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Comparative Effects of Probiotics and Inactivated Cells of *Saccharomyces cerevisiae* Supplementations on Growth Performance, Feed Conversion Ratio and Some Blood Parameters in Quail (*Coturnix japonica*)

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Abstract

In the recent years, the use of probiotics and prebiotics, as a food supplement, has been increased, it appears to be an effective supplement to reduce the use of antibiotics, coccidostats and mycotoxin binder, particularly in the poultry industry. The aims of the study were to investigate the effect of probiotic and prebiotic supplements on growth rate, feed conversion ratio and some blood parameters in Japanese quail. 150 quails were used, and randomly divided at day 3 of age into three equal groups: control groups; without any treatments, probiotic-treated group; treated with probiotic (0.150 g probiotic/l liter of water), and prebiotic-treated group; treated with (1 ml prebiotic/l liter of water) for 4 weeks. The resulted showed that total white blood cells, lymphocytes numbers, total red blood cells, hemoglobin concentration and total platelets were significantly ($p \leq 0.05$) increased in birds, treated with prebiotic when compared to the control and probiotic-treated group. While the granulocytes count, mean corpuscular volume, mean corpuscular hemoglobin and red blood cell distribution width-standard were significantly ($p \leq 0.05$) reduced in both prebiotic and probiotic-treated groups. The white blood mid cells number (monocytes, eosinophils, basophils and blasts cells) and mean corpuscular hemoglobin concentration were significantly increased in the prebiotic-treated birds compared to the control group. In addition, the pre-and probiotics were found to improve the level of serum-phosphor, blood sugar level, serum-creatinine. In conclusion, the prebiotic and probiotic supplementation appears to enhance red blood cell indices, and boost the immune system via the process of leukocytosis.

Introduction

Since 70th of the twentieth century, the concerns about an increase in the level of the antibiotic's resistance is increased, particularly through over-prescription and misapplication of antibiotics in human and using antibiotics as growth promoters in animal and food industry [1]. To reduce the risk of antibiotic resistance, probiotic and prebiotic supplementation in poultry industry has drawn the attention of the researchers as an alternative for antibiotics use to maintain the health and growth in poultry industry [2].

Maintaining the intestinal microflora is important to disrupt the growth of pathogenic bacteria, and to augment the immunity and controlling infection without using antibiotic, the use of feed additives, such as probiotic, prebiotic and synbiotic, are possible alternatives of antibiotics, is urgently requested [2,3,4,5].

Probiotics are living microorganisms; they act positively on the host by enhancing the activities of the intestinal microflora [6]. While, prebiotics are indigestible agents such as oligosaccharides: fructo-oligosaccharides, gluco-oligosaccharides and mannan-oligosaccharides. Similar to that of probiotics, prebiotics stimulate the growth and the activity of the intestinal bacteria. They all improve the gut health and

boost the immune system [7] through enhancing the growth of useful bacteria which are already present in the host organism and adapted to all conditions of the environment [8].

Japanese quail, *Corturnix japonica,* is considered as a commercial bird and it has been used for the egg and meat production since 1910 in Japan [9]. Using quail as a source of eggs and meats production in Kurdistan region of Iraq is rare. While, the Japanese quails have many desirable characteristics, such as the rate of grow is fast, maturity of sex at (5 weeks), rate of egg production is high (300 egg/bird), generation interval is short (3-4 generations a year), needs small floor space (200-250 and 150-200 cm^2 for litter and cage respectively), needs small quantity of feed (20-25 g/adult bird/day), incubation period for hatching egg is short and feed cost is low. Quail meat has low loss of moisture, which might aid in its marketing, also quail are resistant to common chicken diseases. In compared to chicken and duck, quail farming needs lower capital investment with almost the same profits [10, 11, 12]. Quail farming produced less waste compared with other livestock enterprises so it has less effect on environment pollution [13].

Basis on the above-mentioned properties of the prebiotics and probiotics I hypothesized that they induce the growth and health of the Japanese quail. We investigate the influence of probiotic and prebiotic on the growth performance, feed conversion ratio and some blood parameters in Japanese quail.

Hematologic parameters in quail are significantly variable according to different techniques that used by different laboratories [14, 15, 16]. In this study the control groups fed with the basal feed without probiotic or prebiotic were used as reference for the comparison of parameters between the groups.

