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Effects of dietary probiotic supplementation on growth, rumen development and selected blood metabolites of growing calves

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ABSTRACT

Objective: The present study was carried out to evaluate the effects of probiotic in diets of growing calves on performance, blood metabolites and rumen metabolism parameters.
Design: Descriptive study.
Animals: Twelve Holstein calves (~151 kg) were divided randomly into three groups of four animals and were reared in clean well-ventilated boxes for 3 months.
Procedure: In the treatment groups, probiotic (ANKOR ONE)® contained Saccharomyces cerevisiae, Aspergillus oryzae and Kluyveromyces marxianus was supplemented with concentrate mixture at the rate of 0.5 g/kg and 1 g/kg feed. All the calves were offered diet contained roughage and concentrate separately.
Results: The average daily gain of the calves in the control group had significantly lower value (54 kg) as compared to those in the supplemented groups. Furthermore, calves fed unsupplemented diet had significantly the highest fecal score (3 = runny) compared to those fed supplemented diet with probiotic. Supplementation of probiotics in diets of calves did not show any significant differences in the blood glucose, total protein and albumin contents in comparison to those fed unsupplemented diet. Addition of probiotics led to significant higher concentrations of β-hydroxybutyrate and total volatile fatty acids of ruminal fluid in comparison to unsupplemented group. No significant effects for ammonia-N content of ruminal fluid were noted by feeding probiotic-supplemented diet compared to unsupplemented group.
Conclusion and clinical relevance: Probiotic supplementation in diets of calves generally improved average daily gain, reduced the incidence of diarrhea and did not adversely affect the levels of blood metabolites indices.
Keywords: Calves, probiotic, growth, blood metabolites, rumen parameters

1. INTRODUCTION

Achieving high production and growth performance of growing calves is necessary to increase their economic benefits. There are many researches focusing on using feed additives (antibiotics, probiotics and prebiotics) to modify/change the gastrointestinal tract microbial population to enhance animal production and health [1]. However, years ago the use of antibiotics in ruminant feeds has been inhibited due to avoid the occurrence of cross-resistance with pathogens in human. Thus, it is essential to find suitable feed additives to sustain the ruminant productivity and health as probiotics "natural" products [2].
Probiotics are defined as live microorganisms that improve the intestinal microbial balance of the host animal [3]. Probiotics enhance beneficial ruminal microorganisms and stabilize the rumen pH and hence increase the nutrient digestibility [4]. Moreover, addition of probiotics to the diets of ruminants showed enhancement of the immunity and reduction of pathogens in the intestine [5].

Yeast contains some growth factors, as vitamins which help rumen pH stabilization and prevention of acidosis by stimulation lactate utilizing bacteria. Saccharomyces cerevisiae (S. cerevisiae) is considered the most common yeast culture used in ruminant diet [6]. Nevertheless, the positive results observed with yeast of S. cerevisiae varied and differed according to management and diet composition [7].

The cellulolytic rumen microbes establishment is faster in calves and lambs receiving S. cerevisiae on daily basis [8]. Dijkstra et al. [9] observed that yeasts reduced daily fluctuations in pH values and hence a higher stability of rumen environment during the day. Dry matter intake and growth of Holstein Friesian calves were increased significantly by addition of S. cerevisiae in the calf starter [10].

Sullivan and Martin [11] reported that the dietary supplement of a S. cerevisiae improved the utilization of lactate and digestion of cellulose. It could be that S. cerevisiae compete with other starch utilizing bacteria for starch fermentation leading to the prevention of ruminal lactate accumulation [12]. In contrast, other researchers showed that the yeast culture had no effect on counts of cellulolytic bacteria and ruminal digestion [13].

Another probiotic which widely used is Aspergillus oryzae (main source of fungi) being used as a feed additive to stimulate fiber digestion and milk yield [14]. Improving fiber or starch digestion in the rumen could be enhanced by using Aspergillus oryzae and Aspergillus niger [15]. Kluyveromyces marxianus (formerly Kluyveromyces fragilis) is a lactic yeast isolated from different dairy products, mainly kefir [16].

The actions of *K. marxianus* on nutrient utilization in ruminants are not well investigated; however, Nooraee et al. [17] found that in vitro apparent dry matter digestibility of alfalfa, guinea grass and timothy hay improved significantly with addition of the *K. marxianus*. In contrast, Tripathi et al. [18] observed that addition of *K. marxianus* in diets of lambs did not show any positive effects on the performance.
Therefore, this study aimed to determine the effects of probiotics supplementation in diets of growing calves on growth performance, feces quality, some blood metabolites and rumen parameters.

