Effect of different types of probiotic on some physiological parameters and morphological changes in intestine of Japanese Quail diets

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Abstract

The aim of this project is to know the effect on the physiological performance, after adding the different types of probiotics with different concentrations to the quail diets. One hundred and fifty one-day-old quail birds were used in the study, divided into five groups, each group having 30 bird and 3 replications. The experiment extended from one day old to 42 days from its start. The control group was not supplemented with probiotic, while the other groups 2, 3,4 and 5 were fed a basic diet to which the Saccharomyces group was feed on standard ration fortified with *Saccharomyces cerevisiae*, Lactobacillus group was feed on standard ration fortified with *Lactobacillus acidophilus* , Bifidobactrium group was feed on standard ration fortified with *Bifidobacterium bifidum* and Mix probiotics group was feed on standard ration fortified with mixture of above probiotics was added respectively. Cellular characteristics of blood (RBC, Hb and PCV%) and biochemical parameters(Albumin, Globulin, Glucose, Cholesterol, AST, ALT, MCHC, MCH and ALP concentration)in blood was recorded. The results showed non-significant difference among groups and between the intervals in total count of RBC for all studied group. Otherwise, elevation in Hb concentration for the groups treated with probiotic compared with control group that fed on standard ration without adding probiotics for the two intervals period (30 and 45), Whereas, MCV values appeared significantly higher during the period of 30 day of experiment in control and sarco groups than lacto, bifido and mix probiotic groups. While the 45-day period of experiment showed non-significant difference among the groups of the study. The results recorded significant elevation in ALT activity for both control and sacro groups compared with other treatment of the study in 30 day intervals. the fifth group was alone significant in the depth of the crypts compared with other treatment of the study. So, the ratio of length of villus and depth of crypts showed significant increase for mix probiotic group compared with other studied groups. While, control and sacro groups appeared significantly less the other studied groups of the birds.

Keywords: Blood, Quails, Probiotic.

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Background

Poultry is one of the most important sources of animal protein because of its great importance in determining the economic course of countries. Due to the unique characteristics of quail meat that distinguish it from other birds, the demand for it has increased recently due to its high nutritional value. Japanese quail is considered one of the important alternative resources of animal protein, because it has many advantages such as fast growth, early sexual maturity, short incubation period, small size and high egg production, low feed requirements and less housing costs and space, compared with the different species of poultry ((Mountzouris *et al.*, 2017). Various types of feed additives were used to improve growth and maintain the health of birds. Antioxidant is one of diet additives to protect feed from go bad and improve bird growth and enhance the immunity (Luna *et al.*, 2017). Also, Free-flowing agents to prevent interaction between the ingredient, pelleting, feeding enzymes, molds inhibitors, Coccidiostats and antibiotics are all act to protect the diet from oxidation or change to harmful form and enhance the bird growth and immunity (Jacob, 2020). Also, Probiotic has been a potential choice as feed additive due to its beneficial

effects on nutrient digestibility (Mountzouris *et al.*, 2010). All animal necessary maintain the number of microbiota in gastrointestinal tract for proper balance (Aref *et al.,*2019) to stabilize gut environment, compete with pathogenic bacteria for intestinal attach site , enhance epithelium growth and function and reduce the symptoms of diseases(Alagawany *et al.,*2018). There are various types of bacterial strain have been used as probiotic, some of which aerobic while other anaerobic bacteria. Many studies referred to using combination of aerobic and anaerobic bacteria may have improving effect on birds' production, health and welfare (Roose *et al.,* 2018; Abdel Moneim *et al.,* 2020). While Manati *et al.,*(2018)improve performance and enhance gut epithelium when replaces the antibiotic by supplement probiotic Bacillus species and *Saccharomyces boulardii* in broiler chicken. In addition, many types of beneficial bacteria showed improvement in performance of bird and enhance their immunity accompanied with better growth indices as investigate by using *Bifidobacterium bifidum* in broiler chicken (Abdel Moneim *et al.,* 2020). therefore, the present study design to identify the role of each type of probiotics alone and as mixture on the physiological performance in Japanese quails

Materials & Methods

Study design:

