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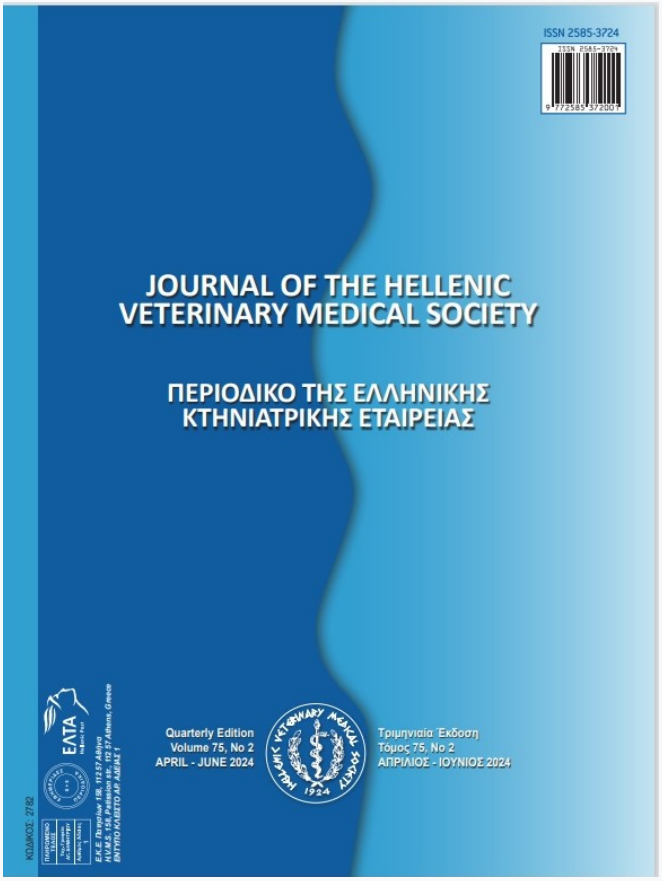

Improvement of serum B12, certain biochemical variables and rumen function indicators after live yeast feeding with or without cobalt in dairy cattle with signs of impaired digestion

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Research article
Ερευνητικό άρθρο

Improvement of serum B12, certain biochemical variables and rumen function indicators after live yeast feeding with or without cobalt in dairy cattle with signs of impaired digestion

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ABSTRACT: The objective of this study was to evaluate under field conditions the effect of live yeast feeding with or without cobalt on rumination time, manure score and serum concentrations of vitamin B12, blood urea nitrogen (BUN), total protein (TP), albumin and fructosamine in dairy cattle with impaired rumen function. The study was conducted in a commercial dairy herd of 94 milking cows and lasted for 75 days. The first 15 days (Day -15 to Day -1) the animals were fed the basal total mixed ration without supplementation of live yeast or Co and served as a control period. The evaluation period lasted for sixty days (Day 0 to Day 60). During days 0 (D0) to 30 (D30), the cows were fed the basal ration supplemented with a commercial live yeast product (4gr/cow/day), and during days 31 to 60 (D60) were fed the same ration supplemented with live yeast and cobalt (cobalt carbonate; 6mg/cow/day). Rumination time was evaluated daily using a commercial monitoring system. Prior to the onset (D0) and at the end of the first (D30) and second month (D60) of the study, manure was scored and blood samples were obtained. Rumination time was higher and manure scores were improved on D60 than D0. Serum B12 concentration was higher on D30 and even higher on D60 compared to D0. BUN was unaffected on D30 but significantly lower on D60 than D0 and D30. Serum albumin was lower on D30 and D60 compared to D0 whereas serum globulins concentration was significantly different between all sampling days and the highest value was recorded on Day 30. Serum fructosamine was significantly higher at the end of the study than D0. Combined in-feed inclusion of live yeast and cobalt improves digestion, serum B12 levels, and energy status of dairy cows with impaired rumen function and provides evidence for possible acute phase reaction by live yeast feeding.

