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Effect of Synbiotic on Performance, Intestinal Morphology, Fecal Microbial Population and Blood Metabolites of Suckling Lambs

Research Article

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ABSTRACT

The effects of synbiotic supplementation to the Moghani suckling lambs diet were investigated using 18 lambs (initial BW 5-6 kg, age 3±2 day) were divided into 3 groups and fed experimental diets for 90 days. The dietary treatments were: 1) control (lambs were fed with mother's milk, along with their normal diets), 2) control + 3 g of synbiotic and control and 3) control + 6 g of synbiotic for each lamb per day. Feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR) were measured monthly throughout the experimental period. Blood samples were taken at the beginning and end of the experiment. Intestinal microflora was assessed monthly in feces, assaying for coliform bacteria, total aerobics and lactic acid bacteria. Also, at the end of study 4 lambs were slaughtered for assaying intestinal morphology changes. The lambs that fed 3 g synbiotic had a higher BW, greater BWG, increased FI and a better FCR compared to the control diet although the differences not statistically significant. Supplementation with 3 g symbiotic significantly reduced ($P<0.05$) serum cholesterol levels. Fecal coliform bacteria were reduced and lactic acid bacteria increased by supplementation. The results indicate that synbiotic can be used as a growth promoter in suckling lamb's diets and can improve their gut health.

KEY WORDS fecal, intestinal microflora, performance, suckling lamb, synbiotic.

INTRODUCTION

Healthy lambs are very important for the rancher from a commercial point of view. In a primary period of life, the growth pattern of intestinal colonies is very important. The first settled colony is very important because the bacteria make intestine a suitable settlement with changing gene expression in epithelial cells (Siggers *et al.* 2007). Artificial changes in the lamb's intestinal flora may change the gut environment to a better condition. The new intestinal colony can play its role as a barrier against antigens from microorganisms and food. The generation of immunophysiologic regulation in the gastrointestinal tract depends on the establishment of indigenous microflora. This has led the researchers to introduce the novel therapeutic interventions based on the consumption of cultures of beneficial live microorganisms that act as probiotics (Isolauri *et al.* 2001). The gastrointestinal tract of a newborn ruminant is sterile; microbes are introduced from the environment and from the dam's birth canal and colonize in the gastrointestinal tract (Ewaschuk *et al.* 2004). Using antibiotics in an animal's diet eliminates profitable bacteria and does not make good support for cellular immunity system (Heinrichs *et al.* 2009) and excess use of antibiotics increases bacterial resistance against them (Langford *et al.* 2003). With the ban of antibiotic growth promoters from animal diets in different

areas of the world, it is necessary to investigate potential alternatives to maintain good growth performance and good intestinal microbial flora as well as controlling the growth of harmful bacteria (Yakhkeshi *et al*. 2011). Thus, replacement of antibiotics with other compounds such as prebiotics, probiotics and synbiotics is considered as a suitable solution. Probiotics may be defined as living microorganism which given to animals, assist in the establishment of an intestinal microbial population, which is beneficial to the animal and have antagonistic properties against harmful microbes (Green and Sainsbury, 2001). Probiotics not only are used as a growth promoter, but also can induce immune system and have protective effects against many diseases (Gibson and Fuller, 2000). Frizzo *et al*. (2010) and Moore (2004) reported that in pre-ruminants, probiotics increase BWG, improve FCR and control diarrhea by balancing the intestinal microbial populations. In adidition, probiotic increased average daily gain (ADG) and FCR in lambs (Lema *et al*. 2001).

Prebiotics are non-digestible food ingredients that modify the microbial ecology of the colon and improve indices of host health (Gibson and Roberfroid, 1995). Most identified prebiotics are carbohydrates and oligosaccharides with different molecular structures normally occurring in the animal diet; dietary carbohydrates such as fiber, are candidate as prebiotics, but the most promising prebiotics are non-digestible oligosaccharides (Patterson and Burkholder, 2003). Kong *et al*. (2007) reported that the using prebiotics increased ADG and FCR, and reduced the incidence of diarrhea in piglets. Deng *et al*. (2007) reported that Cassiae seed can be used as a prebiotic to improve intestinal microflora. They showed that dietary inclusion of this prebiotic increased *lactobacillus* count, and reduced coliform counts in the gastrointestinal tract of piglets.