One hundred and fifty one-day-old quail birds were used in the study, birds were divided into 5 groups randomly, each group containing 3 replicates (10) birds / replicate. The probiotic was added to treated groups, Saccharomyces cerevisiae 250 mg1x107 CFU, Lactobacillus acidophilus 5x108 CFU, *Bifidobacterium bifidum* 30 mg $3x10^8$ CFU, Mix probiotic capsule $5x10^8$ CFU and a control group without addition. The duration of the experiment from start to finish was 6 weeks. Fed and water *add libitum*. The above probiotics were used by weighting the products according to the weight of birds and dose of product by using the following formula:

$$\frac{\text{Mean weight of human}}{\text{Mean weight of birds'}} \quad X \quad \frac{\text{dose of product}}{\text{dose for birds}}$$

Then, probiotics mixed with100mg of carrier materials (wheat bran) and distributed with feed of bird.

. The ration was prepared according to the basic diet during the experiment to provide all other nutrients except for lysine according to the nutrient recommendations from the NRC.

Table 1. bird feed ingredients

Ingredients	Growth diet (%)(1-42) day	Productive diet (%)(45- end of the experiment)
Yellow corn	49	46
Wheat	11.5	24
Protein concentrated (50% protein)	5.3	8.0
Soybean meal (48% protein)	31.2	17
Limestone	1.4	2.5
Vegetable oil	1.6	-
Mixture of vitamins and minerals	-	2.5
Total	100	100
Metabolic energy (kcal/kg)	3049	2930
Protein (%)	22.26	18.38
Calcium (%)	0.8	1.95
methionine%	0.50	0.50
Phosphorus (%)	0.58	0.8

NRC,1994

Studied Parameters

Cellular Characteristics of blood

Hematological parameters analysis was done in Al-Noor veterinary laboratory by using manual procedure as following:

- Total number of red blood cells (RBC)

Red blood cells were counted after dilution with haymes solution consist from 0.5 gm Nacl , 2.5 Na2so4 and Hhcl2 addition to 100 ml of distill water where this solution maintains the shape of the red cells, destroys the others and adds luster to them. The counting process was carried out using a hemocytometer, where the number of red blood cells was calculated in five square millimeters×1000.

Henry *et al,*1974)(

hemoglobin concentration (Hb)

calculated by uses of the cyanomethemoglobin method to calculated of the concentration by using of drabkins solution which contain from potassium cyanide (kcn) and Ferrous potassium cyanide(K3fe(Cn)6 and sodium bicarbonate Which is dissolved in distilled water and kept in a closed container where the solution oxidizes hemoglobin to methemoglobin, which reacts with a solution Cyano-methemoglobin light brown in color the specific density of the spectrophotometer is read at a wavelength of 540 nm and the hemoglobin concentration is extracted based on the standard curve according to the equation :-

HB concentration (gm/ml)=

$$\frac{\text{Sample absorption}}{\text{concentration Standard solution absorption}} \times \text{Standard solution}$$

packed cell volume (PCV)**:**

It was measured by the method (Microhematocrate) was used, which includes drawing blood by capillary action into micro-tubes at one end until two-thirds of the length of the tube is filled with blood. One end of the tube was closed with artificial clay, then the tubes were placed in a centrifuge at 5000 rpm for five minutes, then the readings were recorded by special ruler (Micro-hematocrit reader).

Biochemical parameters:

Biochemical parameters analysis were done in Al-noor veterinary laboratory by spectrophotometers and special kit for each parameters as following :

- **Albumin concentration**

The serum albumin concentration was estimated using a kit manufactured by Biolabo/France, and the reading was carried out using a spectrophotometer at a wavelength of 630 nm. The albumin concentration was calculated according to the following equation:

Serum albumin concentration (g/100 mL) = (for sample photometric reading)/(standard solution for photometric reading) x S.C

Since S.C represents the Standard Concentration = 5 g / 100 ml
(Henry 1974).

- **Globulin concentration**

The globulin concentration was calculated after estimating the concentrations of total protein and albumin according to the following equation:

Globulin concentration = total protein concentration - albumin concentration *(*Henry *et al.,* 1974*)*

- **Glucose concentration**

Blood glucose was measured using a kit supplied by the French company (Biolabo). The analyzes were carried out based on the steps indicated by the company and using a spectrophotometer at a wavelength of 550 nm. Then the absorbance of each sample was measured, then the following equation was applied:

Blood glucose (mg/100ml) = (absorption sample)/(standard absorbance solution) x 100 (Coles, 1986).

