can modulate rumen function by optimizing the rumen pH.

Keywords Endotoxins binders · Rumen pH · Transition period · Dairy cattle

Introduction

With increasing productivity of dairy cows, management of diet for meeting energy required for milk production, becomes more vital for animal health. In many dairy herds after parturition, a diet with high fermentable carbohydrate is often fed to compensate the negative energy balance. Occurrence of sub-acute ruminal acidosis increase when the level of carbohydrate increases in

the diet (Owens et al. 1998). Sub-acute ruminal acidosis (SARA) has a negative effect on animal health and profitability of dairy herds (Kleen et al. 2013). Sub-acute ruminal acidosis occurs when ruminal pH declines below 5.6 for a prolonged period for several hours per day (Kleen et al. 2003; Stone 2004; Gozho et al. 2005); but dairy cattle suffered from sub-acute ruminal acidosis has no typical clinical sign of illness (Tajik and Nazifi 2011). Sub-acute ruminal acidosis is associated with inflammations of different organs and results in economic losses in dairy herds (Kleen et al. 2003; Stone 2004; Plaizier et al. 2008; Zebeli and Ametaj 2009). Tajik et al. (2009) did a survey on prevalence of sub-acute ruminal acidosis in Iranian dairy herds and they reported that the prevalence of sub-acute ruminal acidosis was 27% in early lactation and mid-lactation cows. Newly Kleen et al. (2013) reported that incidence of sub-acute ruminal acidosis was 20% in 315 cows in Northern Germany. Sub-acute ruminal acidosis causes pathological changes in the rumen wall (Steele et al. 2011), leading to the increased absorption of lipopolysaccharide (LPS) released from

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gram negative bacteria in rumen and the lower gut (Gozho et al. 2006; Dong et al. 2011; Plaizier et al. 2008, 2012). Study by Gozho et al. (2006) showed an increase in acute phase protein as a result of sub-acute ruminal acidosis, also liver damage may occur (Ainmalamali et al. 1992). An increase in LPS concentration of blood associated with metabolic disturbances, such as the increase in blood glucose and non-esterified fatty acid which reduce feed intake and affect pattern of hydroxybutyric acid, cholesterol, and minerals, such as Ca, Fe, and Zn (Ametaj et al. 2009; Zebeli et al. 2010). Many studies have addressed different strategies to decrease the incidence of sub-acute ruminal acidosis. A study by Gonzalez et al. (2012) demonstrated that adding feed additives in cows' diets, such as buffers, natural plant extracts, ionophores, yeasts, and organic acids could reduce the prevalence of sub-acute ruminal acidosis. Bentonites are clays belong to smectite group (Trckova et al. 2004). Smectite minerals have a wide-range absorbent ability, which make it a good absorptive material of heavy metals and bacteria (Hassen et al. 2003; Katsumata et al. 2003) and toxic and anti-nutritive agents (Ditter et al. 1983; Pulsipher et al. 1994; Ibrahim et al. 2000; Phillips et al. 2002). Some studies have reported that many strains of lactic acid bacteria and some strain of *Saccharomyces cerevisiae* have probiotic activity (Coeuret et al. 2004). For a long time, yeast products have been fed to animals, and nowadays, many commercial yeast products are being specially produced for animal feeding (Celyk et al. 2003). Yeast cell wall also is able to bind sterols from the medium (Adams and Parks 1967), where the binding molecule was identified as the cell wall mannan (Thompson et al. 1973). Study by Lei et al. (2013) in beef cows reported that dietary supplementation of yeast cell wall and montmorillonite (bentonite) can effectively reduce free LPS concentration in plasma, digesta, and the feces. Therefore, the main objectives of this study is to evaluate potential effect of dietary supplementation of endotoxins binders (bentonite and *S. cerevisiae* cell wall) on serum concentration of BUN, triglyceride, ALP, and Ca in high-producing dairy cows during the transition period.

Material and methods

Animals and experimental design

In this study, 24 multiparous Holstein cows with an initial body weight (BW) of 567.5 ± 40.3 kg (mean \pm SD), body condition score of 3.5 ± 0.26 out of 5 (mean \pm SD), and average milk production of previous lactation of 35.8 ± 1.6 kg/day (mean \pm SD), were assigned in a completely randomized design in four groups ($n = 6$). Treatment groups received balanced ration in accordance with the NRC nutrient requirements based on the cow condition (NRC 2001). Control group received the same ration at similar intervals; except, it was not supplemented with a toxin binder based on bentonite and *S. cerevisiae* cell wall.

