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The Effects of Probiotics and Food Restriction on Relative Growth and Serum Cholesterol and Triglycrides Contents in Broiler Chickens

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Abstract: This study has investigated the effects of addition of probiotic supplements to two types of food restriction programs on ascites, RV/ TV ratio and relative growth of heart also has determined the effects of probitic on total cholesterol and triglycerides concentrations in the blood serum. The first treatment accessed food freely. The second one had a skip-a-day restriction. In the third treatment, there was probiotic added to the second treatment. The fourth one, included bellyful feeding and the fifth one was the bellyful feed with probiotic supplement. Total cholesterol (Chol) and triglyceride (TG) were measured in blood samples of day 45. Results revealed that relative growth of heart reduces as the age increases. As the age goes up, the RV/ TV ratio raises as well and in case of food restriction diets, bellyful feeding may decrease the mortality caused by ascites. It should be noted that adding probiotic in the restriction period, reduced the ascites and overall casualties ($P \leq 0.05$). The amount of total Chol and TG in the serum did showed a significant decline ($P<0.01$) in all dietary groups with probiotc.

Key words: Probiotic · Food Restriction · Heart · Ascites · Cholesterol

INTRODUCTION

To date, various kinds of Antibiotics were used in poultry industry in order to prevention of infectious diseases. In some countries, the usage of antibiotics has been forbidden because of some problems caused by lavish usage of antibiotics like bacterial resistance Probiotics are microbial supplements which can improve host body by microbial balance of intestine. To date, probiotics are one of major food supplements for poultry industry [1].

Numerous studies have found food restriction programs on broilers helpful against metabolic diseases like ascites, sudden death, carcass problems etc. resulted from a swift rate of growth [2-6]. Application of these restriction techniques bring about stress [7, 8] and have unfavorable effects on immune system [9]. There are reports that probiotics can reduce the cholesterol level of blood in broiler chickens [10,11]. Panda *et al.* (2003) reported that probiotics cause the reduction of serum and yolk cholesterol and also increase of egg production [12].

The present study tried to evaluate the effects of probiotic supplements in two types of food restriction regimes on ascites and RV/ TV ratio and we investigated the effect of probiotic on serum cholesterol and triglyceride.

MATERIALS AND METHODS

A total number of 600 hybrid male broilers of Ross 308 was used for the study. After bringing the chickens to the salon, a 5% solvent of sugar was given to them for 10 hours. On the second day, they were weighed and were put into 20 groups of 30 according to their average weight (48.6 ± 0.5). Then they were divided into 2×1.5 meter spaces. The temperature of the salon was 32 ± 1.5 degrees centigrade on the first day but the first week's average was 31 ± 1 degrees. In order to develop ascites, the temperature was brought down to 25 ± 1 degrees on the 10^{th} day. Later on the 19^{th} day, it was reduced down to 21 ± 1 and on the 25^{th} day, it was 17 ± 1 degrees centigrade [10]. The 17 ± 1 degree temperature continued until the end of the raising. The lighting was done 24 hours a day and the other conditions were kept as normal. The following treatments were applied:

- Group 1 (T1) was the control group and had a free access to food.
- Group 2 (T2) was kept hungry from the 10^{th} and 12^{th} day up the next days.
- Group 3 (T3) was kept hungry like group 2 but probiotic was added to their water on the restriction days.

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- Group 4 (T4) went under bellyful feeding. They had 2 meals a day. For the remaining 15 hours of the day, they were under food restriction.
- Group 5 (T5) was like T4 but in the food restriction hours probiotic was added to their drinking water.

The composition of the diets in different stages is shown in Table 1. The probiotic used in the test was *L. casei*, *L. acidophilus*, *Lactobasiloos Plantarum*, *Lactobasilos Delbroki*, *Lactobasilos Acidofilos* and *Lactobasilos Ramnosos*. The broilers recieved 1 miligram in 1 litre of drinking water. At the end of the days 15, 25, 35 and 45, four chickens were randomly selected (after weighing [16] chickens out of each treatment) [16] making an overall 80 and were sent to slaughter. After the slaughter, the chickens' hearts (after removing the vessels and fat) were weighed. The criteria for measuring the heart's relative weight was: the heart's weight / the chicken's weight × 100. On the days 35 and 45 the weight of the left and right ventricles were measured to find the RV/TV ratio. The mortalities of each day were sent to autopsy within 12 hours and the hearts were cut to find the deaths caused by ascites. In cases with a RV/TV ratio more than 0.3, the mortality was supposed to be caused by ascites. All the collected data were analyzed through the SAS (1997) software and the comparison of the averages were done through Dunken method. After 45 days, one chicken from each group randomly selected for blood sampling. Serums were used for further experiments.

