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Research Article

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The effect of probiotics, organic and mineral on some blood serum biochemical parameters of Japanese quail

Hamidreza Khodaei

Department of Animal Science, Golpayegan Branch, Islamic Azad University, Golpayegan, Isfahan, Iran

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ABSTRACT

Probiotics are organisms that by improving conditions for the growth of intestinal bacteria, useful and antagonistic effects against harmful bacteria exert their effect. Evaluate the effects of probiotics, organic and inorganic based on some biochemical parameters in serum Japanese quail. A total of 160 randomly selected quails and chickens were raised over a period of 42 days. The experimental groups: 1: no probiotics, oral injection of normal saline (negative control), 2: no probiotics, oral administration of saline contaminated with Salmonella interic (positive control), 3: Contains probiotics, oral injection of normal saline (negative probiotics), 4: contains probiotics, oral administration of saline contaminated with Salmonella interic (positive probiotics), respectively. Blood samples to measure antibody titer of Newcastle and Gumboro and to determine white blood cell count and serum cholesterol concentration was performed on 21 and 42. SAS statistical software for statistical analysis and a statistical method completely randomized design was used. The results of this study showed that weight gain can indicate that the immune system is strengthened. Some safety parameters chickens that have been exposed to Salmonella infection is increased, which indicates immune system.

Keywords: probiotics, Salmonella, safety systems, quail

INTRODUCTION

One of the objectives in poultry, to prevent the occurrence of the diseases is through proper implementation of insecurity programs and if defects arise in the implementation of Insecurity and patients, Should know that the avian immune system are desirable and are able to defend itself against diseases. At this stage the efficiency of the immune system is to reduce losses. Some non-genetic factors such as diet can be of some nutrients including minerals, Expression of genes responsible for immune response by altering the maturation of the immune system and antibodies against infection rate of change [1].Among these nutrients, minerals such as zinc (Zn) play an important role in the immune system. Is a very great and important physiological function of the body? Zinc is found in all animal tissues. Zinc has many functions in the body in a variety of roles cofactor for many enzymes to control the expression of genes responsible, and its deficiency causes slow growth, high hatchability weak, enlarged knee, flaking of the skin, usually on the legs and feet short and thick bones and increase the hematocrit and delay in growth in poultry. Indirect effects of zinc deficiency on erythrocyte membrane composition and stability have been reported [2]. Diets containing low levels of lead are reduced appetite, which reduces food intake and body weight is. It is known that zinc deficiency leads to increased susceptibility to infectious diseases against which indicates the importance of the immune system [3]. Zinc deficiency can lead to different levels of host defense defect in the body's first defense barrier of the skin to be humeral and [5].Researchers [4], the effects of different levels of zinc and immune function in broilers were investigated. For this purpose, three treatments of 34, 68, 181 mg per kg, zinc was used. Their studies showed that different levels of zinc supplementation had no significant effect on growth performance levels and apparently this was not enough to affect feed intake or weight gain. Today, because of the sense of danger that consumers of livestock products associated with microbial resistance to antibiotics has been seen, a lot of research to find suitable alternatives to antibiotics has been done [6]. The probiotics were considered

over the rest of the diet, including additives that have been recognized by European countries [7]. Probiotics are organisms that by improving conditions for the growth of beneficial bacteria in the intestine and antagonistic effects against harmful bacteria exert their effect. The antagonistic effect of a decrease in pH environment of the gastrointestinal tract, of the lactic acid, acetic acid and other compounds inhibiting growth of harmful bacteria and their toxic secretions [8]. The aim of this study was to investigate the effects of probiotics, organic and inorganic based on some biochemical parameters in serum Japanese quail.

EXPERIMENTAL SECTION

The project consists of two phases field work was conducted in farm and laboratory activities. The study consists of two phases in the farm field operations () and laboratory activities (Laboratory Animal Science Faculty of Agriculture, Tehran University Golpayegan branch, private laboratories and optics doctor Jalayer in Isfahan Science and Research Branch of Tehran) were carried out. 160 quail were prepared. Corn and soybean meal based diets with software WUFFDA set. The chicks were fed diets prepared. Since the introduction of chickens to the hall to the Seventh-day period of breeding, diet opening, the eighth day of the period of breeding the twenty-fifth day, diet, growth, and from 26 to 42 days the period ending breeding, diet final the chickens were. Baltic is now the most commonly used probiotics additives were used as material. Type is used as a probiotic Bacillus subtitles. The amount of probiotics, according to company catalogs 200 grams per ton (0.30 percent).