Our experiment conducted from 4 weeks before the expected calving date until 3 weeks after the calving date. Experimental cows were housed in pen with four stalls ($n = 6$). After calving, experimental animals were moved to another pen near to milking parlor, consisting of four stalls and their diet was changed from close-up diet to fresh cow diet. Pens were bedded with clean wood shavings and dry manure, and bedding was refreshed daily if it was necessary. The cows were selected from a 1000-cow industry dairy herd in Khorasan Razavi province in the northeast of Iran in summer of 2016. The experimental groups consisted of (1) the basal diet (control); (2) the basal diet+ *S. cerevisiae* cell wall (12 g/day/cow); (3) basal diet + bentonite (150 g/day/cow); and (4) the basal diet + bentonite + *S. cerevisiae* cell wall on a dry matter basis.

Diet

The bentonite (particle size less than 37 μm according to manufacturer introduction) was purchased from Zarin Binder (Mashhad, Iran). The *S. cerevisiae* cell wall was supplied from ActiveMOS (dry matter $\geq 94.0\%$, Chitin ≥ 2.0 , β -glucan $\geq 30.0\%$, and mannoprotein $\geq 20.0\%$). (Biorigin, Sao Paulo, Brazil). Dietary doses of endotoxins binder were based on the manufacturer recommendation for dairy cows (12 g/day/cow for SCW and 150 g/day/cow for bentonite). The total mixed ration (TMR) was formulated to meet or exceed the requirements for fresh and close-up cows separately (National Research Council (NRC) 2001). Ingredients and chemical formulation of the experimental diets (DM basis) are showed in Table 1. During the trial, cows were fed the experimental diet as a TMR twice daily at 1000 and 1700 h. The experiment animals had free access to water during the trial.

Sampling procedure

Blood samples were taken five times during the trial period, 2 h before the morning feeding on all cows through tail vein in 10-mL plain tubes. One sample was taken 1 week before adding the additives to the diet. The next sample was collected at 3 and 1 weeks before the parturition and 1 and 3 weeks after the parturition. The samples were cooled down quickly and serum was separated by centrifugation (Sigma 1-15p-Sigma-Laborzentrifugen, Munich, Germany) for 10 min at $\times 3000g$ and stored at -22 °C until being assayed. Biochemical analyses and methods of measuring are presented in Table 2.

Statistical analyses

In our study, data were expressed as the mean \pm deviation (SD). Comparison of quantitative data between the experimental and control groups was done using linear mixed models to compare mean concentrations of different serological factors within similar weeks between our different

Table 1 Ingredients and chemical formulation of the experimental diets (DM basis)

Item	The amount in the diet (%)	
	Close-up cow	Fresh cow
Ingredients:		
Alfalfa hay	17.78	24.6
Corn silage	33.51	15.38
Wheat straw	10.46	0
Linseed seed	2.54	4.31
Whole cottonseed	0	5.54
Barley grain	14.12	12.44
Corn grain	6.21	12.04
Soybean meal	3.15	6.7
Cottonseed meal	1.92	3.09
Wheat barn	2.59	6.16
Soybean meal (treated)	2.57	5.6
Bergafat ^a	0.71	0.51
Sugar beet pulp	0	0.91
Sucrose	0	0.55
Magnesium oxide	0	0.05
Sodium bicarbonate	0	0.31
Calcium diphosphate	0	0.17
Sodium carbonate	0	0.21
Vitamin premix ^b	0.71	0.68
Vitamin D3	0.42	0.48
Vitamin E	0.63	0.27
Anionic salt	2.68	0
Chemical composition (% of DM):		
NEL (Mcal/kg of DM)	1.50	1.61
CP	11.8	16.8
NDF	40.5	32.6
ADF	25.6	20.6
Ether-extract	4.2	6.2
DCAD (mEq/kg)	−98	237
Ca	0.4	0.8
P	0.3	0.5

^a Fractionated palm fatty acid; palmitic acid (C 16:0) min. 98%

^b Contained 4540 mg/kg of Fe, 5000 mg/kg of Cu, 3400 mg/kg of Mn, 43 mg/kg of Co, 6000 mg/kg of Zn, 140 mg/kg of Se, 3600 kIU/kg of vitamin A, 360 kIU/kg of vitamin D, and 3 kIU/kg of vitamin E

CP crude protein, NDF neutral detergent fiber, ADF acid detergent fiber, DCAD dietary cation-anion difference

experimental groups. Statistical analysis was done using SPSS software (SPSS for Windows, version 16, SPSS Inc. Chicago, Illinois). The statistical significance level was set at $P < 0.05$.