- **Cholesterol concentration**

The concentration of cholesterol in the blood serum was measured by using a kit supplied by the French company (Biolabo)according to the attached instructions. A spectrophotometer was used to read the samples at a wavelength of 500 nanometers and the concentrations were calculated according to the following equation:

Cholesterol concentration (mg/100ml) = (form reading)/(standard cholesterol reading×200) (Henry *et al.,* 1974*)*

- **Measurement of AST enzyme activity**

A ready-made assay kit (Kit), manufactured by the French company (Biolabo), was used using a spectrophotometer, at a wavelength of 505 nm, and the activity of AST enzyme (unit/liter) was extracted using a standard curve prepared for this purpose.

- **Measurement of ALT enzyme activity**

A ready-made assay kit (Kit), manufactured by the French company (Biolabo)was used using a spectrophotometer at a wavelength of 505 nm, and the activity of ALT enzyme (units/liter) was extracted using a standard curve prepared for this purpose.

- **Measurement of alkaline phosphatase enzyme ALP activity**

The enzyme activity was estimated by a kit produced by the French company (Biolabo) Reagents, and the absorbance was read by a spectrophotometer at a wavelength of 510 nm, and the enzyme activity was extracted from the following equation:

Enzyme activity (CNC Armstrong unit) = (plank reading - sample reading) / (standard reading) x 20Whereas, Armstrong's kunn = IU/100ml

Intestinal characteristics:

Measuring the height of the villus, the depth of the crypts, and the ratio between them:

Length of villi, depth of crypt and length of villi to depth of the crypts were measured for tissue sections prepared from three regions of the small intestine: duodenum, jejunum and ileum by using a microscope and using a 40x ocular lens after calibrated with the scale stage micrometer. The length of the villi estimated from the top of the villi to its association with the crypts (Shamoto *et al.,* 2000). As well as, the depth of the crypts, which is the immersion distance of adjacent villi (Uni *et al.,* 1998), and measured from the base to the transition zone between the crypts and the villi *(*Aptekmann *et al.,* 2001). The ratio between length of villi and depth of crypts.

Analysis of Data

In order to determine the statistical significances among different variables SPSS program (Statistical package for social sciences) version 21 was used. Analysis of variance tests were applied to analyze the obtained results.

Results

The effect of different types of probiotic in the diet on some blood parameters were established in table (2). This table showed non-significant difference among groups and between the intervals in total count of RBC for all studied group. Otherwise, Hb concentration revealed significant for the groups treated with probiotic compared with control group that fed on standard ration without adding probiotics for the two intervals period (30 and 45).

Table (2) Effect of probiotics addition on some blood parameters of Japanese quail birds during growth stage (mean ± standard error)

parameters / groups	RBC cell/ml×10⁶		Hb mg/dl		PCV %	
	30 DAYS	45 DAYS	30 DAYS	45 DAYS	30 DAYS	45 DAYS
Control	2.12±0.18	2.26 ± 0.14	9.23± 0.30 b	10.62 ±0.43 b	29.75± 1.53	33.18± 2.33 b
Sacra. Group	2.08 ±0.08	2.66±0.03	9.71±0.56 ab	11.22 ±0.20 ab	29.66±1.85 B	36.66± 3.66 A ab
Lacto. Group	2.56 ± 0.57	2.45 ± 0.12	10.13±1.06 ab	9.64±0.26 b	28.74±3.17	32.28 ± 3.88 b
Bifido. Group	2.34 ±0.43	2.97 ±0.26	10.63±0.36 ab	11.44± 0.36 a	30.24± 6.02 B	37.63± 5.36 A a
Mix prob. Group	2.67±0.06	2.96±0.24	10.90±0.06 a	11.73±0.27 a	36.55± 6.01	38.54 ± 5.88 a

