TÜBİTAK

Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Short Communication

Turk J Vet Anim Sci
(2017) 41: 578-581
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doi:10.3906/vet-1701-54

Effective use of probiotic-glyconutrient combination as an adjuvant to antibiotic therapy for diarrhea in rearing dairy calves

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Received: 18.01.2017 • **Accepted/Published Online:** 21.04.2017 • **Final Version:** 21.08.2017

Abstract: Thirty Holstein calves presented with infectious diarrhea during the second week following birth were utilized to evaluate the effects of a standardized product containing a combination of probiotics, β-glucans, and glyconutrients (GLY) in support of antibiotic therapy during the early rearing phase (day 7 to day 45 following birth). Treatments were 1) antibiotic therapy (trimethoprim and sulfas; ANTIB), and 2) ANTIB plus 5 g/calf per day GLY. Treatments did not affect diarrhea duration, averaging 16.7 days. However, GLY support decreased ($P < 0.05$) the mortality rate and increased ($P < 0.05$) live weight gain. Increased weight gain was attributable to enhanced ($P < 0.05$) consumption of calf starter. Supplemental GLY increased ($P < 0.05$) serum glucose and lowered ($P < 0.05$) serum urea nitrogen, nonesterified fatty acids, and cortisol concentration. We conclude that daily provision of 5 g of a standardized product containing probiotics, β-glucans, and glyconutrients to diarrheic calves under antibiotic therapy will promote health and enhance early growth performance.

Key words: Feed additives, diarrhea, dairy calves, glyconutrients, probiotics

The high mortality rate of Holstein calves during the rearing period is a major concern. Infectious diarrhea accounts for 70% of this mortality (1). Successful antibiotic interventions for calves with infectious diarrhea are strongly associated with the physiological and immunological capacity of the affected calf (2). Recently, probiotics are being used as a prophylactic management in rearing calves with positive results on growth and health status (3). Beneficial effects are attributed to facilitated maintenance of intestinal epithelial integrity (4). The use of glyconutrients in combination with β-glucans and probiotics (GLY) is popularized in dietary supplements for humans and some nonruminant species (5). Although not without controversy (6), the increased use of these substances is attributed to enhanced cell signaling, promoting general immune responses, mediating inflammatory responses, and generalized reduction in cellular stress (5). To the best of our knowledge, no information is currently available regarding effects of combining GLY with conventional antibiotic therapy protocols as a supportive treatment for young calves diagnosed with infectious diarrhea. Therefore, an experiment was performed to evaluate the effects (mortality, days of diarrhea, change in body weight, and hematological profiles) of a standardized product containing probiotics, β-glucans, and glyconutrients in support of antibiotic therapy for calves diagnosed with infectious diarrhea in a commercial setting.

The experiment was conducted at a commercial dairy located in the Mexicali valley, Baja California, 34 km south of the city of Mexicali in northwestern Mexico (32°40′7″N; 115°28′6″W, about 10 m above sea level, and under Sonoran desert conditions (*BWh* classification according to Köppen). All animal management procedures were conducted within the guidelines of locally approved techniques for animal use and care. The inclusion criteria for the calves used in the experiment were 1) total serum protein 72 h following birth was adequate (>5.5 g/dL), and 2) calves had symptoms of infectious diarrhea during the second week following birth. Of these, 30 Holstein calves were randomly assigned to two treatment groups (15 calves/treatment) consisting of either a conventional antibiotic therapy protocol (ANTIB) or ANTIB plus