Results

Blood parameters level

The effect of treatment group and time on concentrations of BUN and triglyceride are summarized in Figs. 1 and 2 respectively. Mean BUN concentration was lowest in group SCW + BEN ($136 \pm [124\text{--}147(\text{CL})]$ mg/L) and lower than that in the control group and BEN group ($p < 0.05$). Four outliers were identified and removed prior to analysis as the results were not

Table 2 Biochemical analyses and methods of measuring indices in dairy Holstein calves

Variables	Method of measuring
Calcium	Colorimetric complexometric test (Arsenazo III) using commercially available kits (Biorex Fars, Shiraz, Iran). (Assay range: 2–190 mg/L, sensitivity: 2 mg/L, intra-assay: CV < 1.33% and inter-assay: CV < 1.97%).
Triglycerides	Enzymatic colorimetric method (GPO-PAP) using kit Biorex Fars (Shiraz, Iran) (assay range: 30–11,000 mg/L, sensitivity: 30 mg/L, intra-assay: CV < 1.19% and inter-assay: CV < 1.56%).
Blood urea nitrogen (BUN)	Enzymatic method by the Man company kit (Tehran, Iran) according to the manufacturer's instructions (assay range: 90–3000 mg/L, intra-assay: CV < 3.9% and inter-assay: CV < 4.4%).
Alkaline phosphatase (ALP)	DGKC (Deutsche Gesellschaft Fur Klinische Chemie) method using a commercially available kit (Pars Azmun, Karaj, Iran) according to manufacturer's instructions (sensitivity: 3 U/L, intra-assay: CV < 1.5% and inter-assay: CV < 1.6%).

biologically plausible (BUN concentration > 500 mg/L). All these outliers belong to the group control. Mean triglyceride concentration was lowest in group control ($179 \pm [114\text{--}244(\text{CL})]$ mg/L). Despite the significant interaction, no effect of treatment group was observed for triglyceride at any time point. Mean triglyceride concentration at 1 week after calving was the lowest ($127 \pm [107\text{--}146(\text{CL})]$ mg/L). The p values from the linear mixed models of the effect of treatment group, time and their interaction on the mean concentrations of the measured analyzed are shown in Table 3.

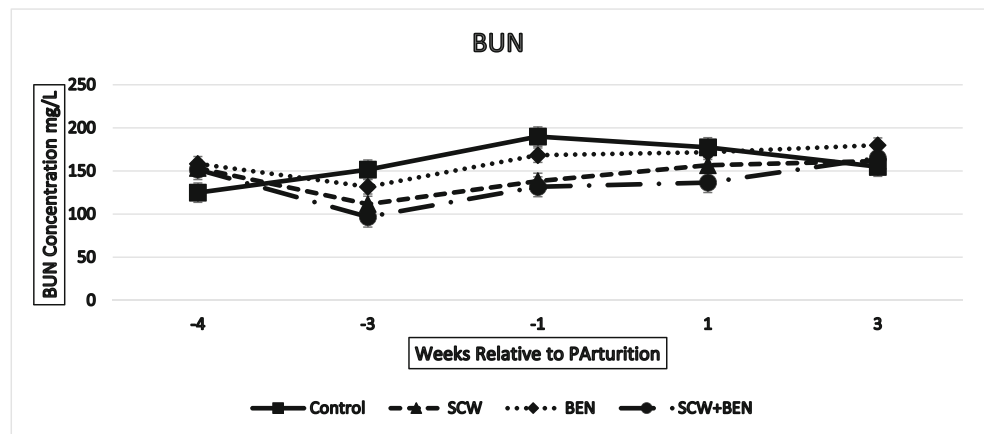
Serum calcium levels

The effect of treatment group and time on concentrations of Ca is presented in Fig. 3. No effect of treatment group was found for Ca at any time point. The mean Ca concentration was lowest at 1 week before calving ($86 [(95\% \text{ CL}) 84\text{--}88]$ mg/L). In that time point, the mean Ca concentration was lower than that at 4 and 3 weeks before calving ($p \leq 0.001$).