Keywords: Cattle; Cobalt; Live yeast; Rumination time; Vitamin B12

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INTRODUCTION

Health and biological functioning of livestock are often prioritized (Fiore et al., 2018). In recent years, nutritional strategies such as live yeast supplementation, have emerged and it has been proposed as a key factor to improve health status and welfare of animals as well as to enhance productivity in livestock (Monteverde et al., 2017). Ruminants depend on microbial fermentation within the rumen to acquire energy from plant compounds. Rumen function has been strongly manipulated by supplementing forage diets with readily fermentable carbohydrates and additives in order to improve animal productivity. However, this type of feeding sometimes induces rumen dysfunction because of an imbalance in the microbial populations (Fernando et al. 2010). Dietary supplementation of yeast cultures has been reported to increase body weight gain and feed conversion efficiency in ruminants (Armato et al., 2016) by improving the ruminal ecosystem; this improvement lies in their contribution on anaerobiosis and is related to the oxygen scavenging properties of yeast in the rumen, which enhances bacterial viability (Newbold, 2003). This is achieved via the stabilization of rumen pH (Al Ibrahim et al., 2013; AlZahal et al., 2014; Krizova et al., 2011; Marden et al., 2008), the optimization of rumen environment for bacterial growth, by the prevention of lactic acid accumulation (Nocek, 1997) and the enhancement of fiber digestion (AlZahal et al., 2014; Chaucheyras-Durand et al., 2008).

Cobalt (Co) is an essential trace element and is considered to be a crucial cofactor for rumen bacterial growth at optimal rates (Lean et al., 2014). The most significant role of Co is that it constitutes a component of vitamin B12. This vitamin is synthesized by the rumen flora in the presence of Co (McDowell, 2003) and according to some reports, the rumen concentration of vitamin B12 in cattle is directly associated with Co content in the ration (Singh and Chhabra, 1995; Stemme et al., 2008). However, apart from the bacteria that synthesize B12 in the rumen, there are also others that require vitamin B12 in ruminal fluid for their normal metabolic function (Franco-Lopez et al., 2020). It has been recently proven that the overall abundance of ruminal B12 is better associated with the absence of the microorganisms that consume, rather than the presence of those that efficiently produce this vitamin (Franco-Lopez et al., 2020). Obviously, any alteration of the rumen microbiota could affect the ruminal and serum concentration of B12.

The impact of yeast and Co feeding on digestion, productivity, and health parameters of dairy cows has been extensively evaluated in many experimental studies (González-Montaña et al 2020; Burdick Sanchez et al., 2021). Nevertheless, there is a lack of evidence for their effects when they are fed to animals with indications of impaired rumen function and digestion, as defined by non-desirable manure scores, under field conditions. It is expected that when added to the diet of such animals, yeast and Co could possibly improve, through the restoration of balance in the rumen ecosystem, the fecal scores, the rumination time and potentially the energy, and protein digestibility to animals, but this hypothesis has to be justified. It is well documented that live yeast feeding alters significantly the rumen flora population (Bach et al., 2019; Dolezal et al., 2012; Ogunade et al., 2019; Pinloche et al., 2013; Uyeno et al., 2017). However, there is a paucity of data in the available literature regarding the possible impact of this shift of the ruminal population on the serum concentration of B12, especially when live yeast is fed to animals with impaired rumen function. It could be hypothesized that live yeast might enhance the microflora that synthesize B12 and/or reduce those that consume this vitamin, when fed to such animals and thus increase serum B12 concentration. In addition, there are reports suggesting that dietary supplementation of live yeast might also be associated with acute phase response elucidated by the rumen flora shift, but the obtained results are controversial (Emmanuel et al., 2007; Garcia Diaz et al., 2018). Since these observations were made under experimental conditions it would be of value to investigate such a possible effect under field conditions.

In this context, the objective of the present study was to perform an on-field evaluation of live yeast supplementation with or without cobalt on rumination time, manure score and serum concentrations of vitamin B12, blood urea nitrogen (BUN), total protein (TP), albumin (ALB) and fructosamine in dairy cattle with impaired rumen function and digestion.