- Experimental groups and design: A total of 160 chicks, quail a day with factorial design 2 × 2 (including both free diet or oral administration of probiotics and two levels of Salmonella or normal saline) on the basis of completely randomized design into four groups (treatments) and each group of four replications (10 chicks per treatment) were divided. Experimental groups were: 1: diet without probiotics, oral injection of normal saline (negative control), 2: diet without probiotics, oral administration of Salmonella-contaminated saline (positive control), 3: diet with probiotics, oral administration of serum Physiology (negative probiotics), 4: diet with probiotics, oral administration of saline contaminated with Salmonella (positive probiotics), respectively.

- Salmonella growing and feeding it to the chickens: The project is part of the water was contaminated with the bacterium Salmonella to the number of PTCC (Persian Type Culture Collection) Culture 1709. The bacteria were cultured in the laboratory of microbiology and after counting and dilution to 20 ml of dilution of 105 chicks all positive control and the experimental groups were fed probiotics positively on a mandatory basis and the Sampler. Was selected for counting bacteria broth liquid medium. It is necessary to count the bacteria from the culture medium used solid. Two types of solid culture media were used: Basic nutrient agar medium (N.A), Salmonella, Shigella specific medium agar (SS.Agar). Preparation of solid nutrient agar medium: Five grams of powdered nutrient agar amount to 250 ml of distilled water to a final volume of completed, the solution was heated to boil, then closed the door and was autoclaved. To avoid the accumulation of vapors in the door petri dish, the solution cooled to a temperature of 45 degrees Celsius and then was poured into Petri dishes. Salmonella Shigella Agar specific medium: 12 g Salmonella Shigella agar powder with 200 ml of distilled water and brought in an autoclave for 15 minutes was 121 degrees Celsius. After sterilization boiled and cooled to a temperature of 45 degrees Celsius and poured into sterile Petri dishes and the refrigerator was moved 4 degrees Celsius. After preparing the petri dish for carefully counted three times (two general media and a special culture medium cultivation of bacteria was performed. Eleven were considered petri dish for each inter action, the total (11 × 3 = 33) petri dish that eleven Salmonella Shigella and the rest of the medium was nutrient media. Each 1.0 ml of the test tube to each of the dishes were inoculated related to that number, and by a curved rod is uniformly distributed in the solid medium; All these steps were taken under the hood Microbiology. For example, in the first three petri dish (a number of SSA and two NA) of the number one test tube is diluted 1 to 10, 1.0 ml and 1.0 mm up to medium SSA L to each of the repeated NA was inoculated culture medium and the medium was broadcast with curved rod. After inoculation medium and close all doors Petri dishes for 48-24 hours at 37 degrees Celsius, respectively. Practice counting was done using a grid. On the eleventh day breeding period, for force-feeding of Salmonella in chickens, saline 9.0% was developed: some distilled water is poured in 100 ml Erlenmeyer flasks and 9.0 grams of sodium chloride in the magnetic stirrer added to the flask and after the solution was brought to a volume of 100 ml. Salmonella of the following methods were used for dilution and administration: Falcon prepare several tubes of 9 ml of saline in each shed 9.0 percent. The pipes numbers 107, 106, 105, 104 entries with pipette solution containing salmonella broth; 1 ml was removed and added to a Falcon 107 Number of pipeting action was to be a uniform solution. Falcon 107, 1 ml removed, and the next Falcon tube (106) and pipeting was added, this was repeated. 105 dilutions with 20 ml pipette away all the chickens in group tested positive (positive control and positive probiotics) were given oral Nodal. After killing the first series with 100 ml of diluted Sampler 107 (9 ml physiological serum + 1 ml of broth containing Salmonella) orally to all groups of chickens were tested positive.

- Slaughter and sampling: On 21 and 42 breeding period randomly selected from each replicate a chick, weight and were killed by decapitation. On the tenth day of the period of rearing a chick under the wing vein of each pen were selected randomly by 1.5 ml of blood were taken from each bird, Blood in the syringe at 4 degrees Celsius were to separate serum. By Sampler serum isolated and poured into ependorf in (3000 rpm) was centrifuged for 10 minutes. The salmonella sampling was done before giving up a basis for comparison of headline immunoglobulins and the amount of white blood cells, is. Preparation of plasma to measure white blood cell count, randomly selected and blood samples were taken from each replicate was a chick. To prevent blood clots from reaching the lab, before blood was drawn into the syringe ml sodium citrate. This solution ratio of 1: 9 (1 sodium citrate to 9 bloods) is drawn into the syringe and the syringe containing sodium citrate blood samples were taken and the base was sent to a lab flask containing ice. Cholesterol in serum samples using enzymatic CHOD-PAP and commercial kits at a wavelength of 546 nm was estimated Pars and test. In this way, antibodies against all lipoprotein VLDL, LDL, chylomicrons block and only for specific HDL cholesterol, the cholesterol measuring enzyme is calculated. Triglycerides in serum samples using an enzyme GPO-PAP method and commercial kits at a wavelength of 546 nm Pars-test and measured. HDL- cholesterol in serum samples using enzymatic CHOD-PAP and commercial kits at a wavelength of 546 nm Pars test and measured.