2. MATERIALS AND METHODS

2.1. Animal housing and experimental design

Twelve Holstein calves (~151 kg ± 1.16) were divided randomly into three groups of four calves according to their body weights and reared for 3 months (duration of the experiment) at Abdel Monaem Abdel Aziz Farm, Tanta Province, Egypt. The calves were reared individually in clean well-ventilated boxes. A clean, fresh drinking water was provided *ad libitum* for all calves individually. Before the start and each four weeks throughout the experimental period, the calves were weighed.

2.2. Feeding regime

All the calves in control and experimental groups were offered diet contained roughage and concentrate separately to meet the requirements [19]. The calves control group fed diets without any supplementation of probiotic (0 Pro). In the treatment groups, probiotic was supplemented with concentrate mixture at the rate of 0.5 g/kg feed (recommended levels) as well as at higher level of 1 g/kg feed (0.5 Pro and 1 Pro, respectively). The composition of the concentrate mixture fed to calves and its calculated composition are shown in Table (1). Calves were fed concentrates twice daily (2 kg/calve/d) and chopped hay was offered *ad libitum*. On the next day morning, any residues were weighed.

2.3 Source of yeast

The thermo tolerant probiotic (ANKOR ONE, AKRON GLOBAL, Turkey) contained *Saccharomyces cerevisiae* (NCYC Sc47, CFU 4x1011), *Aspergillus oryzae* ak7001 (DSM 1862) and selected cultures of *Kluyveromyces marxianus*. The chemical analysis of ANKOR ONE is: 14% moisture, 17% protein, 25% ash, 4% fiber, 3% fat, 1.7% calcium, 3% magnesium, 0.5% sodium.

2.4. Sample collection of feces

The fecal consistency was scored bi-weekly before feeding. A scoring system was used from one to four (1= firm, well-formed feces; 2= soft pudding like feces; 3= runny pancake batter (beginning of diarrhea); 4= watery liquid like substance feces that can be described as severe diarrhea as described by Larson et al. [20].

2.5. Rumen metabolism profile

Every four weeks the rumen fluid was collected via stomach tube for analysis of ammonia nitrogen and volatile fatty acids (VFA) throughout the experimental period at four hours post-feeding. Rumen fluid was analyzed for pH immediately and for further analysis, rumen fluid was stored frozen (-20°C). According to Coverdale et al. [21], for analysis of VFA the samples were thawed then centrifuged for 10 min at 3500×g. Samples were then filtrated through two layers of cheesecloth and were treated with 25% meta-phosphoric acid at a ratio of 5:1 (rumen fluid to acid). Then the tubes were mixed and allowed to stand for 30 min and the fluid was then centrifuged for 10 h at 3500×g. The clear supernatant was removed and frozen at -20°C. Total VFA components were separated and quantified by gas chromatography (model GC-2014, Shimazu, Kyoto, Japan) Ammonia-N levels in ruminal fluid were determined by the steam distillation method using an automatic-N analyzer (Kjeltec auto sampler system 1035 Analyzer, Tecator, Sweden) as described by Qadis et al. [5].

Table 1. Composition of the concentrate mixture fed to growing calves and its calculated composition

Ingredient	Unit	Value
Cotton seed cake	%	50
Rice bran	%	30
Wheat bran	%	15
Limestone	%	3
Salt	%	2
Calculated composition		
Dry matter	g/kg	858
Metabolizable energy	Mcal/kg	2.7
Crude protein	g/kg	297
Crude fat	g/kg	61.6
Neutral detergent fiber	g/kg	142
Acid detergent fiber	g/kg	162
Lignin	g/kg	56.3
Crude ash	g/kg	74.2
Calcium	g/kg	12.2
Phosphorus	g/kg	12.9
Magnesium	g/kg	6.3
Sodium	g/kg	8.1
Potassium	g/kg	14.9

2.6. Blood samples and analytical procedures

Blood samples were collected (four calves/group) every four weeks via jugular vein puncture. Blood was collected in 10 mL tubes containing potassium oxalate and sodium fluoride for glucose and total protein analysis and sodium heparin for plasma urea nitrogen (PUN) and β-hydroxybutyric acid (BHBA) analyses. The blood was centrifuged for 15 min at 3000 rpm, then plasma was collected and stored in deep freeze at -20ºC until following analyses glucose; total protein and albumin; BHBA and PUN according to Quigley and Bernard [22]; Dumas and Biggs [23]; Quigley et al. [24]; Hayashi et al. [25], respectively.

