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Effect of *Sangrovit* on Performance and Morphology of Small Intestine and Immune Response of Broilers

Mohsen Karimi*, F. Foroudi and M.R. Abedini

Department of Animal Science, Islamic Azad University, Varamin - Pishva Branch, Iran.

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This study was designed to investigate the effects of *Sangrovit* on performance, small intestinal morphology, and immune response of 300 commercial hybrid broilers, Ross 308. The study was conducted in the Agricultural Research Station of Islamic Azad University, Varamin, in the spring 2010 for 42 days. The broilers were kept in a completely randomized design with 3 treatments, 4 replicates, 12 experimental units each containing 25 broilers. Treatments used in this experiment included controls, 0.05 % and 0.1 % *Sangrovit* of total ration. The test results showed no significant difference between different treatments during the breeding period on feed intake, feed conversion, and small intestinal morphology. However, treatments containing 0.1% *Sangrovit* showed significant differences on weight gain in the early rounds between the other treatments ($P<0.05$). Statistically, these differences were not significant during the breeding period. Regarding the effects of different treatments on the carcass components, the diet containing 0.05% *Sangrovit* could significantly increase the weight of the thigh ($P<0.05$) than the other diets. In this treatment, the mean serum antibody titers against Newcastle disease by HI method showed significant differences at 35 and 42 days of the breeding period ($P<0.05$). Based on the results of this experiment, *Sangrovit* can obtain the desired results in improving immune system, performance and carcass yield.

Key words: *Sangrovit,* Phytobiotic, Small Intestine Histology, Immune System, Broilers.

In the past few decades, antibiotics growth promoters have been popular due to their positive effects on weight gain, feed conversion and mortality. In January 2006, the Europe Union completely prohibited antibiotics growth promoters in livestock feed. Afterwards, antibiotics has been allowed in treatment feed or prophylactic additives as a medicine (Berghmann *et al*., 2005; Casewell *et al*., 2003; Mohan wt al., 1996). Since the past years, kinds of probiotics, prebiotics and Synbiotic have been used as microbial additives in animal feed. Phytobiotics are new generation of additives with plant origin (*Sangrovit*). The present study used a plant phytobiotic (*Sangrovit*, Phytobiotics Futterzusatzstoffe GmbH. Eltville, Germany) to investigate the mechanism of its effect on the performance of broiler in Iran. Isabel and Santos (2009) studied the effect of organic acids and herbal extracts (cloves and cinnamon) on broiler carcass characteristics. They found no influence on weight gain; while they found a significant improvement in feed conversion of organic acids or a combination of organic acid and plant extracts. These additives did not influence the carcass weight; however breast weight as a percentage of carcasses in the plant extracts was more than other groups.

Buchanan *et al*., (2008) demonstrated that herbal biostrong stimulants improved feed conversion and increased breast meat yield. Garcia

* To whom all correspondence should be addressed.
Tel.: +989122161868;
E-mail: mohsenkarimi62@gmail.com

et al., (2007) studied the effect of formic acid on plant extracts, cinnamon, pepper, oregano and Avilamycin antibiotics on the morphology of the small intestine, carcass yield and performance traits. These additives improved feed conversion and addition of formic acid increased the height of the intestinal villi. These additives had no effect on carcass yield. Studying broilers, Hernandez *et al*., (2004) found that plant extracts of thyme, cinnamon, pepper and rosemary enhanced the digestibility of dry matter and fat; however, it had no effect on digestibility of protein. Hosseini *et al*., (1387) studied the effect of probiotics containing Bifidobacterium and Streptococcus on broiler performance. Average live weight and feed conversion significantly improved in 0-21 days in the probiotic groups compared with the control group ($P<0.05$); however, this effect was not significant in the whole course of the experiment. Huang *et al*., (2004) studied the effects of Lactosyl and acidophilic fungus on the production performance and immune response of broilers. Evidence suggests that inactive probiotics can have beneficial effects similar to the live probiotics. The findings also suggests an increase in feed intake, body weight and feed conversion ratio.

Denli *et al*., (2003) investigated the effects of additives different from probiotic in the diet of broilers. The results showed that the group receiving flavomycin %0.2 + %0.15 genex main diet show weight gain. In addition, feed intake, carcass weight, and feed efficiency of the control group significantly increased compared to the other groups. However, live weight, intestinal pH and abdominal fat weight are not highly treated with probiotics, antibiotics and organic acids.

Kabir *et al*., (2002) studied the effect of probiotics in relation to live weight gain, carcass yield, meat cutting weight and immune response. The obtained results caused by breast weight gain, leg weight and increased spleen weight.

