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Effects of Four Probiotics on the Gastrointestinal Microflora in Ostrich Chickens

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This experiment has been done for considering the effect of four types of commercial probiotics (Bioplus 2B, Primalac, Thepax and Protexin) on gastrointestinal microflora of ostrich chickens during 6 weeks (from third week to eighth week of breeding ostrich chickens). The experiment was done in the form of full random design with 5 treatments and 5 replication. The treatments included (1) 0.04 percent of Bioplus 2B, (2) 0.09 percent of Primalac (3) 0.1 percent of Thepax, (4) 0.03 percent of Protexin (5) without probiotics (control treatment). Data was analyzed by SPSS software and means were compared by Tukey test. The result showed that adding probiotics have significant effect on the microbial population of bacteria of *Lactic acid*, *Escherichia coli* and *coliforms* ($p<0.05$). Generally amongst different treatments, treatment 1 (having 0.04 percent of Bioplus 2B and attendance 2 (having 0.09 percent of Primalac) have had the greatest effect on gastrointestinal microflora.

Key words: Probiotics, Microbial population, Intestine, Ostrich chicken.

Nowadays ostrich chicken regarding its advantages has been paid attention as one of new procedures at animal husbandry industry. This bird eats everything and lives easily in arid and low water environment. In terms of resistance against natural adversity, versus lack of food and water it is like camel and look likes birds. These days ostrich products have been spread in meat, leather, feather and fat. After recognizing diseases of the cow, ostrich meat has been known as the rich and pure source without having any common pathogen between human and animal. Ostrich meat unlike other animal doesn't absorb bacteria such as *Escherichia coli* and *Salmonella*[1]. Ostrich meat is red that have less calories, cholesterol and fat than chicken, turkey and beef, this is while its iron and protein is higher. In this case health organization such as: America heart association, America cancer association and diabetes association suggested ostrich meat as an important source of protein[2].

Digesting fiber of NFD that is digestible to the 5 to 6 percent by poultry, in nutrients of ostrich chicken can be used at the third week. The ability of digesting NFD consist a percent of fiber exists at food (cellulose, hemicelluloses and lignin) that has the potential of digestion. In growing ostriches, during the first 10 weeks this ability increases linearly to 51% and about fully grown ostriches it is over 60 percent. This strong power of digesting fiber food can be found in ruminant herds. Such ability in ostrich found by microflora existed at digesting system of animal. Therefore considering effective elements on microbial population has high importance at ostrich productions.

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Fermentation activity that is done by bacteria in large intestine enables ostrich to use large amount of fiber in diet. Growing ostriches can achieve 76% of their required metabolic energy from breaking cellulose[3].

Lactic acid bacteria are usually considered as useful probiotics bacteria that these bacteria are known as limiting *Escherichia coli*, *Proteus*, *Staphylococcus* and *Salmonella*[2]. Manipulating intestine microbes through using prebiotics and probiotics increases the ability of digesting nutrients, resistance to disease and health of poultry and livestock. Combining favorable microbial population of the intestine and eliminating pathogens are distinguished as the important effective elements on performance, health and growth. Prebiotics are such added non-living components and are not located at the group of nutrients of the ration. These compounds are used for changing and balancing microbial population, increasing growth of useful bacteria and creating healthy intestine environment for better and more absorption of nutrients. Probiotics are microorganisms that when fed to animals, they provide the environmental condition for the growth of useful intestinal bacteria.

Probiotics by changing gastrointestinal flora release enzymes (amylase level increases until 40 days after probiotic consumption) and other useful precursors in gastrointestinal system.

Anatoly[4] reported that using *Bifidobacters* at ration of fleshy chickens leads to the overcome of protein-degrading bacteria to carbohydrate degrading bacteria that digestion of protein will increase and its analysis will decrease[5, 6].

Hajaj *et al.*,[7] have also reported that feeding fleshy chickens with *Aspergillus aryza* have significant effect on the increase of performance of factors such as increase of body weight and nutrition and was effective at producing Ammonia gas and microbial population in a method.

Therefore the aim of using live microbial products, is its effect on microbial activities of gastrointestinal system through stabilizing favorable bacteria and preventing concentration of harmful bacteria and fallowing that helping to keep animal's health[8]. Change at microbial population due to adding probiotic to ration leads to proliferation and sticking pathogenic microorganisms and reducing disease. it became obvious that the products (a) causes useful change at gastrointestinal flora and reduce the population of *Escherichia coli* (b) by producing lactate it changes pH of digestive system (c) produces material of antibiotics (d) decrease toxins[9]. Therefore considering methods of improving microbial population of digestive system of ostrich, have high importance at improvement of the power of production of this animal. Hence, the aim of this experiment is considering the effect of using 4 types of commercial probiotics on microflora of ostrich.

MATERIALS AND METHODS

This research was done for considering the effect of 4 types of commercial probiotics of Bioplus 2B, Primalac, Thepax and Protoxin on microflora of ostrich chickens.

