CIBTech Journal of Zoology ISSN: 2319–3883 (Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/cjz.htm
2016 Vol. 5 (2) May-August, pp.25-32/Nejat et al.
Research Article

THE EFFECT OF USING THREE KINDS OF PROBIOTICS ON PERFORMANCE, SKELETAL GROWTH, AND NUTRIENT DIGESTIBILITY OF DAIRY HOLSTEIN CALVES

***Ali Nejat, Seyed Nouroaldin Tabatabaei and Amir Davar Forouzandeh Shahraki**
Department of Animal Science, Khorasgan Branch (Isfahan), Islamic Azad University, Isfahan, Iran
**Author for Correspondence*

ABSTRACT
The present study was undertaken to evaluate the effect of three kinds of probiotics on performance, skeletal growth, and nutrient digestibility of dairy Holstein calves. A total of 100 one day old Holstein calves were randomly assigned to the 4 experimental treatments with 25calves each. Experimental treatments consisted of basal diets (control), basal diet supplemented with 1 g *Bacillus coagulans* containing 5×10^8 cfu/g, basal diet supplemented with 1 g *Saccharomyces cerevisiea*, basal diet supplemented with *Bacillus subtilis* containing 1×10^8 cfu/g as a completely random design that fed during a 60-d feeding trial. Results showed that dietary treatments especially *Bacillus coagulans* supplementation resulted in higher starter intake at 15 (P<0.01), 60 (P<0.0001) d of age and also total period of trial (P<0.001). Furthermore, administration of *Bacillus coagulans* led to a significant (P<0.01) increase in body weight at 45 d of age, a marked rise in weight gain at 45 (P<0.001), 60 (P<0.0001) d of age, as well as total period of experiment (P<0.0001) as compared to control group in dairy calve. Feed conversion ratio at 15 d of age and total period of trial was significantly (P<0.05) improved in calve receiving 1 g of *Bacillus coagulans* when compared to control group. Compared to control group, addition of 1 g of *Bacillus coagulans* resulted in increased body height at 45 (P<0.05) and 60 (P<0.01) d of age and also breast girth at 45 (P<0.001) d of age in calve. Supplemental *Bacillus coagulans* significantly (P<0.05) increased digestibility of crude protein, digestible protein and crude fat in dairy calve. However, digestibility of starch, NDF and organic matter was unaffected by dietary treatments. In general, inclusion of probiotics particularly *Bacillus coagulans* could increase performance, growth skeletal, and some nutrient digestibility in dairy calve.

Keywords: *Probiotics, Performance, Skeletal Growth, Nutrient Digestibility, Dairy Calves*

INTRODUCTION
Diarrhea is the main cause of mortality and huge economical losses in rearing neonatal calves as a result of lactose feeding. Thus, the high levels of lactose supplemented to the animals resulted in a significant microbial imbalance in the intestinal of calves (Frizzo *et al.*, 2011). A typical case of osmotic diarrhea is induced by lactose malabsorption in lactase deficiency (Heyman and Menard, 2002).
In addition, Frizzo *et al.*, (2010) observed that coliforms are mainly factors causing diarrhea in calves. Jones and Rutter (1972) indicated that adherence *E. coli* producing enterotoxin is crucial to stimulate diarrhea. So, antibiotics are generally applied as feed additives to milk replacer to avoid calve diarrhea (Constable, 2004), but researchers followed the alternatives to antibiotics due to increased risk of bacterial residua in meat and milk (Phillips *et al.*, 2004) as well as enhanced antibiotic-resistant bacteria of cattle industry (Fey *et al.*, 2000; Langford *et al.*, 2003).
Probiotic is one of the most important substitutions of antibiotics (Callaway *et al.*, 2004) that has been known as microbes affecting the host animal and identified as growth promoters in calves (Frizzo *et al.*, 2011).
Probiotics have been administrated to avoid or treat diarrhea in human (Sazawal *et al.*, 2006) as well as animals (Reid and Friendship, 2002) due to their efficacies such as improved intestinal microbial balance (Kaur *et al.*, 2002) and calf enteric environment, growth-promoting activities and also increased intestinal digestive capacity (Khuntia and Chaudhary, 2002), improved animal health and protection against infectious agents because of the beneficial effects on immunity (Schiffrin and Blum, 2002). Lactic acid

bacteria, *Bifidobacterium*, yeast and bacilli are the most common microbes used as probiotics (Abu-Tarbush *et al.,* 1996; Agrawal *et al.,* 2002). Frizzo *et al.,* (2011) exhibited that the animals supplemented with probiotic had the most lactic acid bacteria numbers in intestine, because it has been found that intestinal ecosystem is influenced by the healthy status of host affected by its environment (Khuntia and Chaudhary, 2002; Frizzo *et al.,* 2011).