The purpose of this study was to investigate the potential influence of plant Phytobiotics (Sangrovit) on the performance, morphology of the small intestine and immune system of broilers and to identify a proper alternative for growth stimulator antibiotics in order to introduce the effectiveness of these additives to the feed mills as well as poultries.

MATERIALS AND METHODS

To determine the effect of phytobiotic on performance, small intestinal morphology, and immune response of broilers, this study used 300 Ross hybrid broilers (308). The study was conducted in the research farm of College of Agriculture, Islamic Azad University, Varamin in 2010 for 42 days. The broilers were randomly divided into 3 groups and 4 replicates in 12 experimental units with 25 broilers in each experimental unit in the completely randomized design; so that, 0.12 m^2 was considered for per broiler. Broilers of each experimental unit were fed using separate water and feeding with the diet related to their treatments from day 1. Water and feed was freely provided (Ad libitum) for broilers. Conditions of the hall was completely under control in terms of temperature, humidity and ventilation. In this study, the energy level was equivalent to the standard requirements of broilers recommended in the Ross 308 Manual. This study also used a brand phytobiotic commercially called as *Sangrovit* (the active ingredient of the poppy plume (MacleayaCordata)) in 0.05% and 0.1% in per ton. Tables 1 and 2 show diet composition and requirements of broilers during the starter (0-10 days), grower (10-22 days) and finisher (22-42 days)

1. Per Kilogram of vitamin premix contains: vitamin A 3600000 IU, D_3 800000, E 3600000 IU, K_3 800 mg, B_1 720 mg, B_2 2640 mg, B_3 4000 mg, B_5 12000 mg, B_6 1182 mg, B_9 400 mg, B_{12} 6 mg, H_2 40 mg, chloride 100000 mg, anti-oxidant 150 mg.
2. Per Kilogram of mineral premix contains: manganese 40000 mg, zinc 37000 mg, iron 20000 mg, copper 4000 mg, iodine 400 mg, choline chloride 100000 mg, selenium 80 mg.

Table 1 shows the composition of diets for starter, grower, and finisher periods separately for the three diets (for each treatment) in each column, as well as plant phytobiotic *(Sangrovit)* added to the columns below the table.

Evaluation of Morphological Characteristics of Intestine

Intestinal samples (jejunum) taken in the day 42 were fixed in the 10% formalin (by volume) and embedded in paraffin. Five-micron-thick serial

sections of jejunum were prepared on glass slides. Samples were deparaffinised by corroding solution; then, it was dehydrated in graded alcohol solutions. The specimens were collared by hematoxylin and eosin and examined by Leica system, Senso Additive GmbH. Weizlar, Germany and Leica Qween 550. Assessments included the length and width of the villi, crypt depth and goblet cell count in 250 microns (Tako *et al*., 2004).

Result Analysis

Statistical design was categorized in a completely randomized block with three treatments and four replicates by Excel software; then, analysis of variance was performed by statistical software MSTATC.The means were compared with polynominal Duncan test. Statistical modeling scheme was $X_{ij} = \varepsilon_{ij} + \tau_j + \delta_i$.where: X_{ij} = individual observations, ε_{ij} = error, τj = treatment effect, δi = effect of replicate, μ = population mean.

RESULTS

Results of data analysis are presented in Tables 3 to 6.

Average feed intake, weight gain and feed conversion in broilers have been reported in Table 3 for the starter, grower and finisher periods of the breeding period (0-42 days).The results obtained from performance of broilers showed that *Sangrovit* had no significant effect on average

Table 1. Composition of base diets for broiler chicks in 3 periods

Ingredient	Starter(0-10)			Grower(10-22)			Finisher(22-42)		
Corn	48.43	48.50	48.68	59.29	59.40	59.50	67.63	67.73	67.84
Soybean meal	44.09	44.06	44.10	34.18	34.16	34.14	26.92	26.90	26.88
Oil	2.97	2.49	2.71	2.44	2.40	2.37	1.45	1.41	1.38
DL-Methionine	0.27	0.27	0.27	0.23	0.23	0.23	0.21	0.21	0.21
L-LysinHCl	0.07	0.07	0.07	0.09	0.09	0.10	0.15	0.15	0.15
L-Threonine	0.00	0.00	0.00	0.02	0.02	0.02	0.03	0.03	0.03
DCP	2	2	2	1.77	1.77	1.77	1.64	1.64	1.64
Oyster	1.23	1.23	1.24	1.04	1.04	1.04	1.02	1.02	1.02
NaCl	0.33	0.33	0.33	0.34	0.34	0.34	0.35	0.35	0.35
Vitamin Premix	0.3	0.3	0.3	0.25	0.25	0.25	0.25	0.25	0.25
Mineral Premix	0.3	0.3	0.3	0.25	0.25	0.25	0.25	0.25	0.25
Sangrovit %0.05	0.05	0.00	0.05	0.00	0.05	0.00			
Sangrovit %0.1	0.10	0.00	0.10	0.00	0.10	0.00			