2.7. Statistical analysis

One-way ANOVA was used to test the effects of using probiotics supplementation in diets of growing calves on growth performance, feces quality, some blood metabolites and rumen parameters. Data were analyzed using statistical SPSS v20 (SPSS Inc., Chicago, IL, USA). Differences between dietary groups' means were compared using Duncan's multiple range test. Differences were considered significant if P-value for the effect was < 0.05.

3. RESULTS AND DISCUSSION

Optimal calf health either pre-or post-weaning is essential for production. It is well known that growth performance and health of the calves are affected mainly by GIT function as well as immune function, which could be altered at periods of infection and/or stress [26]. Therefore, prebiotic supplementation was given to determine its effects on performance and health of the post-weaned calves.

3.1. Effects of probiotic on body weight

Average daily gain of calves affected by probiotic supplementation is shown in Table (2). At beginning of the experiment, body weight (BW) of calves was averaged 151 kg. The calves fed unsupplemented diet (control group) have lower significantly final BW than those fed supplemented diets (Table 2). Average daily gain of all supplemented groups showed a significant difference compared to unsupplemented ones. The average daily gain of the calves in the control group (0 Pro) had the lowest value (54 kg) as compared to those in the supplemented groups 0.5 Pro (58.7 kg) and 1 Pro (64.8 kg).

According to Miles [27] the desirable effects of probiotics supplementation to diets of ruminants are mainly due to improvement in nutrient absorption and reduction in the counts of pathogens.

Table 2. BW development of calves raised on experimental diets (Mean±SE)

Dietary treatment			
Period (week)	Control	0.5 Pro	1 Pro
0	151±1.11	151.3±1.28	151.2±0.78
4	172.5^{c}±3.23	181.5^{b}±2.64	185.7^{a}±2.50
8	189.4^{c}±4.38	193.7^{b}±3.27	197.3^{a}±2.35
12	205^{c}±3.62	210^{b}±3.30	216^{a}±3.07
Wight gain (kg) in 90 d	54.0^{c}±2.51	58.7^{b}±2.02	64.8^{a}±2.29
Wight gain (g/d)	600	652	720
Feed:gain	7.15	6.99	6.93
Dry matter intake (kg/d)			
Concentrate	2.21±0.11	2.32±0.23	2.55±0.21
Straw	2.08±0.14	2.24±0.20	2.44±0.18
Total	4.29	4.56	4.99

^{a,b}Means in the same row with different superscripts are significantly different (p<0.05)

Furthermore, the digestibility of the nutrients and improved rumen microbial growth have been found upon *Aspergillus oryzae* supplementation via cellulases and proteases secretion [28]. These current results are in agreement to the results of Hossaini et al. [29] who stated that using probiotic and antibiotic as feed additives led to higher BW ($p < 0.05$) than the control group. In addition, Abdala et al. [30] found that in groups fed diets supplied with probiotics resulted in a significant higher growth rate in comparison to unsupplemented group.

In contrast, Gorgulu et al. [31] reported that supplied groups with probiotics showed no significant difference in average daily gain compared to unsupplied groups. Similarly, in another study of Vishal and Baghel [32] the addition of *Lactobacillus acidiphilus* to diets of developed rumen calves the utilization of crude fiber is not improved. Nevertheless, many factors should be considered regarding the differences in the results of previous studies and our results as the composition of animal diets, growth stage, environment, supplementation level, etc.

3.2. Effect of probiotic on diarrhea

Influence of probiotic supplementation on mean fecal score according to Larson et al. [20] in calves is shown in Figure (1). Briefly, the fecal scoring was done as follows: 1 = normal, 2 = soft, 3 = runny and 4 = watery. Generally, in this study if the fecal score is =3, it indicated diarrhea. There was significant difference ($p < 0.05$) in the fecal score between the treatment groups and the control. The highest fecal score (3 = runny) was recorded in the control (0 Pro) and the lowest score (2 = soft) was noted for group (0.5 Pro). Suggesting that calves in the supplemented groups had constant fecal score and never exceeded the normal value (Figure 1). This could be explained due to improvement of intestinal bacterial flora in calves supplemented with probiotic. Timmerman et al. [33] stated that addition of probiotics to milk replacer fed by calves reduced the incidence of diarrhea by stabilizing their intestinal flora. Probiotics can reduce diarrhea in neonatal calves after weaning and as well as morbidity cases [34]. Furthermore, Gorgulu et al. [31] also reported that calves supplemented with probiotics were superior with respect to diarrhea than the control groups. Their findings are in agreement with this present study. In contrast, no marked effects were noted with probiotic supplementation to milk replacer regarding incidence of diarrhea [35].