MATERIALS AND METHODS

Animals and management

The study was conducted in a commercial dairy herd with indications of impaired rumen function and digestion as defined by the presence of loose manure and of undigested feed particles in feces in a considerable number of animals. The herd was consisting of

94 lactating Holstein cows (approximate weight 600-650 kg; median parity 4, median days in milk 183) and had an average daily milk yield of 27.9 (SD: 0.8) kg/day according to the records of the farm for the last 15 days prior to the onset of the study. The animals were also free of internal parasites according to the results of fecal examination that was performed 35 days prior to the onset of the study. All lactating animals were housed as a single group in a free-stall barn with access to an open yard. They were milked twice daily and were fed a total mixed ration (Table 1) which was offered after each milking.

Experimental design

The whole group of the 94 lactating cows enrolled in this prospective cohort study lasted for 75 days. In the first 15 days (Day -15 to Day -1) the animals were fed the basal total mixed ration without supplementation of live yeast or Co (control period; Figure 1). The evaluation period lasted for sixty days (Day 0 to Day 60). (Figure 1). During days 0 to 30, the cows were fed the basal diet supplemented with live yeast product (ProB.I.O.-Sacc® Biochem, GmbH, Germany). The yeast was fed at the rate of 4 g/d, providing approximately 60 billion cfu of live yeast/cow per day. For the next 30 days, the animals were offered the same diet supplemented with live yeast and cobalt. Cobalt was incorporated in the premix of vitamins and trace minerals used, to provide daily 6 mg/cow of cobalt as cobalt carbonate.

The day prior to the onset of the control period (D-15), and on days 0 (D0), 30 (D30) and 60 (D60) the manure of a minimum of 30 animals was scored by the same observer (PDK) for consistency (FC) and for an undigested fraction (UF) using 5-point scales as described previously (Zaaijer and Noordhuizen, 2003) at the milking parlor. On the same days, blood samples were collected from certain 15 animals. Those obtained on D-15 were used only for the monitoring

Table 1. Total mixed ration composition in kg as fed per cow per day

Ingredients	Quantity (kg as fed)
Corn silage	27
Alfalfa hay	2.7
Wheat straw	1.3
Maize grain	3.5
Soybean meal	3.6
Wheat bran	1.3
Beet pulp molasses	1.5
Limestone	0.15
Natural zeolite	0.25
Salt	0.12
Premix of vitamins and trace minerals*	0.03
Analysis	
Crude proteins (g/kg dry matter)	154.8
Net energy for lactation (Mcal/kg dry matter)	1.48
Ca (% dry matter)	0.68
Phosphorus (% dry matter)	0.36

* The premix of vitamins and trace minerals contained per kg: 4500000 IU vitamin A, 1000000 IU vitamin D3, 30 g vitamin E, 50 g niacin, 220 g Ca, 50 g Zn, 20 g Fe, 20 g Mn, 10 g Cu, 1 g I, 0 g Co and 0.2 g Se.

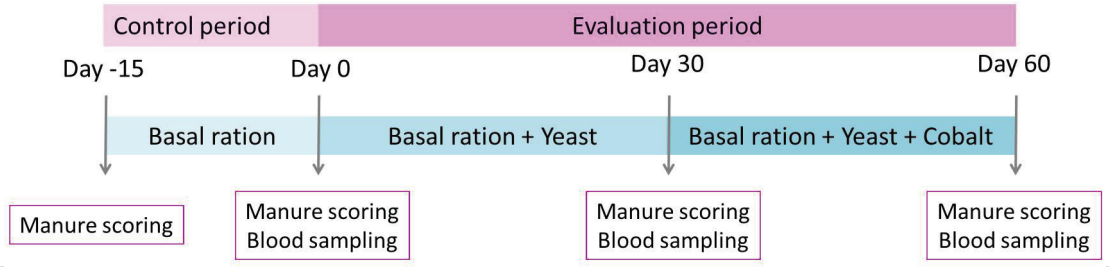

Figure 1: Study design.

