IJFANS
International Journal of Food And Nutritional Sciences

Volume 3, Issue 4, Jul-Sep 2014, www.ijfans.com e-ISSN: 2320-7876

INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES

IMPACT FACTOR ~ 1.021

Official Journal of IIFANS

INTERNATIONAL JOURNAL OF FOOD
AND NUTRITIONAL SCIENCES

e-ISSN 2320 –7876 *www.ijfans.com*
Vol.3, Iss.4, Jul-Sep 2014

Research Paper *Open Access*

EFFECTS OF *BACILLUS SUBTILIS* AND *BACILLUS LICHENIFORMIS*-BASED PROBIOTIC ON PERFORMANCE, HEMATOLOGICAL PARAMETERS AND BLOOD METABOLITES IN LAMBS

Hossein-Ali Arab[1*], Alireza Mashhadi-Esmaeil[1], Mohammad Rezaeian[2] and Majid Mohtasebi[3]

[1]Department of Pharmacology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran, [2]Department of Animal Health and Nutrition, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran, [3]Razak Pharmaceutical Laboratories Company, Tehran, Iran.

[*]Corresponding Author: harab@ut.ac.ir

Received on: 29th July 2014 **Accepted on: 12th September 2014**

ABSTRACT

This study designed to test the effects of a probiotic containing *Bacillus subtilis* and *Bacillus licheniformis* (Bioplus 2B) on performance and blood parameters. 32 lambs were randomly divided into 4 experimental groups. One group was chosen as control and remainders received 0.25, 0.5 or 1gr Bioplus/kg of feed. The Body Weight Gains (BWG) and Feed Conversion Ratio (FCR) were determined each week, and bloods were taken to analysis hematological parameters and glucose, triglyceride, cholesterol, total proteins, albumin and urea changes. An increased BWG was shown in groups received 0.5 and/or 1gr (40.3±5.1and 39.0 ±6.6kg respectively) compared to the control (38.1±4.2kg) with a decrease in FCR from 7.94 (control) to 6.80 and 7.24 in those receiving Bioplus, respectively. Bioplus caused a significant ($p<0.05$) decrease in the level of red blood cells (RBCs), hemoglobin (Hb), Packed Cell Volume (PCV) and increase in Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin (MCH) levels. The level of total proteins, albumine, glucose, triglyceride and cholesterol were also significantly decreased in groups received 0.5 or 1g Bioplus. In conclusion, Bioplus can promotes animal performance by increasing BWG and decreasing FCR and improve health status and quality of animal products by changing the level of blood metabolites.

Key words: Bioplus, probiotic, lambs, glucose, cholesterol, triglyceride.

INTRODUCTION

Probiotics are generally defined as live and non-pathogenic microbial products which have different beneficial effects on host performance and health (Fuller, 1989; Gibson and Roberfroid, 1995). Increased Body Weight Gain (BWG), stimulation of immune responses and reduction of morbidity or mortality are the most beneficial effects of these products (Kabir et.al., 2004; Min et.al., 2004; Kritas et.al., 2006; Wang et.al., 2009; Riddell et.al., 2010). These feeding supplements are also useful for the consumers by improving quality and quantity of milk, meat and egg (Haddadin et.al., 1996; Kurtoglu et.al., 2004; Ceslovas et.al., 2005; Kritas et.al., 2006). Besides, probiotics have been recommended for prevention or treatment of many disorders; mainly gastrointestinal dysfunction and improving different body fluid components including blood metabolites. The beneficial effects of these microbial products are related to the improvement of intestinal microbial balance, feed conversion rate and the release of indigenous products (Kalbande et.al., 1992; Vandenbergh, 1993; Mcfarlane & Cummings, 1999; Andersson et.al., 2002; Bai & Ouyang 2006; Rioux & Fehdorak 2006).

The usefulness of *Lactobacillus* consumption as a useful method to improve the health status has been suggested for the first time by Metchnikoff (1907). Since then, many studies disclosed different effects of probiotics on animals and human beings. As such, there is evidence that the consumption of probiotics can change some blood metabolites including sugar and fat (Al-Salami et.al., 2008; Chiu et.al., 2006). For many years, it has been known that increased serum cholesterol can be as a risk factor for different cardiovascular complications including atherosclerosis and coronary diseases. Owing to the recent findings due to the beneficial effects of probiotics on health status, attention has been increased toward the use of these products as a new procedure to improve different disorders occurred because of the imbalance in the level of serum cholesterol and sugar. It is found that the oral administration of *Lactobacillus* decreased the level of cholesterol in hypercholesterolemic mice (Taranto et.al., 2003) and blood glucose in a noninsulin-dependent diabetic mouse model (Matsuzaki et. al., 1997). Al-Salami et al (2008) reported that the use of a microbial probiotic decreased gliclazide absorption in healthy rats, while it increased the absorption of the drug in diabetic rats. The administrated probiotic also induced a hypoglycemic effect on type 1diabetic rats when it was used at early stage of the disease (Al-Salami et.al., 2008).Furthermore, there is evidence that the concentration of haemoglobin and heamatocrit, red blood cell (RBC) count and phagocytic activity of leukocytes were significantly increased after the use of *Enterococcus faescium* strain in piglets (Strompfová et.al., 2006). Similarly, an increased white blood cells (WBC) count has been reported in birds given a diet supplemented with probiotics (Rahimi & Khaksefidi 2006).