Materials and Methods

A total of 150 Japanese quails (Two-days old) were obtained from a commercial seller in Kirkuk market, each weighed about 11.079 g. The birds were kept on starter diets for quails bought from Kirkuk market with nutritional contents: crude protein 22%, fat 7%, crude fiber 7%, ash 14%, Calcium 3% and Phosphorus 0.8%. The temperature of the room, where the quails were kept, was tightly controlled (set on 36°C for the 1st week, then, it was gradually reduced (3 degrees per week) till fixed at 27°C) with 24 hours' light. All hygienic measures were considered to avoid the possibility of a disease outbreak among the birds. The birds had not been on any vaccination or treatment throughout the course of the study.

Experimental Design

The birds were randomly divided on day three of age on three equal groups, 50 birds per a group. Control groups; without any treatment. Probiotic-treated group; Treated with 0.150 g of probiotic/1 liter of drinking water) (MiaClost, MIAVIT). Prebiotic-treated group; Treated with 1 ml of prebiotic /liter of drinking water (Xanthan gum-E 415, Medro- Doxal). The birds had been treated for 4 weeks.

Sampling

At the end of the experiment, day-30, the blood samples were randomly collected from six birds per each group through the heart-puncture. The blood, which was collected from each bird, was divided between two tubes. One milliliter of blood was kept with anticoagulant (EDTA-Na2) tubes to be used for complete blood counts (CBC). Five milliliters of the blood was kept in non-anticoagulated tubes for collecting serum, and the serum extracted by laboratory was used for calcium, phosphorus, blood glucose level, total cholesterol, triglyceride (TAG), low-density cholesterol (LDL), high-density cholesterol (HDL), very low density lipoprotein (VLDL) and creatinine determination. The activities of enzymes, alanine aminotransferase (ALT), asparagine aminotransferase (AST) and alkaline phosphatase (ALP) were estimated; as well we looked for total serum protein (TP), albumin and globulin. The levels of enzymes were measured by following the manufacturer's instructions, which was provided with the enzymatic kits. All hematological and bio chemical tests were done in laboratory in Sulaimani city by using a hematological analyzer device type SIEMENS Dimension Xpand plus.

The feed conversion ratio

Feed intake and body weighed were measured weekly, then they were used to calculate the feed conversion ratio (FCR) using this equation: FCR= weight of total feed consumption per / (final weight – starting weight)

Statistical analysis

A complete randomized design (CRD) with three treatments and six replicates was used for conducting this study. Analysis of variance and Duncan's multiple rang test ($p \leq 0.05$) were used for comparing treatments means [17] by using XLSTAT excel data analysis program, version 2017.

Results and Discussions

The results showed that none of the treatment had an effect on the bird's body weights (Fig. 1A), cumulative feed consumption (Fig. 1C) and the ratio of consumption/total weight (Fig. 1D) in Japanese quail. While, the probiotic-treated group was found to increase ($p<0.05$) the feed consumption (Fig. 1B) at week-2 in comparison to the prebiotic and control group. However, the increase in the feed consumption found to have not any effects on the bird`s body weight (Fig. 1A). In agreement with my finding, previous studies were reported that mannan oligosaccharide, probiotic and live yeast (*Saccharomyces cerevisiae*) had no effect on the body weight, feed conversions and feed in take in broilers [18,19], in contrary, Tohid et.al. (2011)[20] found that *Saccharomyces cerevisiae* in quail feed enhanced body weight and feed conversion ratio, Nikpiran et al. (2013)[5] reported elevation in body weight by using prebiotic *Thepax*. A transit increase in the food consumption, which was seen in the second-week of the probiotic-treatment, might be related to the presence of the Bacillus subtilis as an active component of the probiotic, which is well-known for its stimulatory impacts on the gut health [21] and the activity of the digestive enzymes [22]. While, Tengfei et al. (2019) [23] found that dietary addition of probiotics, including *Bacillus subtilis, Bacillus licheniformis,* and *Saccharomyces cerevisiae*, had the similar effects as chlorotetracycline in improving growth performance.

Lack of a significant difference between the treatment groups, and a smooth and satisfactory growth rate of the birds throughout the course of the treatment might be related to a good sanitary precaution, which had been taken during our experiment.