Synbiotics are defined as a mixture of probiotics and prebiotics that beneficially affect the host by improving the survival and implantation of the live microbial dietary supplements in the gastrointestinal tract (Gibson and Roberfroid, 1995).

Therefore, the objective of the present study was investigating the effect of synbiotic on performance, intestinal morphology, blood metabolites and fecal microbial population in suckling lambs.

MATERIALS AND METHODS

Experimental design and husbandry

Eighteen 3 ± 2 day old Moghani's Race suckling lambs, with an initial BW of 5-6 kg, were selected for the experiment. They were grouped based on their BW. The lambs were removed from their ewes and housed individually in a lamb house with separated pens, each of which was equipped with feeding and watering trough as required for lambs. All pens were located in the same lamb house and the lambs were randomly allocated. The lamb house was equipped with controlled ventilation and the bedding in the pens was chopped straw. Suckling lambs were fed 1 L of fresh milk from ewes (Table 1) by nipple bottle three times per day for 3 months.

Table 1 Ewes milk composition

Composition	Value
Total dry matter (%)	18.32
Crude protein (% of dry matter)	26.93
Fat (% of dry matter)	28.85
Metabolizable energy (Mcal/kg)	3.7

Lambs were assigned randomly to one of 3 treatments. Treatments included: 1) control treatment (lambs were fed with mother's milk), 2) control treatment + 3 g of synbiotic for each lamb per day and control treatment, 3) control + 6 g of synbiotic for each lamb per day. Also, after 2 weeks from birth, lambs were fed *ad libtum* with a diet (Table 2) formulated to meet their requirements according to NRC (1985). Feed Intake (FI), body weight gain (BWG) and feed efficiency ratio (FCR) were measured monthly throughout the experiment period.

The Biomin IMBO (Biomin GmbH, Herzogenburg, Austria) that is a combination of the probiotic strain *E. Faecium* (DSM 3530), a prebiotic (derived from chicory) and immune-modulating substances (derived from sea algae) was used as a commercial symbiotic.

Microbial sampling and incubation

On days 1, 30, 60 and 90, fecal samples were obtained from 4 lambs in each group. Sterile disposable gloves were used to collect the samples into sterile tubes. The tubes were kept cool during transfer to the laboratory (Stella *et al*. 2007). Then serial dilutions (10^{-4} to 10^{-8}) were made. Thereafter, the selective media of Plate Count Agar (Merck, Germany), De Man Rogosa Sharpe Agar (MRS) (Merck, Germany) and MacConkey Agar (Merck, Germany) were used for assaying total aerobics; lactic acid bacteria and coliforms, respectively. Microbial populations for total aerobics and *E. coli* were counted after aerobic incubation at 37 ˚C for 24 h and lactic acid bacteria after anaerobic incubation at 37 ˚C for 48 h (Walter *et al*. 2000).

Intestinal morphology assay

At the end of the study, 4 lambs in each experimental group were slaughtered and duodenum, jejunum, and illeum tissue samples were collected to evaluate intestinal morphologic changes. The histological indices were measured according to Iji *et al*. (2001) and Garcia *et al*. (2007). Intestinal tissue samples were fixed in 10% formalin and after fixation they were dehydrated by graded alcohol in ascending order and

then cleared in Xylol. The processed tissue blocks were embedded in paraffin. The paraffin blocks were cut in 6µm serial sections using a LEICA RM 2145 rotary microtome. The sections were floated in warm water (55-60 ˚C) prior to mounting on 10% poly L-lysine coated slides. The slides were stained by haematoxylin and eosin. Histological indices were determined by using a computer-aided light microscopic image analyzer (Analysis Starter, Olympus, Japan). The villi height and crypt depth were measured and calculation was made for villi height: crypt depth rate. Mean values of 10 adjacent, vertically oriented villous-crypt units per section were considered for analysis.