These numerous evidence shows the potential beneficial effects of probiotics *in vitro* or mostly in laboratory animals and to a lesser extent in human beings. However, there are concerns about the validity of these findings if probiotics are used in target animals or healthy people. On the other hand, although the probiotics may have the beneficial effects on animals or human beings, they may alter the components of body fluids which may cause undesirable effects on the host (Boyle et.al., 2006). In addition, authorization to marketing of these natural products needs many studies to test the efficacy and safety of microbial contents in target species. This study aimed to examine the effects of a probiotic containing *Bacillus subtilis* and *Bacillus licheniformis* on performance, hematology parameters and different blood metabolites including sugar, cholesterol, triglyceride, total proteins, and urea in growing lambs.

MATERIALS AND METHODS

ANIMALS, HOUSING AND DIET

Thirty two male lambs 4-5 months-old were purchased from a commercial livestock center and housed in a closed pen. Research procedures were conducted in compliance with Institutional Animal Care and Use Committee guidelines. To be assured of animal health, a complete physical examination including general condition, respiratory and heart rate and rectal temperature, was performed on the animals before and during the experiments. The lambs were fed with a diet containing 176 g/kg DM barley straw, 132 g/kg alfalfa hay and 692 g/kg concentrate. The concentrate composition was (g/kg DM) barley 432, wheat bran 189, molasses 54, limestone 7, salt 3.5 and a vitamin-mineral premix 7. One kilogram of vitamin-mineral premix contained 500000 IU Vit. A, 100000 IU Vit. D, 0.1 g Vit. E, 0.19 g Ca, 90 g P, 19 g Mg, 50 g Na, 2g Mn, 3 g Fe, 0.3g Cu, 3 g Zn, 0.1 g Co, 0.1 g I and 0.001g Se. The foods were divided into two equal portions and were fed to the animals twice a day at 8:00 am and 16:00 pm. Water was offered for *ad libitum* intake throughout the trial. The amounts of feed refusal were recorded daily and intakes of each group were then calculated. Samples of feed ingredients were taken before commencement and during the trial, and the chemical compositions of each ingredient including crude protein (CP), ether extract (EE), crude fiber (CF) ash, calcium (Ca) and phosphorus (P) was determined. Table 1 shows the chemical analysis of the feed before consumption and those left at the end of each day.

Table 1- Chemical composition of different feed ingredients used as a normal diet in different experimental group. The analysis was performed before consumption and those left at the end of each day

		g/kg DM					
Feed ingredients	**DM**	**Ash**	**CP**	**Fat**	**CF**	**Ca**	**P**
Alfalfa hay	930.0	90.20	130.4	10.47	330.22	10.10	2.2
Barley straw	970.0	60.4	18.0	14.0	443.0	3.2	0.8
Barley grain	922.5	28.3	98.0	13.6	48.1	0.8	3.1
Wheat bran	891.6	50.5	145.0	16.2	85.3	1.1	6.5
Molasses	949.3	52.0	257.0	60.3	242.0	2.8	5.4

DESIGN OF THE STUDY

The lambs were divided into four separated groups (n=8) so that the total weight of each group was nearly equal (216 ± 0.5 kg). The first group (control) was fed with a normal diet, but the second, third and fourth groups received the normal diet plus 0.25, 0.5 or 1g of the probiotic/kg diet, respectively. The commercial name of the probiotic supplement used in this study is additive Bioplus 2B®. The active constituents of this product is a mixture of two strains of *Bacillus*, *B. subtilis CH 201* (1.6 billion colony-forming unite (CFU)/g) isolated from a soybean fermentation and *B. licheniformis CH 200* (1.6 billion CFU/g) isolated from soil. The product is intended for use with sows, pigs for fattening, chickens for fattening, turkeys for fattening and calves up to 6 months for fattening. Animals were weekly weighted and their weight gain was calculated from the difference between two consecutive weight measurements. Feed conversion ratio in each group was measured by the amount of total feed consumption divided by body weight gain.