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5 g/day of Glycozyme (Maxcell Global Co. Ltd, Seoul, South Korea), consisting of a standardized mixture of glyconutrients, β-glucan, probiotics, and enzymes (ANTIB + GLY). The daily dose of Glycozyme was weighed using a precision scale (Ohaus, AS612 mod, Pine Brook, NJ, USA) and administered combined with milk replacer at the morning feeding. Following detection of diarrhea, the ANTIB protocol consisted of 3 daily intramuscular applications of sulfa/trimethoprim (10 mg sulfas/kg + 2 mg trimethoprim/kg live weight; Sulfa-Jet, Laboratories Norvet, Torreón, Mexico). If the diarrhea did not subside within 72 h of therapy, the calves were administered enrofloxacin intramuscularly (2.5 mg/kg body weight per day; Floxi-Jet 5%, Laboratories Norvet, Torreón, Mexico) until recovery. In addition to antibiotics, all calves were provided with oral electrolytes (1 L/day; Electrodex Calves, Pisa Agropecuaria, Mexico) along with an antispasmodic containing prifinium bromide (Prifinial, Vetoquinol de México, Mexico City). In the case of death, the calves were necropsied and samples of abnormal tissues of intestines were taken, cooled (in an ice chest), and immediately sent to the laboratory for histologic analysis. In the calves that survived feed intake and changes in body weight were registered from birth to day 45 of age. All calves included in the study underwent routine procedures of newborn calf management, including 4 L colostrum ingestion (2 L shortly after birth followed by 2 L 6 to 8 h following birth), and 4 L/day transition milk during the first 7 days. From day 8 through day 45, calves were fed twice daily a commercial milk replacer (4 L/day; Nodricina-200, Agriband Purina de México, Mexicali, Mexico), and allowed ad libitum access to a commercial calf starter (AMPLI-Calf starter 22, Purina Animal Nutrition, LLC, USA) and fresh water. The calf starter was formulated to meet or exceed the requirements for calves during the period of the study (7). The calves were housed in individual pens (100 cm wide, 150 cm long, and 50 cm high) in an indoor facility, with individual waterers and feed bunks. The calves were daily monitored from 0600 to 1400. Diarrhea was identified through the quality of feces deposited on the floor or those attached to the perianal area of the calf. Only cases of diarrhea diagnosed by a staff veterinarian as infectious were included. At birth, all calves were individually weighed (electronic scale, Salter Breknell, Fairmont, MN, USA). Approximately 72 h following birth (1000 hours), blood samples (10 mL) were obtained via the jugular (Venojet, Terumo Europe, Belgium). Once the clot retraction was present, the samples were centrifuged (Eppendorf, model 5804 R) for 10 min at 1300 × *g* at 5 °C. Total protein concentration was assayed on freshly extracted serum using a refractometer (Mark Atago, model SPR-N). The remaining serum was stored at −20 °C until subsequent determination of glucose, urea N (BUN), nonesterified fatty acids (NEFA), and cortisol concentrations. Upon completion of the study (day 45), surviving calves were individually weighed and blood sampled approximately at 1000 hours. Glucose was determined (spectrophotometric method; UV-2100, Shimadzu, Kyoto, Japan) using a diagnostic kit (Sigma-Aldrich, USA). Serum insulin (Cisbo Assays, USA) and cortisol (MP-Biomedicals, USA) concentration were determined via RIA. Readings for the cortisol samples were performed at 450 nm (MPR4, PerkinElmer Inc., Life Sciences, Germany). Serum NEFAs were determined according to Abeni et al. (8). Individual feed delivery and feed refusals were measured daily. Dry matter was determined by oven drying [method 930.15 (9)]. The trial was analyzed as a completely randomized design using the MIXED procedure of SAS software (10). The treatment means were separated using the least significant difference test (Tukey's test). Treatment effects were considered significant when the value of $P \leq 0.05$, and were identified as trends when the value of $P > 0.05$ and ≤ 0.10.

Treatment effects on mortality, and body weight change and diarrhea duration of surviving calves are shown in Table 1. All cases of diarrhea were confirmed as cryptosporidiosis by microscopically examination of stool samples using acid-fast staining and were confirmed by histopathological findings in the necropsied calves. There was no treatment effect on duration of diarrhea in surviving calves, averaging 16.7 days. However, the supporting antibiotic protocol with GLY reduced calf mortality (43%, $P < 0.05$), and increased (59%, $P < 0.05$) weight gain of the surviving calves. The increased weight gain was associated with enhanced starter consumption (44%, $P < 0.05$). Although health and productivity advantages have been demonstrated when probiotics are used as a prophylactic measure in rearing calves (3,11), there is limited information of probiotics' effects on growth performance and health parameters when calf health is compromised by infectious diarrhea. However, similarly to our results, scouring calves that received probiotics (consisting of *Lactobacillus plantarum*, *Enterococcus faecium*, and *Clostridium butyricum*) showed fewer days of scouring and higher immune response (greater peripheral leukocytes and cytokine mRNA expression levels) compared with the controls (12). The advantages observed here for GLY supported calves may be due a positive effect of GLY on cell stability (5).

Treatment effects on blood metabolites are shown in Table 2. Serum glucose, urea-N, NEFA, and cortisol concentration were not different among calves at 3 days of age (prior to presentation of clinical diarrhea). All serum metabolite values recorded at 3 days of age are within the normal range for healthy calves, as recorded in previous trials (13–15). Upon completion of the study (45 days

Table 1. Treatment effects on mortality and 45-day growth performance and diarrhea duration of surviving calves.