Time and location of project

This research project was implemented for 2 months at the farm of growing ostrich at Darejir in the village of Ahandan in Lahijan city in 2012. Geographical situation of Ahandan is 49^0 and 58 minutes and 37^0 and 10 minutes latitudes. The first and second weeks of chickens birthday were devoted to adaptation of chickens and the main experiment was done from third to eight weeks (For 6 weeks).

Physical characteristics of the field

The area of farm of growing ostrich was 5000 m that was devoted to generator ostriches. The space of breeding ostrich includes 4 rooms with the dimension of 3×3 m, open area with the dimension of 12×6 m and a roofed space with the dimension of 8×8 m. For each ostrich chicken, the closet space was 2×1.7 and open space was considered 1×1 m. The size of each line was considered 3×1.7 m that the space between lines was separated by block wall until the age of 5^{th} weeks and after that they changed to plastic mesh. Also a room had incubators, Hatcher device, a room devoted to ostrich chicken aged below 2 weeks and two other rooms devoted to ostrich chickens aged 2 weeks.

Rearing management

Ostrich chickens used in this project were African race having blue and black neck that these ostrich chickens were from the eggs of generator

of the same farm.

The first step of breeding was cleaning and disinfecting the environment. Before starting the work, waste materials remained from previous period were collected and removed and the area was washed by high pressure. Then the rooms and Setter and Hatcher machines were disinfected by formalin-feeding dishes and water fountains were disinfected by water and detergents.

Eggs after being collected and disinfected were transferred to the setter machine and remained at the machine for 40-44 days (until chickens came out). Then they were transferred to Hatcher machine and remained there for 24 hours. After they went out of Hatcher machine they were transferred to a room having bed and artificial mother. Ostrich chickens remain on plastic bed and under artificial mother until the age of 2 weeks. Since the second day of their birthday the chicken fed up with multivitamin syrup for 3 weeks. The first 3 days of their breeding, water and multivitamin was given to them in order to help yolk sac to be absorbed. After some days a small amount of grain milled several time was given to chickens. At the age of 2 weeks after being sure about the complete absorption of yolk sac, the number of treatment and repeat was fasten to chicken's leg by metal clamp. Of course at the end of each week, regarding the growth of any ostrich chicken, the diameter of metal clamps was increased. Inside rooms artificial mothers were installed in order to transfer ostrich chickens to the rooms at night or rainy days and cold weather. Water fountain became full of water several times during each day and if necessary the grain were poured into feeding dishes.

Studied treatments

Treatment 1: ration having probiotics Bioplus 2B at the amount of 0.04 percent that was determined regarding the amount suggested by the company. Bioplus 2B is a probiotic having superior probiotics strains of two kinds of bacilli called *Bacillus subtilis* and *Bacillus licheniformis* that exist in equal proportion in this compound.

Treatment 2: ration having probiotics Primalac at the amount of 0.09 percent that was determined regarding the amount suggested by the company. Primalac have 4 live strains of *Lactobacillus acidophilus, Lactobacillus casei, Streptococcus facium, Bifidobacterium termophilum.*

Treatment 3: ration having probiotics Thepax at the amount of 0.01 percent that was determined regarding the amount suggested by the company. Thepax is controlled yeast, in active and covered cells of *Saccharomyces cerevisiae* that have minerals, amino acids and vitamins B.

Treatment 4: ration having probiotics Protexin at the amount of 0.03 percent that was determined regarding the amount suggested by the company. Protoxin includes 9 strains of *lactobacillus bacteria, fungi* and *yeast* like: *Lactobacillus plantrum, Lactobacillus thermophilus, Bifidobacterium bifidium, Streptococcus thermophilus, Enterococcus faecium, Aspergillus oryzae* and *Candida pintolopesii.*

Treatment 5: basic ration without probiotics (control). The fifth treatment of this experiment is the control treatment (especial ration of the same farm) that in this ration experimental matter that is probiotic has not been used in order to be able to compare the result of other attendances with the result of other treatments with the result of this treatment.

Dietary ingredient and composition

Food items and composition of ration's nutrients used in 5 treatments were exactly similar and they just had difference from the view of probiotics.

Preparation of microbial samples

Sampling chicken's feces (through soaping method) was done at the end of 8th week. After feeding, the faeces of ostrich chickens was taken by using a sterile soap and inside sterile plate transferred to laboratory for culturing. For growth of bacteria and counting the number of its colonies firstly microbial culturing environment was prepared. The dilution was in this way that one gram of content of ostrich chicken's intestine was completely mixed with one CC of peptone water solution, then this one CC was poured into a pipe NO1 and were mixed completely and again one CC of it was removed and transferred to other tube and was discarded. Finally from pipes No 5, 7, 9 and 11 one CC was taken and transferred to culturing environment beside flame and was cultured. Step of preparing culturing environment was done one day before sampling and microbial culturing.

Studied traits

Factors that were considered in this experiment are as fallow: the number of colony of *Escherichia coli* bacteria, the number of colony of *coliforms*, the number of colony of total population of aerobic bacteria and the number of colony of *Lactic acid bacteria*.

