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RESEARCH ARTICLE

The effects of supplementation of milk with sodium butyrate on calf performance, some blood parameters and fecal *Escherichia coli (E. coli)* presence

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Abstract

The present study was conducted to determine the effects of supplementation of milk with sodium butyrate (SB), on calf performance, some blood parameters and *Escherichia coli (E.coli)* presence in feces. 10 male and 10 female Holstein calves of 7 days of age and 40-45 kg live weight were selected for the trial which lasted 50 days. The milk given to the trial group in the morning feeding was supplemented with SB at a dosage of 3 g/day from day 7 to 21 and 5 g/day from day 21 to 49. Water was provided *ad libitum*. On days 7 and 50, blood samples were drawn from 6 randomly selected calves from each group for Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), β-Hydroxybutyric Acid (BHBA), Immunoglobulin A (IgA) and Growth Hormone (GH) determinations. Fecal samples were also collected for *E.coli* counts. At the end of the study, it was observed that SB supplementation had a positive effect on IgA and GH throughout the trial, as well as on GCAA from day 21 to 35 ($p<0.05$). Whereas BHBA, AST and ALT concentrations, body weight, feed consumption and feed conversion parameters remained unaffected ($p>0.05$). *E.coli* analysis in feces, revealed %33.33 less pressure in trial group calves.

Introduction

Calves undergo significant physiological and metabolic changes in the time frame from birth to weaning. During this period, also referred to as pre-ruminant stage, calves are similar to monogastric animals in many aspects of digestion and metabolism (Heinrichs, 2005). The feature that distinguishes calves from monogastric is the presence of rumen, reticulum and omasum in their digestive system (Quigley *et al.*, 1991). However, these organs are not functional in the early stages of life and do not participate in the digestive process as they have not completed their development yet (Jiao *et al.*, 2015). The rumen constitutes %25 of the total stomach in newborn calves (Sato *et al.*, 2010) and its development begins around 2-3 weeks after birth, continuing until 6 months of age. For the stimulation of rumen development, inoculation and establishment of the anaerobic microbial ecosystem, initiation of solid feed intake and subsequent fermentation processes and absorption mechanisms are essential (Baldwin *et al.*, 2004). If volatile fatty acids (VFAs) can be produced in the rumen by means of adequate forage intake, the calves can use the energy obtained therefrom for organ and digestive system development. Besides, the growth in number and height of papillae as the rumen develops, increases the total absorption surface. Therefore, nutrient absorption through the rumen wall is enhanced (Govil *et al.*, 2017). Of the rumen VFAs, butyric acid (BA) provides the energy necessary for the thickening of the rumen wall, papilla formation and increased capillary development (Suarez *et al.*, 2007), whilst acetic and propionic acids provide the energy required for the growth of the calf. The effect of milk and milk replacers (MRF) on the rumen development of calves is limited, paving the way to digestive problems, metabolic acidosis or villus atrophy (Heinrichs, 2005; Berends *et al.*, 2012).

As excessive milk and milk replacer feeding causes a decrease in solid feed intake, VFA production gets impaired, negatively affecting the development of the anterior stomach, post-weaning feed intake and body weight gain (BWG) (Khan et al., 2007b; Laborde, 2008).