Table 2 Ingredient and chemical composition the diet fed to the suckling lambs

Ingredient	%
Corn	27
Barley	40
Wheat bran	16.4
Soybean meal	14.9
Mineral and vitamin premix*	0.5
Calcium carbonate	0.5
Di calcium phosphate	0.3
Salt	0.3
Crude protein	17.11
Calcium	0.58
Phosphorus	0.34
Metabolizable energy (Mcal/kg)	2.95

* Compsition of mineral and vitamin premix (per kg feed): Zn: 4.9 mg; Mn: 4.05 mg; Cu: 0.45 mg; I: 0.075 mg; Se: 0.1 mg; vitamin A: 2500 IU; vitamin D: 400 mg and vitamin E: 2.5 IU.

Blood metabolites

Blood samples were taken via the jugular vein at 1 and 90 days and stored at -20 ˚C. Serum glucose, triglyceride, cholesterol, albumin, total protein and globulin were measured by using the specific kits (Pars Azmoon, Tehran, 2012) and spectrophotometry was done by a UV spectrophotometer in 546 nm wavelength.

Statistical analysis

A completely randomized design (CRD) was employed. One-way analysis of variance was performed using the general linear model procedure of SAS (2001) software. Duncan's multiple range test was used for means comparison ($P<0.05$).

RESULTS AND DISCUSSION

Performance

No significant difference was found in FI in the 3 g synbiotic group compared to control group at all the days while the 6 g synbiotic group showed a significant decrease in FI compared the two other experimental groups ($P<0.05$). Moreover, there was no significant difference ($P>0.05$) in BWG between control group and 3 g synbiotic group in 1-30, 60-90 and 1-90 days of age. The BWG for the 6g symbiotic group was significantly lower ($P<0.05$) compared to the other groups. Also, significant differences were found in FCR between treatments at 30-60 and 60-90 days of age with better performance in the 3 g symbiotic group (Table 3).

Fecal microbial population

The population of bacteria in fecal samples was affected by synbiotic treatments (Table 4). When compared over all day, lambs on 3 g synbiotic treatment had more *Lactic acid* bacteria in their feces especially at 60 days of age. Populations of *Lactic acid* bacteria were similar for 3 g and 6 g synbiotic groups for all day. Coliform bacteria was reduced ($P< 0.01$) by supplementation. Populations of coliform bacteria in 6g synbiotic group were lower compared to the other two groups. Total aerobic bacteria were higher ($P<0.05$) for the supplemented groups compared to the control. All bacteria populations in this experiment demonstrated significant changes over time. However, no interaction of time and treatment was observed.

Blood metabolites

In general results of blood plasma parameters for lambs fed the experimental diets indicated that all treatments had little effect on blood plasma parameters as the differences in all parameters due to treatment effect were not significant except for cholesterol levels (Table 5). Cholesterol levels were lower ($P<0.05$) for lambs fed 3 g compared to the control and 6g group.

Intestinal morphology

Synbiotic treatment increased ($P<0.05$) jejunum and ileum villus height as compared to control diet (Table 6). The highest and lowest villus height in the duodenum and jejunum were observed in 6 g synbiotic group ($P<0.05$). Moreover, no significant difference ($P<0.05$) was observed between control and 3 g synbiotic treatments in the villus height in duodenum and jejunum, In addition, no significant difference ($P>0.05$) was found between treatments in the crypt depth in jejunum and ileum in all ages. However the crypt depth in the duodenum was higher ($P<0.05$) for the 6g group than the 3 g group. The 6 g synbiotic supplementation decreased ($P<0.05$) the villus height: crypt depth ratio compared to 3 g synbiotic and control group in the jejunum.

Performance

The improvement in performance of suckling lambs fed 3 g symbiotic *vs*. 6 g synbiotic seen in these experiments can be attributed to retention of beneficial microbial population in digestive tract.