of subclinical ketosis, on D0 for monitoring of ketosis and serum parameters determination and those collected at the other sampling points only for the determination of serum parameters. Animal selection was based on the stage of lactation (5 were at early lactation, 5 at middle and 5 at late lactation), the absence of subclinical ketosis (blood β-hydroxybutyrate < 1.2 mmol/l) on days -15 and D0, the milk production so as to represent the average daily milk production of the herd and the absence of health issues from calving to D0 of the study based on the health records of the farm. Blood was sampled by jugular venipuncture from each cow after the morning milking and before feeding into plain vacuum tubes (BD Vacutainer, Mississauga, ON). Blood β-hydroxybutyrate concentration was determined immediately after sampling by using Precision Xceed® (Abbott, Abbott Diabetes Care Ltd., Oxon, UK). After clotting, the serum was separated by low-speed centrifugation (1,000 × g, 4°C, for 10 min), transferred into plastic vials, and forwarded on ice to an ISO-certified commercial veterinary laboratory for analysis. Serum B12 concentrations were determined using an automated chemiluminescence immunoassay (Immulite 1000, Siemens Healthcare Diagnostics, Deerfield, USA). Serum total protein (TP) concentration was measured refractometrically with a temperature compensated refractometer (Reichert TS Meter refractometer, Model 1310400A, Reichert Scientific Instruments Buffalo, NY, USA) as previously described and validated (Katsoulos et al., 2017). The serum concentrations of albumin, blood urea nitrogen, and fructosamine were determined in an automatic biochemical analyzer (Advia® 1800 chemistry analyzer - Siemens Healthineers) using the commercial diagnostic kits, ADVIA® Chemistry Albumin BCP Assay, ADVIA® Urea Nitrogen, ADVIA® Chemistry Fructosamine (FRUC) Assay reagents respectively).

The rumination time was evaluated using the Alflex® SenseHubTM Dairy monitoring system. At the end of the study, the data regarding the average daily rumination time of all lactating cows from day -15 until day 60 were recorded and the comparisons were made at 15 days intervals.

Ethics

Samples were acquired for diagnostic or monitoring purposes under informed farmer consent. Protocol was approved by the Ethics Committee, University of Thessaly. Moreover, all procedures were done according to the ethical standards in the Helsinki Declaration of 1975, as revised in 2000, as well as the national law inducing minimal stress in animals.

Statistical Analysis

The data were analyzed with the statistical software IBM SPSS 25. The normality was evaluated with the Kolmogorov-Smirnov test. With the exception of manure scores, the data for all the other parameters evaluated were normally distributed. Kruskal-Wallis and Mann-Whitney tests were used for the comparison of manure scores obtained at the different timepoints. Univariate ANOVA was run to analyze the data for rumination time whereas, the data obtained for serum biochemical parameters evaluated were analyzed with repeated measures ANOVA. Bonferroni test was used for the adjustment of the confidence interval. In all comparisons, a value of $P \leq 0.05$ indicated a significant difference.

RESULTS

Manure scores for FC and UF were not significantly different ($P>0.05$) between D0 and D30 (Table 2). However, FC was significantly higher ($P<0.05$) and UF was significantly lower ($P<0.05$) on D60 compared to D0 and D30.

Rumination time was not significantly different ($P>0.05$) among the 15-days intervals until D45 of the study (Figure 2). However, the average rumination time between days 45 to 60 of the study was significantly higher than the previous periods ($P<0.05$).

Table 2. Mean±SE manure scores for consistency (FC) and for undigested fraction (UF), milk yield, serum blood urea nitrogen (BUN) and serum fructosamine concentrations on days -15, 0, 30 and 60 of the study.

	Manure score		BUN	Fructosamine
	FC	UF	(mmol/l)	(μmol/l)
Day-15	2.61±0.09*	2.30±0.09*	N/D	N/D
Day 0	2.60±0.11*	2.30±0.10*	5.22±0.22*	213.13±3.05*
Day 30	2.64±0.08*	2.14±0.09*	5.21±0.20*	N/D
Day 60	2.87±0.06†	1.70±0.12†	4.31±0.21†	222.00±4.39†

*, †Different superscripts at the same column denote significant difference ($P<0.05$); N/D: not determined