Liver enzyme activity

The effect of treatment group and time on concentrations of ALP is shown in Fig. 4. The overall effect of treatment group on ALP activity was statistically significant at 5% level; besides, an interaction between group and time was found. Mean ALP concentration in the control group serum was highest ($97 [(95\% \text{ CL}) 84.6\text{--}109.3]$ U/L). Mean ALP concentration in control group was higher than that in group BEN ($p < 0.05$). Mean ALP concentration in serum was lowest at 1 week

Fig. 1 Mean (\pm SEM) serum concentrations of BUN (mg/L) 4, 3, and 1 weeks before and 1 and 3 weeks after parturition ($n = 6$ six in each group, $*p < 0.05$) (BUN references value in cattle 60–270(mg/L)) (Constable et al. 2017)



before parturition (52.5 [95% CL] 33.9–71.1) U/L, and it was lower than 4 and 30 weeks before parturition ($p < 0.05$).

Discussion

A study by Fairfield et al. (2007) on dairy cattle after calving demonstrated that prevalence of sub-acute ruminal acidosis increased when cows received diet with 54% forage DM (34% NDF and 39% NFC, DM basis). A study by Penner et al. (2007) also showed that the occurrence of sub-acute ruminal acidosis raised in early lactation cows fed with a diet containing 47% forage DM (29% NDF, DM basis). The experimental diet of our study contains 51.29% forage (DM basis) for close-up cows and 39.98% forage (DM basis) for fresh cows, and thus the concentrate levels were 48.71 and 60.02% respectively. When the levels of carbohydrate increase in diet, it is predisposed cows to sub-acute ruminal acidosis due to the accumulation of organic acids produced by microbial fermentation and the consequence is a decrease in rumen pH (Owens et al. 1998). Study by Chiquette et al. (2012, 2015) showed milk urea nitrogen (MUN) decreased in cows during sub-acute ruminal acidosis. Milk urea nitrogen (MUN) is originated from blood urea nitrogen (BUN) in ruminants (Jonker et al.

1998). A low concentration of MUN can be attributed to better utilization of $\text{NH}_3\text{-N}$ in rumen due to a suitable environment for higher stimulation of ruminal microbial protein synthesis which leads to a declined $\text{NH}_3\text{-N}$ concentration in rumen and BUN (Chiquette et al. 2015). BUN might be changed during water deprivation, diarrhea, urinary disease, pregnancy toxemia, and acidosis (Patra et al. 1996; Singh et al. 1992; Nozad et al. 2012), which were not case in experimental animals of this study. In the current study, the level of carbohydrate in the experimental diet was similar between experimental groups but BUN concentration was lower in SCW + BEN group. This result might be attributed to suitable environment for better utilization of $\text{NH}_3\text{-N}$ and better stimulation for ruminal microbial protein synthesis in the rumen. BUN level was higher in the control group that did not receive endotoxin binders, probably because of the lower number of cellulolytic ruminal bacteria that use $\text{NH}_3\text{-N}$ as source of nitrogen (Atasoglu et al. 2001). Although we did not measure rumen pH, we think that difference in the rumen pH results in different ruminal bacterial population. Khafipour et al. (2009) reported a decrease in ruminal $\text{NH}_3\text{-N}$ concentration during a sub-acute ruminal acidosis challenge. Chaucheyras-Durand et al. (2008) did a survey on the impact of yeasts on ruminal $\text{NH}_3\text{-N}$ concentration and reported that type of diet, host, and microbial-related factors has

Fig. 2 Mean (\pm SEM) serum concentrations of triglyceride (mg/L) 4, 3, and 1 weeks before and 1 and 3 weeks after parturition ($n = 6$ six in each group, $*p < 0.05$) (Triglyceride references value in cattle 10–140 (mg/L)) (Constable et al. 2017)