Dietary inclusion of *Saccharomyces serevisiae* culture has been known as a growth promoting feed additives in animal production (Tripathi and Karim, 2010). The main effects of addition yeast culture on ruminants include an improvement of gastrointestinal health and microbial balance, stabilization of rumen pH and an interaction with lactate-used bacteria (Yang *et al.,* 2004) and growth-promoting of fibrolytic and cellulytic bacteria (Chaucheyras-Durand *et al.,* 2008) attributed to the capacity of yeast to use oxygen (Mosoni *et al.,* 2007).

Furthermore, beta glucan derived from yeast cell wall has been shown to adjust innate immunity in many species (Rodriguez *et al.,* 2003; Lowry *et al.,* 2005). As a result, feeding of *Saccharomyces serevisiae* suppressed diarrhea by reducing gut colonization of pathogenic microorganism (Jouglar *et al.,* 2000).

Haddad and Goussous (2005) reported that addition of yeast culture at 3 or 6 g/d to calves had no influence on feed intake of Awassi lambs.

However, Lesmeister *et al.,* (2004) indicated that the high levels of *Saccharomyces serevisiae* led to increased dry matter intake and weight gain.

Bacillus subtilis containing four species of bacillus such as *Bacillus amyloliquefaciens*, *Bacillus licheniformis, Bacillus pumilus, Bacillus subtilis* (Priest *et al.,* 1988) that secrets protease, amylase and lipase; in turn, it improved performance in animal (Santoso *et al.,* 2001).

Kritasand Morrison (2005) found that administration of *Bacillus subtilis* had favorable ability to improved micro flora balance in gastrointestinal tract and subsequently, increased animal performance. Kowalski *et al.,* (2009) observed that health status and fecal score were unaffected by feeding 1.32×10^9*Bacillus subtilis* spore in Holstein dairy calve.

Additionally, supplementation of *Bacillus coagulans* at 1.8×10^{10} led to higher lactic acid bacteria to *Escherichia coli* ratio and lower diarrhea occurrence in calve as compared to control group (Agazzi *et al.,* 2014).

Since there is limiting information about dietary addition of *Bacillus coagulans* and *Bacillus subtilis* on performance, health and nutrient digestibility of Holstein calves, the objective of present study was to evaluate and compare the effects of three kinds of probiotic son health and performance of dairy Holstein calves.

MATERIALS AND METHODS

Treatments and Animal Management

A total of one hundred one day old Holstein calves were used. The calves were randomly attributed to individual box among different dietary treatments. Twenty five calves assigned into each of 4 experimental diets. Dietary treatments consisted of basal diets (control), basal diet supplemented with 1 g *Bacillus coagulans* containing 5×10^8 cfu/g, basal diet supplemented with 1 g *Saccharomyces cerevisiea*, basal diet supplemented with *Bacillus subtilis* containing 1×10^8 cfu/g as a completely random design that fed during a 60-d feeding trial. Starter was formulated to meet all the nutritional requirements for calves based on NRC 1998. Starter ration and water were offered for ad libitum intake during the experiment (Table 1).

Feed Intake and Weight Gain

The calves weighed on d 1, 15, 30, 45 and 60 of experiment. Additionally, they were separately weighed every week on a sensitive digital scale (25 g). Feed intake was measured daily and fresh food was given each calf in every day.

Skeletal Growth Parameters

At the first of trial, body height from floor to withers and breast girth were recorded. Then, they were measured on d 15, 30, 45 and 60 of trial by meter.

Nutrient Digestibility

Samples of concentrates, hay and feces were oven-dried at 60°C to determine dry matter (DM), ash, crude protein, digestible protein, crude fat, neutral detergent fiber (NDF), and starch as described by Jansen *et al.,* (2000) using acid insoluble ash as internal marker.