BLOOD SAMPLING AND HEMATOLOGICAL AND BIOCHEMICAL ANALYZING

Blood samples were taken from each lamb at four stages; the first one was before the administration of the probiotic (on day 20) and the others were on days 25, 50 and 87 of the trial. Blood was collected by jugular venipuncture using EDTA-k and plain vacutainers and divided into two parts. The first part was used to measure total and differentiated white blood cells (WBCs), red blood cells (RBCs), hemoglobin (Hb), packed cell volume (PCV%), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). Hb was determined using a cyanomethoglobin method, packed cell volume (PCV %) by microhematocrit, centrifuge method and WBCs were estimated by hematocytometer. The second part of the blood samples were centrifuged at 2500g and the sera were obtained and kept at -20^{0}C until analyzing. Sera were used to determine the levels of glucose, triglyceride, cholesterol, urea and total proteins. The level of glucose was determined using the Eppendorf Auto analyzer (Epos5060). Triglyceride and cholesterol levels were measured by the enzymatic colorimetric method using the same autoanalyzer. The concentration of total serum protein (TP) was estimated by the Biuret method and the level of total urea was measured spectrophotometrically using urea assay reagent kits.

STATISTICS

Data obtained from various parameters are expressed as Mean±SD. Analysis of variance (ANOVA) procedure and a post hoc Duncan's test were used to compare the effects of probiotic on each parameter using a SPSS statistic program (Version 20). Significance was declared if $p < 0.05$.

RESULTS

BODY WEIGHT GAIN AND FEED CONVERSION RATIO

There was an increase in BWG and a decrease in the feed conversion ratio in lambs receiving the diet supplemented with 0.25 and/or 0.5 g/kg probiotic. The results of body weight gain, feed consumption and feed conversion ratio during the 86 day experimental period are summarized in table 2. As this table shows, while there wasn't any difference between total feed consumption in different groups, the BWG increase in lambs received 0.5 and 1g probiotic/kg feed (group 3 and 4) was higher than the others, although the differences were not statistically significant. The final BWG of the lambs in these groups was 40.30±5.10and 39.0 ±6.60 kg, respectively*;* while in the non-treated group (control) it was 38.10±4.20 kg. The changes in BW and BWG in Bioplus received groups are shown both individually and in groups (table 2). Ascending increase in the BWG of lambs used in different experimental groups is shown in figure 1. As this figure shows, there was a noticeable but not statistically significant, difference in BWG between lambs received 0.5 g Bioplus /kg feed with other groups in the second week thereafter. The reduction in feed conversion ratio was also noticeable in lambs of groups 3 and 4 received 0.25 or 0.5 g Bioplus/kg feed, respectively (7.24 and 6.80 vs. 7.94 in control). However, the differences in the BWG and feed conversion ratio in the group given 0.5 g of Bioplus was higher than the others

Table 2: The mean body weight, weight gain (BWG), total feed consumption and feed conversion ratio (FCR) of control group and those treated with 0.25g (group2), 0.5g (group3) or 1g (group 4) bioplus/kg of diet. The values are expressed as Mean ±SD obtained from at least eight lambs in each group

Test Parameters	Group 1 (control)	Group 2 (0.25g BP*/kg)	Group 3 (0.25g BP/kg)	Group 4 (0.25g BP/kg)
Initial BW (Kg)	27.1±3.5	27.1±3.8	27.1±3.3	27.0±4.5
Final BW (Kg)	38.1±4.2	38.1±5.7	40.3±5.1	39.0 ±6.6
Daily gain (g)	138	138	166	150
Feed consumption (Kg DM/d)	1.10	1.12	1.13	1.09
FCR (kg feed/kg gain)	7.94	8.10	6.80	7.24

*BP= Bioplus 2B

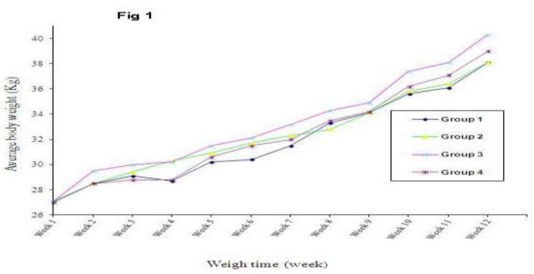

Figure 1: Ascending increase in the average body weight of different experimental groups over a period of 87 days trial. The group 1 was fed with a normal diet, but the groups 2, 3 and 4, received the normal diet plus 0.25, 0.5 or 1g of the Bioplus/kg feed, respectively

HEMATOLOGICAL PARAMETERS AND BLOOD METABOLITES

A summary of data obtained from hematology tests are shown in table 3. The level of total and differentiated WBCs was not significantly affected by the use of Bioplus in different treatment groups. However, the use of this probiotic caused significant changes in the level of most of the hematological parameters measured in the study. There was a significant decrease on the level of RBCs, hemoglobin and PVC in lambs which received 0.5 and/or 1 g bioplus/ kg feed (groups 3 and 4). However, the amount of MCV and MCH were significantly increased in those treatment groups (table 3).

