Effect of Probiotic Bacteria on Immunoglobulin G Concentration and Other Blood Components of Newborn Calves

M.Y. Al-Saiady

Department of Animal Production, College of Food Science and Agriculture,
King Saud University, P.O. Box 2460, Riyadh 11451, Saudi Arabia

Abstract: Twenty-four, 3-4 days old male calves were used in this study. All animals were clinically normal. They were weighed within 24 h of arrival and divided into 3 groups A-C matched for body weight. Group A (control) calves were given a basal diet containing a combination of whole pasteurized milk and reconstituted milk replacer. In group B, a commercial culture of *L. acidophilus* and *L. plantarum* was added to the basal diet. In group C a culture of *L. acidophilus* 27 sc was added to the basal diet. Serum total proteins, albumin and total globulins were comparable in all three groups as were the serum concentrations of urea, BUN and triglycerides. Serum inorganic constituents also showed no significant differences between the control and treated groups. A significant increase in serum Ig G concentration was recorded in the probiotic-supplemented groups, which also showed a significant increase in body weight at 5 weeks and during the entire experimental period.

Key words: Probiotic, *L. acidophilus*, *L. plantarum*, hematology, serum Ig G, serum total proteins

INTRODUCTION

Neonatal diarrhea is a major cause of calf mortality worldwide. Its morbidity in individual herds can be as high as 80% (Cornaglia *et al.*, 1992; Wright *et al.*, 1995), while its mortality ranges between 1.5 and 8% (Bendali *et al.*, 1999; NAHMS, 2007). According to Davis and Drackley (1998) and NAHMS (2007) gastrointestinal infections and subsequent diarrhea and dehydration account for the majority of health problems affecting calves during the pre-weaning period and are the primary reason for poor development and death in the first 60 days of age.

Treatment of calf diarrhea is commonly based on broad-spectrum antibiotics or other antimicrobials agents. In addition to preventing infection by pathogenic bacteria in newborn calves, antibiotics have also been used as growth promoters in livestock. However, the frequent use of antibiotics in livestock can have serious consequences such as the development of resistant populations of bacteria. Subsequent use of the same antibiotics for therapy might therefore be ineffective. Also, residual antibiotics in dairy foods, meat, eggs and milk are unacceptable. Probiotic treatment is currently attracting increasing attention as a means of reducing the risk of infection, while enhancing the immune system in livestock (Abe *et al.*, 1995; Avita *et al.*, 1995; Kyriakis *et al.*, 1999). A probiotic is defined as a live microbial feed supplement, which beneficially affects the host animal by improving its intestinal microbial balance (Fuller, 1989). Some species of Lactic Acid Bacteria (LAB) have been claimed as probiotics, such as *Lactobacillus acidophilus*, *L. delbrueckii* sub sp. *bulgaricus*, *L. casei*, *L. fermentum*, *L. plantarum* and *L. reuteri*.

To be considered as probiotics, these bacteria should become part of the normal microbial flora in the intestine, survive the gastrointestinal passage and be able to adhere and colonize the intestinal tract (Havenaar *et al.*, 1992). Previous studies have demonstrated the importance of LAB to human and animal health (Bengmark, 1998; Fuller, 1989; Goldin and Gorbach, 1980). Some types of LAB can affect protective immunity against pathogens and tumors and have the ability to increase the mucosal immune response (Goldin and Gorbach, 1980; Isolauri *et al.*, 1994; Kimura *et al.*, 1997; Majamaa *et al.*, 1995). Antimicrobial activity is thought to be an important means by which probiotic bacteria competitively exclude or inhibit the activities of harmful intestinal microbes. Antimicrobial compounds produced by probiotic bacteria include organic acids (lactic and acetic acid), hydrogen peroxide (in environments where abundant oxygen is present), β-hydroxypropionaldehyde (produced by *L. reuteri*) as well as bactericidal or bacteriostatic peptides and proteins (De Vuyst and Vandamme, 1994). Donovan *et al.* (2002) reported that growth and performance of calves receiving probiotics are equivalent to those of calves fed antibiotics during the first 5 weeks of age. Direct-Fed Microbial (DFM) products used in

calves provide benefits by several mechanisms, e.g., restricting adherence of pathogenic microbes to mucosal surfaces (La Ragione *et al.*, 2001), stimulating immune responses (Hong *et al.*, 2005) and proliferation of other beneficial microorganisms (Abe *et al.*, 1995) and producing antimicrobial substances (Hong *et al.*, 2005). The objective of the present study was to investigate the effect of probiotics on neonatal calf health as reflected in their blood cellular, biochemical and chemical constituents and IgG levels.