Table 1: Ingredients and chemical analyses composition of the starter and grower diets

Ingredients (g/kg)	Starter	Grower
Maize	557	300
Wheat	--	330
Soybean meal	370	300
Soybean oil	30	40
Fish meal	20	--
Limestone	10	--
Oyster shell	--	12
Dicalcium phosphate	5	15
Vitamin-mineral mix[2]	5	5
dl-methionine	1	1
Sodium chloride	2	2
Vitamin E (mg/kg)	--	100
Analyzed chemical composition (g/kg)		
Dry matter	892.2	893.5
Crude protein	222.3	200.7
Fat	62.4	62.9
Fiber	36.1	35.6
Ash	61.7	57.0
Calcium	8.22	8.15
Phosphorus	5.48	5.57
Selenium (mg/kg)	0.53	0.58
ME by calculation (MJ/kg)	12.78	12.91

Starter diet fed to birds from 0 to 21 days. [2]Provides per kilogram of diet: vitamin A, 9,000 IU; vitamin D3, 2,000, IU; vitamin E, 18 IU; vitamin B1, 1.8 mg; vitamin B2, 6.6 mg B2,; vitamin B3, 10 mg; vitamin B5, 30 mg; vitamin B6, 3.0 mg; vitamin B9, 1 mg; vitamin B12, 1.5 mg; vitamin K3, 2 mg; vitamin H2, 0.01 mg; folic acid, 0.21 mg; nicotinic acid, 0.65 mg; biotin, 0.14 mg; choline chloride, 500 mg; Fe, 50 mg; Mn, 100 mg; Cu, 10 mg

RESULT AND DISCUSSION

According to Table 2, on the 15th day, the relative weight of the heart in the treatment with bellyful feeing and the treatment with a bellyful feeding and probiotic supplement (T4 and T5) was significantly different from the treatment with a skip-a-day feed with probiotic (T3) ($P \leq 0.05$) and the highest weight was for the T4 and T5. On the 25nd day, the relative weight of the heart does not differ greatly. On the day 35, the broilers with a higher amount of probiotic feed had higher relative weight of heart and the control group possessed the lowest weight of heart compared to other treatments (1.09) so that its difference with the T5 was meaningful. It could be said that as the age grows, relative weight of the heart decreases and as it is seen in Table 2, the trend of the relative weight of heart is a decreasing one. It seems that after the 35nd day, the growth of the relative weight of the body becomes faster than the growth in the relative weight of heart. According to Table 3, RV/TV ratio on the 35nd day was shown among the treatments, in T4 and T5 were higher than the other groups ($P \leq 0.05$). Among them, the control group had the lowest ratio. On the 35nd day, it seems as if the trend of changes in this ratio has a specific direction. It could be inferred from this trend that, since the relative weight of heart in treatments with bellyful feeding was more than the other treatments on the 15th, (and this may be because of the long period of feeding originated by the retarded growth of body organs causing higher metabolic activities in the body for it had been observed that while food restriction diets are applied [13, 14] and right after them [4] the level of serum thyroid hormone in T4 increases significantly) and because high metabolic activity needs higher amount of oxygen, this may have necessitated the larger relative weight of heart. It had already been reported that the heart weight grows as a percentage of the overall body weight [15, 16]. Other researchers have not reported this effect after the food restriction regimes and between the 5th and 12th days but they have reported that high ratios of RV/TV and the increased rate of growth of liver, heart and lung in birds

Table 2: Effect of meal feeding and probiotics on growth of heart in different days

Days	T1	T2	T3	T4	T5
15	1.56[a]	1.62[a]	1.38[ab]	1.62[a]	1.69[a]
25	1.11[a]	1.21b	1.32[b]	1.65[ab]	1.71[ab]
35	1.09[a]	1.17[a]	1.21[a]	1.39[b]	1.41[b]
45	0.71	0.82	0.89	0.81	0.83

Dissimilar indexes have significant differences in 5% level

Table 3: The changes in RV/TV ratio from the 15th day to the 45th days

Days	T1	T2	T3	T4	T5
15	0.16	0.18	0.19	0.19	0.21
25	0.21	0.22	0.23	0.22	0.24
35	0.22[a]	0.23[a]	0.24[a]	0.30[b]	0.31[b]
45	0.39[ab]	0.25[a]	0.25[a]	0.31[b]	0.31[b]

Dissimilar indexes have significant differences in 5% level

Table 4: Percent of total and ascites mortality between different groups

Mortality	T1	T2	T3	T4	T5
Ascites mortality (%)	1.6[a]	5.8[b]	1.6[a]	14.1[ab]	0[a]
Total mortality (%)	4.1[a]	8.3[b]	5.8[a]	19.1[ab]	2.5[a]

Dissimilar indexes have significant differences in 5% level

Table 5: Effects of diet supplemented probiotics on cholesterol and triglycerides levels