In recent years, alternative organic acid (OA)-derived feed additives are being developed with a view to improving gastrointestinal flora in animals. Although they come under the Generally Recognized as Safe (GRAS) category, palatability issues (Moss and Newbold, 2002), inconsistent responses on ruminal pH and cost considerations limit their use (Newbold and Rode, 2006). One of these acids, the BA, has been the subject of considerable research work in the last few years. Butyric acid (systematic name: butanoic acid, chemical formula: CH3 (CH2) 2 COOH) is found in all biological fluids and tissues as a natural component of cellular metabolism and has a pKa value of 4.82. BA is present in the digestive tract contents, milk, and also in the perspiration and feces of most mammals, and in the initial stages of life it is supplied first by colostrum, then by milk (Ceballos *et al.*, 2009; Garcia *et al.*, 2014). BA used for feed additive purposes consists of water-soluble and odorless sodium (Na), potassium (K), magnesium (Mg) and calcium (Ca) butyrate, usually supplied in powder or protected form (Gorka *et al.*, 2009; Nazari *et al.*, 2012). These forms have the advantage of being odorless and having less solid and volatile properties which facilitate their use (Fernández-Rubio *et al.*, 2009; Guilloteau *et al.*, 2010). Third generation butyrate are produced with a specific oil coating process to protect the active ingredient, which at the same time may eliminate the odor problem and help reduce the level of pathogenic bacteria, especially *Salmonella* (Fernández-Rubio *et al.*, 2009). Studies on bacterial colonization of the intestine during the calf period have focused on *E. coli* which has been identified as the most common pathogen implicated in diarrhea cases (Chanter *et al.*, 1984; Janke *et al.*, 1989). Frequent diarrhea problems can be significantly reduced by preventing the attachment of pathogens such as *E. coli* to the intestinal wall (Diebold and Eidelsburger, 2006).

Sodium butyrate (SB) supplementation has been reported to affect calf performance and health status positively by stimulating intestinal cell proliferation, villus development and digestive enzyme activity (Guilloteau *et al.*, 2010; Valentine, 2016). In a study, it was observed that the addition of %0.3 SB to the milk replacer (MRF) of 46-day-old calves did not alter the ruminal flora and pH but improved the reticulo-rumen epithelial structure and increased its mass (Gorka *et al.*, 2009). The addition of SB to the milk replacer fed to newborn calves had positive effects on the development of rumen papillae (Gorka *et al.*, 2009; Ślusarczyk *et al.*, 2010), butyrate added to milk and calf starter feed (CSF) improved calf health by reducing diarrhea in the early weeks of life (Gorka et al, 2009), accelerates the maturation process of the intestinal mucosa (Kotunia *et al.*, 2004) and delays gastric evacuation (Guilloteau *et al.*, 1981; Zabielski *et al.*, 1998). It has also been reported that SB can be an effective growth promoter when added to the MRF and CSF of calves aged 3-26 days at an inclusion rate of %0.3 of dry matter (DM) (Guilloteau *et al.*, 2009b). Furthermore, there are also studies indicating a more efficient feeding and rumen development with the addition of SB to CSF (Gorka *et al.*, 2009; Gorka *et al.*, 2011a). It was reported that the growth performance of animals increased with the addition of butyrate to the feed, the most effective SB dose being %1 to 4 of DM, and that the protection of the butyrate molecule by microencapsulation in a lipid matrix to ensure slow release increases the effectiveness of the molecule, by preventing its rapid metabolism in the stomach and increasing its availability in the upper sections of the small intestine (Claus *et al.*, 2007; Gorka *et al.*, 2009).

The purpose of this study was to determine the effects of adding SB, a form of BA protected with Na salt, to the milk on calf performance, some blood parameters and fecal *E. coli* presence.

Materials and Methods

Ethic approval

This study was approved by Animal Experiments Local Ethics Committee of Ankara University with the letter of consent dated 05/07/2017, protocol number 2017-14-115. The trial was conducted at a commercial dairy farm in Adana, Turkey (Sarıçam Alibaba Süt Sığırı İşletmesi, 37°07'04.1"N 35°35'49.6"E), between August the 5th and October the 1st, 2017.

Animals, feeds and feed additive

A total of 20 Holstein calves, 10 males and 10 females with an average body weight (BW) of 40-45 kg at 7 days of age, were used in the study. A period when high calving frequency was expected in the farm was chosen for the trial and births were monitored. Calves were fed in the infirmary during the first week of life to obtain consistent animal

material. Immediately after birth, calves were weighed individually to record their birth weights. Calves were weighed again on day 7, just before they were placed in individual hutches, to record their initial trial weights. Attention was paid to ensure that the distribution of male and female calves in the groups and their 7th day body weights were close to each other.