Table 1: Feed Ingredients and Nutrient Composition of Starter Diets

Item	Starter
Ingredients (%)	
Corn	30
Barley	30
Soybean meal	25
Corn gluten	4
Wheat bran	6
Sugar beet by-product	1.5
Di calcium phosphate	0.5
Calcium carbonate	0.9
Salt	1
Bicarbonate	0.1
Mineral-vitamin premix	0.4
Vitamin A	0.6
Nutrient Composition	
Dry matter (%)	92
Crude protein (%)	19.3
ME (Mcal/kg)	2.79
NDF (%)	14.6
Fat (%)	4
Ca (%)	0.6
P (%)	0.5

Statistical Analysis

All data were subjected to ANOVA using the mixed procedure of SAS software (SAS institute, 1999) based on completely random design. The treatments were separated by Duncan's tests at $P<0.05$ statistical level.

The initial weight of calves, initial body height, and breast girth were used as covariate for performance, body height, and breast girth data in statistical model, respectively.

The used statistical analysis in this trial was as below:

$Y_{ijk}= \mu + T_i+ \beta (X_i-X) + e_{ijk}$

Where Y_{ijk}=observed value for a particular trait, μ= overall mean of the population, T_i= fixed effect of level of probiotics, $\beta (X_i-X)$= covariate variable, e_{ijk}= random error associated with the ijkth recording.

RESULTS AND DISCUSSION

One of the important factors influencing their performance during following rearing is growth of calves during first few weeks of age affected by disease (Frizzo *et al.*, 2011). Zhou *et al.*, (2000) observed that feed consumption, weight gain and diarrhea incidence have been applied to assess acute toxicity by probiotic strains and they are the most susceptible indices of calf health.

Performance

Table 2: The Effect of Three Kinds of Probiotics on Performance in Dairy Calve

Variable	Treatments				SE	P Value
	Control	*Saccharomyces Cerevisiae*	*Bacillus Coagulans*	*Bacillus Subtilis*		
Starter Intake (g/d)						
15 d	38.43[b*]	34.64[b]	61.81[a]	52.24[ab]	7.35	0.0100
30 d	153.12	150.40	200.40	148.11	16.65	0.1321
45 d	353.95	352.71	481.95	393.54	34.44	0.1602
60 d	478.05[c]	556.44[bc]	977.47[a]	663.11[b]	60.17	0.0001
Total period	231.27[b]	253.03[b]	384.48[a]	290.07[b]	22.38	0.0010
Body Weight (kg)						
15 d	49.56	51.28	53.92	53.48	0.71	0.742
30 d	55.28	59.08	60.84	59.56	0.92	0.747
45 d	66.12[b]	69.28[b]	75.48[a]	68.60[b]	1.27	0.004
60 d	81.44	84.20	85.20	84.28	1.58	0.253
Weight Gain(kg/d)						
15 d	0.32	0.34	0.52	0.31	0.048	0.739
30 d	0.38	0.52	0.46	0.41	0.048	0.225
45 d	0.60[b]	0.68[b]	0.98[a]	0.70[b]	0.068	0.001
60 d	0.65[b]	0.99[a]	1.02[a]	1.05[a]	0.082	0.0001
Total period	0.61[b]	0.63[b]	0.86[a]	0.65[b]	0.048	0.0001
Feed Conversion Ratio (kg/kg)						
15 d	1.98[a]	1.87[a]	1.74[b]	1.85[a]	0.04	0.049
30 d	2.05	2.01	1.86	1.95	0.05	0.082
45 d	2.12	2.06	1.92	1.97	0.11	0.229
60 d	2.13	2.04	1.99	1.98	0.09	0.152
Total period	2.06[a]	1.99[a]	1.89[b]	1.93[ab]	0.04	0.046

*Means within a row with no common letter are significantly different (P<0.05)