Table (3) Effect of probiotics addition on some blood indices of Japanese quail birds during growth stage (mean ± standard error)

parameters / groups	ALT U/L		AST U/L		Urea mg/dl	
	30 DAYS	45 DAYS	30 DAYS	45 DAYS	30 DAYS	45 DAYS
Control group	34.08±0.86 a	34.083±0.86 ab	22.43±1.22 c	22.43±1.22	20.46±1.12 b	20.46±1.12
Sacra. Group	38.30±2.13a	24.22±2.08 b	32.45±3.36 ab	25.12±2.44	22.08±1.64 b	23.00±0.42
Lacto. Group	25.63±1.00 b	38.42±3.47 a	25.43±0.42 bc	24.57±1.40	27.44±1.42 b	22.82±0.19
Bifido. Group	32.41±1.41 ab	35.63±2.81 a	27.76±1.42 abc	28.57±2.46	36.05±2.46 a	22.83±0.08
Mixprob. Group	26.20±1.89 b	30.75±2.08 ab	35.98±1.98 a	27.33±5.01	35.45±1.65 a	20.97±1.32

Liver enzymes activities were represented by ALT and AST enzymes and kidney function was indicated by urea concentration in serum of Japanese quail fed on different type of probiotic established by table (4). The results recorded significant elevation in ALT activity for both control and sacro groups compared with other treatment of the study in 30 day intervals. Whereas, the same group (control and sarco) appeared less significant from the other treated groups. Whoever, mix probiotic group showed significant increase in AST activity compared with other studied groups during 30 day of experiment but there were non-significant differences recorded during 45 day of experiment among the group. In contrast, urea concentration showed high significant values for bifido and mix probiotic groups compared with other studies groups during the 30 day of experiment, but there were no differences among groups during 45 day of experiment.

Table (4) Effect of probiotics addition on liver enzymes activities and kidney function of Japanese quail birds during growth stage (mean ± standard error)

MCHC and MCH of blood indices. Whereas, MCV values appeared significantly higher during the period of 30 day of experiment in control and sarco groups than lacto, bifido and mix probiotic groups. While the 45-day period of experiment showed non-significant difference among the groups of the study.

indices / groups	MCHC%		MCH /pg		MCV/ft	
	30 DAYS	45 DAYS	30 DAYS	45 DAYS	30 DAYS	45 DAYS
Control group	30.30±0.45	34.29±1.01	44.29±1.39	46.13±1.39	149.50±1.50 a	146.17±4.55
Sacra. Group	30.26±1.25	34.42±2.03	43.15±0.65	41.62 ±0.65	155.66±2.33 Aa	137.51±2.19 B
Lacto. Group	30.45±0.72	33.61±1.08	39.85±3.73	40.45 ±1.04	118.16±1.33 c	133.48±3.41
Bifido. Group	29.74±1.22	29.74±2.44	38.46±2.45	40.22 ±2.99	120±2.12 c	136.86±14.17
Mix prob. Group	30.27±0.41	30.27±2. 12	40.89±0.94	39.48±2.21	131.64±3.21 b	130.42±7.32

Table 5: Effect of probiotic additive and their mixture to the diets on morphological changes in the intestines of Japanese quail birds at 42 days of age (mean ± standard deviation)

In Table 5 the fifth group was alone significant in the depth of the crypts compared with other treatment of the study. So, the ratio of length of villus and depth of crypts showed significant increase for mix probiotic group compared with other studied groups. While, control and sacro groups appeared significantly less the other studied groups of the birds.

Intestinal traits	Groups	(mean ± standard deviation)
Length of Villus	Control group	370.00 ± 80.00 b
	Sacro. group	380.00 ± 55.68 b
	Lacto.group	403.00 ± 51.32 a
	Bifido.group	393.33 ± 40.41 ab
	mix. group	420.00 ± 43.59 a
	Mean effect	**393.26 ± 54.2**
The Depth of the Crypts	Control group	36.67 ± 3.51b
	Sacro. group	37.33 ± 4.93b
	Lacto.group	37.50 ± 3.97ab
	Bifido.group	38.08 ±3.37 ab
	mix. group	40.33 ±3.79 a
	Mean effect	**37.98 ± 3.91**
Length of Villus/ The Depth of the Crypts	Control group	7.86±1.15 c
	Sacro. group	8.19 ± 0.88 c
	Lacto.group	9.95 ± 0.79 b
	Bifido.group	10.42±0.25 b
	mix. group	12.11±0.71 a
	Mean effect	**9.71 ± 0.75**