Sodium butyrate in powder form, soluble in milk and MRFs, protected with sodium salts of distilled palm fatty acids (SB %70, palm fatty acid %30) was used as trial material. Dry matter (DM) and ether extract (EE) of regular and SB supplemented milks were determined by gravimetrically while crude protein (CP) levels were measured by Kjeldahl method (Table 1) (Akyıldız, 1984).

Calf feeding and vaccination program

Calves were allowed to receive colostrum for the first two days of life. Subsequently, each calf was given a total of 5 L of milk from day 2 to 25, and 6 L from day 25 to 49. This amount was bottle fed in two accurately measured and controlled feedings at 7:30 AM and 5 PM. The animals were divided in two groups of 10 mixed sex calves each: one control, and the other trial (SB supplementation) group. Calves in the trial group received 3 g/day SB from day 7 to 21 and 5 g/day from day 21 to 49, added into the morning milk. Butyric acids were dosed by weighing on scales with a sensitivity of 0.1 gr and added in the milk of the trial group calves. To ensure homogeneous dispersion and maximum dissolution, SB supplemented bottles were shaken well before feeding. On the seventh day the calves were removed from the infirmary and transferred to individual calf hutches, where, alongside milk, CSF, Alfalfa hay and water were offered free choice according to the trial design. Drinking water was refreshed 3 times during the day, considering local temperatures and relative humidity levels. Ademin (vitamin) and Yeldif (B group vitamins and selenium) injections were administered on days 3 and 53, as well as Pasteurella vaccine on days 15 and 45 days, as per the routine practice of the farm.

Performance parameters

Body weights (BW) were recorded individually by weighing at birth, day 7 and thereafter every two weeks until the end of the trial. Calves were weighed before the morning feeding and at the same hours. Body weight gain (BWG) was determined over the difference between weighing and then daily body weight gain (DBWG) was calculated by division. Individual daily solid feed intake of the calves was calculated by weighing the feeds before distribution in the morning and subtracting the leftover feed collected the next morning. Feed conversion ratio (FCR) was calculated using the aforementioned data. Performance data set was thus compiled.

Blood parameters

Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), β-Hydroxybutyric Acid (BHBA), Immunoglobulin A (IgA) and Growth Hormone (GH) determinations were performed on the blood samples drawn on days 7 and 50, from 6 calves randomly selected from each group (12 in total). To ensure that SB is fully transferred to blood circulation, the samples were taken 4 hours after the milk feeding from the vena jugularis by means of a catheter. Serum was separated from the blood samples by centrifuge for 5 minutes at 5000 rpm in

Table 1. Chemical compositions of feeds and milk fed to the calves

	DM (%)	CP (%)	EE (%)	CC (%)	ADF (%)	NDF (%)	Starch (%)	Sugar (%)	ME (kcal/kg)
Feed									
AH	94.64	14.3	2.4	20.5	33.7	46.0	1.1	2.4	1927
CSF	88.00	23.58	1.25	9.75	10.65	26.27	30.60	5.0	2437
Milk									
Regular	11.63	3.18	3.40						
+3 gr SB	11.67	3.20	3.40						
+5 gr SB	11.75	3.19	3.40						

DM: Dry matter, CP: Crude protein, EE: Ether extract, CC: Crude cellulose, ADF: Acid detergent fiber, NDF: Nort detergent fiber, ME: Metabolizable energy, AH: Alfalfa hay, CSF: Calf starter feed, SB: Sodium butyrate

a NUVE NF 200 bench-top device. Separated sera were then placed in Eppendorf tubes using Finnpipet. On the sera thus obtained, GH was determined by Ferritin Electro-chemiluminescence immunoassay (ECLIA) method using a Roche E-170 device with Roche diagnostic kit; IgA by NEF (nephelometric) method (Aksu *et al.*, 2006), whilst ALT and AST were determined spectrophotometrically using commercial kits (Herbos Dijagnostika).