The figures are in percentage

Table 2. Feed nutrients in 3 periods(%)

Finisher(22-42)	Grower(10-22)	Starter(0-10)	Nutrients
3050	3000	2900	ME(kcal/kg)
18.01	20.17	23.81	Cp (%)
0.93	1.05	1.25	Lys (%)
1.07	1.26	1.52	Arg (%)
0.71	0.78	0.90	Met+Cys (%)
0.46	0.50	0.59	Met (%)
0.83	0.88	1.03	Ca (%)
0.41	0.44	0.49	P (%)
0.16	0.16	0.16	Na (%)
0.73	0.85	1.01	K (%)
1.42	1.56	1.77	Leu (%)
0.66	0.76	0.92	ILe (%)
0.18	0.22	0.27	Tre (%)
0.61	0.68	0.79	Thr (%)

Table 3. Means of feed intake, weight gain and feed conversion ratio in different periods

Age period					
Overall(1-42)	Finisher(22-42)	Grower(10-22)	Starter(0-10)	Treatments	Performance
4085	2943	920	222	Control	Feed intake
4224	3029	970	225	Sangrovit0.05%	
4177	2999	951	227	Sangrovit 0.1%	
1967	1421	425	121[b]	Control	Weight gain
2043	1463	452	128[ab]	Sangrovit0.05%	
2068	1469	466	133[a]	Sangrovit 0.1%	
2.08	2.07	2.19	1.83	Control	FCR
2.07	2.07	2.16	1.76	Sangrovit0.05%	
2.02	2.04	2.06	1.70	Sangrovit 0.1%	

a-b: Means in a column with a different common superscript are significantly different (P<0.05)

Table 4. Effect of different treatments on carcass characteristics (d 42)

Treatment	Carcass	Breast	Thigh	Liver	Abdominal fat	Gizzard	Heart
Control	72	25	19.8[b]	1.9	1.7	2.3	0.5
Sangrovit 0.05%	72	24	20.6[a]	1.8	1.6	2.3	0.4
Sangrovit 0.1%	72	23	20.3[ab]	1.9	1.7	2.3	0.5
SEM	0.33	0.72	0.17	0.04	0.16	0.12	0.03

a-b: Means in a column with a different common superscript are significantly different (P<0.05)

Table 5. Effect of different treatments on small intestine morphology characteristics (d 42)

Treatment	Villus length	Villus width	Crypt depth	Gablet cells	Villus length : width	Villus length : depth
Control	1253	188	197	25	6.78	6.58
Sangrovit 0.05%	1253	187	179	30	6.75	7.10
Sangrovit 0.1%	1326	181	181	30	7.31	7.24
SEM	113.96	9.64	14.16	2.15	0.65	0.67

a-b: Means in a column a common superscript are significantly different (P<0.05)

Table 6. Means of antibody titer blood HI method

Treatment	Age period				
	(d 15)	(d 21)	(d 28)	(d 35)	(d 42)
Control	1.5	2.5	5	4.9[b]	4.9[b]
Sangrovit0.05%	2.1	2.8	5.5	6[a]	6[a]
Sangrovit 0.1%	2.3	2.9	5.5	5.3[ab]	5.9[a]
	0.31	0.26	0.41	0.31	0.28

a-b: Means in a column a common superscript are significantly different (P<0.05)

feed intake and feed conversion ratio ($p<0.05$); however, the treatment containing 0.1% *Sangrovit* was significantly different from weight gain in the starter period ($P<0.05$). Although the difference was not significant in the total period.

As the results in Table 4 show, 0.05% *Sangrovit* significantly increased tight weight in days 1-42 ($P<0.05$); while no statistically significant difference was observed on other parts of the carcass ($P>0.05$).

Morphologic study of intestines in Table 5 shows that the addition of *Sangrovit* to the diet of broilers did not show significant differences in the morphology of the small intestine ($P>0.05$).

Table 6 compares the mean serum antibody titers against Newcastle disease by determining HI. There is a significant difference among different treatments in days 35 and 42 days of treatment with 0.05% *Sangrovit*.

Newcastle vaccines during the breeding period is used in the eleventh day as the Newcastle B1 vaccine using eye drops as well as dual oil Newcastle by subcutaneous injection and in the twenty-first day as La sota Newcastle vaccine as a beverage.