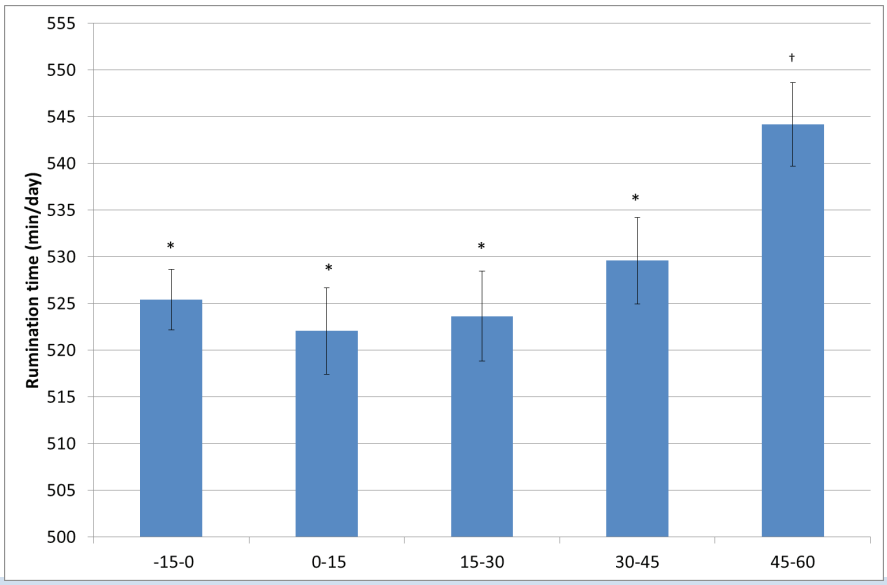

Figure 2: Mean±SE rumination time (min/day) at 15 days intervals before and throughout the study period.

*,†Different symbols denote significant differences (P<0.05)

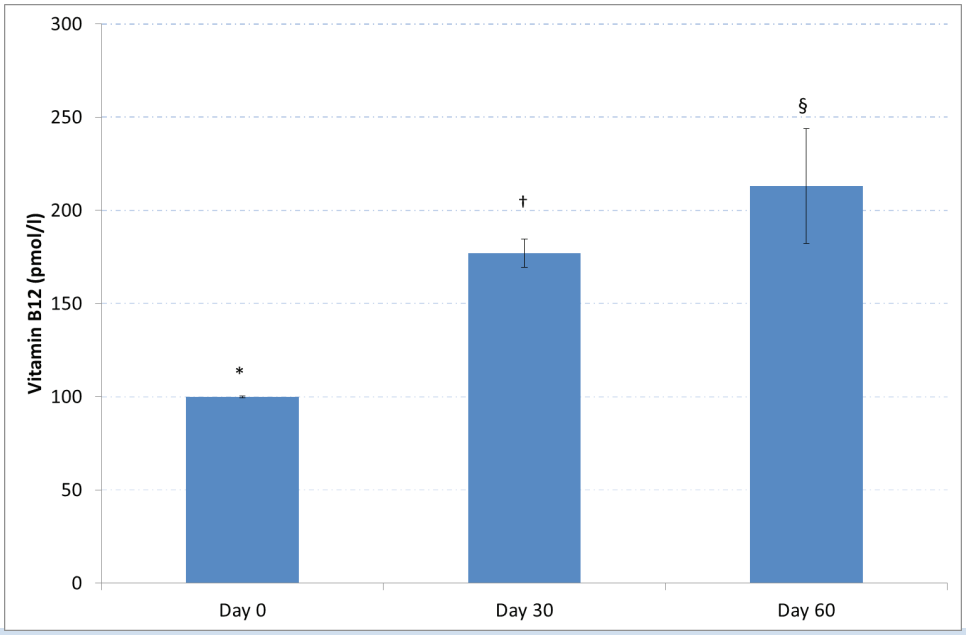

Figure 3: Mean±SE serum vitamin B12 concentration (pmol/l) prior to the onset of the study (Day 0) and on days 30 and 60

*,†,§Different symbols denote significant differences (P<0.05)

On D0 serum vitamin B12 concentration was below the method's lowest detection limit of 100 pmol/l at 14 of the 15 samples. In order to perform the statistical analysis, the value of 100 pmol/l was used for all these samples. As it is shown in Figure 3, on D30 average serum vitamin B12 was significantly higher compared to Day 0 (P<0.05) and on D60 significantly higher than both previous sampling days (P<0.05). The alterations observed on serum TP, ALB and globulins are presented in Figure 4. Average TP concentration was not significantly different between D0 and D30 (P>0.05) but the values recorded on D60 were significantly higher (P<0.05). Average serum ALB was significantly lower on D30 and D60 than Day 0 (P<0.05) whereas no significant difference was detected between days 30 and 60 (P>0.05). Serum globulins concentration was significantly different (P<0.05) between all sampling days; the average values were significantly higher on Day 30 (P<0.05) and even higher on Day 60 compared to Day 0 (P<0.05). The average fructosamine concentration (Table 2) was significantly higher on D60 than D0 (P<0.05) and the average