Fecal *E. coli* presence

Escherichia coli presence in feces was determined on fecal samples collected on days 7 and 50 from a total of 12 calves randomly selected from the trial (n=6) and control (n=6) groups. Fecal samples were taken rectally to evade the risk of contamination of the samples. The samples were placed in gel tubes and stored in deep freezer to prevent increases or decreases in the bacterial load of the feces over time. The samples were subjected to microbiological analysis by culture method and *E. coli* presence was determined as positive or negative (present - absent).

Statistical methods

The data obtained in the trial are descriptive statistics related to the variables studied in the trial and control groups of 10 subjects each. The correspondence of the data to a normal distribution (goodnes-of-fit) was evaluated with Saphiro Wilk's test. It was observed that the data structure was not distributed normally. Change over time was analyzed with Wilcoxon Sign test (Wayne, 1987).

Results and Discussion

Performance parameters

The average BW, BWG, DBWG, feed intake (AH, CSF, TF) and FCR results of the groups are given below (Tables 2, 3 and 4). As shown in Tables 2 and 3, the supplementation of SB to milk did not create any difference in BW, BWG and DBWG ($p>0.05$), except for the period from day 21 to 35, during which BWG and DBWG increased with SB addition in milk ($p<0.05$). Alfalfa hay (AH), calf starter feed (CSF), total feed (TF = AH + CSF) intakes and feed conversion ratio (FCR) remained unchanged ($p>0.05$) (Table 4). Until day 21 calves did not demonstrate any propensity to consume the AH offered free choice.

In earlier studies of similar nature, it was reported that SB supplementation generally increased (Gorka *et al.*, 2011; Nazari *et al.*, 2012; Zahao *et al.*, 2013; Serbester *et al.*, 2014), decreased (Gorka *et al.*, 2009; Ślusarczyk *et al.*, 2010; Araujo *et al.*, 2015; Wanat *et al.*, 2015) or, as is the case in this study, did not alter the performance parameters (Kato *et al.*, 2011; Guerrero, 2015; Hiltz and Laarman, 2019). There are also studies evaluating calcium as a butyrate source, which report no advantage in performance parameters over control (Serbester *et al.*, 2014), improved performance (Nazari *et al.*, 2012) or, increased average daily gain and feed efficiency although feed intake (CSF, alfalfa) remained unaffected (Davarmanesh *et al.*, 2015). There is even a study recommending SB over calcium butyrate (CaB) as it improved performance parameters (Serbester *et al.*, 2014).

According to this, the discrepancies with their results can be attributed to the supplementation of SB in MRF or CSF instead of milk unlike the present study, different SB feeding concentrations and protocols, differences in the amount of SB per calf, not including forage in the calculations, not offering forage to calves, differences in the ingredients and chemical composition of calf starter feed or its presentation (restricted or ad libitum), differences in weaning age and breed differences.

Blood parameters

The averages of serum BHBA, IgA, AST, ALT and GH results obtained from blood samples taken from

Table 2. Effects of sodium butyrate supplementation on body weight of calves

Day						
Group	n	0.day	7. day	21. day	35. day	49. da
BW, kg						
Control	10	38.80 ± 0.61	42.61 ± 0.98	47.31 ± 0.83	56.13 ± 0.89	65.81 ± 1.20
Trial	10	38.64 ± 0.69	41.68 ± 0.93	48.21 ± 1.45	59.48 ± 2.11	69.65 ± 2.77

BW: Body weight

Table 3. Effects of sodium butyrate supplementation on body weight and daily weight gain of calves