Prebiotic-treated group was found to induce a significant increase in the number of total WBCs, lymphocytes, RBCs, Hgb and platelets, concentration in comparison to the control and probiotic-treated group (Tables 1, 2 and 3). While the granulocytes, MCV, MCH and RDW-SD were significantly reduced in the probiotic-treated groups, and each of blood mid number and MCHC increased significantly in the prebiotic-treated group compared to the control group. Probiotic treatment was reported to be associated with a reduction in the MCV value, and an increase in the level of the total number of RBCs and MCHC value in broiler [24]. However, other studies were found that prebiotic and probiotic supplementations had not a significant effect on the blood parameters in birds [25].

The results showed that the probiotic-treatment was found to induce a significant ($p<0.05$) increase in the level of serum-phosphorus in comparison to the control and prebiotic-treated groups. However, none of the treatments was found to have an effect on the serum-calcium level. Those results were in agreement with the Siadati et al (2017) [26] who found the same pattern in the quail by using commercial probiotic had a significant effect on serum phosphorus but no difference in calcium.

In the current study, high levels of TP and ALB in male quail may be associated with positive effects on protein metabolism.

In contrast to that of the control-group, the probiotic-treated group was found to reduce ($P<0.05$) the blood sugar level. Meanwhile, the level of serum creatinine was significantly ($P<0.05$) reduced in both prebiotic- and probiotic-treated groups. Whereas, the serum level of the cholesterol, TAG, LDL, HDL, VLDL, ALT, AST, ALP, TP and albumin were not affected by any of the treatments, these results are indicator of healthy liver state in all groups [27], not affected serum level of TP and albumin mean that protein metabolism not

affected by different treatments [28]. The same result was reported by Siadati et al., (2017) [26] in quail by adding commercial probiotic with two different concentrations while they found low density in LDL with using selected native Lactobacillus strain. However, using Saccharomyces *cerevisi ae* in broiler feed was found to increase the blood-glucose level [29], and decreased cholesterol and TGA level [19, 29, 30] reported significant decreased in ALT and AST but Aluwong et al (2012)[31] showed that ALT and ALP levels in serum significantly decreased whereas AST value was non-significant by adding probiotic in broiler chicken