Groups	Triglyceride	Cholesterol
T_1	101.22[a]	199.76[a]
T_2	98.00[a]	188.75[a]
T_3	73.04[b]	161.00[b]
T_4	87.98[a]	181.23[a]
T_5	66.38[b]	152.57[ab]
SEM	9.26	9.058

P-value * *

[a-b]Values in the same row and variables with no common superscript differ significantly.*: P<0.05

suffering ascites may be caused by larger right ventricle and edema in viscera organs [17]. The RV/TV ratio growth with aging has an increasing trend (Table 3). The important point worth mentioning is that on the days between the 35nd and 45nd, the RV/TV ratio in the control treatment was the lowest (0.16) among all other treatments at first and became the largest (0.39) on the 45nd day. This high increase in control treatment compared to the experimental groups may be a clue for the highly active physiologic system of the body and the overactive heart in parts of the remedial growth period. Thus, it could be said that on the 35nd day, treatments under food restriction had the largest RV/TV ratio in contrast to the others and after the 45nd day, the heart returns to its normal activity and this means that this ratio in control group is higher than other treatments. Further studies should be conducted to reveal the changes in the ratio over the whole growth period and the effects dieting regimes have on it.

Table 4 shows that there were big differences between groups. The control group had a less total mortality rate compared to other treatments (except for treatment 5). The percent of mortality in treatment T5 with a one-week probiotic supplement beside its food restriction program is 2.5. Bellyful feeding has increased overall mortality but the probiotic supplement has decreased it. In other words, addition of probiotic is effective only if the birds are under stress for several reasons including weak growth management and poor environmental condition. Other experiments [18] have reported that in spite of the decrease in mortality caused by probiotic supplement, the main reason for this is the stable condition for the broilers' growth [19]. Comparison of the control group and experimental groups experiencing food restriction regimes demonstrates that mortality goes up as the food restriction is imposed and as the length of this program increases, the mortality caused by ascites grows as well. Approving this, Jones [20] reported an increase in ascites with food restriction program. He used hematologic data from the birds to examine his theory. However, his finding contradicts results of similar studies [21, 22]. In the present study, temperature decreased right after the restriction regime ended in order to create ascites. This decrease in temperature reduced the metabolic activity. Experiencing this downfall right at the time when birds' physiologic system has increased its activity to gain more oxygen [8, 23] to make up for basic metabolism, caused ascites mortality. Ozkan et.al. reported such a result and found that food restriction from the 5th to the 11th day reduces overall mortality and ascites mortality in broiler chickens. In other words, simultaneity of the decrease in the temperature and the increase in the growth rate of the birds after the food restriction may cause lack of oxygen and development of ascites. Besides, they found the relative weight of heart, lung and liver in chickens suffering ascites (RV/ TV > 0.28) to be more than healthy chickens and that the food restriction program did not have any effect on the above-mentioned features.

The control group and experimental treatments receiving probiotic while food restriction period (T3 and T5) have afew ascites mortality but, treatments receiving no probiotic in the food restriction regime (T2 and T4)

had 5.8 and 14.1% ascites mortality. Since the number of birds tested was not big and the rate of mortality caused by ascites was not large, no statistical analysis was conducted about them so a definite conclusion seems to be illogical. Cuevas *et al.* conducted a similar research and found that overall and ascites mortality in treatment shaving no food restriction was larger and probiotic supplement reduced the rate significantly. They reported that using probiotic decreases urease activity in the broiler chickens' digestive system [24, 25]. The fall in the urease activity of the digestive system, especially in early ages, improved the birds' overall health, affected its growth positively [26- 27] and reduces ascites [28, 29]. Finally it is suggested that if the food restriction program is to reduce ascites and mortality caused by it, the stress of this program should be lowered as well. One of the outcomes of probiotic supplements is reducing stress. There is a possibility that this hinder in probiotic activity is resulted from a simultaneous food restriction and cold salon. So, probiotic supplements may have a positive effect in case4 there are low levels of stress.

The cholesterol level of serum significantly decreased in groups supplemented with probiotics in compared to the other groups (Table 5). There are many reports that are in agreement with presented results in the current study. *L. acidophilus* is capable to deconjucate glyco cholic and taurocholic acids under anaerobic condition [30]. Deconjucation of gallbladder acids in small intestine can affects control of serum cholesterol, while deconjucated acids are not capable to solve and absorb fatty acids as conjucated acids.As a consequence, they prevent from absorption of cholesterol. Also free gallbladder acids attach to bacteria and fibres and this can increase the excretion of them.

There is a significant decrease in the serum level of triglycerides between groups treated with probiotics supplemented in broilers diet in combination with water or alone. Moharrery *et al.* [31] reported that fat digestion rate is linked to rate of gallbladder acids in digestion latex and subsequently the lipid concentration. probiotics in diet or water cause a decrease in gallbladder acids in digestion latex and this resulted in a reduction in ability of fat digestion and therefore decreasing lipid level of blood [32].

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