	Weight gain, kg			
	BW		**DBW**	
Day	Control	Trial	Control	Trial
0-7	3.81 ± 0.63	3.28 ± 0.49	0.54±0.09	0.43±0.07
7-21	4.70 ± 0.44	5.72 ± 0.62	0.34±0.03	0.47±0.07
21-35	8.82 ± 0.53b	11.27 ± 1.02a	0.63±0.04b	0.80±0.07a
35-49	9.68 ± 0.66	10.17 ± 0.87	0.69±0.05	0.73±0.06
7-49	23.20 ± 1.07	27.97 ± 2.11	0.55±0.03	0.67±0.05
0-49	27.01 ± 1.02	31.01 ± 2.41	0.55±0.02	0.63±0.05

a,b: Means within a row with different letters differ significantly ($p<0.05$).
BW: Body weight; DBW: Daily body weight

Table 4. Effects of sodium butyrate supplementation on feed intake and feed conversion ratios of calves

	AH		**CSF**		**TF**		**FCR**	
Day	Control	Trial	Control	Trial	Control	Trial	Control	Trial
7-21	-	-	1.04 ± 0.10	1.22 ± 0.27	1.04 ± 0.10	1.22 ± 0.27	2.00 ± 0.49	2.27 ± 0.43
21-35	0.89±0.2	1.31± 0.19	3.27± 0.48	4.11± 0.55	3.89 ± 0.56	5.29 ± 0.67	4.28 ± 0.71	4.47 ± 0.27
35-49	1.10± 0.11	1.53 ± 0.19	5.88± 0.92	7.62± 1.26	6.98 ± 1.00	10.56 ± 2.4	7.72 ± 1.31	9.97 ± 2.15
7-49	1.73± 0.25	4.18 ± 1.52	10.20± 1.41	12.90± 1.91	11.93± 1.57	17.09± 3.17	4.65 ± 0.45	5.28 ±0.55

AH: Alfalfa hay, CSF: Calf starter feed, TF: Total feed, FCR: Feed conversion ratio

both groups at the beginning (day 7) and end (day 50) of the trial are presented in Table 5.

Sodium butyrate supplementation did not lead to any difference in serum BHBA levels between the groups ($p>0.05$), however BHBA tended to increase over time in both control and trial groups ($p<0.05$). The finding of the present study that SB supplementation did not create any difference in BHBA between the groups was found to be consistent with the results of some studies (Ferreira and Bittar, 2011, SB in commercial starter feed at 150 g/kg DM; Guerrero, 2015, in MRF; Araujo et al., 2015 and Hiltz and Laarman 2019, 2.5%/BW in colostrum). The fact that in the present study, unlike others, SB was evaluated independently from CSF consumption, renders its results unique in this respect. On the other hand, the finding that BHBA concentrations of calves increased with the addition of SB (0, 0.03, 0.06 and 0.09 g isobutyrate/kg BW/calf) pre- and post-weaning (Wang et al., 2016) is inconsistent with the results of the present study, in which serum BHBA increased over time in both control and trial groups. The difference from Wang et al., (2016) is presumed to be arising from the difference in SB concentrations.

While no difference was found between the groups in serum IgA levels over the 7th day samples ($p>0.05$), serum IgA increased with SB supplementation in the trial group ($p<0.05$) by day 50. In a study, SB supplementation (15, 30 and 45 g/day) did not alter serum IgA, IgG and IgM concentrations, but improved performance and antioxidant capacity in pre-weaned calves (Wenhui et al., 2020). In that study as well, SB supplementation did not result in a difference compared to the control group, but increased serum IgA in the trial group ($p<0.05$). In their studies, the authors added SB to the milk, as in the present trial, but they tested higher levels of SB supplementation. Nevertheless, the findings of those studies were in line with the findings of the present trial. The report that the addition of sodium butyrate (%1 of DM) increased serum IgA, IgG and IgM levels ($p<0.05$) (Zhao et al., 2013) also supports the serum IgA results of the present study.