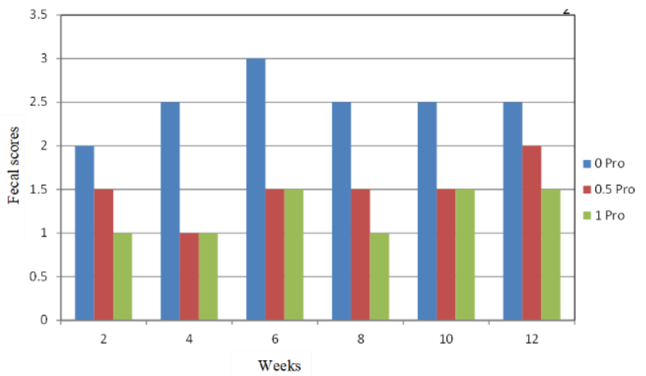

Figure 1: Mean values of fecal scores for calves fed diets with with or without probiotics; Fecal score scale: 1 = normal, 2 = soft, 3 = runny and 4 = watery

3.3. Blood metabolites

Effect of probiotic supplementation on some blood metabolites in calves is shown in Table (3). Supplementation of probiotics in diets of calves did not show any significant differences in the blood glucose, total protein and albumin in comparison to those fed unsupplemented diet. It was observed from the above table that 1 Pro and 0.5 Pro recorded the lowest values of blood glucose (74.2 mg/dL) and (70.7 mg/dL), respectively whilst the highest value was recorded in 0 Pro (78.4 mg/dL) but the difference observed was not statistically significant ($P > 0.05$).

Table 3. Effects of probiotic supplementation on some blood levels in calves (Mean±SE)

Parameters	Dietary treatment		
	Control	0.5 Pro	1 Pro
Glucose, mg/dL	78.4^{a}±1.34	74.2^{a}±1.14	70.7^{a}±1.14
Total protein, g/dL	7.51^{a}±0.21	7.57^{a}±0.11	7.64^{a}±0.04
Albumin, g/dL	3.64^{a}±0.06	3.71^{a}±0.23	3.74^{a}±0.07
PUN, mmol/L	3.13^{a}±0.05	2.89^{a}±0.18	2.73^{a}±0.17
Β-Hydroxybutyrate, mmol/L	0.318^{b}±0.05	0.422^{a}±0.04	0.486^{a}±0.08

^{a,b}Means in the same row with different superscripts are significantly different (p<0.05)

According to Radostits [36] the values obtained in this study were within the normal references for cow 59-105 mg/dL which indicates a good health condition. The physiological state of the ruminant animals could be indicated by the glucose level in the blood. It is well stated that the main functions and/roles of proteins inside animal body are building all cells/tissues as well as for growth performance and production of animals [37]. Thus, it could be concluded that low protein level in the body resulted in decrease production, immunity and the animal is become to be more susceptible to pathogens [37]. Two classes of blood proteins were enumerated: albumin and globulin. Albumin keeps fluid from leaking out of blood vessels while; globulin proteins stimulate the immune system [37]. The results in this study are in consistent with the findings of Adams et al. [38] who found no significant differences in blood protein levels between calves fed diets supplemented with probiotics and control animals. However, calves fed diets supplied with probiotic had lower levels of urea in the blood, which could be due to better utilization of nitrogen in the rumen.

Addition of probiotics led to significant higher concentration of β-hydroxybutyrate in comparison to control group. The ketone β-hydroxybutyric acid produced in the rumen epithelial cells from the oxidation of the butyrate, thereafter, absorbed into the blood vessels [39]. Additionally, the metabolic development of the rumen might be indicated by the capacity of rumen epithelial cells to produce β-hydroxybutyric acid (as a predictor) from butyrate as undeveloped rumen at birth is unable to oxidize butyrate [39].

3.4. Rumen parameters

The effects of different levels of probiotic on some biochemical parameters of ruminal fermentation are presented in Table (4). The pH values are important parameters reflecting ruminal environment. According to Chiquette [40], probiotics in ruminants are of special interest whereas there is microbial imbalance. It is well known that concentrates are rapidly fermented in the rumen with a rapid accumulation of VFAs. Prolonged period with low rumen pH for can lead to acidosis and hence, affect negatively on feed intake and microbial metabolism and decreased activity of cellulolytic bacteria [8].

Interestingly, it was observed that with using yeasts in diets of ruminant animals led to regulate the ruminal pH and limit risks of acidosis by its role in interactions with lactate producing bacteria [41]. *S. cerevisiae* are able to utilize soluble sugars more efficiently than lactate producing bacteria and can provide nutrients, which may be used by the lactate-utilizing bacteria [42]. In the current study, all pH values of rumen fluid were within the normal range and were not affected by probiotic addition. In this study, differences in the pH values of rumen fluid sampled from experimental calves were statistically insignificant. These results are in consistent with results of previous studies [11].