MATERIALS AND METHODS

Twenty four, clinically normal 3-4 days old, Holstein male calves were purchased from a commercial dairy farm in Riyadh (central province-Saudi Arabia). The calves were weighed within 24 h of arrival and divided into 3 groups, A-C matched for Body Weight (BW). They were placed individually in cool, well-ventilated houses.

Group A (Control) calves were given a basal diet containing a combination of whole pasteurized milk and reconstituted milk replacer. The latter contained 24% protein, 19% fat, 10% mineral, 5% crude fiber and 42.5% carbohydrate (United Company, Saudi Arabia). The diet was non-medicated and was reconstituted in 13% dry matter. For group B, commercial culture of *L. acidophilus* and *L. plantarum* (Bio Saver, Kemin Industries Inc. Des Moines, IA, USA) was added to the basal diet at manufacturer's recommended amounts (1.25 g/100 kg of milk). For group C a culture of *L. acidophilus* 27 sc (obtained form the Animal Science Department of Oklahoma State University, OK, USA and propagated in our laboratories) was added to the basal diet prior to feeding at concentration of 1.85×10^7 colony forming Unit/liter Cfu 1-1). All calves received liquid diet at 8% of initial BW twice daily with maximum of 2 kg/feeding for 9 weeks and were restricted to 1.25 kg/feeding during 10 weeks and then weaned.

The calves were offered pelleted starter feed *adlibitum* consisting of 17% crude protein, 15.4% digestible protein, 2% ether extract, 8.5% crude fiber, 0.8% Ca, 0.45% P and 0.85% NaCl on dry matter (Grain silo and flour mill organization-Saudi Arabia). A 0.20 kg chopped alfalfa hay containing 91.06% DM (19.63% crude protein, 1.75% ether extract, 30.29% acid detergent fiber, 1.4% Ca) and 0.22% P was also fed *ad libitum*.

The animals were allowed free access to water and were weighed individually every 2 weeks. Two 10 mL blood samples were obtained from each calf by jugular venipuncture using EDTA-K and plain vacutainers (Becton, Dickinson and Co., USA) for hematological and serum analyses, respectively. These samples were collected 1 week before treatment and at 2 week intervals thereafter for a total of 15 weeks HemogloBin (Hb) was determined by a cyanomethoglobin method, Packed Cell Volume (PCV) by hematocrit centrifuge and white Blood Cell Count (WBCs) by hemocytometer. The sera were separated by centrifugation at 1500 g from clotted blood samples, dispensed into vials and stored at -20°C until used.

Total serum Protein (TP) concentration was determined by Biuret method (Henry, 1974), Albumin (ALB) by bromocresol green method (Rodkey, 1965) and total Globulin (GLOB) as the difference between TP and ALB. The albumin/globulin ratio was calculated. Total cholesterol, glucose, total lipids, triglycerides and urea concentration were determined Spectrophotometercally (Dr. Lang-LP3-West Germany) using commercial reagent kits (Randox Laboratories Ltd-Diamond Road-UK). Blood Urea Nitrogen (BUN) was calculated from urea. Inorganic serum constituent were determined using an atomic absorption spectrophotometer (Perkin-Elmer Ltd, UK). For Fe and Zn determination the serum was diluted 5 folds in demineralized water; for Mg, Ca and K 50 folds and for Na 500 folds in 0.25 strontium chloride. Immunoglobulin G (IgG) was determined by indirect sandwich Elisa method using commercial kits (Immunological and biochemical test system Gmbh-west Germany).

Statistical analysis: The data were subjected to statistical analysis using the SAS program (SAS, 2000). The GLM and least-Square means (LSmeans) were applied to the data.The effect of treatment on animal's health and blood parameters was tested using the following model:

$$Y_{ij} = \mu + T_j + e_{ij}$$

Where:
Y_{ij} = The ith observation of the jth treatment
μ = The population mean
T_j = Treatment (j = 1-3)
e_{ij} = The residual error

RESULTS AND DISCUSSION

Blood hematology, biochemistry and serum inorganic: The results are summarized in Table 1 and 2. Both probiotic treated groups had higher Hb and PCV values than the control although the difference was significantly significant only in group C calves. To the knowledge, there are no previous studies on the effect of probiotic supplementation on the hematology of calves. However, increased hemoglobin concentration, RBC and PCV were previously reported in kids (Sayed, 2003) and Turkeys