Table 5. Effects of sodium butyrate supplementation on some blood parameters in calves

Parameter	Days	n	Control	Trial	P
BHBA (mmol/l)	7	6	0.12 ± 0.01aA	0.11 ± 0.07aA	0.935
	50	6	0.24 ± 0.03bA	0.26 ± 0.02bA	0.470
P			0.027	0.027	
IgA (mg/dl)	7	6	5.89 ± 0.25aA	5.15 ± 0.28aA	0.092
	50	6	5.35 ± 0.45aA	6.12 ± 0.27bA	0.200
P			0.345	0.028	
AST (IU/l)	7	6	48.66 ± 8.75aA	42.83 ± 4.86 aA	0.749
	50	6	32.00 ± 2.98aA	35.50 ± 5.61aA	0.629
P			0.116	0.293	
ALT (IU/l)	7	6	9.00 ± 1.03aA	7.16 ± 1.51aA	0.226
	50	6	8.00 ± 1.15aA	7.00 ± 1.78aA	0.688
P			0.357	0.750	
GH (ng/ml)	7	6	6.23 ± 0.50aA	5.88 ± 0.15aA	0.748
	50	6	6.94 ± 0.85aA	9.64 ± 0.60bB	0.025
P			0.500	0.028	

a,b: Means within a column with different letters differ significantly ($p<0.05$). A, B: Means within a row with different letters differ significantly ($p<0.05$). BHBA: Beta hydroxy butyric acid, IgA: Immunoglobulin A, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, GH: Growth hormone

It was reported that the addition of SB to colostrum (at %2.5 of BW) decreased IgG absorption due to forming bonds with IgG (Hiltz and Laarman, 2019). On the basis of that study, it would be more appropriate to add SB to milk rather than colostrum in order to positively affect immunoglobulin absorption and reinforce the immune system of calves.

It has been concluded that addition of SB to milk had no effect on serum AST and ALT levels ($p>0.05$). In a study conducted to determine the effects of essential oils on calf performance and blood parameters, it was found that essential oils improved butyrate concentration in calves but did not lead to any increase in AST and ALT levels (Vakili et al., 2013). The data obtained in the present trial(AST 35.50 and ALT 7.00IU/L) are similar to the results of the above-mentioned study.

It was reported that ALT and AST (IU/L) in a healthy calf respectively ranged between 3.3-12.1 and 0.0-60.0 at 2 weeks of age and between 4.3-19.5 and 19.0-178 at 5 weeks of age (Yu et al.,2019). The figures of the present study were found to vary within the reference values (Huang et al., 2006; Hsueh et al., 2011; Yu et al., 2019).

However, the 7th day AST value (48.66IU/l) of the control group found in the present study was slightly above the reference range of 5-40 IU/L for maximum AST of Huang *et al.*, (2006) and Hsueh *et al.*, (2011). This can be explained by the presence of *E. coli* pressure in the control group, which will be described below. In the context of the scientific literature, the fact that the AST and ALT values found in this study were within the reference ranges may be interpreted as an indication that the calves were healthy during the trial. The ALT and AST values determined in this study are in congruence with the reports that ALT and AST are lower ($p\leq0.05$) in calves with respiratory problems(Almujalli *et al.*, 2015), ALT is generally higher than AST in liver diseases (Kaneko *et al.*, 1997), and serum AST and Alanine Aminotransferase concentrations are higher in calves with diarrhea compared to healthy ones

(Sing and Sodhi, 1992; Albayrak and Kabu, 2016).