Table 3 Suckling lambs performance in response to different treatments

Measurement	Treatments			SEM
	Control	3 g synbiotic	6 g synbiotic	
Feed intake (g/day)				
1-30 day	122.9[a]	117.11[a]	99.44[b]	10.87
30-60 day	424.34[a]	517.66[a]	399.03[b]	104.15
60-90 day	1212.8[a]	1258.45[a]	1088.8[b]	84.81
1-90 day	608.77[a]	634.61[a]	529.4[b]	151.05
Weight gain (g/day)				
1-30 day	266.67[a]	246.94[a]	154.94[b]	54.81
30-60 day	265.00[a]	279.44[ab]	176.4[b]	74.88
60-90 day	192.5[a]	206.93[a]	145.95[a]	59.44
1-90 day	225.06[a]	244.66[a]	160.42[b]	120.3
Feed conversion ratio				
1-30 day	0.55[ab]	0.48[b]	0.67[a]	0.14
30-60 day	2.28[a]	2.12[a]	2.28[a]	0.79
60-90 day	7.93[a]	6.38[a]	8.04[a]	3.56
1-90 day	2.85[b]	2.74[b]	3.36[a]	0.47

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 4 Effects of synbiotic on faecal flora (log10/g of faeces) at 0, 30, 60 and 90 day

Measurement	Treatment	Day 0	Day 30	Day 60	Day 90	SE	P-value		
							Treatment	Day	Treat × day
Total coliforms									
	Control	9.54	10.45	9.23	9.83				
	3 g synbiotic	9.14	9.89	7.99	8.76	0.45	**	**	NS
	6 g synbiotic	9.38	9.27	7.62	7.74				
Lactic acid bacteria									
	Control	4.94	7.49	5.37	8.89				
	3 g synbiotic	9.96	9.37	10.03	9.99	0.16	**	NS	NS
	6 g synbiotic	9.00	10.02	9.52	9.55				
Total aerobic									
	Control	8.04	8	9.53	7.71				
	3 g synbiotic	9.4	10.27	11.47	8.92	0.17	**	**	NS
	6 g synbiotic	9.5	10.91	12.01	10.13				

* (P<0.05) and ** (P<0.001).
NS: non significant.
SE: standard error.

Table 5 The effects of treatments on some blood parameters of suckling lambs

Items	Day	Treatments			SEM
		Control	3 g synbiotic	6 g synbiotic	
Total protein (g/dL)	0	6.09	5.84	6.99	0.44
	90	5.95	7.81	7.08	0.68
Albumin (g/dL)	0	4.96	4.81	4.28	0.3
	90	5.11	4.87	4.1	0.53
Glubulin (g/dL)	0	1.13	1.03	2.71	0.57
	90	0.84	2.94	2.98	0.94
Glucose (mg/dL)	0	76.78	83.1	81.38	2.29
	90	74.20	73.3	79.52	2.98
Triglyceride (mg/dL)	0	19.13	21.34	20.96	2.43
	90	20.98	18.41	19.76	2.37
Cholesterol (mg/dL)	0	53.24	55.232	54.11	1.2
	90	52.29[a]	48.66[b]	50.65[a]	1.68

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 6 The Intestine histomorphological parameters of suckling lamb at 90 days age

Intestine morphology	Treatments			SEM
	Control	3 g synbiotic	6 g synbiotic	
Villus height (µm)				
Duodenum	358.15^{b}	381.37^{b}	493.00^{a}	19.89
Jejunum	365.5^{a}	396.7^{a}	295.81^{b}	15.91
Ileum	324.12^{b}	335.63^{b}	402.87^{a}	11.55
Crypt depth (µm)				
Duodenum	239.75^{ab}	213.11^{b}	265.54^{a}	9.17
Jejunum	231.17	217.93	259.33	6.67
Ileum	197.71	219.08	240.20	31.22
Villus width (µm)				
Duodenum	157.48	150.68	165.89	4.69
Jejunum	133.32	146.99	142.17	6.21
Ileum	132.5	168.787	151.68	5.7
Villus height/crypt depth (µm)				
Duodenum	1.50	1.80	1.85	0.087
Jejunum	1.58^{a}	1.82^{a}	1.14^{b}	0.075
Ileum	1.63	1.65	1.68	0.29