The levels of VFA in the rumen affected by addition of probiotic is presented in Table 4. Increased ruminal VFA concentrations are often assumed a result of microbial fermentation of carbohydrates. According to Kristensen [43] the VFA fermented in the rumen are considered as an energy source and as a factor in increasing the surface, length and width of rumen papillae [44]. Physiological reference range of total VFA of rumen fluid were between 80 and 120 mmol/L [45]. Our data regarding increasing total VFA of rumen fluid by supplementing probiotic are in agreement with Frumholtz et al. [46]. However, Caton et al. [47] found that total VFA were not affected by probiotic supplementation to the diets.

The content of ammonia in the rumen depends mostly upon the dietary protein breakdown and the uptake of ammonia by bacteria [48]. The rumen microflora starts to convert a part of ammonia-N into microbial proteins, which represent an essential source of nitrogen for the ruminant animal, and another part is recycled in form of urea by the animal [48]. In this study, no significant effects for ammonia-N were noted by feeding probiotic-supplemented diet compared to control group.

Similarly, Jouany et al. [49], who reported that ammonia-N in vitro, did not affected by adding probiotic. Also, Monnerat et al. [50] found that addition of S. cerevisiae did not influence the rumen ammonia-N concentration of cattle fed high concentrate diet with two different levels of starch.

Table 4. Some rumen parameters affected by probiotic supplementation in diets of calves (Mean±SE)

Parameters	Dietary treatment		
	Control	0.5 Pro	1 Pro
pH	6.68^{a}±0.34	6.63^{a}±0.12	6.57^{a}±0.24
NH_3-N, mg/dL	15.2^{a}±1.14	14.7^{a}±1.06	13.5^{a}±0.83
Total VFA, mmol/L	87.6^{b}±1.55	93.4^{ab}±1.71	104.2^{a}±1.26

^{a,b}Means in the same row with different superscripts are significantly different (p<0.05)

Moreover, Al Ibrahim et al. [51] stated that addition of *S. cerevisiae* to the diet of Holstein cows decreased the concentration of ammonia-N in the rumen. It is well known that low dietary roughage and/or also higher easily degradable protein in rumen is often associated with a higher content of ammonia in rumen fluid. The obtained results corroborate earlier observations that revealed that added yeast product had no significant effects on the ammonia content in rumen [52]. However, Ghasemi et al.

[53] reported that rumen ammonia-N concentration did not affect by feeding bulls of yeast.

Conclusion

Probiotic supplementation in diets of calves generally improved average daily gain, reduced the incidence of diarrhea and did not adversely affect the levels of blood metabolites indices compared with the unsupplemented groups.

Conflict of interest

The authors declare that they have no conflict of interest.

Research Ethics Committee Permission

All methods used in the study were performed in accordance with the ethical guidelines and recommendations of the Research Ethics Committee, Faculty of Veterinary Medicine, Mansoura University.

Authors´ contribution

Tarek Abbas performed the experiment and drafted the manuscript, Abd EL-Hady Orma designed the model and revised the manuscript, Tarek Ibrahim designed the model and revised the manuscript, Amr Abd El-Wahab supervised the work, analyzed the data and revised the manuscript

4. REFERENCES

[1] Allen MS, Bradford BJ, Harvatine KJ. The cow as a model to study food intake regulation. Annu Rev Nutr 2005; 25: 523-547. DOI: 10.1146/annurev.nutr.25.050304.092704. https://doi.org/10.1146/annurev.nutr.25.050304.092704

[2] Arowolo MA. Jianhua He. Use of probiotics and botanical extracts to improve ruminant production in the tropics: A review. Animal Nutrition 2018; 4 (3): 241-249. https://doi.org/10.1016/j.aninu.2018.04.010.

[3] Newbold CJ. Probiotics for animals. Ann. Zootech 1996; 45:329-335. https://doi.org/10.1051/animres:19960664

[4] Qiao GH, Shan AS, Ma N, Ma QQ, Sun ZW. Supplemental Bacillus culture on rumen fermentation and milk yield in Chinese Holstein cow. Journal of Animal Physiology and Animal Nutrition 2009; 94: 429-36. https://doi.org/10.1111/j.1439-0396.2009.00926.x.