- Statistical analysis:

Antibody titers with split test and the rest were analyzed using 2 × 2 factorial. All data collected during the breeding period in Excel recorded and analyzed with SAS software.

RESULTS AND DISCUSSION

Table 1.4 the effect of treatments on feed conversion ratio of chickens in three phases' starter, grower, and finisher

treatment	Starter	Grower	Finisher
Negative control	1.47	1.75^{ab}	2.05^{ab}
Positive control	1.47	1.87^{a}	2.13^{a}
Negative probiotic	1.43	1.66^{b}	1.92^{b}
Positive probiotic	1.44	1.74^{b}	1.99^{ab}
SEM	0.027	0.591	0.086
Effects of probiotic	n.s	*	*
Effects of Salmonella	*	*	*
interaction	n.s	n.s	n.s
Model	n.s	*	n.s

Posts with different letters are significantly different.

The comparison between experimental groups FCR significant difference was observed in the beginning period of growing time (P< 0.05), however the best feed conversion probiotic group were negative. Probiotics, Salmonella and interaction between the two on feed conversion ratio was not significant. Feed conversion ratio during the growth of chickens showed significant differences between the experimental groups (P<0.05); the conversion rate between the experimental groups in the probiotic group and positive control group had the fewest negative. Probiotics, Salmonella showed a highly significant effect (P<0.05). However, a significant interaction effect between the two feed conversion ratios was not significant. FCR in comparison between the experimental groups was no significant difference between groups in the final period showed the positive and negative probiotics (P<0.05). The best feed conversion ratio was negative and probiotics. Probiotics, showed a highly significant effect (P<0.05). The effect of Salmonella and interaction on feed conversion ratio was not significant. In all groups a better conversion rate for the whole period of growing negative probiotics showed (Table 4-1).

Table 2.4 the effect of the experimental groups and subtract the total number of white blood cells 21 days (data for all of the traits expressed in percent)

treatment	Total	Heterophil/ Lymphocytes	Heterophil	Lymphocytes	Eosinophils	Monocytes
Negative control	23467^{b}	0.31^{ab}	22.6^{b}	71	0.33	2
Positive control	24867^{b}	0.35^{ab}	25.6^{ab}	74	0.33	2
Negative probiotic	27933^{a}	0.29^{b}	22.6^{b}	76	0.33	2
Positive probiotic	24600^{ab}	0.4^{a}	29^{a}	72	0.66	2.33
SEM	1555.633	0.051	2.952	3.311	0.76	0.917
Effects of probiotic	**	n.s	n.s	n.s	n.s	n.s
Effects of Salmonella	n.s	*	*	n.s	n.s	n.s
interaction	n.s	n.s	n.s	n.s	n.s	n.s
Model	*	n.s	n.s	n.s	n.s	n.s

Posts with different letters are significantly different.

In the comparison between the experimental groups in the number of monocytes, eosinophils and lymphocytes was no significant difference (P>0.05). But the number of monocytes and eosinophils in the experimental group had the highest number of positive probiotics. The largest number of probiotic treatment groups in the number of lymphocytes was negative. Probiotics and Salmonella and interaction between these two levels in the number of monocytes, eosinophils and lymphocytes had no significant effect. The positive probiotic groups compared to other groups heterophil largest number of exams (P<0.05). In comparison, no significant differences between the experimental groups without probiotics (P>0.05). But the largest number of heterophil in comparison to the control group was positive. The comparison between experimental groups probiotic diet groups was significant difference (P<0.05) and the largest number in the experimental group showed positive probiotics. In the comparison between the experimental groups in terms of the absence of contamination and pollution of significant differences between the groups were observed (P>0.05). The number of heterophil related to the absence of pollution. Probiotics plasma had no significant effect on the number of heterophil. Salmonella showed a highly significant effect and interaction of the test had no significant effect on the number of heterophil. Heterophil to lymphocyte ratio between the experimental groups showed significant differences (P<0.05). Probiotics significant effect on the proportion of Salmonella showed a highly significant effect on the ratio. The interaction between two surfaces tested had no significant effect. Probiotics have a significant effect on the number of white blood cells were examined for Salmonella and interaction between the two levels had no significant effect.