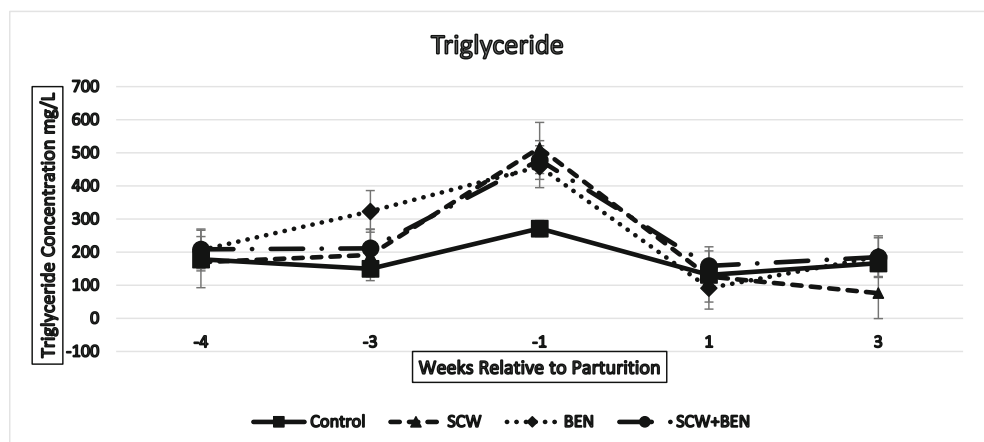


Table 3 Effect of the experimental groups, time and group × time on the mean concentration (*p* values)

Item	Group	Time	Group × time
BUN	0.006	<0.001	0.009
Triglyceride	0.3	<0.001	0.003
ALP	0.001	<0.001	0.02
Ca	0.4	<0.001	0.08

BUN blood urea nitrogen, ALP alkaline phosphatase

very important role. One study showed that actively dried yeast supplementation to dairy cows with sub-acute ruminal acidosis is able to improve rumen function, considering a tendency for an improved ruminal pH and greater abundance of *M. elsdenii* within the rumen (Malekkhani et al. 2016). We used yeast cell wall and due to a lower concentration of BUN in treatment groups, which might be because of better utilization of NH₃-N: we suggest that endotoxin binders, especially combined feeding of endotoxin binders, can improve rumen function as indicated by optimizing rumen pH. Chiquette et al. (2008) demonstrated that supplementing early lactating cattle with probiotics (*P. bryantii* 25A) can result in reduction of lactate concentrations in the rumen which contributes to prevention of rumen acidosis. We are not certain whether supplementation of endotoxin binders can prevent sub-acute ruminal acidosis, but we suggest that it can improve rumen function in some aspect. A study of Guo et al. (2013) showed that triglyceride decreased in animals with sub-acute ruminal acidosis. A study by Van Nevel and Demeyer (1996) demonstrated that lipolysis is much more sensitive to low pH value in rumen and lipolysis and biohydrogenation decreased when the pH is low in animals fed high concentrate diet. Results of this study demonstrated that, triglyceride concentration in serum was lower in the control group, which might be due to reduction in lipolysis in the control group and also the rumen pH might have been lower in that group. Serum triglyceride concentration was high in all

groups. It might be due to negative energy balance and also lipolysis was pronounced in them. Danscher et al. (2015) showed that blood-ionized calcium concentrations were lower in sub-acute ruminal acidosis group compared to animals without sub-acute ruminal acidosis. A study by Zebeli et al. (2010) in healthy lactation cows showed the negative correlations between Ca and rumen LPS and plasma serum amyloid a (SAA). Also, a study by Wenz et al. (2001) showed a decrease in the Ca concentration in lactating cows affected by coliform mastitis. The results of this study showed no treatment effect on Ca concentration in blood. An explanation for this observation might be the fact that, we did not induce acidosis in experimental animals and thus should not expect a significant decrease in Ca concentration. Although Ca concentration in serum was lower than references range in all experimental animals, especially from 1 before calving but there was not any sign of milk fever in them. Kalaitzakis et al. (2007) observed that, moderate to severe cases of fatty change of the liver had a higher level of activity of ALP compared to normal cows though ALP mostly indicating the cholestasis. Results of the current study suggested that, ALP concentration was in references range, suggesting that the experimental animals did not have cholestasis or moderate to the severe fatty liver. Due to a lower BUN concentration that could be a result of better stimulation of ruminal microbial protein synthesis in treatment groups and also due to lower triglyceride in the control group which might be associate to the lower pH in the rumen, we suggest that endotoxin binder (especially combined feeding of endotoxin binders) can modulate rumen function by optimizing rumen pH. Authors of the current study are not sure that with which mechanism endotoxin binders are able to optimize rumen pH but according to other study (Chiquette et al. 2008; Malekkhani et al. 2016). It might be because of the declined lactate concentrations in the rumen which contributes to prevention of rumen acidosis or by improved ruminal pH as a result of changing in ruminal microbial population. We suggest conducting further research to verify this theory.