The supplementation of Bioplus resulted in significant changes in the level of most of the blood metabolites except urea, measured in this study. The changes induced by Bioplus in the blood metabolites are shown in table 4 and figure 2. The results showed that the level of most of the tested metabolites including glucose, cholesterol triglyceride, total protein and albumin were significantly reduced in the lambs fed with a diet supplemented with 0.5 and/or 1g Bioplus/kg feed (groups 3 and 4). However, the differences between these groups were not statistically significant. No significant changes were shown in the level of blood urea in any groups of lambs given probiotic.

Table 3- Total (×10[6]/ml) and differentiated (in percent) white blood cells and hematology parameters in the serum of lambs of control group (group 1) and those received 0.25g (group2), 0.5g (group3) or 1g (group 4) bioplus/kg diet

Test parameters	Group 1 (control)	Group 2 (0.25g BP*/kg)	Group 3 (0.25g BP/kg)	Group 4 (0.25g BP/kg)
White blood cells				
Total WBC	10.82±3.7	12.29±4.1	11.61±3.3	11.60±3.4
Lymphocytes	64.25±15.3	63.37±8.4	63.43±11.2	61.40±12.7
Neutrophil	32.50±11.6	34.47±10.0	34.03±10.0	34.12±14.0
Monocytes	0.97±1.0	1.00±0.8	1.10±1.0	1.12±1.0
Eosinophil	1.20±1.0	1.40±1.0	0.80±1.0	1.10±1.0
Hematology				
RBC†(10⁹/ml)	9.74± 1.3[a]	8.86±1.1[ab]	8.69±0.9[b]	8.10±2.0[b]
Hb (g/dl)†	8.72±1.0[a]	8.23±1.0[ab]	7.95±0.9[b]	8.00±1.2[b]
PCV† (%)	28.61±3.3[a]	26.98±3.9[ab]	25.83±3.0[b]	26.07±4.3[b]
MCV†(fl)	29.18±1.8[a]	31.70±4.9[ab]	30.06±3.5[ab]	32.65±5.0[b]
MCH† (pg)	8.94±0.56[a]	9.66±1.35[ab]	9.36±1.26[ab]	10.13±1.58[b]
MCHC† (g/dl)	30.55±1.8	30.62±1.8	30.80±2.0	31.03±2.6

Values in the same row with different superscript letters (a, b…..x) are significantly different (p<0.05, n=8).*BP=Bioplus 2B †: RBC=red blood cells, Hb= hemoglobin, PCV= packed cell volume, MCV= Mean corpuscular volume (Mean volume of red cell), MCH= mean corpuscular hemoglobin (mean percentage of hemoglobin) and MCHC= mean corpuscular hemoglobin concentration (mean percentage of hemoglobin concentration)

Table 4 - Blood metabolites in different experimental groups of lambs used in the trial. The Values are expressed as Mean ±SD obtained from at least from eight lambs in each group and the values with different superscript letters (a, b…) in the same row are significantly different (p<0.05)

Parameter (Mg/Dl)	Group 1 (Control)	Group 2 (0.25g Bioplus/Kg)	Group 3 (0.25g Bioplus/Kg)	Group4 (0.25g Bioplus/Kg)
Glucose	72.71± 8.6[a]	65.31±9.4[a]	57.00±12.0[b]	56.31±12.0[b]
Triglyceride	24.67±6.8[a]	22.59±6.3[a]	17.09±5.6[b]	20.66±7.5[ab]
Cholesterol	65.27±10.08[a]	63.48.4±11.5[a]	53.50±10.6[b]	49.37±13.8[b]
Total protein	6.27±1.0[a]	6.15±1.0[a]	5.74±0.3[b]	5.58±0.34[b]
Albumin (mg/ml)	3.06±0.19[a]	2.90±0.22[b]	2.82±0.24[b]	2.73±0.25[b]
Urea	24.71±4.8	26..68±7.3	25.53±5.8	25.75±6.0

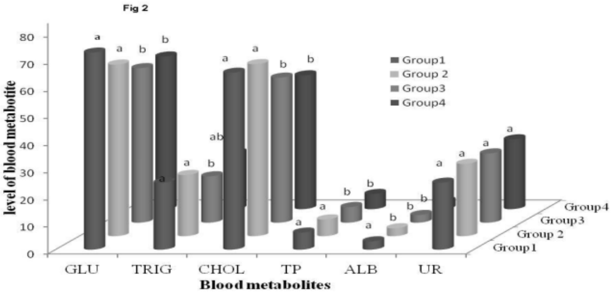

Fig 2: Changes in blood metabolites in different experimental groups of lambs used in the trial. Values in the same row with different superscript letters (a, b…..x) are significantly different (p<0.05). The group 1 was fed with a normal diet, but the groups 2, 3 and 4, received the normal diet plus 0.25, 0.5 or 1g of the probiotic/kg feed, respectively