Table 4-3-effect of the experimental groups and subtract the total number of white blood cells 42 days (data for all of the traits expressed in percent)

treatment	Total	Heterophil/ Lymphocytes	Heterophil	Lymphocytes	Eosinophils	Monocytes
Negative control	21833	0.28	20	69.33	0	1.66
Positive control	23467	0.31	23.66	76.66	0	4
Negative probiotic	23933	0.35	25.33	72	0.66	2.33
Positive probiotic	24300	0.25	20	77.33	0	3.66
SEM	2.43.017	0.066	3.761	4.697	0.578	2.299
Effects of probiotic	n.s	n.s	n.s	n.s	n.s	n.s
Effects of Salmonella	n.s	n.s	n.s	*	n.s	n.s
interaction	n.s	n.s	n.s	n.s	n.s	n.s
Model	n.s	n.s	n.s	n.s	n.s	n.s

Posts with different letters are significantly different.

Overall, in comparison between the experimental groups in the number of monocytes, eosinophils, lymphocytes, was no significant difference heterophil to lymphocyte ratio (P>0.05); Statistics largest number of monocytes to control positive and lowest negative control group, respectively. The only negative was the number of eosinophils in the probiotic group and the other groups did not mention numbers. The highest and lowest number of lymphocytes positive probiotics, prebiotics score was negative. Heterophil negative probiotic has the highest number compared with other tests. The results showed that at 21 days the period of breeding white in the blood plasma cells (monocytes, eosinophils, lymphocytes and heterophile) was not significant (P>0.05). Heterophil significant effect on Salmonella (P<0.05). The total number of white blood cells in the blood plasma (WBC) was significant (P<0.05) and in groups with an increase in the total number of salmonella infection (WBC) was evident.

Table 4-4-effects of treatments on cholesterol, triglycerides and HDL (21 days)

treatment	Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)
Negative control	165.33^{b}	1.93^{b}	382.3^{b}
Positive control	206.66^{a}	2.20^{a}	318.6^{b}
Negative probiotic	147.66^{c}	1.93^{b}	902.3^{a}
Positive probiotic	161.00^{bc}	1.90^{b}	343.0^{b}
SEM	7.901	0.122	68.913
Effects of probiotic	***	n.s	**
Effects of Salmonella	***	n.s	**
interaction	**	n.s	*
Model	***	n.s	***

Posts with different letters are significantly different

The effect of the experimental groups showed a significant difference in reducing serum cholesterol (P<0.05). In the comparison between groups showed that the lowest amount of cholesterol in the blood serum of the probiotic group a negative (with prebiotics in the diet and oral injection of normal saline), The maximum value of the positive control (no probiotic and oral injection of Salmonella bacteria to number 10,000) compared with the lowest level of cholesterol in the blood serum showed a significant difference (P<0.05). Triglycerides in the positive control group evaluated in the experimental group was significantly different from other experimental groups (P<0.05). The

highest amount of triglycerides in the positive control group and the lowest was positive probiotics. The amount of blood serum HDL negative probiotic groups compared with others the maximum amount of HDL in the blood serum showed a significant difference compared to the other experimental groups (P<0.05). Probiotics, Salmonella and interaction between the two levels of serum HDL chickens tested in the study were statistically significant at 21 days (P<0.05).

Table 4-5-effects of treatments on cholesterol, triglycerides and HDL (42 days)

treatment	Cholesterol (mg/dl)	Triglycerides(mg/dl)	HDL(mg/dl)
Negative control	136.3^{b}	1.9	783.3
Positive control	150.3^{ab}	2	746.3
Negative probiotic	114.6^{c}	1.76	786.3
Positive probiotic	155.3^{a}	1.86	686
SEM	8.285	0.166	58.451
Effects of probiotic	n.s	n.s	n.s
Effects of Salmonella	***	n.s	n.s
interaction	*	n.s	n.s
Model	***	n.s	n.s

Posts with different letters are significantly different

The trial compared the serum cholesterol chickens in 42 days, a significant difference was observed between the experimental groups (P<0.05). The amount of cholesterol in the blood serum of experimental groups of Probiotics Probiotics negative and the most positive. There was no significant effect on the comparison of probiotics, but Salmonella and interaction between two surfaces tested showed a significant effect. In the experimental group, a significant difference in triglycerides was observed chicks (P>0.05). Statistics highest amount of triglycerides in the positive control group and the lowest negative probiotic group. In the comparison between the experimental groups and the interaction between probiotics and Salmonella in chickens tested had no significant effect on triglycerides. The results at 21 and 42 days showed growing period in the total amount of triglycerides and HDL period of 42 days with no statistically significant difference; But the amount of HDL cholesterol in 21 days and showed no significant difference in the whole period.