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

If the synbiotics are added to animal feed in appropriate amounts they will lead to improve digestive and absorptive functions. Very high dietary levels of synbiotic (6 g for each lamb per day), introduce serious challenges to the gastrointestinal tract environment such as a decrease in gut pH, which in turn decreases FI and lowers the performance in 6 g synbiotics group. The positive effects of adding 3 g for each lamb per day synbiotic to suckling lamb's diet on their performance obtained in this experiment are in agreement with the other researchers report (Hillal *et al*. 2011; Tripathi and Karim, 2011; Frizzo *et al*. 2010). Sudies about dietary supplementation including probiotic, prebiotic and synbiotic have reported growth promotion, nutrient digestibility enhancement, and feed efficacy mechanisms in ruminant (Ellinger *et al*. 1980; Salama *et al*. 2002). Results of studies by Krehbiel *et al*. (2003) and Fleige *et al*. (2009) indicated beneficial effects of using *Enterococcus faecium* in the small ruminants. These data support the benefits of using synbiotic on BWG and FCR observed in our experiments. Furthermore, synbiotics help to eliminate the harmful bacteria and thus increase the utilization of nutrients in the gut and improve FCR.

Fecal microbial population

Synbiotics improve the intestinal microbial balance of suckling lambs. Furthermore, it has been known that probiotics change gene expression in intestinal cells (Siggers *et al*. 2007). The changes in the function of the intestinal tract also affect bacterial population and colonization. Synbiotic cultures of *Lactobacillus* spp. cause a challenge in pathogenic bacteria for obtaining nutrients. Synbiotics reduce nutrient availability for harmful bacteria and increase lactic acid bacteria colonies in the intestine. The results of the present study have shown further that synbiotic increase lactic acid and *Enterobacteria* population, and reduce the number of coliforms counts in the intestinal contents. Mann *et al*. (1980) showed that a strain of *E. coli*, which causes illness and death in young lambs, could be tolerated in the presence of *Lactobacilli* and also it has been reported that coliforms decrease in calves when an increase of *Lactobacilli* was found (Bruce *et al*. 1979). This might result from a reduction in pH, which can preventthe growth of many pathogens (Fuller, 1977). Moreover, *Enterococcus faecium* is a normal inhabitant in the gastrointestinal tract (Willard *et al*. 2000) and it shows anti- enteropathogenic effects against some bacteria, such as *E. coli*, *Salmonella typhimurium*, *Staphylococcus aureus* and *Clostridium perfringens* (Willard *et al*. 2000). By decreasing the gut pH (Huang *et al*. 2004) and increasing the population of *Lactobacilli* bacteria in the gastrointestinal tract, synbiotics can cause a reduction in competition for microbial nutrients in the host's gastrointestinal tract and thereby increases availability of nutrients. This can improve FCR and increase BWG in lambs (Lubbadeh *et al*. 1999; Tripathi *et al*. 2007) as shown for the 3 g synbiotic group.

Blood metabolites

All the blood metabolites investigated were within the normal range for pre-ruminants (Tripathi *et al*. 20011; Keithly *et al*. 2011; Dayani *et al*. 2011). Serum cholesterol and triglyceride levels also were reduced by use of synbiotic in

this study, which agrees with results of Antunovic *et al.* (2006) and Collins and Gibson (1999). Probably synbiotics influence blood cholesterol level by inhibition of cholesterol synthesis, or decrease its level directly by assimilation. Also lactobacilli can absorb cholesterol in their membranes and therefore reduce cholesterol levels in blood. Some gram-positive bacteria such as *Lactobacillus* and *Bifidobacteria* cause deconjugation of bile acids; this causes reduction of blood cholesterol and reduces the amount of triglycerides. Sadik (1989) found that *lactobacillus* concentrate supplement significantly increased plasma globulin concentrations. Synbiotics can improve lactose absorption and also increase gluconeogenesis to increase blood glucose levels (De Valdez *et al*. 1997), However the higher serum glucose obtained in synbiotic treatment groups was not significant in our study ($P<0.01$). Theresults however, indicated no differences in total serum protein among groups. Similar results were obtained by Lather, (1975) and Salem *et al.* (2001). This indicates that, treatment with synbiotic did not affect utilization of dietary proteins or protein synthesis in liver.