[5] Qadis AQ, Goya S, Ikuta K, Yatsu M, Kimura A, Nakanishi S, Sato S. Effect of a bacteria-based probiotic on ruminal pH, volatile fatty acids and bacterial flora of Holstein calves. Journal of Veterinary Medical Science 2014; 76: 877-885. https://doi.org/10.1292/jvms.14-0028

[6] Rossi F, Luccia AD, Vincenti D, Cocconcelli PS. Effects of peptidic fractions from Saccharomyces cerevisiae culture on growth and metabolism of the ruminal bacteria Megasphaera elsdenii. Anim Res 2004; 53:177-186. https://doi.org/10.1051/animres:2004009

[7]Bitencourt LL, Silva JRM, Oliveira BML, Dias Júnior GS, Lopes F., Siécola Júnior S, et al. Diet digestibility and performance of dairy cows supplemented with live yeast. Sci. Agric 2011; 68: 301-307. http://dx.doi.org/10.1590/S0103-90162011000300005

[8]Chaucheyras-Durand F, Chevaux E, Martin C. Forano E. Use of Yeast Probiotics in Ruminants: Effects and Mechanisms of Action on Rumen pH, Fiber Degradation, and Microbiota According to the Diet In: Probiotic in animals. Chapter 7. 2012. http://dx.doi.org/10.5772/50192.

[9]Dijkstra J, Ellis JL, Kebreab E, Strathe AB, López S, France J. Bannink A. Ruminal pH regulation and nutritional consequences of low pH. Anim Feed Sci Technol 2012; 172: 22-33. https://doi.org/10.1016/j.anifeedsci.2011.12.005

[10] Lesmeister KE, Tozer PR, Heinrichs AJ. Development and analysis of a rumen tissue sampling procedure. J. Dairy Sci 2004; 87:1336-1344. https://doi.org/10.3168/jds.S0022-0302(04)73283-X

[11]Sullivan HM, Martin SA. Effects of Saccharomyces cerevisiae culture on in vitro mixed ruminal microorganism fermentation. J Dairy Sci 1999; 82: 2011-2016. https://doi.org/10.3168/jds.S0022-0302(99)75438-X.

[12]Strohlein H. Back to nature. Live yeasts in feed for dairy cows. DMZ, Lebensm. Ind. Milchwirtsch 2003; 124: 68-71.

[13] Putnam DE, Schwab CG, Socha MT, Whitehouse NL, Kierstead NA, Garthwaite BD. Effect of yeast culture in the diets of early lactation dairy cows on ruminal fermentation and passage of nitrogen fractions and amino acids to the small intestine. Journal of Dairy Science 1997; 80: 374-384. https://doi.org/10.3168/jds.S0022-0302(97)75947-2

[14] Harris BJr, Van Hom HH, Manookian KE, Marshal SP, Taylor MJ, Wilcox C.J. Sugarcane silage, sodium hydroxide and steam pressure-treated sugarcane bagasse, com silage, cottonseed hulls, sodium bicarbonate, and Aspergillus oryzae product in complete rations for lactating cows. J Dairy Sci 1983; 66:1474. https://doi.org/10.3168/jds.S0022-0302(83)81962-6.

[15] McAllister TA, Hristov AN, Beauchemin KA, Rode LM, Cheng KJ. Enzymes in ruminant diets. In: BEDFORD, M.R.; PARTRIDGE, G.G. (Eds.) Enzymes in farm animal nutrition. Oxon: Cab International: 273-298. 2001. https://doi.org/10.1079/9780851993935.0273

[16]EFSA. Scientific Opinion on Safety and efficacy of Sel-Plex® (organic form of selenium produced by Saccharomyces cerevisiae CNCM I-3060) for all species. EFSA Journal 2011; 9(4):2110. https://doi.org/10.2903/j.efsa.2011.2110

[17]Nooraee SE, Alimon AR, Ho YW, Abdullah N. Characterization of Kluyveromyces marxianus as a potential feed additive for ruminants. Lett Appl Microbiol 2010; 50: 578-584. https://doi.org/10.1111/j.1472-765X.2010.02836.x

[18] Tripathi MK, Karim SA, Chaturvedi OH, Verma DL. Effect of different liquid cultures of live yeast strains on performance, rumen fermentation and microbial protein synthesis in lambs. Journal of Animal Physiology and Animal Nutrition 2008; 92: 631-639. https://doi.org/10.1111/j.1439-0396.2007.00759.x

[19] NRC (National Research Council). Nutrient Requirements of Dairy Cattle, 7th rev. ed. Natl.Acad. Press, Washington, DC. 2001.