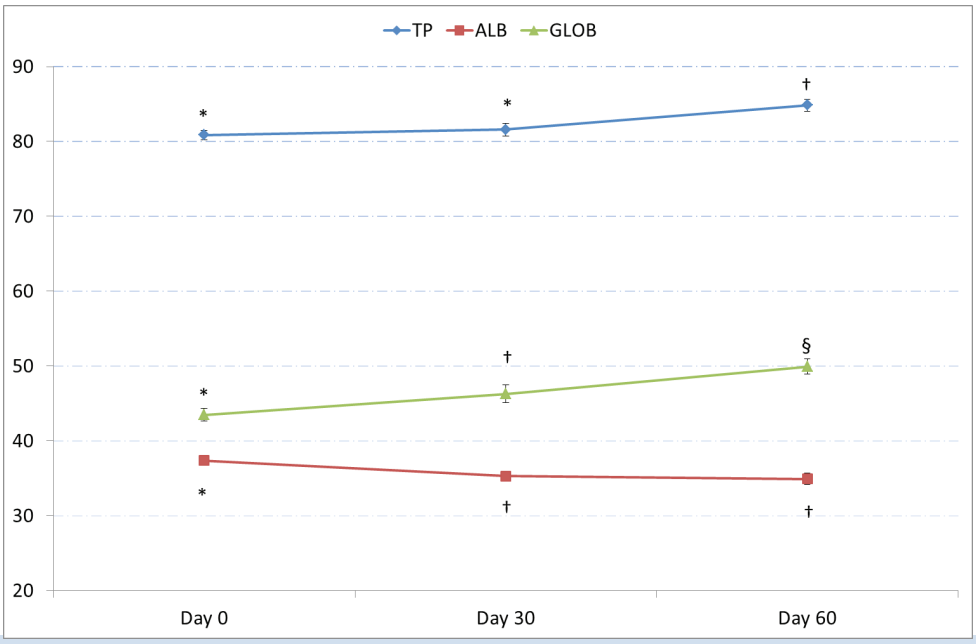

Figure 4: Mean±SE serum total protein (TP), albumin (ALB) and globulin (GLOB) concentrations (g/l) prior to the onset of the study (Day 0) and on days 30 and 60.

*,†,§Different symbols denote significant differences (P<0.05)

BUN concentration (Table 2) was significantly lower on D60 in comparison to D0 and D30 (P<0.05).

DISCUSSION

The impaired rumen function and digestion were justified at the study herd by the inappropriate manure scores for dairy cows (Zaaijer and Noordhuizen, 2003) on D-15 and D0 and the very low vitamin B12 concentration, below the assay's detection limit for the vast majority of the animals, recorded on D0. It was selected not to leave untreated a considerable amount of these animals and use them as control group because it would be against animal welfare since a health issue was identified. Instead, the values obtained prior to the onset of the evaluation period for all the parameters tested were used as a substitute of a control group. This study design is not expected to bias our conclusions given that i) the composition of the rumen microbiome is tremendously impacted by diet, despite it continues to evolve and mature as the dairy cow advances in lactation cycle (Pitta et al., 2016), ii) the total mixed ration remained the same throughout the study with the exception of live yeast and Co supplementation, iii) the fecal scores are only affected by rumen function and digestion and remained constant between D-15 and D0, iv) rumination time depends on the ration and reflects rumen function without being affected by the stage of lactation, v) serum vitamin B12 concentration only depends on the Co supply and the rumen flora population, vi) none of the selected animals for blood sampling was on significant negative energy balance prior to the onset of the evaluation period, based on the absence of subclinical ketosis and vii) the duration of evaluation period is relatively small to significantly affect the concentration of the other serum parameters evaluated. Another limitation is that at the second phase of the evaluation period, it was not clear whether the recorded effects were due to the Co supplementation alone or to the interaction between Co and live yeast. To minimize the latter, it was selected to incorporate live yeast alone during the first month of the study, a time period that is regarded sufficient in order to estimate the effects of yeast.