Intestinal morphology

In this study, intestinal morphological characteristics were affected by dietary treatments. The results showed that the use of synbiotic improved intestinal morphological characteristics, which can lead to increased feed utilization and improve performance. Awad *et al*. (2009) reported that a synbiotic containing *Enterococcus faecium* increased the villi height in jejunum compared to control group. Caspary (1992) reported that increasing the villus height leads to increased surface area capable of greater absorption of available nutrient. Crypts are the principal site of cellular proliferation in the intestinal tissue (Potten, 1977) and the crypt is considered as the villi factory and deeper crypts indicate fast tissue turnover to permit renewal of the villi in the gastrointestinal tract (Yason *et al*. 1987). Also Xu *et al.* (2003) reported that deepening of the crypts depth and shortening of the villi can lead to a decrease in nutrient absorption and increase the secretory function in the gastrointestinal tract leading to lower performance. An improvement in gut morphology is not only likely to benefit feed utilization and absorption, but may help to prevent opportunistic indigenous bacterial infections by the maintenance of an intact, healthy mucosal epithelium (Dimitroglou *et al.* 2009).

CONCLUSION

Synbiotics are probably most relevant to suckling lambs since these animals have a low immunity to enteric diseases and require time to develop a functional and balanced intestinal microflora for the effective utilization of nutrients and the inhibition of coliform bacteria. In this study, it is concluded that synbiotics can have a positive effect on lamb performance, including improved lamb health and increased growth rate. Generally, the growth performance of suckling lambs was improved when 3 g synbiotic were supplemented in the diet, which increased the beneficial bacteria and decrease *E. coli* population in feces during the time of the trial. Except for cholesterol, there was no significant difference between the different blood parameters in each symbiotic treatment group's *vs*. control. More studies are needed to elucidate the role of dietary synbiotics during the first days of life in lambs when they are more susceptible to disease and death. The findings of the current study explain the improved growth performance and may help the rancher for improve economic efficiency.

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REFERENCES

Antunovic Z., Speranda M., Amidzic D., Seric V., Steiner Z., Doma-Cinovic N. and Boli F. (2006). Probiotic application in lambs nutrition. *Krmiva.* **4,** 175-180.

Awad W.A., Ghareeb K., Abdel-Raheem S. and Böhm J. (2009). Effects of dietary inclusion ofprobiotic and synbiotic on growth performance, organ weights and intestinal histomorphology of broiler chickens. *Poult. Sci.* **88,** 49-55.

Bruce B.B., Gilliland S.E., Bush L.J. and Staley T.E. (1979). Influence of feeding cells of *Lactobacillus acidophilus* on the fecal flora of young dairy calves. *Okla. Agric. Exp. Stn.* **104,** 207-209.

Caspary W.F. (1992). Physiology and pathophysiology of intestinal absorption. *Am. J. Clin. Nutr.* **55,** 299-308.

Collins M.D. and Gibson G.R. (2012). Probiotics, prebiotics and synbiotics: approaches for modulating the microbial ecology of the gut. *Am. J. Clin. Nutr.* **69,** 1052-1057.

Dayani O., Dadvar P. and Afsharmanesh M. (2011). Effect of dietary whole cottonseed and crude protein level on blood-parameters and performance of fattening lambs. *Small Rumin. Res.* **97,** 48-54.

Deng Z.Y., Zhang J.W., Li J., Fan Y.W., Cao S.W., Huang R.L., Yin Y.L., Zhong H.Y. and Li T.J. (2007). Effect of polysaccharides of Cassiae seeds on the intestinal microflora of piglets. *Asia Pacific J. Clin. Nutr.* **16(1),** 143-147.

De Valdez D.F., Martos G., Taranto M.P., Lorca G.L., Oliver G. and De Ruiz Holgado A.P. (1997). Influence of bile on b-

galactosidase activity and cell viability of *Lactobacillus reuteri* when subjected to freeze-drying. *J. Dairy Sci.* **80,** 1955-1958.

Dimitroglou A., Merrifield D.L., Moate R., Davies S.J., Spring P., Sweetman J. and Bradley G. (2009). Dietary mannan oligosaccharide supplementation modulates intestinal microbial ecology and improves gut morphology of rainbow trout, Oncorhynchus mykiss (Walbaum). *J. Anim. Sci.* **87,** 3226-3234.

Ellinger D.K., Muller L.D. and Glantz P.J. (1980). Influence offeeding fermented colostrum and *Lactobacillus acidophiluson* fecal flora of dairy calves. *J. Dairy Sci.* **63,** 478-482.