As shown in Table 2, feed intake at 15 (P<0.01), and 60 (P<0.0001) d of age and throughout of trial (P<0.001) was higher in calve receiving probiotics especially *Bacillus coagulans* than control group. Higher feed intake might be due to improvement of intestinal nutrient absorption (Simpsons, 1989), and development of rumen function (Beharka *et al.*, 1991). Feeding of *Saccharomyces serevisiae* led to a slight increase in feed intake as compared to control calve at 60 d of age and throughout of trial. This might be occurred because *Saccharomyces serevisiae* intake stimulates cellulose degradation resulting in increased feed intake (Chaucheyras Durand and Fonty, 2001). However, feed intake at 30 and 45 d of age was unaffected by supplemental treatments. Our results are in line with those of Agazzi *et al.*, (2014), who found that feed intake was increased in calves fed on Bacillus coagulans when compared to control. Similarly, Kowalski *et al.*, (2009) observed that feeding of 1.32×10^9 spore of *Bacillus subtilis* to calve led to higher feed intake than control group. In contrast to ours, it was reported that addition of probiotic mixtures containing *Saccharomyces serevisiae*, *Lactobacillus acidophilus* and *Bacillus subtilis* had no significant effect on feed intake when compared to control diet (Higginbotham *et al.*, 2012). Furthermore, Lesmeister *et al.*, (2004) studied feeding of 0, 1 and 2% of yeast culture to dairy calve; they showed that starter intake was increased in calves given 2% of yeast culture when compared to those on basal diet.

Although additional probiotics particularly *Bacillus coagulans* highly significant (P<0.01) increased body weight at 45 d of age, it had no influence on body weight at 15, 30 and 60 d of age (Table 2). This might be attributable to either higher feed intake (Fuller, 1976; Table 2) or rumen microflora alteration causing an improvement in nutrient digestibility and absorption (Table 4). Our observations are contrary to those of Agazzi *et al.*, (2014) who reported that supplemental *Bacillus coagulans* at 1.8×10^{10} raised body weight as compared to control calve. Furthermore, in disagreement with our findings, Philips and Vontungeln (1985) reported that body weight was elevated after *Saccharomyces serevisiae* intake in dairy calve.

Table 2 summarizes the effect of three kinds of probiotics on weight gain of calve. Supplementation of probiotics had no significant effect on weight gain at 15 and 30 d of age. However, weight gain at 45 (P<0.001), and 60 (P<0.0001) d of age and total period of trial (P<0.0001) was increased after feeding *Bacillus coagulans* in dairy calve as compared to control group. This might be related to increasing of protease, amylase and lipase secretion leading to higher nutrient digestion. Additionally, feeding of probiotics especially bacillus in diet improved microflora balance in gastrointestinal tract causing an improvement in food digestion and absorption; subsequently, it increased animal performance (Kritas and Morrsion, 2005). Our findings are in line with those of Kowalski *et al.*, (2009) who showed that feeding 1.32×10^9 spore of *Bacillus subtilis* increased weight gain in dairy calve in comparison to control group. However, Higginbotham *et al.*, (2012) studied probiotic mixture containing *Saccharomyces serevisiae*, *Lactobacillus acidophilus* and *Bacillus subtilis* in diet; they observed that probiotics supplementation had no influence on weight gain in dairy calve as compared to basal diet. Moreover, Haddad and Goussous (2005) reported that weight gain was improved by feeding of 3 g per d of yeast culture to Awassi lambs.

Inclusion of probiotics especially *Bacillus coagulans* resulted in improved feed conversion ratio at 15 d of age (P<0.05) and also total period of experiment (P<0.05). However, it had no influence on feed conversion ratio at 30, 45 and 60 d of age (Table 2). Improved feed conversion ratio as a result of probiotics intake might be associated with higher weight gain (Table 2) resulting from an improvement of nutrient digestion and absorption (Table 4). Our results are in contrast to those of Higginbotham *et al.*, (2012) who found no influence of feeding of probiotic mixtures containing *Saccharomyces serevisiae*, *Lactobacillus acidophilus* and *Bacillus subtilis*in terms of feed efficiency in dairy calve. Furthermore, Haddad and Goussous (2005) showed that feed conversion ratio was improved after administration of 3 g/d yeast culture in Awassi lambs.

Skeletal Growth

Table 3: The Effect of Three Kinds of Probiotics on Skeletal Growth in Dairy Calve

Variable	**Treatments**				**SE**	**P Value**
	Control	***Saccharomyces Cerevisiae***	***Bacillus Coagulans***	***Bacillus Subtilis***		
Body Height (cm)						
15 d	79.92	79.36	80.86	80.40	0.47	0.302
30 d	82.44	83.00	83.64	82.76	0.88	0.543
45 d	86.40^b	86.96^b	88.64^a	87.12^b	0.56	0.022
60 d	91.04^b	90.80^b	93.40^a	91.36^b	0.65	0.007
Breast Girth (cm)						
15 d	79.72	80.68	81.76	81.84	0.47	0.931
30 d	83.12	84.84	85.96	85.28	0.50	0.424
45 d	88.56^b	89.76^b	93.24^a	89.16^b	0.66	0.0002
60 d	95.88	95.96	98.00	97.52	0.77	0.788