	ANTIB[a]	ANTIB+GLY[b]	SEM	Significance
Calves				
Initial	15	15		
Surviving	8	11		
Mortality, %	46.7	26.7		<0.05
Live weight, kg				
Initial	38.89	39.28	1.51	NS
Final	42.85	45.58	1.90	NS
Weight gain, kg	3.96	6.30	0.563	<0.05
Calf starter intake (DM), kg	45.34	81.14	10.92	<0.05
Diarrhea duration, days	15.88	17.45	12.65	NS

[a] The antibiotic therapy protocol consisted of a daily intramuscular application of sulfa/ trimethoprim (10 mg sulfas/kg + 2 mg trimethoprim/kg live weight at the time of detecting the problem for up to 72 h. If diarrhea did not subside within 72 h of therapy, then continued with intramuscular application of enrofloxacin (2.5 mg/kg body weight/24 h until recovery. Oral electrolytes and antispasmodic containing prifinium bromide were administered in all cases.

[b] Protocol of antibiotic therapy reinforced with daily intake of 5 g of a standardized compound of the combination of yeasts and sugars/calf per day. Oral electrolytes and antispasmodic containing prifinium bromide were administered in all cases.

Table 2. Effect of treatments on the profile of some serum parameters related to energy balance and stress in calves at day 3 of age and at day 45 of the experiment.[a]

	ANTIB[b]	ANTIB+GLY[c]	SEM	Significance
Days of test	45	45		
Glucose, mg/dL				
Day 3	87.74	92.78	0.38	NS
Day 45	54.30	70.26	0.28	<0.05
Urea nitrogen, mg/dL				
Day 3	5.62	6.26	1.16	NS
Day 45	5.78	4.18	0.76	<0.05
Cortisol, µg/dL				
Day 3	1.79	2.06	0.25	NS
Day 45	1.48	0.95	0.15	<0.05
NEFA, mmol/L				
Day 3	0.534	0.510	0.06	NS
Day 45	0.267	0.149	0.02	<0.05

[a] Serum profiles reported correspond to calves surviving through day 45.

[b] The antibiotic therapy protocol consisted of a daily intramuscular application of sulfa/ trimethoprim (10 mg sulfas/kg + 2 mg trimethoprim/kg live weight at the time of detecting the problem for up to 72 h. If diarrhea did not subside within 72 h of therapy, then continued with intramuscular application of enrofloxacin (2.5 mg/kg body weight per 24 h until recovery. Oral electrolytes and antispasmodic containing prifinium bromide were administered in all cases.

[c] Protocol of antibiotic therapy reinforced with daily intake of 5 g of a standardized compound of the combination of yeasts and sugars/calf per day. Oral electrolytes and antispasmodic containing prifinium bromide were administered in all cases.

of age), surviving calves that received ANTIB+GLY had serum glucose, urea-N, cortisol, and NEFA concentrations within a normal range recorded for healthy calves 4 to 10 weeks of age (13,14,16). In contrast, surviving calves that received ANTIB alone had lower serum glucose (29%, $P < 0.05$) and greater NEFA (44%, $P < 0.05$) concentrations. Serum urea-N was also greater (29%, $P < 0.05$) in calves treated solely with ANTIB, although those values were not outside the normal range (14). High plasma levels of NEFA accompanied by a lowered glucose concentration reflect greater mobilization of body lipids mediated by a negative energy balance and/or sustained stress (17). The greater levels of urea-N for the ANTIB treatment could reflect a decreased rate of anabolism. Surviving calves that received ANTIB+GLY also showed decreased (36%, $P < 0.05$) plasma cortisol concentration, resulting in concentrations approximating that recorded for unstressed calves (18). This decrease is consistent with differences in serum glucose and NEFA as well as growth performance, reflecting a mediation of generalized stress. It is concluded that the inclusion of GLY along with conventional antibiotic therapy promotes health and enhances early growth performance of calves diagnosed with infectious diarrhea. However, it is important to consider that the product additionally contains β-glucans, which also function as activators of the immune system, and the magnitude of their role in this study is not considered. It is necessary to continue research that contributes to elucidate more deeply the role of glyconutrients in the health and productivity of livestock.

Acknowledgments

The authors are grateful for the support received by the owner (Mr José Luis Nevarez Borquez) and personnel of the commercial dairy farm "Establo Nevarez" during development of the experiment.

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