Ewaschuk J.B., Naylor J.M., Chirino-Trejo M. and Zello G.A. (2004). *Lactobacillus rhamnosus* strain GG is a potential probiotic for calves. *Canadian J. Vet. Res.* **68,** 249-253.

Fleige S., Preibinger W., Meyer H.H.D. and Pfaffl M.W. (2009). The immunomodulatory effect of lactulose on *Enterococcus faecium* fed preruminant calves. *J. Anim. Sci*. **87,** 1731-1738.

Frizzo L.S., Sotto L.P., Zbrun M.V., Bertozzi E., Sequeira G., Armesto R.R. and Rosmini M.R. (2010). Lactic acid bacteria to improve growth performance in young calves fed milk replacer and spray-dried whey powder. *Anim. Feed Sci. Technol.* **157,** 159-167.

Fuller R. (1977). The importance of *Lactobacilli* in maintaining normal microbial balance in the crop. *Br. Poult. Sci.* **18,** 85-94.

Gibson G.R. and Roberfroid M.B. (1995). Dietary modulation of the human colonic microbiota. Introducing the concept of prebiotics. *J. Nutr.* **125,** 1401-1412.

Gibson G.R. and Fuller R. (2000). Aspects of *in vitro* and *in vivo* research approaches directed toward identifying probiotics and prebiotics for human use. *J. Nutr.* **130,** 391-395.

Green A.A. and Sainsbury D.W.B. (2001). The role of probiotic in producing quality poultry products. Pp. 245-251 in Proc. 15th European Symp. Qual. Poult. Meat. Kusadasi, Turkey.

Heinrichs A.J., Jones C.M., Elizondo-Salazar J.A. and Terrill S.J. (2009). Effects of aprebiotic supplement on health of neonatal dairy calves. *Livest. Sci.* **125,** 149-154.

Hillal H., El-Sayaad G. and Abdella M. (2011). Effect of growth promoters (probiotics) supplementation on performance, rumen activity and some blood constituents in growing lambs. *Inst. Farm. Anim. Biol*. **6,** 607-617.

Huang M.K., Choi Y.J., Houde R.J., Lee W., Lee B.H. and Zhao X. (2004). Effects of *Lactobacilli* and an *Acidophilic fungus* on the production performance and immune responses in broiler chickens. *Poult. Sci*. **83,** 788-795.

Iji P.A., Saki A.A. and Tivey D.R. (2001). Intestinal development and body growth of broiler chicks on diets supplemented with non-starch polysaccharides. *Anim. Feed Sci. Technol.* **89,** 175-188.

Isolauri E., Sutas Y., Kankaanpaa P., Arvilommi H. and Salminen S. (2001). Probiotics: effects on immunity. *Am. J. Clin. Nutr.* **73,** 444-450.

Keithly J.I., Kott R.W., Berardinelli J.G., Moreaux S. and Hatfield P.G. (2011). Thermogenesis, blood metabolites and hormones, and growth of lambs born to ewes supplemented with algae-derived docosahexaenoic acid. *J. Anim. Sci.* **89,** 4305-4313.

Kong X.F., Wu G.Y., Yin Y.L., Liu H.J., Yin F.G., Li T.J., Huang R.L., Kang P., Xing F., Fan M.Z., Yang C.B. and He Q.H. (2007). Dietary supplementation with Chinese herbal ultrafine 3 powder enhances cellular and humoral immunity in early weaned piglets. *Livest. Sci.* **108,** 94-98.

Krehbiel C.R., Rust S.R., Zhang G. and Gilliland S.E. (2003). Bacterial direct-fed microbials in ruminant diets: performance response and mode of action. *J. Anim. Sci.* **81,** 120-132.

Langford R.M., Weary D.M. and Fisher L. (2003). Antibiotic resistance in gut bacteria from dairy calves: a dose response to the level of antibiotics fed in milk. *J. Dairy Sci.* **86,** 3963-3966.

Lather A.L. (1975). In Cantarow and Trumper, Clinical Biochemistry. Souders, Co., Toranto.

Lema M., Williams L. and Rao D.R. (2001). Reduction of fecal shedding of enterohemorrhagic *Escherichia coli O157:7* in lambs by feeding microbial feed supplement. *Small Rumin. Res.* **31,** 31-39.