The manure scores were not significantly affected by the live yeast supplementation; they were significantly improved, with scores close to ideal for FC and at acceptable levels for UF (Zaaijer and Noordhuizen, 2003), after the combined feeding of live yeast and Co. It is uncertain whether the period of 30 days was enough in order to observe the beneficial dietary effects of live yeast in the manure of dairy cows that had impaired rumen function and digestion. However, in the context of this study, the synergistic action between yeast and Co on improving rumen function and digestion appears to be needed for the improvement of manure scores. Live yeast optimizes rumen metabolism by stabilizing rumen pH, increasing fiber

digestibility, reducing the rate of lactic acid production, and releasing vitamins and growth factors that stimulate bacterial growth (Armato et al, 2016; Singh and Chhabra, 1995; Garcia Diaz et al, 2018). On the other hand, Co is an important cofactor for the optimal growth of rumen bacteria (Lean et al., 2014) and increases the digestion of the fibers in the rumen (Lopez-Guisa and Satter, 1992; Waterman et al., 2017) by acting as a divalent cation, increasing the affinity between the fiber particles and microbes (Lopez-Guisa and Satter, 1992). So, it can be supported that the live yeast improved the rumen environment and that Co addition further enhanced the yeast effects on rumen function and digestion something that is reflected on the optimized manure scores at the end of the study period.

The synergistic action of live yeast and Co is also denoted by the obtained results regarding rumination. In line with our findings, it has been observed in former studies that feeding live yeast alone for 3-4 weeks caused a non-significant increase in rumination time (Ambriz-Vilchis et al., 2017; DeVries and Chevaux, 2014). In the present study, rumination time started to increase only after the simultaneous incorporation of Co in the ration so as to be significantly higher at the last two weeks of the study. Considering that rumination activity is decreased at lower rumen pH values (DeVries et al., 2009), it could be assumed that Co supplementation enhanced the stabilizing role of live yeast on ruminal pH resulting, probably, in higher pH values and, consequently, higher rumination time.

The inclusion of the live yeast in the cows' ration resulted in a significant increase of serum vitamin B12 concentration, even before the addition of Co. This finding suggests that the ration already provided an adequate amount of Co to support vitamin B12 production but the rumen flora either was not able to produce significant levels of B12 or this vitamin was consumed by bacteria in the rumen. It is well documented that the plethora of B12 vitamin in the rumen is controlled by the composition of the microbiota, since it has been observed that different populations of bacteria are present in larger amounts in cows with high and low ruminal vitamin B12 concentrations (Franco-Lopez et al., 2020). The exact mechanism by which live yeast enhanced serum B12 concentration is currently unknown; it seems that live yeast shifted the groups of microorganisms in a way that favors the production and/or reduces the consumption of vitamin B12 in the rumen. Since it has been proven that the overall abundance of ruminal B12 is better associated with the absence of microorganisms that consume rather than the presence of those that efficiently produce this vitamin (Franco-Lopez et al., 2020), the second explanation seems to be the most possible. In support of this point of view, it has been observed that the in-feed inclusion of live yeast significantly decreases the ruminal population of *Bacteroidetes phylum* (Pinloche et al., 2013; Uyeno et al., 2017). This phylum is excellent at acquiring vitamin B12 from the environment and some of its members can readily bind vitamin B12, rendering it unavailable for intestinal absorption (Wexler et al., 2018). Thus, by reducing the numbers of these bacteria, live yeast could enhance ruminal vitamin B12 levels and consequently increase the serum B12 concentration.

Having ensured a favorable ruminal environment for B12 after live yeast supplementation, the further increase of serum B12 concentration observed at the present study after the addition of Co in the ration was rather expected. It has been proven in many studies that the increase of dietary Co supply results in an increase of the ruminal and duodenal concentration of B12 in cattle and sheep (Ambriz-Vilchis et al., 2017; Chaucheyras-Durand et al., 2008; Lean et al., 2014) and that, until a certain level of Co supply, the association between them is linear (Singh and Chhabra, 1995).