Discussion

Effect of probiotics addition on some blood parameters of Japanese quail birds during growth stage

Hematological indices are regards very important tools for clinical diagnosis and important indication for the health of animals. Table (2) showed non-significant difference among groups and between the intervals in total count of RBC for all studied group. Otherwise, elevation in Hb concentration for the groups treated with probiotic compared with control group that fed on standard ration without adding probiotics for the two intervals period (30 and 45) days. The significant increase in PCV confluent blood cells is attributed to the weight increase that occurred in the average body weight in those treatments, and because it mostly linked to a positive relationship, the improvement in production associated with the increase in body weight needs processing and also related to RBC and HGB status. In the form of red blood cells, PCV increased the body with high percentages of compacted blood cells occurred by the support or enhancement of lactic acid bacteria present in the manufactured bacterial preparation for the digestive system to provide a suitable environment for the activity of the intestinal flora to carry out its work in the

optimal way as well about giving way to it, due to the exclusion of pathogenic bacteria outside the digestive canal, which ensures that the integrity of the small intestine, thus improving the efficiency of utilization of nutrients, especially iron, Responsible (Hemoglobin), which is one of the necessary elements in the construction of hemoglobin molecules erythropoietin for the formation of red blood cells. It is also attributed to stimulating the kidneys to secrete the hormone which stimulates the bone marrow to produce red blood cells, which increases the volume of PCV (Al-Hasani, 2000). This result is in agreement with Odeh (2017) when using kefir milk as a probiotic in drinking water for broilers, and recorded non- significant differences in blood cells, HGB, PCV. Also, agreement with Al_ Sardary and Beski (2015) that used probiotic at the level of (5.2, 5) g / kg, does not affect the rate of PCV and other physiological parameters. Non-significant differences among groups and between the intervals for all studied groups for MCHC and MCH of blood indices. Whereas, MCV values appeared significantly higher during the period of 30 day of experiment in control and sarco groups than lacto, bifido and mix probiotic groups. While the 45 days period of experiment showed non-significant difference among the groups of the study. This result is in agreement with Hassan *et al*. (2019) In a study conducted on 360 experimental units of laying hens (Lohmann Light) for a period of 21 weeks, the results showed that the use of the probiotic at a level of 0.1% did not affect the concentration of cholesterol in the blood serum and liver enzyme got compared with the control factor. Revealed significant decrease in total protein level for birds fed on diet supplemented with lactobacillus for the 30 and 45 intervals of the experiment compared with other probiotic addition groups and control group. In the other hand, control and bifido group of birds showed significant increase in their values at 45 days of experiment compared with the same group at 30 days of experiment. In contrast, albumin level in the serum of birds for all study groups and the two intervals of experiment appeared non-significant. Otherwise, globulin levels that showed significant elevation in their levels for the mix and bifido groups in the two intervals of the experiment compared with other studied groups. The globulin level in bifido and sacro groups appeared significantly higher in 45day of experiment than 30 days of this study. while the other treatment group showed without difference. The reason for the decrease in the concentration of cholesterol is attributed to the treatments of probiotics that carry an enzyme hydrolase, which is made by lactic acid bacteria. It helps to unbind the bile salts, which reduces their solubility in the acidic medium and thus inhibits the process of cholesterol molecule formation, after which it is removed from the body with the feces. When measuring the glucose concentration, it was noticed that there were no significant differences between the age of (48) All trial transactions. This result is in agreement with Adli and Sjofjan (2020) was used Weighing 80 grams per 100 kg of broilers feed that no significant differences in the biochemical parameters of blood that included total protein, albumin, globulin, cholesterol, glucose and liver enzymes. As well agreement too with Khabirov *et al,* (2021) probiotic that contains two types of bacteria 10^6 and 10^7(cfu/g) for each type, with the number of Lactobacillus and Enterococcus respectively. Constricted In improving the biochemical blood parameters of broilers. Lactobacillus improves the coefficient of digestion and the coefficient of food conversion, by increasing the readiness of some nutrient compounds such as proteins, fats, carbohydrates, minerals and vitamins (Humam *et al.,* 2019). Table (4) observed significant elevation in bifido and mix probiotic groups compared with other treatment and control groups of birds. Also, there were significant increase in all treatment and control groups in glucose levels at 45 days of experiment when compared with the same group during the interval of 30 days, decrease in total protein level for birds feed on diet supplemented with lactobacillus for the 30 and 45 intervals of the experiment compared with other probiotic addition groups and control group. In the other hand, in contrast, albumin level in the serum of birds for all study groups and the two intervals of experiment appeared non-significant. The moral improvement that occurred in serum proteins, which included(Albumin, Globulin and total Protein) was caused by mix bacteria present in probiotics, which increases the production of Protein, by increasing the availability of nutrients, including protein, as well as increasing absorption the amino acid