Statistical analysis

This experiment was carried out based on completely randomized design (CRD). There were 5 treatments and each treatment included 3 replications. Each replication included one ostrich chicken. For removing the effect of gender, in all treatments the proportion of gender (male: female) was similar. Data were analyzed by SPSS statistical software and the mean were compared with each other by Tukey test.

Statistical model of the project was: $X_{ij} = \mu + T_j + e_{ij}$

In this formula X_{ij} shows numerical value of each observing at experiment, μ is the mean of whole society through which samples were considered by null hypothesis, Tj shows the effect of each group with experimental ration, eij shows the effect of error. Therefore numerical value of each observing of total effect of attendance, experiment error and mean of the whole society was obtained. Also before doing statistical analysis of data, all data was considered by normalized test and if necessary, the appropriate conversion was used.

RESULTS AND DISCUSSION

The result of this experiment has been presented in table 1. It showed that using probiotics can have positive effect on microbial population of ostrich chickens. Generally among different treatments, treatment 1 (0.04 percent of Bioplus 2B) and treatment 2 (having 0.09 percent of Primalac) has had the highest effect on microbial population.

Probiotics are living organisms and unlike chemical compound, determining the dose and way of their operation is difficult. Unlike antibiotics, consumption of probiotics by poultry and livestock doesn't have any remaining tissue and doesn't create microbial resistance. Microorganisms that are added as probiotics to ration of poultry are usually part of gastrointestinal microbes[10].

Table 1. Effects of four commercial probiotics on mean (± standard error) of microbial population in 8th week of age in ostrich chicks*

Treatment Trait	Treatment 1 (0.04% Bioplus 2B)	Treatment 2 (0.09% Primalac)	Treatment 3 (0.1% Tipax)	Treatment 4 (0.03% Protexin)	Treatment 5 (Without probiotics)
Acid lactic bacteria	$1410000000^{a}\pm$ 523300000	$1440000000^{a}\pm$ 148500000	$485000000^{ab}\pm$ 77780000	$880000000^{ab}\pm$ 282800000	$874000000^{b}\pm$ 56570000
Total aerobic bacteria	$5950000000^{a}\pm$ 20510000000	$9500000000^{a}\pm$ 1414000000	$1880000000^{a}\pm$ 229800000000	$2810000000^{a}\pm$ 154900000000	$1620000000^{a}\pm$ 70710000000
Escherichia coli	$95000000^{a}\pm$ 35360000	$1030000000^{a}\pm$ 49500000	$895000000^{a}\pm$ 77780000	$1700000000^{a}\pm$ 127300000	$3610000000^{a}\pm$ 3578000000
Coliforms bacteria	$2830000000^{a}\pm$ 240400000	$1830000000^{b}\pm$ 14140000	$2850000^{c}\pm$ 3535000	$6750000000^{c}\pm$ 374800000	$500000000^{c}\pm$ 14140000

*Means in each row followed by the same letters are not significantly different at α=0.05.

Manipulating microbes of intestine through using prebiotics and probiotics increases the capability of digesting nutrients, resistance to disease and health of livestock and poultry. Combining favorable microbial population and eliminating pathogens are recognized as the important factors effective on performance, health and growth. Prebiotics are such added inorganic compounds and are not located at the group of nutrient of the ration. These compounds are used for changing and balancing microbial population, increase of the growth of useful bacteria and creating healthy intestine environment for better and more absorption of nutrients. Probiotics are microorganisms that when the animal is fed up with them it provides environmental condition for growth of useful microbes of digestive system[10].

Probiotics are living organisms and unlike chemical compounds, it is difficult to determine the dose and way of their operation. Unlike antibiotics consuming probiotics by poultry and livestock will not have any tissue remains and doesn't create microbial resistance. Microorganism that are added to ration of poultry as probiotics, are usually part of microbes of digestive system. These kinds of microorganisms are *Lactobacillus*, *streptococcus*, *probiobacters*, *bifidobacter* and some *Basils*.

Mechanism of improving food efficiency by probioticsare : change at intestine's flora, increase of the growth of anaerobic bacteria and positive gram that produce lactic acid and hydrogen peroxide, preventing growth of intestinal pathogens, increasing digestion and break down of food in intestine (Kabir, 2009). Totally the occurrence of cases that was mentioned above, leads to improvement of the growth, improvement of food efficiency and reduction of losses[11].

Lactobacillus besides balancing gastrointestinal microflora of poultry, decreases nitrogen by breaking down the protein and changing them[12]. It can be generally concluded that using probiotics and prebiotics usually lead to the microbial balance of intestine[13,14].

Like the result of our experiment, other different experiments have shown the positive effect of using probiotics at improvement and keeping population of useful bacteria of intestine of fleshy chickens[15, 16].

Also it is said that the action of prebiotics and probiotics is through connecting and eliminating pathogens and stimulation of immune system[17].

CONCLUSION

Based on the result of this research, probiotics lead to the increase of useful microbial population of digestive system of ostrich. However for making a conclusion about the effect of using probiotics on microbial population of digestive system of ostrich chickens and health of livestock, more research is required.

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