DISCUSSION

The present study was designed to examine the effects of Bioplus 2B, a probiotic containing *Bacillus subtilis* and *Bacillus licheniformis*, on performance, hematological parameters and blood metabolites. The consumption of this probiotic did not cause any changes in the values of white blood cell counts in any group of lambs fed with diet supplemented with different doses of probiotic. However, the levels of RBCs, Hb, PCV, MCV and MCH were significantly changed in the group received 0.5 and 1g Bioplus /kg feed. While it seems that the consumption of probiotic improved the animal performance by increasing BWG and reducing FCR, it caused a significant decrease in levels of total protein, albumin, glucose, cholesterol and triglyceride in the serum of the lambs receiving 0.5 and 1g Bioplus. It was interesting that the effects of probiotics at a dose of 0.5g/ kg feed, the recommended dose by the manufacturer, was bigger than the other doses in terms of BWG and FCR alterations.

The improvement of lactose intolerance, decreasing fat level, increasing natural resistance, suppression of gastrointestinal disorders and the stimulation of growth and BWG are the potentially useful functions suggested for probiotics (Chiofalo et.al., 2004; Li et. al., 2006; Casey et.al., 2007; Samli et.al., 2007; Torres-Rodriguez et.al., 2007). Different mechaisms attributed to the beneficial effects of probiotics include antagonism to pathogenic organisms by production of antimicrobial substances, competition with the pathogen for adhesion sites or nutritional sources, stimulation of host defense mechanisms and inhibition of bacterial toxins (Vandenbergh, 1993; Bernet *et.al.*, 1994; Brandao RL, 1998; Walker, 2008). The use of probiotics is associated with the increased release of different endogenous substances including antibacterial substances, nutrients, antioxidants, growth factors and coagulating agents resulting in the stimulation of gut motility and modulation of innate and adaptive immune defense mechanisms (Bielke *et.al.*, 2003; Patterson and Burkholder 2003).

Data regarding the impact of probiotics on hematology and blood metabolites are controversial. Çetin et al (2005) reported that the use of probiotic supplementation in turkey induced significant increase in RBC count, hemoglobin concentration and haematocrit values. However, they did not find any changes in the total and differentiated leukocyte count in the sera of the animals receiving probiotic supplementation. The use of probiotics containing *L. acidophilus* and *L. plantarum* in 3-4 day old-male calves caused a risise but not statistically significant in the levels of Hb and PCV and WBC (Al-Saiady, 2010). Similar results are found in other species including goat kids and buffalo-calves fed with probiotic supplementations (Sayed, 2003; Bakr et.al., 2009). The results of the present study in terms of hematological parameters are consistent with some of the above mentioned findings. The reduction in the level of RBC, Hb and PCV may be indicative of anemia in the present study, but the increase in the level of MCV and MCH can contravene it. In addition, these changes are seen at the early stages of sampling, where lambs were exposed to a large number of live microorganisms, causing a semi septicemia and temporary anemia in lambs receiving probiotic. It seems that Bioplus 2 B not only caused any observes-adverse effects on hematological parameters, but also had the improvement effects on different blood parameters.

The improvement of blood metabolites is another aspect of beneficial effects of probiotic products attracted the interests of many investigators. The use of fermented milk to treat intestinal disorders and improving the health status goes back to the ancient times. Yoghurt is as a traditionally fermented dairy product containing different microorganisms including *Lactobacilli* present in digestive system as intestinal microflora. Gilliland et al (1985) showed that the administration of *Lactobacillus* to pigs fed with a high fat diet inhibited in serum cholesterol increasing. In a feeding trial carried out on the ewes in late pregnancy and lactation feeding, it was found that the application of Bioplus had positive effects on decreasing lamb mortality, milk yields, fat and protein contents (Wang *et.al.*, 2009). It is suggested that the absorption of cholesterol or deconjugating of bile salts by bacteria may be the possible mechanisms attributed to these functions (Klaver & Meer 1993; De Smet et.al., 1998). Short chain fatty acids produced by bacterial probiotic may also inhibit hepatic cholesterol synthesis and/or redistribution of cholesterol from plasma to the liver (Pereira & Gibson, 2002). There is evidence that the use of *Lactobacilli* or probiotics containing these microorganisms can alter the level of some blood metabolites including glucose and fats. It is reported that administration of probiotics to diabetic rats for 3 days inhibited the elevation of blood glucose. This hypoglycemic effect is suggested to be due to the bacterial stimulation of the gut and subsequently the release of insulinotropic polypeptides and glucagon-like peptide-l (Drucker, 2001 and 2007). However, Samanya and Yamauchi (2002) found that the concentration of blood glucose in chickens which consumed a diet supplemented with dried *Bacillus subtilis* did not significantly changed in comparison to the control group. However, the results of our study showed a significant reduction in the level of blood glucose in the lambs fed with the diet supplemented with Bioplus 2B. It seems that the lack of changes in blood glucose of chicken that received probiotic may be attributed to the experimental condition, the components of the probiotic and the species discrepancy. Rodas *et al* (1996) observed a hypocholesterolemic effect in pigs received large doses of *L. acidophilus* that were many times higher than the *Lactobacillus reuteri* doses (104 cells/d) used in hypercholesterolemic mice in their previous study (Taranto et al. 1998). Similar effects had been found by Grunewald (1982) in rats that received large amounts of fermented dairy products. However, Gilliland & Walker (1990) did not find any changes in blood fat of people who consumed probiotics. However, in the present study we showed that the level of glucose, cholesterol and triglyceride was significantly reduced in the blood of the lambs administered with 0.5 and/or 1g probiotic/kg feed.