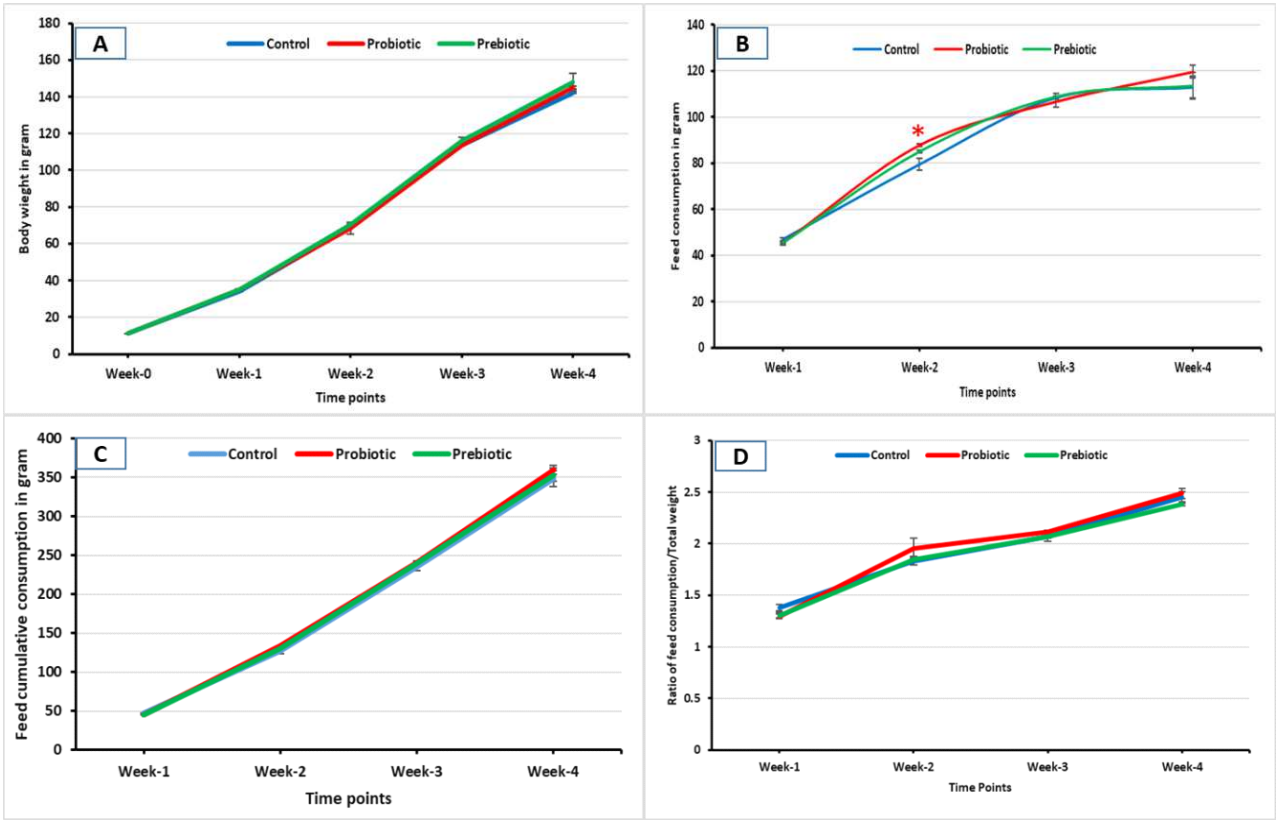

Figure-1: The effect of probiotic and prebiotic on the body weight (A), feed consumption (B), cumulative feed consumption (C) and the ratio of consumption/total weight (D) in Japanese quail. Values are mean±SE for N= 6. P<0.05.

Table-1. Effects of probiotic and prebiotic treatments on RBCs in Japanese quail.

Treatments	RBCs X10^6/µL	Hgb g/l	Hct%	MCV fl	MCH pg	MCHC g/L	RDW-CV %	RDW-SD fL
Control	^{b}3.013± 0.04	^{a}136.833± 2.18	^{a}53.133± 0.77	^{a}176.633± 2.76	^{a}45.350± 0.31	^{a}257.167± 3.7	^{a}9.933± 0.31	^{a}90.233± 10.8
Probiotic	^{b}3.138± 0.09	^{a}135.833± 4.03	^{a}51.500± 2.01	^{b}165.867± 4.91	^{b}43.650± 0.58	^{ab}263.833± 4.28	^{a}9.600± 0.17	^{b}62.817± 9.14
Prebiotic	^{a}8.068± 4.65	^{b}150.333± 6.61	^{a}55.700± 2.39	^{b}165.450± 2.33	^{ab}44.550± 0.49	^{b}269.167± 1.4	^{a}10.217± 0.34	^{b}65.217± 7.08

* Means followed by the same letters within column are not significantly different at $p \leq 0.05$ according to the Duncan test.

** Red blood cells (RBC), hemoglobin (Hgb), hematocrits (Hct), mean corpuscular volume (MCV fl), mean corpuscular hemoglobin per pictograms (MCH pg), mean corpuscular hemoglobin concentration (MCHC), Red blood cell distribution width-coefficient variation (RDW-CV), red blood cell distribution width-standard deviation per femtoliter (RDW-SD fl).

Table 2. Effects of probiotic and prebiotic treatments on WBCs in Japanese quail.

Treatments	WBCs X10³/µL	Lymph X10³/µL	Mid X10³/µL	Gran X10³/µL	Lymph %	Mid %	Gran %
Control	^{a}226.383 ± 3.72	^{a}20.800 ±2.18	^{a}72.933± 2.07	^{a}132.650±1.22	^{a}9.150± 0.87	^{a}32.200± 0.69	^{a}58.650± 0.87
Probiotic	^{a}225.567 ± 4.05	^{a}24.117± 1.54	^{ab}74.933± 2.18	^{b}126.517± 1.8	^{a}10.717± 0.59	^{a}33.183± 0.42	^{a}56.100± 0.89
Prebiotic	^{b}241.367± 4.9	^{b}33.917±3.52	^{b}79.317± 1.54	^{ab}128.13± 1.73	^{b}14.017± 1.16	^{a}32.833± 0.39	^{b}53.150± 1.12

* Superscripts on the left of the means indicate statistical differences between the groups.
** White blood cells (WBC), lymphocytes (Lymph), Mid cells (Mid), granulocytes (Gran) numbers and percent.