Fig. 3 Mean (± SEM) serum concentrations of Ca (mg/L) 4, 3, and 1 weeks before and 1 and 3 weeks after parturition (*n* = 6 six in each group, **p* < 0.05) (Ca references value in cattle 97–124(mg/L)) (Constable et al. 2017)

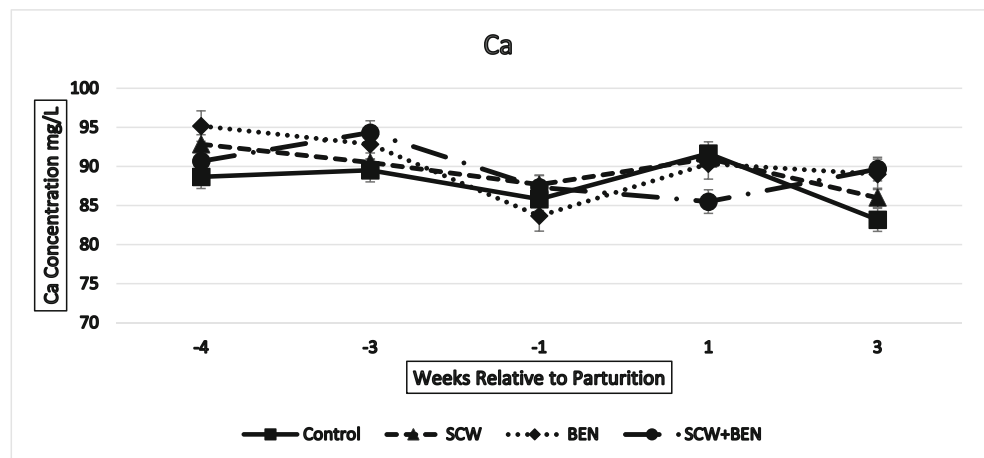
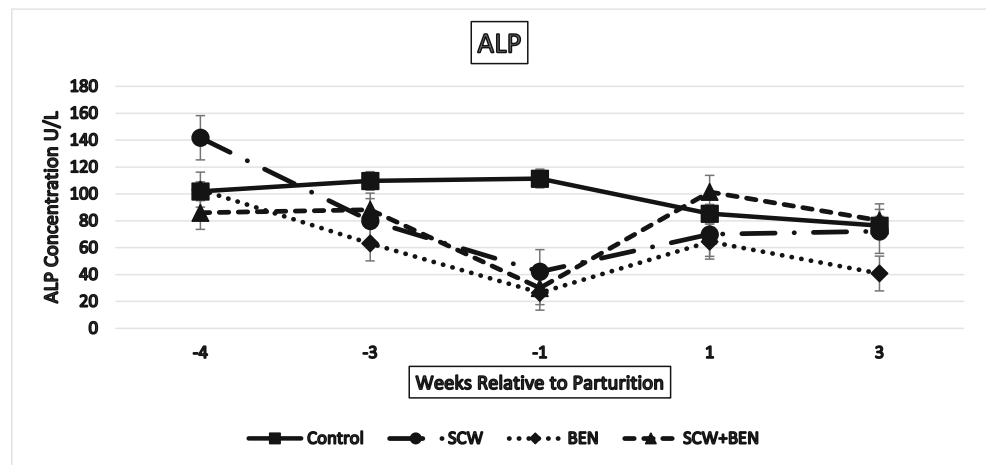


Fig. 4 Mean (\pm SEM) serum concentrations of ALP (U/L) 4, 3, and 1 weeks before and 1 and 3 weeks after parturition ($n = 6$ six in each group, $^*p < 0.05$) (ALP references value in cattle 0–500(units/L)) (Constable et al. 2017)



Conclusion

In conclusion, BUN was lower in treatment groups. It might be due to the better stimulation of ruminal protein synthesis bacteria and better utilizing $\text{NH}_3\text{-N}$ in the rumen. Besides, pH of the rumen was suitable for cellulolytic bacteria which utilize $\text{NH}_3\text{-N}$ especially in SCW + BEN group. Furthermore, considering a lower triglyceride in control group that might have associated with lower pH in the rumen, we think that toxin binders can modulate rumen function by optimizing rumen pH. Nevertheless, further research is required to prove this claim.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Statement of animal right All animals were treated in accordance with the regulations on the guidelines of the Iranian Council of Animal Care (1995), and the experiment was approved by the Institutional Animal Care Committee for Animals Used in Research.

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