lysine is secreted by some types of lactic acid bacteria, which is reflected in an increase in total protein in the blood serum of quail, which in turn increases albumin concentrations and globulin. This result does not agree with Hassan *et al*. (2019) In a study conducted on 360 experimental units of laying hens (Lohmann Light) for a period of 21 weeks, the results showed that the use of the probiotic at a level of 0.1% did not affect the concentration of cholesterol in the blood serum and liver enzyme got compared with the control factor. The results recorded significant elevation in ALT activity for both control and sacro groups compared with other treatment of the study in 30 days intervals. Whereas, the same group (control and sarco) appeared less significant from the other treated groups. Whoever, mix probiotic group showed significant increase in AST activity compared with other studied groups during 30 days of experiment but there were non-significant differences recorded during 45 days of experiment among the group. In contrast, urea concentration showed high significant values for bifido and mix probiotic groups compared with other studies groups during the 30 days of experiment, but there were no differences among groups during 45 days of experiment. Odeh (2017) using kefir milk as a probiotic with three different levels (12,8,4) ml / liter of drinking water for broilers, with no significant differences in blood cells PCV, albumin, glucose, AST and ALT liver enzymes with decreased Significant in the ratio of heterogeneous cells to lymphocytes L/H, cholesterol, and a significant increase in the concentration of total protein and globulin in comparison with the control. As well due to addition of Lactobacillus it works to reduce the concentration of cholesterol in the blood serum, by secreting the enzyme salt bile hydrolyse, which uncouples bile salts, increasing their excretion with the stool and thus contributes to lowering the concentration of cholesterol (Albano *et al*.,2018).

Effect of probiotic additive and their mixture to the diets on morphological changes in the intestines of Japanese quail birds at 42 days of age:

The moral improvement in the t that has occurred in the rate of the length of the villi and the depth crypts increases, Due to the improvement of gut conditions, after achieving microbial balance, by the action of the probiotics contained in different types of bacteria that act synergistically, the displacement of pathological bacteria, which impede the work of the gastrointestinal tract; Because it competes with the device the digestive system of quail depends on the nutrients that are released during the metabolism process, and their exclusion outside the body, it allows the host to benefit more from the absorption of nutrients, and as a result .Therefore, the rate of the length of the villi and the depth of the crypts increases, and the increase in the length of the villi contributes to a narrowing of the diameter the alimentary canal, which causes a slow passage of the food mass into the jejunal and ileal regions, and this It will also contribute to increasing the utilization of nutrients and improving the digestion coefficient. The reason is due to addition of probiotics increases the effectiveness of the beneficial bacteria in the small intestine by lowering the pH (increasing the acidity of the contents of the small intestine), (Mathlouthi *al et,*.2003). Al-Nuaimi (2015) broilers 42-day study in it, kefir milk was used as a prebiotic, it was added to drinking water at four different levels (5, 10, 15 and 20) g / liter, as it showed a significant increase in the average length of villi and depth of crypts in the jejunum, region compared to the control treatment. As well this reason may due to addition of lactobasillus this bacterium has superior ability to adhere to the inner cavity of the lining of the small intestine, and is considered a characteristic of adhesion is an important trait; Because it contributes to the settlement of lactic acid bacteria in the Gastrointestinal system (Akalu *et al*.,2017). broilers, probiotic was used with a weight of 80 g / 100 kg of food, a significant improvement was observed in the height of the staves and the depth of the crypts compared to the control treatment. It was found in a separate study that the exposure of broiler birds to the enhancer contributes to an increase in the height of villi and depth of crypts compared to the control treatment. Adli and Sjofjan (2020)

Conclusion

Our finding of study appear that physiological performance was improved when used different type of probiotic

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