Probiotics in primary education courses do not have a great effect on feed intake, but with aging, may be through the effects of the probiotic microbial populations and processes that affect digestion, increase feed intake. On the other hand the probiotic to improve digestion and absorption of nutrients may cause the bird food needs. In general, probiotics through effects on nutrient digestion and absorption processes are increased feed intake. The results of this trial with results Balachandar(2003) was adapted, but the results Kannan et al (2007) did not conform [9]. These results are consistent with findings due to lack of staff and colleagues may be due to differences in the types of compounds used and the experimental conditions. As shown in the table, in the initial period, probiotics had no significant effect on weight gain. But in grower and finisher significant difference in weight gain between treatments was observed. From these results it can be concluded that probiotics could lead to weight gain in the final period. Probiotic effect may be due to competition between the probiotic bacteria in the digestive system is a microbial populations, the probiotic bacteria have more time, you may be more successful in competition with intestinal microbial populations. On the other hand due to the lack of full development of the microbial population in the gut of birds at an early age, the probiotic can deploy as appropriate in the digestive tract and by eliminating competition reduces their activity and the growth of harmful bacteria. No significant effect of probiotics on body weight in the initial period may be due to the need of probiotic bacteria in the digestive tract is a long time to deploy. These results Kannan et al (2007) were adapted [9]. The feed conversion ratio, probiotic caused no significant difference between treatments in the early growth was final. The use of probiotics in poultry diets improved feed conversion ratio, which is likely to increase due to favorable bacteria in the gastrointestinal tract, which is especially lactobacilli and pathogenic bacteria such as E. coli development through the production of organic acids and prevents bacteriocin and toxins resulting from them inert. The presence of these toxins in the gastrointestinal tract to reduce digestion of proteins, breaking them into nitrogen [9].Mountzouris et al (2010) improved feed conversion ratio of broiler chicks fed with probiotics because as they increase nutrient digestibility [10]. Including the harmful enzymes in the digestive system of birds is causing health problems can be traced to urease. Lactobacilli bind to intestinal epithelial tissue, bacteria producing urease activity decreased; leading to improved feed conversion is possible [10]. According to the results in 42 days rearing period separately types of white blood cells was not significant, But groups that Salmonella challenged had the effect of stimulating the immune system and the subsequent increase in the number of white blood cells, blood plasma, the group without Salmonella and groups with probiotics than in those without probiotics that it would in effect stimulation of beneficial bacteria in the gut (probiotics) are concerned. Salmonella impact as a harmful bacteria and pathogenic factors inducing effect is intensified. As it was determined, probiotics are increasing the total number of white blood cells indicating that stimulate the immune system of the host. Since the percentage of any white blood cells (eosinophils, lymphocytes, neutrophils, Mono Phil) has not changed, it can be concluded that the increase of the share of all white blood cells, white blood cells and thus stimulate the immune

system of chickens is the same . Bird's body's mucosal surfaces in direct contact with the environment and subsequently antigens, and internal secretion levels are involved in host defense. Rising food antigens, including antigens probiotics and subsequently influence the migration of cells to the tissue in the digestive tract. The lymph node cells through the blood stream to find. This forms the cell migration IgA production. IgA is found in secretions and tissues of the body's first line of defense against viruses and is the main immunoglobulin and other microorganisms can. As shown in tests, the probiotic reduces cholesterol; reducing plasma cholesterol in diets by Mohan et al (1996) have been reported [9]. According to the findings of Mohan and cooperation between lowering cholesterol and reducing plasma cholesterol in eggs and cholesterol in the body there is a significant positive relationship, this means that reducing plasma cholesterol levels, lowers cholesterol in the broiler chicken carcasses and eggs. Gilliland et al (1985) the mechanism of cholesterol lowering cholesterol, attributed to digest and modernization [13]. Grunewald (1982) believed that lowering cholesterol into bile acids, which may result in a break followed by the rebuilding of cholesterol can be prevented [12]. Serum triglyceride and HDL are not affected by probiotics. In testing Panda et al (2000) also report on other probiotics have been reported. The reason for this may be that probiotics are effective in less dietary fat metabolism, bacteria Group B are more carbohydrates and less fat is used as a substrate feed affect the day and this causes the fat absorbed from the diet control and experimental diets and Metabolism against them is the same.

CONCLUSION

According to the results, some of the chickens' immune parameters that have been exposed to disease, Salmonella, all this confirms immune system is increased.

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