The effects of Bioplus 2 B on some farm animals including pigs, calves and lambs have been tested by a few researches, but there is inconsistency in the results of these studies. Some have reported that supplementation of the diets of nursery pigs with Bioplus did not improve the growth performance (Kritas & Morrison 2004 and Min et al. 2004). Similarly, Wang et al (2009) reported that supplementation of diet with this probiotic did not significantly improve the growth performance in grower pig, but it could reduce the slurry NH3 emission. However, Gracia *et al* (2004) reported that pigs fed with the diet supplemented with 0.04% Bioplus 2B had promotion effects on the average daily gain and the average daily feed intake during both the prestarter and the finishing periods. It is also found that the addition of 0.05% Bioplus 2B to the finishing diet of pigs caused an improvement in the average daily feed intake, but had no effect on the average daily gain or the gain:feed ratio (Munoz *et.al.*, 2007).

This study showed that the administration of a bacterial probiotic containing *Bacillus subtilis* and *Bacillus licheniformis* with the trade name of Bioplus 2B, improved animal performance through increasing body weight gain and decreasing feed conversion rate in ambs. The use of this probiotic also promoted the health status and the quality of animal products by reducing the level of blood sugar, cholesterol and triglyceride in the treated animals.

ACKNOWLEDGEMENTS

We are grateful to the **Biochem Company** for supplying the probiotic and financial support.

REFERENCES

- Al-Saiady, M.Y., 2010. Effect of Probiotic Bacteria on Immunoglobulin G Concentration and Other Blood Components of Newborn Calves. *Journal of Animal and Veterinary Advances* 9:604-609
- Al-Salami, H., Butt, G., Fawcett, J.P., Tucker, I.G., Golocorbin-Kon, S., and Mikov, M., 2008. Probiotic treatment reduces blood glucose levels and increases systemic absorption of gliclazide in diabetic rats. *European Journal of Drug Metabolism and Pharmacokinetics* 33:101-6
- Andersson H., Asp, N-G., Bruce, A., Roos, S., Wadström, T., and Wold, A.E., 2002. Health effects of prebiotics and probiotics: a literature review on human studies. *Scandinavian Journal of Nutrition/Näringtorskning* 45:58-75.
- Bakr, H.A., Said, E.M., Abd El-Tawab M.M., and Hassan, M.S., 2009. The impact of probiotic (Biovet®) on some clinical, hematological and biochemical parameters in buffalo-calves. *Beni-Suef Veterinary Medical journal* 19:1-10.
- Bai, A-P and Ouyang, Q., 2006. Probiotic and inflammatory bowel disease. *Postgraduate Medical Journal* 82:376–382.
- Bernet, MF., Brassart, D., Neeser, JR., and Servin, AL. 1994. Lactobciluus acidophilus LA1 binds to culture human intestinal cell lines and inhibits cell attachment and cell invasion by enterovirulent bacteria. *Gut* 35:483-89.
- Bielke, L.R., Elwood, A.L., Donoghue, D.J., Donoghue, A.M., Newberry, L.A., Neighbor, N.K., and Hargis B.M., 2003. Approach for selection of individual enteric bacteria for competitive exclusion in turkey poultries. *Food Addit Contam* 82:1378–1382.
- Boyle, R.J., Robins-Browne, L.K., and Tang, RM. 2006. Probiotic use in clinical practice: what are the risks? *American Journal of Clinical Nutrition* 83:1256–64
- Brandao R.L., Castro I.M., Bambirra E.A., Amaral S.C., Fietto L.G., Tropia M.J., et al. 1998. Intracellular signal triggered by cholera toxin in Saccharomyces boulardii and Saccharomyces cerevisiae. *Applied and Environmental Microbiology* 64:564–568.
- Casey, P.G., Gardiner, G.E., Casey, G., Bradshaw, B., Lawlor, P.G., Lynch, P.B., et al. 2007. A 5-strain probiotic combination reduces pathogen shedding and alleviates disease signs in pigs challenged with Salmonella enterica Serovar Typhimurium. *Applied and Environmental Microbiology* 73:1858-1863.
- Ceslovas, J., Vigilijus, J.,and Almantas, S., 2005. The effect of probiotic and phytobiotics on meat properities and quality in pigs. *Veterinarija ir Zootechnika* 29:80-84.
- 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 Development* 44:449-457.
- Chiu, C.H., Lu, T.U., Tseng, Y.Y., and Pan, T.M., 2008. The effects of Lactobacillus-fermented milk on lipid metabolism in hamsters fed on high-cholesterol diet. *Applied Microbiology and Biotechnology* 71:238–245.
- Çetin, N., Güçlü, B.K., and Çetin, E., 2005.The effects of probiotics and mannanoligosaccharide on some haematological and ımmunological parameters in Turkeys. *Journal of Veterinary Medicine Series A* 52:263-267.
- De Smet, I., De Boever, P., and Verstraete, W., 1998. Cholesterol lowering in pigs through enhanced bacterial bile hydrolase activity. *British Journal of Nutrition* 79:185–194.
- Drucker, D.J., 2001. Mini review: the glucagon-like peptides. *Endocrinology* 142:521-527.
- Drucker, D.J., 2007. The role of gut hormones in glucose homeostasis. *Journal of Clinical Investigations*117:24-32.
- Fuller, R. 1989: Probiotics in man and animals. *Journal of Applied Bacteriology* 66:365–378.