Mortality

The results show that phytobiotic (*Sangrovit*) diet has no significant effect on mortality among broilers fed by the diet. In studies conducted by other researchers (Antogiovani, 2005; Baurhoo *et al*., 2007; Khan *et al*., 2006; Lesson *et al*., 2006; Odea *et al*., 2006; Teo and Tan, 2006), no significant differences was found in mortality rates between control treatments and the treatments containing butyric acid, prebiotic, antibiotics, organic acids, probiotics and formalin.

DISCUSSION

The results obtained in the Table 3 on performance of broilers show no significant difference in feed intake, weight gain and FCR at the end of the period. In most of the studies conducted by Hossain *et al.,* (2008) and Denli *et al*., (2003); Ignatova *et al*., (2009); Isabel and Santos (2009); Midilli *et al*., (2008); Mohan *et al*., (1996) on broiler performance, improvement was not significant which is consistent with results of this study. Biernasiak and Slizewska (2009) studied the effect of a new probiotic combination on the performance and faecal microflora of broiler. Final body weight and feed conversion in all groups were on average 2.4 kg and 1.63 kg, respectively. The body weight gain and feed conversion were not influenced by supplement added. Testing broilers, Yeo and Kim (1997) found no significant difference in feed intake by adding 0.1% *Lactobacillus* casei as a probiotic. The results obtained for the effect of different treatments on carcass components at day 42 days showed no significant effect on carcass yield, breast, liver, abdominal fat, gizzard and heart. While the percentage of thigh weight to body weight showed a significant difference between different treatments in the present experiments; however, treatment with 0.05% *Sangrovit* accounted for the highest proportion. In the trial by Garcia *et al*., (2007), the treatment with organic acids led to more thigh weight than the control and Avilamycin treatment, which is consistent with Table 4; because treatments with 0.05% *Sangrovit* led to more thigh weight than control and 0.1% *Sangrovit*. This difference was also significant ($P<0.05$). Denli *et al*., (2003) studied the effects of probiotic-separated supplements in the diet of broilers. Receiving main diet with genex % 0.2 +% 0.15 flavomycin, abdominal fat weight was not highly under influence of probiotics, antibiotics and organic acid treatment; this was consistent with the results of the present experiment.Incontrast, Hensa *et al*., (2009) showed that effects of kefir as a probiotic on growth performance and carcass characteristics of geese caused a gradual increase in the amount of abdominal fat, which is inconsistent with our results. Mark *et al*., (2009) conducted a study to evaluate the effect of dietary supplement, *Sangrovit*, on liver function, blood parameters and intestinal bacteria on Tilapia fish; their results showed a positive influence on the growth performance of tilapia fish, but no significant influence on liver function and health status.

The results obtained from the small intestinal morphology of broilers showed that adding plant phytobiotic (*Sangrovit*) to diet of broilers had no significant effect on different weeks of breeding. Owen *et al*., (2008) showed that the treatments containing prebiotic (MOS[1]) and antibiotics (Avilamycin) led to greater villus length than control treatments; these differences were not significant. On the width of villus, however, Owen *et al*., (2008) found that the greatest width of the

villus was related to treatment with antibiotics and there was no difference between treatments containing prebiotic and control treatments. On the crypt depth, the lowest depths was related to treatments with Avilamycin; however, there was no significant difference between control and mouse-containing treatments. Although the difference between Avilamycin treatment and these two treatments was not statistically significant. Roth *et al*., (1998) also showed that essential oils and Avilamycin caused no changes in mucosal morphology in the jejunum, ileum and large intestine (villus, hole width); actually any of these two additives had no effect on intestinal mucosal tissue. This is consistent with experimental results of the present study.

Ceslovas *et al*., (2005) showed that receiving probiotics is associated with the beneficial effects on the immune system, including improved resistance to diseases and a reduced risk of allergies. Probiotic in organisms of a healthy animal stimulates the non-specific immune response and improves immune protection system. Rowghani *et al*., (2007) studied the effects of a probiotic and other feed additives on performance and immune response of broilers. Experimental treatments were added to the basic diets (starter and finisher) as a control, 0.15% probiotic, 0.1% Toxiban, 0.1% Formycin, mixed 0.15% probiotic and 0.1% Toxiban. These additives, except Toxiban, significantly increased blood antibody Newcastle ($P<0.05$). Regarding the influenza antibody titer, there were significant differences among treatments, except Formycin. Only probiotic significantly increased serum bronchitis antibody titers ($P<0.05$).

CONCLUSIONS

Considering the information obtained from different treatments as alternatives to antibiotic growth stimulators in the present experiment and taking into account the performance, small intestinal morphology and immune system, it can be concluded that *Sangrovit* improves immune system, carcase yield and weight gain in the starter period.

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