[20] Larson LL, Owen EG, Albrigh JL, Appleman RD, Lamb RC, Muller LD. Guidelines towards more uniformity in measuring and reporting calf experimental data. J Dairy Sci 1977; 60:989-991. DOI: https://doi.org/10.3168/jds.S0022-0302(77)83975-1

[21]Coverdale JA, Tyler HD, Quigley JD. Brumm JA. Effect of various levels of forage and form of diet on rumen development and growth in calves. J. Dairy Sci 2004; 87: 2554-2562.https://doi.org/10.3168/jds.S0022-0302(04)73380-9

[22]Quigley JD, Bernard JK. Effects of nutrient source and time of feeding changes in blood metabolites in young calves. J Anim Sci 1992; 70:1543-1549. https://doi.org/10.2527/1992.7051543x.

[23] Dumas BT. Biggs HG. Standards methods of clinical chemistry. 7: Academic Press; New York: p. 175. 1972.

[24] Quigley JD, Caldwell LA, Sinks GD, Heitmann RN. Changes in blood glucose, non-stratified fatty acids, and ketones in response to weaning and feed intake in young calves. J Dairy Sci 1991; 74:250. https://doi.org/10.3168/jds.S0022-0302(91)78167-8

[25] Hayashi H, kawai M, Nonaka I, Terada F, Katoh K, Obara Y. Development changes in the kinetics of glucose and urea in Holstein calves. J Dairy Sci 2006; 89:1654-1661. https://doi.org/10.3168/jds.S0022-0302(06)72232-9.

[26] Hulbert LE, Moisá SJ. Stress, immunity, and the management of calves. Journal of Dairy Science 2016; 99 (4): 3199-3216. https://doi.org/10.3168/jds.2015-10198.

[27]Miles RD. Manipulation of the microflora of the gastrointestinal tract natural ways to prevent colonization by pathogens. Proceeding of Alltechs Ninth Annual Symposium. In: Biotechnology in the Feed

Industry. T. P. Lyons, Ed. All tech Technical Publications. Nicholasville, Ky, USA; pp :133-150. 1993.

[28] Wiedmeier RD, Arambel MJ, Walters JL. Effect of yeast culture and Aspergillus oryzae fermentation extract on ruminal characteristics and nutrient digestibility. Journal of Dairy Science 1987; 70 (10): 2063-2068. https://doi.org/10.3168/jds.S0022-0302(87)80254-0

[29] Hossaini SMR, Bojarpour M, Mamouei M, Asadian A, Fayazi J. Effects of probiotics and antibiotic supplementation in daily milk intake of newborn calves on feed intake body weight gain, fecal scores and health condition. Journal of Animal and Veterinary Advance 2010; 9:872-875. https://doi.org/10.3923/javaa.2010.872.875

[30] Abdala AA, Zimmerman G, Calvinho LF, Gianre VR, Vottero, D et al . Efficacy of a probiotic added to whole milk and to a milk substitute. Revista de Medicina Veterinaria (Buenos Aires) 2002; 83: 196-198.

[31] Gorgulu M, Siuta A, Yurtseven S, Ongel E, Kutlu HR. Effect of probiotics on growing performance and health of calves. Cuban J Agric Sci 2003; 37: 125-129. https://doi.org/10.3923/pjbs.2003.651.654

[32] Vishal M, Baghel RPS. Effect of probiotic supplementation on growth performance of pre-ruminant buffalo calves. Buffalo Bulletin 2010; 29(3):225-228.

[33] Timmerman HM, Mulder L, Everts H, van Espen DC, Van der Wal E, Klaassen G, Rouwers S M, Hartemink R, Rombouts FM, Beynen AC. Health and growth of veal calves fed milk replacers with or without probiotics. J Dairy Sci 2005; 88:2154-2165. https://doi.org/10.3168/jds.S0022-0302(05)72891-5.

[34] Krehbiel CR, Rust SR, Zhang G, Gilliland SE. Bacterial direct-fed microbials in ruminant diets: Performance response and mode of action. Journal of Animal Science 2003; 81: 120-132. https://doi.org/10.2527/2003.8114_suppl_2E120x.

[35] Cruywagen CW, Jordaan I. Venter L. Effect of lactobacillus acidophilus supplementation of milk replacer on pre-weaning performance of calves. J Dairy Sci 1996; 79:483-486. https://doi.org/10.3168/jds.S0022-0302(96)76389-0.

[36] Radostits OM. The Merck Veterinary Manual, 8th Edition. The Canadian Veterinary Journal 2000; 41(4): 334.

[37] Aderemi FA. Effect of replacement of wheat bran with cassava root alleviate supplemented or unsupplemented with enzyme on the haemotology and serum biochemistry of pullet chicks. Tropical Journal of Animal Science 2004, 7: 147-153.