- Gibson, G. R., and Roberfroid, M.B., 1995. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *Journal of Nutrition* 125:1401–1412.
- Gilliland, S.E., Nelson, C.R., Maxwell, C., 1985. Assimilation of cholesterol by Lactobacillus acidophilus. *Applied and Environmental Microbiology* 49:377–381.
- Gilliland, S.E., and Walker, D.K., 1990. Factors to consider when selecting a culture of Lactobacillus acidophilus as a dietary adjunct to produce a hipocholesterolemic effect in humans. *Journal of Dairy Sciences* 73:905–911.
- Gracia, M.I., Hansen, S., Sanchez, J., Medel, P., Imasde Agropecuaria, S.L., 2004. Efficacy of addition of B. licheniformis and B. subtilis in pig diets from weaning to slaughter. *Journal of Animal Sciences* 82 (Suppl. 1):26.
- Grunewald, K.K., 1982. Serum cholesterol levels in rats fed skim milk fermented by *Lactobacillus acidophilus*. *Journal of Food Sciences* 47:2078–2079.
- Haddadin, M.S.Y., Abdulrahim, S.M., Hashlamoun, E.A.R., and Robinson, R.K., 1996. The effects of Lactobacillus acidophilus on the production and chemical composition of hen's eggs. *Poultry Sciences* 75:491-494.
- Kabir, S.M L., Rahman, M.M., Rahman, M.B., Rahman, M.M., and Ahmed, S.U., 2004. The dynamics of probiotics on growth performance and immune response in broilers. *International Journal of Poultry Sciences* 3:361–364.
- Kalbande V.H., Gaffar M.A., and Deshmukh, S.V., 1992. Effect of probiotic and nitrofurin on performance of growing commercial pullets. *Indian Journal of Poultry Sciences* 27:116-117
- Klaver, F.A.M., and Van der Meer, R. 1993. The assumed assimilation of cholesterol by Lactobacilli and Bifidobacterium bifidum is due to their bile salt-deconjugating activity. *Applied and Environmental Microbiology* 59:1120-1124.
- Kritas, S.K.,and Morrison, R.B. 2004. Can probiotics substitute for sub therapeutic antibiotics? A field evaluation in a large pig nursery. Proceedings of the 18th IPVS Congress, Hamburg, Germany, Volume 2.
- Kritas, S.K., Govaris, A., Christodoulopouls, G., and Burriel, R. 2006. Effect of Bacillus licheniformis and Bacillus subtilis Supplementation of Ewe's Feed on Sheep Milk Production and Young Lamb Mortality. *Journal of Veterinary Medicine Series A* 53:170-173.
- Kurtoglu, V., F. Kurtoglu, E. Seker, B. Coskun, T. Balevi, and Polat, E.S., 2004. Effect of probiotic supplementation on laying hen diets on yield performance and serum and egg yolk cholesterol. *British Journal of Poultry Sciences* 21:817-823.
- Li, X.L., Yin, J.D., Li, D.F., Chen, X.J., Zang, J.J., and Zhou, X., 2006. Dietary supplementation with zinc oxide increases IGF-I and IGF-I receptor gene expression in the small intestine of weanling piglets. *Journal of Nutrition* 136:1786–1791.
- Matsuzaki, T., Yamazaki, R., Hashimoto, S., and Yokokura, T., 1997. Antidiabetic effects of an oral administration of Lactobacillus casei in a non-insulindependent diabetes mellitus (NIDDM) model using KK-Ay mice. *Endocrine Journal* 44:357–365.
- Min, B.J., Kwon, O.S., Son, K.S., Cho, J.H., Lee, W.B., Kim, J.H., Park, B.C., and Kim, I.H., 2004. The effect of Bacillus and active yeast complex supplementation on the performance, fecal Bacillus counts and ammonia nitrogen concentrations in weaned pigs. *Journal of Animal Sciences* 82 (Suppl. 1):26.
- Munoz, V.D., Lanz, A.G.E., Lucero, P.M., Soria, F.A., Renteria, F.J.A., Cuaron, I.J.A., Correa, N.S., Martinez, S., 2007. Strategies for enhancing microbiological gut's barrier: BMD y BioPlus 2B. *Journal of Animal Science* 85:150.
- Patterson J.A., and Burkholder, K.M. 2003. Application of Prebiotics and Probiotics in Poultry Production. *Poultry Science* 82:627–631.
- Pereira, D.I.A., and Gibson, GR. 2002. Effects of Consumption of Probiotics and Prebiotics on Serum Lipid Levels in Human. *Critical Review in Biochemistry and Molecular Biology* 37:259–281.
- Rahimi, S.H., and Khaksefidi, A. 2006. A comparison between the effects of a probiotic (Bioplus 2B) and an antibiotic (Virginiamycin) on the performance of broiler chickens under heat stress condition. *Iranian Journal of Veterinary R*esearch 7:23-28.
- Riddell, B., Gallegos, A.J., Harmon, D.L., and McLeod, K.R., 2010. Addition of a Bacillus based probiotic to the diet of preruminant calves: Influence on growth, health, and blood parameters. *Intern Journal of Applied in Research Veterinary Medicine* 8:78-84.
- Rioux, K.P., and Fedorak, R.N., 2006. Probiotics in the treatment of inflammatory bowel disease. *Journal of Clinical Gastroenterology* 40:260-263.
- Rodas, B.Z., Gilliland, and S.E., Maxwell. C.V., 1996. Hypocholesterolemic action of Lactobacillus acidophilus ATCC 43121 and calcium in swine with hypercholesterolemia induced by diet. *Journal of Dairy Sciences* 79:2121–2128.
- Samanya, M., and Yamauchi, K., 2002. Histological alterations of intestinal villi in chickens fed dried