Mann S.O., Grant C. and Hobson P.N. (1980). Interactions of *E. Coli* and lactobacilli in gnotobiotic lambs. *Microb. Let*. **15,** 141-144.

Moore J. (2004). The use of probiotics in the calf: an overview. *Cattl Pract*. **12,** 125-128.

NRC. (1985). Nutrient Requirements of Sheep. 6th Ed. National Academy Press, Washington, DC, USA.

Patterson J.A. and Burkholder K.M. (2003). Application of prebiotics and probiotics in poultry production. *Poult. Sci.* **82,** 627-631.

Potten C.S. (1977). Extreme sensitivity of some intestinal crypt cells to X and Y irradiation. *Nature. London*. **269,** 518-521.

Sadik M.F. (1989). Effect of *Lactobacillus* concentrate (LBC) as a new growth promoter on the performance of growing buffalo heifers raised on milk replacer. MS Thesis. Ain Shams Univ., Cairo, Egypt.

Salama A.A.K., Caja G., Garin D., Albanell E., Such X. and Casals R. (2002). Effects of adding a mixture of malate and yeasts culture (*Saccharomyces cerevisiae*) on milk production of Murciano-Granadina dairy goats. *Anim. Res.* **51,** 295-303.

Salem F.A., El-Amary H. and Hassanin S.H. (2001). Effect of bentonite supplementation on nutrients digestibility, rumen fermentation, some blood physiological parameters and perfromance of growing lambs. *Egyptian J. Nutr. Feeds.* **4,** 179-191.

SAS Institute. (2001). SAS®/STAT Software, Release 9.1. SAS Institute, Inc., Cary, NC. USA.

Siggers R.H., Thymann T., Siggers J.L., Schmidt M., Hansen A.K. and Sangilda P.T. (2007). Bacterial colonization affects early organ and gastrointestinal growth in theneonate. *Livest. Sci.* **109,** 14-18.

Stella A.V., Paratte R., Valnegri L., Cigalino G., Soncini G., Chevaux E., Dell'Orto V. and Savoini G. (2007). Effect of administration of live *Saccharomyces cerevisiae* on milk production, milk composition, blood metabolites and faecal flora in early lactatingdairy goats. *Small Rumin. Res.* **67,** 7-13.

Tripathi M.K. and Karim S.A. (2011). Effect of yeast cultures supplementation on live weight change, rumen fermentation, ciliate protozoa population, microbial hydrolytic enzymes status and slaughtering performance of growing lamb. *Livest. Sci.* **135,** 17-25.

Tripathi M.K., Karim S.A., Chaturvedi O.H. and Verma D.L. (2007). Effect of different liquid cultures of live yeast strains

on performance, ruminal fermentation and microbial protein synthesis in lambs. *J. Anim. Physiol. Anim. Nutr.* **92,** 631-639.

Walter J., Tannkock G.W., Tilsala-Timsjarvi A., Rodtong S., Loach D.M., Munro K. and Alatossava A. (2000). Detection and identification of gastrointestinal lactobacillus species by using denaturing gradient gel electrophoresis and species-specific PCR primers. *Appl. Environ. Microbiol.* **66,** 297-303.

Willard M.D., Simpson R.B., Cohen N.D. and Clancy J.S. (2000). Effects of dietary fructo-oligosaccharide on selected bacterial populations in feces of dogs. *Am. J. Vet. Res.* **61,** 820-825.

Xu Z.R., Hu C.H., Xia M.S., Zhan X.A. and Wang M.Q. (2003). Effects of dietary fructooligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. *Poult. Sci.* **82,** 1030-1036.

Yakhkeshi S., Rahimi S. and Gharib Naseri K. (2011). The effects of comparison of herbal extracts, antibiotic, probioticand organic acid on serum lipids, immune response, GIT microbial population, intestinal morphology and performance of broilers. *J. Med. Plant*. **37,** 80-95.

Yason C.V., Summers B.A. and Schat K.A. (1987). Pathogenesis of rotavirus infection in various age groups of chickens and turkeys: pathology. *Am. J. Vet. Res.* **6,** 927-938.