[38] Adams MC, Luo J, Rayward D, King S, Gibson R, Moghaddam GH. Selection of a novel direct-fed microbial to enhance weight gain in intensively reared calves. Anim Feed Sci Technol 2008; 145: 41-52. https://doi.org/10.1016/j.anifeedsci.2007.05.035.

[39] Lane MM, Morrissey JP. Kluyveromyces marxianus: a yeast emerging from its sister's shadow. Fungal Biol Rev 2010; 24: 17-26. https://doi.org/10.1016/j.fbr.2010.01.001

[40]Chiquette J. The role of probiotics in promoting dairy production. WCDS Adv. Dairy Technol 2009; 21:143-157.

[41] Lynch HA, Martin SA. Effects of Saccharomyces cerevisiae culture and Saccharomyces cerevisiae live cells on in vitro mixed ruminal microorganism fermentation. J Dairy Sci 2002; 85:2603- 2608. https://doi.org/10.3168/jds.S0022-0302(02)74345-2.

[42]Girard ID. Characterization of stimulatory activities from Saccharomyces cerevisiae on the growth and activities of ruminal bacteria. PhD Dissertation, University of Kentucky. 1996.

[43] Kristensen NB. Rumen microbial sequestration of 2-13C acetate in cattle. J Anim Sci 2001; 79: 2491-2498. https://doi.org/10.2527/2001.7992491x.

[44] Shen JS, Chai Z, Song LJ, Liu JX, Wu YM. Insertion depth of oral stomach tubes may affect the fermentation parameters of ruminal fluid collection in dairy cows. J Dairy Sci 2012; 95:5978-5984. https://doi.org/10.3168/jds.2012-5499.

[45] Vrzgula L, Alijev A, Bartko P. Poruchy látkového metabolizmu hospodárských zvierat a ich prevencia. Príroda, Bratislava: 487. 1990.

[46] Frumholtz PP, Newbold CJ. Wallace RJ. Influence of Aspergillus oryzae fermentation extract on the fermentation of a basal ration in the rumen simulation technique (Rusitec). J Agric Sci Camb 1989; 113: 169-172. DOI: https://doi.org/10.1017/S002185960008672X.

[47] Caton JS, Erickson DO, Carey DA. Ulmer DL. (1993). Influence of Aspergillus oryzae fermentation extract on forage intake, site of digestion, in situ degradability, and duodenal amino acid flow in steers grazing cool-season pasture. J Anim Sci 1993; 71: 779-787. https://doi.org/10.2527/1993.713779x

[48] Bach A, Gimenez A, Juaristi JL. Ahedo J. Effects of physical form of a starter for dairy replacement calves on feed intake and performance. J Dairy Sci 2007; 90:3028-3033. https://doi.org/10.3168/jds.2006-761

[49] Jouany JP, Demeyer DI, Grain J. Effect of defaunating the rumen. Anim Feed Sci Technol 1988; 21: 229-266. https://doi.org/10.1016/0377-8401(88)90105-8.

[50]Monnerat JP, Paulino PV, Detmann E, Valadares-Filho SC, Valadares RD, Duarte MS. Effects of Saccharomyces cerevisiae and monensin on digestion, ruminal parameters, and balance of nitrogenous compounds of beef cattle fed diets with different starch concentrations. Trop Anim Health Prod 2013; 45(5):1251-1257. https://doi.org/10.1007/s11250-013-0356-9

[51]Al Ibrahim RM, Kelly AK, O'Grady L, Gath VP, McCarney C, Mulligan F J. The effect of body condition score at calving and supplementation with Saccharomyces cerevisiae on milk production, metabolic status, and rumen fermentation of dairy cows in early lactation. J Dairy Sci 2010; 93(11):5318-5328. https://doi.org/10.3168/jds.2010-3201.

[52] Kamra DN, Chaudhary LC, Neeta-Agarwal Singh R, Pathak NN, Agarval N. Growth performance, nutrient utilization, rumen fermentation and enzyme activities in calves fed on Saccharomyces cerevisiae supplemented Diet. Indian J Anim Sci 2002; 72: 472-475.

[53] Ghasemi S, Naserian AA, Valizadeh R, Tahmasebi AM, Vakili AR, Behgar M. Ghovvati S. Inclusion of pistachio hulls as a replacement for alfalfa hay in the diet of sheep causes a shift in the rumen cellulolytic bacterial population. Small Ruminant Research 2012; 104(1-3):94-98. https://doi.org/10.1016/j.smallrumres.2011.09.052.