*Means within a row with no common letter are significantly different (P<0.05)

As given in Table 3, body height on d 15 and 30 of trial was not affected by additional probiotics in dairy calve. Nevertheless, supplementation of 1 g of *Bacillus coagulans* led to an increase in body height on d 45 (P<0.05) and 60 (P<0.01) of experiment when compared to control group. Compared to control group, breast girth was remarkably (P<0.001) increased in calves supplemented with 1 g of *Bacillus coagulans* only on d 45 of trial (Table 3). This might be because of an increase in bioavailability of minerals such as calcium, magnesium, and phosphorous as a consequence of probiotic intake (Khuntia and Chaudhary, 2002). Additionally, probiotics elevated growth skeletal including body height via improving nutrient digestion and absorption (Hopper *et al.*, 2001). In agreement with ours, Chiofalo *et al.*, (2004) found that supplementation of probiotics in diet increased breast girth and withers height in kids in comparison with control diet.

Nutrient Digestibility

Table 4: The Effect of Three Kinds of Probiotics on Nutrient Digestibility in Dairy Calve (Percentage)

Variable	Treatments				SE	P Value
	Control	***Saccharomyces Cerevisiae***	***Bacillus Coagulans***	***Bacillus Subtilis***		
Crude Protein	66.60^{c}	68.35^{bc}	75.71^{a}	72.61^{ab}	0.002	0.013
Digestible Protein	79.46^{b}	79.65^{b}	85.94^{a}	82.38^{ab}	0.003	0.022
Crude Fat	75.92^{b}	86.22^{a}	89.64^{a}	87.03^{a}	0.007	0.041
Starch	81.97	84.25	87.87	85.75	0.005	0.287
NDF	29.63	30.19	38.11	42.50	0.009	0.089
Organic Matter	59.80	69.46	82.30	79.66	0.013	0.186

*Means within a row with no common letter are significantly different (P<0.05)

Table 4 represents the effect of three kinds of probiotics on nutrient digestibility of dairy calves. Addition of probiotics especially *Bacillus coagulans* resulted in a significant (P<0.05) rise in digestibility of crude protein, digestible protein, and crude fat in dairy calve. This might be attributable to an increase in enzyme secretion including protease, amylase and lipase leading to higher nutrient digestibility (Santoso *et al.,* 2001).

On contrary to our findings, Alexopoulos *et al.*, (2004) and Kritasand Morrison (2005) found that supplementation of *Bacillus subtilis* in diet had ability to maintain microflora balance in gastrointestinal tract and nutrient digestibility in calve.

Nevertheless, digestibility of starch, NDF, and organic matter was not affected by inclusion of probiotics in calve.

Conclusion

In general, results indicated that supplementation of probiotics especially *Bacillus coagulans* could raise performance, and skeletal growth via increasing nutrient digestibility in dairy calve.

REFERENCE

Abu-Tarboush HM, Al-Saiady MY and Keir El-Din AH (1996). Evaluation of diet containing Lactobacilli on performance, fecal coliform, and Lactobacilli of young milk calves. *Animal Feed Science and Technology* **57** 39–49.

Agarwal N, Kamra DN, Chaudhary LC, Agarwal I, Sahoo A and Pathak NN (2002). Microbial status and rumen enzyme profile of crossbred calves fed on different microbial feed additives. *Letters in Applied Microbiology* **34** 329–336.

Agazzi A, Tirloni E, Stella S, Maroccolo S, Ripamonti B, Bersani C, Caputo JM, Dell Orto V, Rota N and Givonni S (2014). Effects of species specific probiotic addition to milk replacer on calf health and performance during the first month of life. *Annals of Animal Science* **14** 101-115.

Alexopoulos C, Georgoulakis IE, Tzivar A, Kritas SC, Siochu A and Kyriakis SC (2004). Field evaluation of the efficacy of a probiotic containing *Bacilluslicheniformis* and *Bacillussubtilis* spores, on the health status and performance of sows and their litters. *Journal of Animal Physiology and Animal Nutrition* **88** 381-392.