Table 1: Effect of treatment on Means+SE for blood hematology and biochemistry

Parameters	A (N:8)	B (N:8)	C (N:8)	Initial mean value* (N:24)
TP g L^{-1}	51.68±0.70	53.69±0.77	51.72±0.77	55.78±1.59
AL g L^{-1}	31.33±0.27^b	32.39±0.27^a	32.92±0.27^a	33.61±0.40^a
GL g L^{-1}	20.35±0.70^{ab}	21.31±0.70^a	18.75±0.70^b	22.51±1.80^a
UR mmol L^{-1}	4.42±0.12^b	4.72±0.12^{ab}	4.80±0.12^a	8.24±0.12^c
BUN mmol L^{-1}	2.06±0.05^b	2.21±0.05^{ab}	2.24±0.05^a	3.85±0.05^c
TLI mmol L^{-1}	4.84±0.12^a	4.13±0.12^b	4.46±0.12^b	5.27±0.12^c
CHLO mmol L^{-1}	3.07±0.08^a	2.67±0.08^b	2.80±0.08^b	2.04±0.07^b
TRI mmol L^{-1}	0.36±0.03^b	0.37±0.03^b	0.41±0.03^b	0.46±0.04^a
GLU mmol L^{-1}	0.91±0.08^a	0.58±0.08^b	0.69±0.08^b	1.12±0.26^a
PCV%	30.98±0.66^b	32.98±0.66^a	34.73±0.66^a	29.38±0.66^c
HB mg dc^{-1}	9.49±0.20^b	9.96±0.20^{ab}	10.43±0.20^a	9.27±0.21^b
WBC x10^9 L^{-1}	6.87±0.31^b	7.55±0.31^a	7.80±0.31^a	6.57±0.31^b
A/G	1.69±0.07^b	1.65±0.07^b	1.94±0.07^a	1.80±0.07^a
IgG ng mL^{-1}	159.51±51.0^b	259.89±5.72^a	273.49±6.36^a	247.53±7.81^a

Different letters within row mean significant difference (p<0.01); TP = Total Protein, AL = Albumen, GL = Globulin, UR = Urea, BUN = Blood Urea Nitrogen, TLI = Total Lipids, CHLO = Cholesterol, TRI = Triglyceride, GLU = Glucose, PCV = Packed Cell Volume, HB = Hemoglobin, WBC = White Blood Corpuscle, A/G = Albumen/Globulin; *Initial mean values determined 1 week prior to treatment

Table 2: Treatment effect on Means+SE of blood electrolytes

Parameters	A (No:8)	B (No:8)	C (No:8)	Initial mean value* (No:24)
K mmol L^{-1}	5.08±0.14^b	5.40±0.14^b	4.78±0.14^c	6.21±0.14^a
NA mmol L^{-1}	134.44±2.94	136.66±2.90	135.50±2.90	137.53±2.90
CA mmol L^{-1}	3.92±0.18^a	3.47±0.18^{ab}	3.28±0.18^b	3.80±0.18^{ab}
MG mmol L^{-1}	0.58±0.03^b	0.63±0.03^b	0.62±0.03^b	0.84±0.06^a
ZN umol L^{-1}	16.85±0.39^b	17.04±0.39^b	18.30±0.39^a	17.24±0.39^b
CU umol L^{-1}	8.43±0.33^b	9.48±0.33^a	7.95±0.33^b	10.19±0.34^a
FE mg L^{-1}	63.16±2.54^a	56.57±2.54^b	66.79±2.54^a	64.23±2.48^a

Different letters within row mean significant difference (p<0.01); * Initial mean values determined one week prior to treatment

(Cetin *et al.*, 2005) supplemented with probiotic. A significantly higher WBC count was also recorded in the study in calves of groups B and C compared to the controls.

A similar increase in WBC count was reported in birds given probiotic-supplemented diets (Rahimi and Khaksefidi, 2006). Furthermore, CHLO and TLI values were significantly reduced in the calves of groups B and C receiving probiotic supplement in comparison to control. This phenomenon is widely documented in the literature both in animals and humans (Mohan *et al.*, 1990; Zacconi *et al.*, 1992; Agerholmlarsen *et al.*, 2000; Liong and Shah, 2005; Salma *et al.*, 2007; Vasiljevic and Shah, 2008; Bakr *et al.*, 2009; Ataie-Jafari *et al.*, 2009; Ignatova *et al.*, 2009). Serum proteins (TP, ALB and GLOB) were comparable in all three groups (A-C) as were the serum concentrations of urea, BUN and triglycerides.