Table 3: Effects of probiotic and prebiotic treatments on platelets in Japanese quail.

Treatments	Platelet X10³/µL	MPV fl	PDW	PCT %
Control	^{a}17.000± 1.21	^{a}9.000 ± 0.2	^{a}15.333± 0.18	^{a}0.015± 0.0
Probiotic	^{a}17.500± 2.42	^{a}8.567± 0.16	^{a}15.083± 0.17	^{a}0.014± 0.0
Prebiotic	^{b}23.500± 3.44	^{a}8.683± 0.22	^{a}15.217± 0.2	^{a}0.020± 0.0

* Superscripts on the left of the means indicate statistical differences between the groups.
** platelets, mean platelet volume per femtoliter (MPV fl), platelet distribution width (PDW), plateletcrit (PCT).

Table 4: Effects of probiotic and prebiotic treatments on serum calcium and phosphor level in Japanese quail.

Treatments	Calcium mmol/L	Phosphor mmol/L
Control	^{a}3.092± 0.065	^{a}6.482± 1.515
Probiotic	^{a}3.010± 0.083	^{b}8.995± 1.604
Prebiotic	^{a}2.988± 0.079	^{a}6.808± 1.724

* Superscripts on the left of the means indicate statistical differences between the groups.

Table 5: Effects of probiotic and prebiotic treatments on sugar, creatinine and lipid profile in Japanese quail.

Treatments	Sugar mmol/L	Cholesterol mmol/dl	TAG mmol/dL	LDL mmol/dl	HDL mmol/dl	VLDL mmol/dl	Creatinine Umol/dl
Control	^{a}19.368± 0.88	^{a}4.498± 0.23	^{a}2.883± 0.1	^{a}1.372± 0.17	^{a}2.550± 0.12	^{a}0.577± 0.02	^{a}11.197± 3.44
Probiotic	^{b}16.207± 0.68	^{a}4.360± 0.18	^{a}2.967± 0.26	^{a}1.402± 0.12	^{a}2.367± 0.16	^{a}0.593± 0.05	^{b}5.303± 1.63
Prebiotic	^{a}19.585± 1.32	^{a}4.830± 0.44	^{a}2.450± 0.19	^{a}1.757± 0.33	^{a}2.583± 0.21	^{a}0.490± 0.04	^{b}4.738± 1.41

* Superscripts on the left of the means indicate statistical differences between the groups.
** Triglyceride (TAG), low-density cholesterol (LDL), high-density cholesterol (HDL), and very low density lipoprotein (VLDL).

Table 6: Effects of probiotic and prebiotic treatments on ALT, AST, ALP,TP, Albumin and globulin levels in Japanese quail.

Treatments	ALT U/L	AST U/L	ALP U/L	TP g/L	Albumin mmol/L	Globulin g/L
Control	^{a}5.180 ± 2.15	^{a}229.145± 24.43	^{a}1189.100± 96.5	^{a}27.950± 0.98	^{a}6.008± 0.27	^{a}21.942± 0.74
Probiotic	^{a}3.937 ± 1.08	^{a}220.892± 11.65	^{a}1175.428± 101.79	^{a}28.903± 0.68	^{a}5.423± 0.43	^{a}22.647± 0.93
Prebiotic	^{a}5.740 ± 1.45	^{a}239.267± 12.79	^{a}1188.973± 49.97	^{a}28.225± 1.35	^{a}5.452± 0.37	^{a}22.773± 1.01

* Superscripts on the left of the means indicate statistical differences between the groups.
** Alanine aminotransferase (ALT), asparagine aminotransferase (AST), alkaline phosphatase (ALP) and total serum protein (TP).

Conclusion

It is concluded that supplementation of these probiotic and prebiotics have no much effects on body weight, feed consumption and feed conversion ratio. They might have an effect on body immunity system as the level of the inflammatory cells was found to be affected by the treatments. In addition, the treatments might improve the process of hematopoiesis.

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