Beharka AA, Nagaraja TG and Morrill JL (1991). Performance and ruminal function development of young calves fed diet with Aspergillus oryzae fermentation extract. *Journal of Dairy Science* **74** 4326–4336.

Callaway TR, Anderson RC, Edrington TS, Genovese KJ, Bischoff KM, Poole TL, Jung YS, Harvey RB and Nisbet DJ (2004). What are we doing about Escherichia coli O157:H7 in cattle? *Journal of Animal Science* **82** E93-E99.

Chaucheyras-Durand F and Fonty G (2001). Establishment of celluloytic bacteria and development of fermentative activities in the rumen of gnotobiotically-reared lambs receiving the microbial additive Saccharomyces cerevisiae CNCM I-1077. *Reproduction and Nutrition Development* **41** 57–68.

Chaucheyras-Durand F, Walker ND and Bach A (2008). Effect of dry yeasts on the rumen microbial ecosystem: past, present and future. *Animal Feed Science and Technology* **145** 5–26.

Chiofalo V, Liotta L and Chiofalo B (2004). Effects of the administration of Lactobacilli on body growth and on the metabolic profile in growing Maltese goat kids. *Reproduction Nutrition and Development* **44** 449–457.

Constable PD (2004). Antimicrobial use in the treatment of calf diarrhea. *Journal of Veterinary Internal Medicine* **18** 8–17.

Fey PD, Safranek TJ, Rupp ME, Dunne EF and Ribot E (2000). Cefriaxone-resistant salmonella infection acquired by a child from cattle. *The New England Journal of Medicine* **432** 1242-1249.

Frizzo LS, Soto LP, Zbrun MV, Signorini ML, Bertozzi E, Sequeira GJ, Armesto RR and Rosmini MR (20110. Effect of lactic acid bacteria and lactose on growth performance and intestinal microbial balance of artificially reared calves. *Livestock Science* **140** 246-252.

Frizzo LS, Sotoa LP, Zbruna MV, Bertozzia E, Sequeiraa G, Rodríguez Armesto R and Rosmini MR (2010). Lactic acid bacteria to improve growth performance in young calves fed milk replacer and spray-dried whey powder. *Animal Feed Science and Technology* **157** 159-167.

Frizzoa LS, Zbruna MV, Sotoa LP and Signorinib ML (2011). Effects of probiotics on growth performance in young calves: A meta-analysis of randomized controlled trials. *Animal Feed Science and Technology* **169** 147-156.

Haddad SG and Goussous SN (2005). Effect of yeast culture supplementation on nutrient intake, digestibility and growth performance of Awassi lambs. *Animal Feed Science and Technology* **118** 343–348.

Heyman M and Ménard S (2002). Probiotic microorganisms: how they affect intestinal pathophysiology. *Cellular and Molecular Life Sciences* **59** 1151–1165.

Higginbotham GE, Robison JD, Atwill ER and Pereira DGM (2012). Effect of a direct-fed microbial product on calf performance and fecal flora. *The Professional Animal Scientist* **14** 108-113.

Hopper LV, Wong MH, Thelin A, Hansson L, Falk PG and Gordon JI (2001). Molecular analysis of commensal host-microbial relationship in the intestine. *Science* **291** 881–884.

Jansen WL, Van der Kuilen J, Geelen SN and Beynen AC (2000). The effect of replacing nonstructural carbohydrates with soybean oil on the digestibility of fiber in trotting horses. *Equine Veterinary Journal* **32** 27-30.

Jones GW and Rutter JM (1972). Role of K88 antigen in the pathogenesis of neonatal diarrhea caused by *Escherichia coli* in piglets. *Infection and Immunity* **6** 918–927.

Jouglar JY, Durand H and Dussert L (20000. Evaluation of *Saccharomyces cerevisiae* I-1079 as a potential alternative to antibiotic growth promoters for piglet feeding. In: *Proceedings of16th International Pig Veterinary Society Congress* Melbourne, Australia 17–20.

Kaur IP, Chopra K and Saini A (2002). Probiotics: potential pharmaceutical applications. *European Journal of Pharmaceutical Sciences* **15** 1–9.

Khuntia A and Chaudhary IC (2002). Performance of male crossbred calves as influenced by substitution of grain by wheat bran and the addition of lactic acid bacteria to diet. *Asian-Australasian Journal of Animal Sciences* **15** 188–194.