The supplementation of live yeast resulted in a significant but not clinically important reduction of serum albumin concentration. It was a rather unexpected finding since the protein content of the ration was constant and evidence in the literature suggests that serum albumin either remains unaffected (Ayad et al., 2013; Yalçın et al., 2011) or even increases (Aung et al., 2019) with the addition of yeast. However, similarly to the results obtained here, it has been noticed that yeast-fed dairy cattle (Bakr et al., 2015) and lambs (Raghebian et al., 2016) had non significantly lower serum albumin concentration compared to the controls. Taking into account that serum albumin is considered as a negative acute-phase protein for ruminants (Tóthová et al., 2016), an acute phase response caused by yeast feeding cannot be excluded. In support of this hypothesis, it has been proven that feeding feedlot steers with *Enterococcus faecium* and *Saccharomyces cerevisiae* resulted in a significant increase of plasma concentrations of acute phase proteins evaluated but not when *Enterococcus faecium* was fed alone without yeast (Emmanuel et al., 2007).

They attributed this effect to the lysis of coliform bacteria when yeast was added, possibly via the production of bacteriocins that can kill certain gram-negative bacteria (Nes and Holo, 2000), and the release of endotoxins that stimulate the production of cytokines. The results obtained here further provide indications for an inflammatory response due to yeast supplementation since the serum globulin concentration was significantly higher at the end of the first month and at the end of the study period compared to that before yeast feeding.

Serum total protein concentration was unaffected by live yeast feeding mainly because of the simultaneous increase of serum albumins and decrease of serum globulins. Similar to this finding, serum total protein concentration was shown to be unaffected by yeast feeding in dairy cattle (Iwanska et al., 1999; Yalçın et al., 2011) and rams (Galip, 2006) but significantly reduced when fed to heifers (Kowalik et al., 2016). The significant increase of serum total proteins at the end of the study period was due to the even higher serum globulins, which is in accordance with former observations (Bakr et al., 2015). In other studies

It was selected to determine fructosamine as an indicator of energy supply rather than glucose in order to avoid the variability associated with the diurnal fluctuation of glucose (Jensen et al., 1993). Moreover, fructosamine provides a more retrospective record of blood glucose (Pattullo and Kidney, 2014). Considering that fructosamine reflects the glucose levels of cattle over the last 2-3 weeks (Pattullo and Kidney, 2014) it was decided to measure fructosamine only on D60 and not on D30. It is believed that the detected increase in serum fructosamine concentration at the end of the study period is associated, apart from the possible increase of volatile fatty acid concentrations in the rumen, to the increase of vitamin B12 availability. This vitamin is essential for propionate metabolism, which is a major precursor of glucose in ruminants. It is well documented that the ability of animals to utilize propionate is impaired when vitamin B12 deficiency occurs (Elliot, 1980) due to its action as a cofactor for the enzyme methylmalonyl-CoA mutase, which catalyzes the conversion of succinyl CoA to methylmalonyl-CoA during the formation of glucose (Nagaraja et al., 1997).

Live yeast feeding had no significant effect on BUN concentration, which is in accordance with former reports (Ayad et al., 2013; Nagaraja et al., 1997; Nes and Holo, 2000). However, the combined administration of live yeast and Co resulted in a significant decrease in BUN. Since BUN in ruminants is related to rumen ammonia content (Staples et al., 1992) and the protein content of the ration remained constant, this finding suggests an improved activity of rumen flora stimulated by live yeast and Co that enhanced the incorporation of ammonia into microbial protein at the end of the study period (McAllister et al., 2011).

CONCLUSIONS

In the context of this study, the obtained results show that a combination of live yeast and cobalt feeding is necessary to improve the fecal scores and rumination time in cattle with impaired rumen function and digestion. However, the supplementation of live yeast alone is capable to increase serum vitamin B12 concentration suggesting that the alterations caused in the ruminal environment enhance the availability of this vitamin. In addition, the observations regarding serum albumins and globulins provide evidence for possible acute phase response, triggered by live yeast feeding but further investigation is required to confirm this hypothesis.

CONFLICTS OF INTEREST:

The authors declare no conflict of interest.

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