Bacillus subtilis var. natty. *Comparative Biochemistry and Physiology-part A* 133:95-104.

- Samli, H.E., Senkoylu, N., Koc, F., Kanter, M., and Agma, A., 2007. Effects of Enterococcus faecium and dried whey on broiler performance, gut histomorphology and intestinal microbiota. *Archives of Animal Nutrition* 61:2-49
- Sayed, A.S., 2003. Studies on the influence of pronifer as a probiotics on the clinical, hematological, and biochemical status of the goat's kids. *Assiut Veterinary Medicine Journal* 99:131-143.
- Strompfová, V., Marcináková, M., Simonová, M., Gancarcíková, S., Jonecová, Z., Sciranková, L., Koscová, J., Buleca, V., Cobanová, K., and Lauková, A., 2006. Enterococcus faecium EK13--an enterocin a-producing strain with probiotic character and its effect in piglets. *Anaerobe* 2:242-8.
- Taranto, M.P., Medici, M., Perdigon, G., Ruiz Holgado, A.P., and Valdez, G.F. 1998. Evidence for hypocholesterolemic effect of Lactobacillus reuteri in hypercholesterolemic mice. *Journal of Dairy Sciences* 81:2336-2340.
- Taranto, M, P., Medici, M., Perdigon, G., Ruiz Holgado, A.P., and Valdez, G.F., 2003. Effect of Lactobacillus reuteri on the Prevention of Hypercholesterolemia in Mice. *Journal of Dairy Science* 83:401-403
- Torres-Rodriguez, A., Donoghue, A.M., Donoghue, D.J., Barton, J.T., Tellez, G., and Hargis, B.M., 2007. Performance and condemnation rate analysis of commercial turkey flocks treated with a Lactobacillus sp.-based probiotic. *Poultry Science* 86:444-446.
- Vandenbergh, PA., 1993. Lactic acid bacteria, their metabolic products and interference with microbial growth. *FEMS Microbiology Reviews* 12:221–38.
- Walker, W. A., 2008.: Mechanisms of Action of Probiotics. *Clinical Infectious Diseases* 46:S87–91.
- Wang, Y., Cho, J.H., Chen, Y.J., Yoo, J.S., Huang, Y., Kim, H.J., and Kim, I.H. 2009. The effect of probiotic BioPlus 2B® on growth performance, dry matter and nitrogen digestibility and slurry noxious gas emission in growing pigs. *Livestock Science* 120:35–42.