On the other hand, serum inorganic constituents did not show a definite trend and no significant differences were recorded between the control and treated groups with regards to K, Na and Mg levels.

Slight but significant increases in Zn and Cu and decreases in Fe and Ca levels were recorded in groups B and C, respectively, although their values were still within normal ranges for calves. A significant finding in the study is the marked increase in serum Ig G concentration in the probiotic-supplemented groups, particularly group C (259.9 and 273.5 ng mL^{-1}, for groups B and C) calves versus the controls (159.5 ng mL^{-1}). Probiotic *Lactobacillus* has also been reported to stimulate mucosal immunity and augment the production of secretory antibodies (IgA) in humans and animals (Kimura *et al.*, 1997; Perdigon *et al.*, 1999).

Recent studies have also shown that probiotic therapy in critically ill patients with multiple organ dysfunction syndrome greatly enhanced their immune activity with significant increases in Ig A and Ig G antibodies and reduced incidence of diarrhea in comparison to placebo-controls (Alberda *et al.*, 2007). A significant increase in serum Ig G concentration was also reported in adult healthy dogs receiving *L. acidophilus* supplement indicating that this probiotic bacterium might enhance the health and immune functions of these animals (Bailon *et al.*, 2004).

Similarly, administration of probiotic bacteria in chickens was shown to enhance specific, systemic antibody response and to stimulate the production of natural antibodies such as serum Ig G and Ig M (Haghighi *et al.*, 2006). Previous studies have indicated that the modulation of innate and adaptive immunity by probiotics is a dose and strain-dependent phenomenon (Perdigon *et al.*, 1999, 2002; Galdeano and Perdigon, 2004; Alberda *et al.*, 2007).
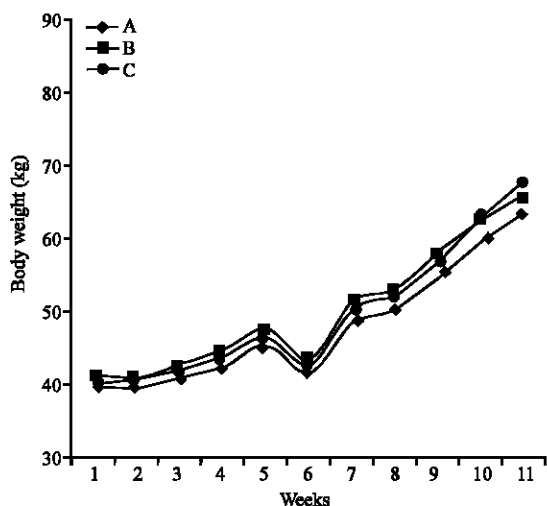
Fig 1: Effect of treatment on Mean+SE body weights of probiotic-fed versus control calves

Body weight gain: Mean bodyweight of the calves prior to probiotic treatment was 46.6+0.68 kg (range 45.50-47.56 kg). Both probiotic treated groups (B and C) gained more weight ($p<0.05$) during the experiment than the control group (Fig. 1) indicating that probiotic treatment using *L. acidophilus* or a combination of *L. acidophilus* and *L. plantarum*, particularly the latter was beneficial to the growth of these animals. At the conclusion of the experiment, the mean body weight gain in groups B and C was 41.75+1.25 and 37.25+1.25, respectively versus 35.63+1.25 in the controls. These findings are consistent with the previous studies (Abu-Tarboush *et al.*, 1996) and those of many other investigators (Abe *et al.*, 1995; Ghorbani *et al.*, 2002; Krehbiel *et al.*, 2003; Isk *et al.*, 2004; Timmerman *et al.*, 2005; Bakr *et al.*, 2009). Further studies should however, be undertaken to investigate this aspect and other beneficial effects of probiotics in growing calves and to determine optimal probiotic bacterial strains and doses to enhance performance and health in these animals.

CONCLUSION

The increase in bodyweight, serum Ig G concentration, Hb and PCV and the decrease in CHO and TLI presently recorded in calves receiving probiotic supplementation are all signs of improved health indicating the potential benefits of *Lactobacillus* bacteria. Further studies concerning beneficial effects of probiotics in growing calves should be pursued using different doses and strains of probiotic bacteria.

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