Growth hormone level did not change with age in the control group but increased in the SB supplemented trial group ($p<0.05$). The increase in GH level with the addition of SB to milk found in the present study was consistent with Wang *et al.*, (2016), reporting that isobutyrate (0, 0.03, 0.06 and 0.09 g/kg BW/calf) added to calf feeds before and after weaning increased GH level ($p<0.05$), and similar to the GH data reported by Kato *et al.*, (2011), for SB and control group calves. However, the drop in GH concentration in the SB group following the first feeding observed in Kato *et al.*, (2011), was not detected in the present study. The authors attributed this decrease to the ability of SB to alter plasma hormone concentration and suppress cellular calcium ion concentration when administered via jugular vein or directly into the rumen. In the present study, blood samples were taken 4 hours after milk feeding to ensure that SB was fully absorbed into the blood circulation, which is presumed to be the reason of the divergence from the aforementioned study. The report that the basal serum GH level varies between 11 - 16 µg/L in the first day of life, increasing 7-folds within a few hours following colostrum feeding, and serum GH levels vary between 10-20 µg/L depending on age and level of animal development (Kühne *et al.*, 2000) supports the results of the present study. It has also been reported that butyrate exerts positive effects on growth, digestibility and utilization efficiency of feeds by means of modulating mucosal epithelial cells and defense systems (barrier function, immune system and antimicrobial effect), cell proliferation, differentiation and function in the digestive system, in both sick and healthy animals (Pouillart, 1998; Partanen ve Mroz, 1999; Manzanilla *et al.*, 2006; Mazzoni *et al.*, 2008). The results of the present study are also backed by the reports that calves receiving SB supplementation displayed a much faster growth (Ślusarczyk *et al.*, 2010), stronger immune functions (Zhao *et al.*, 2013), and that performance and growth were positively affected depending on the amount of bacteria associated with intestinal health in the digestive system (O'Hara *et al.*, 2018).

Fecal *E.coli* presence

The results of *E. coli* presence/absence analysis of fecal samples taken from the groups on days 7 and 50 are given in Table 6. At the start of the trial on the 7th day of life, no *E. coli* was found, neither in the control nor in trial groups, while on day %50, %33 less *E. coli* pressure was found in the feces of the calves receiving SB supplementation.

This finding is aligned with the reports that the addition of %0.3 SB to MRF and CSF decreases the pH in abomasal fluid ($p=0.02$) (Gorka et al, 2009), the growth of numerous microorganisms such as *E. coli*, Salmonella and Clostridium stops at pH levels below 5, and at the same time a barrier against pathogens is formed in the ileum and large intestine due to low pH, that this decrease in pH alters cell integrity and enzyme activity, thus inhibiting the intraluminal microbial growth of pathogens in the stomach (Kluge et al, 2004) and duodenum (Hebeler *et al.*, 2000) by the use of OA (Abhishek and Biswadeep, 2014), and that calves supplemented with SB in MRF and CSF had a lower incidence of diarrhea, requiring less treatment and electrolyte administration than the control group (Gorka *et al.*, 2009; $p=0.01$, Gorka *et al.*, 2011a; $p=0.08$).

It was also observed in this study that calves receiving SB supplemented milk consumed it readily without refusal. This finding is in parallel with the reports indicating that the unpleasant odor problem has been eliminated in third generation butyrate produced by a specific oil coating process which protect the active ingredient (Fernández-Rubio *et al.*, 2009) and that therefore such products can be recommended (Guillotetau *et al.*, 2010).

Table 6. E. coli presence in the groups

***E coli* presence**			
	n	Day	*E. coli**
Control	6	7.	- - - - - -
	6	50.	++- - - -
Trial	6	7.	- - - - - -
	6	50.	- - - - - -

*+ (positive): indicates *E. Coli* presence

Conclusion

As a result, sodium butyrate (SB) supplementation to milk did not affect calves' BW, FCR parameters and CSF, AH, TF (AH + CSF) consumptions in this study. However, SB supplementation did increase daily body weight gain between days 21-35. SB supplementation had positive effects on serum IgA and growth hormone levels of calves, but did not change BHBA, AST and ALT. Fecal *E.coli*presence/absence analysis revealed that SB supplementation reduced *E.coli* pressure by %33.33.

In conclusion, SB supplementation in milk had positive effects on immunity, growth performance and manure *E. coli* pressure in pre-weaned calves. It is

a well-known fact that a healthy calf performance in early life stages will be carried over to post-weaning growth and productivity later on. Therefore, it may be recommended to add SB to the milk fed to calves during the pre-weaning period, in which foundations of a healthy and robust herd are laid.

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Conflicts

No potential conflict of interest was reported by the authors.

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