Khuntia A and Chaudhary IC (2002). Performance of male crossbred calves as influenced by substitution of grain by wheat bran and the addition of lactic acid bacteria to diet. *Asian Australian Journal of Animal Science* **15** 188–194.

Kowalski ZM, Gorka P, Schlagheck A, Jagusiak W, Micek P and Strzetelski J (2009). Performance of Holstein calves fed milk replacer and starter mixture supplemented with probiotic feed additive. *Journal of Animal and Feed Sciences* **18** 399-411.

Kritas SK and Morrison RB (2005). Evaluation of probioticsas a substitute for antibiotics in a large pig nursery. *Veterinary Recommendations* **156** 447–448.

Langford FM, Weary DM and Fisher L (2003). Antibiotic resistance in gut bacteria from dairy calves: A dose response to the level of antibiotics fed in milk. *Journal of Dairy Science* **86** 3963–3966.

Lesmeister KE, Heinrichs AJ and Gabler MT (2004). Effects of Supplemental Yeast (Saccharomyces cerevisiae) Culture on Rumen Development, Growth Characteristics, and Blood Parameters in Neonatal Dairy Calves. *Journal of Dairy Science* **87** 1832-1839.

Lowry VK, Farnell MB, Ferro PJ, Swaggerty CL, Bahl A and Kogut MH (2005). Purified β-glucan as an antibiotic feed additive up-regulates the innate immune response in immature chickens against *Salmonella enterica* serovar *Enteritidis. International Journal of Food Microbiology* **98** 309–318.

Mosoni P, Chaucheyras-Durand F, Béra-Maillet C and Forano E (2007). Quantification by real time PCR of cellulolytic bacteria in the rumen of sheep after supplementation of a forage diet with readily fermentable carbohydrates: Effect of a yeast additive. *Journal of Applied Microbiology* **103** 2676–2685.

Phillips I, Casewell M, Cox T, De Groot B, Friis C, Jones R, Nightingale C, Preston R and Waddell J (2004). Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data. *Journal of Antimicrobial Chemotherapy* **53** 28–52.

Phillips WA and VonTungelln DL (1985). The effect of yeast culture on the postress performance of feeder calves. *Nutrition Reproduction International* **32** 287.

Priest FG, Goodfellow M and Todd C (1988). A numerical classification of the genus Bacillus. *Journal of Genetic Microbiology* **134** 1847-1882.

Reid G and Friendship R (2002). Alternatives to antibiotic use: probiotics for the gut. *Animal Biotechnology* **13** 97–112.

Rodriguez A, Cuesta A, Ortuo J, Esteban MA and Meseguer J (2003). Immunostimulant properties of a cell wall-modified whole *Saccharomyces cerevisiae* strain administered by diet to seabream (*Sparus aurata* L.). *Veterinary Immunology and Immunopathology* **96** 183–192.

Santoso U, Tanaka K, Ohaniand S and Saksida M (2001). Effect of fermented product from Bacillus subtilis on feed efficiency, lipid accumulation and ammonia production in broiler chicks. *Asian-Australian Journal of Animal Science* **14** 333-337.

Sazawal S, Hiremath G, Dhingra U, Malik P, Deb S and Black R (2006). Efficacy of probiotics in prevention of acute diarrhoea: a meta-analysis of masked, randomized, placebo-controlled trials. *The Lancet Infectious Diseases* **6** 374–382.

Schiffrin EJ and Blum S (2002). Interactions between the microbiota and the intestinal mucosa. *European Journal of Clinical Nutrition* **56** S60–S64.

Tripathi MK, Karim SA, Chaturvedi OH and Verma DL (2008). Effect of different liquid yeast cultures of live yeast strains on performance, rumen fermentation and microbial protein synthesis in lambs. *Journal of Animal Physiology and Animal Nutrition* **92** 631–639.

Yang WZ, Beauchemin KA, Vedres DD, Ghorbani GR, Colombatto D and Morgavi DP (2004). Effects of direct-fed microbial supplementation on ruminal acidosis, digestibility, and bacterial protein synthesis in continuous culture. *Animal Feed Science and Technology* **114** 179–193.

Zhou JS, Shu Q, Rutherfurd KJ, Prasad J, Gopal PK and Gill HS (2000). Acute oral toxicity and bacterial translocation studies on potentially probiotic strains of lactic acid bacteria. *Food and Chemical Toxicology* **38** 153–161.