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Effect of Supplementation of Organic Acids on Biochemical Status and Gut Health of Japanese Quail (*Coturnix coturnix japonica*)

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A B S T R A C T

The present research work was conducted to study the performance of Japanese quail (*Coturnix coturnix japonica*). A total number of 300 birds were reared for a period of thirty five days with dietary treatments; T0 - control diet as per ICAR (2013), T1 - T0+ 0.25% Acetic acid, T2 - T0+ 0.25% Butyric acid, T3 - T0+ 0.25% Propionic acid and T4 - T0 + 0.25% Acetic acid +0.25% Butyric acid+0.25% Propionic acid. Each treatment consist of sixty birds with four replicates containing fifteen birds per replicate. Two birds from each replicate of average body weight were selected and slaughtered and blood samples at the end of 35 days were collected for estimation of blood biochemical parameters and for gut health measurement. Blood biochemical parameters viz., total protein, albumin, globulin and cholesterol were found to be significant and triglyceride found non-significant. However there was decreased level of cholesterol in all treatment groups than control. In case of Gut health, ileal pH, intestinal weight, gut thickness, *E. Coli and Salmonella* count found to be significant.

Introduction

Globally, production of the primary poultry products (meat and eggs) has been rising rapidly. India is ranking third in egg production and fourth in meat production which indicates the need for increasing production to combat the problem of malnutrition (Poultry sector in India 2017). The growth is 6-8% in layers and 10-12% in broilers per annum against the growth of agriculture as a whole. Poultry birds are domesticated avian species that can be reared for eggs and meat. The term “poultry” covers a wide range of birds, from indigenous and commercial breeds of chickens to Muscovy duck, Chickens, ducks, guinea fowl, geese and turkeys can be found in all types of poultry systems, both large and small. But pheasants, quail and ostriches are almost exclusively found in large-scale systems (FAO, 2019). Introduction of quail is one of the critical milestones to fulfill the nutritional requirement by means of eggs and meat along with the broilers and the layers. Due to an increasing demand of meat and eggs, use of antibiotics in poultry as a growth promoter is still a common practice in developing countries (Aziz-Mousavi *et al.,* 2012; Shamlo *et al.,* 2014). The ban on the use of antibiotics as growth promoters in EU since 2006 permitted the finding of alternative to antibiotics in farm animal nutrition (Attia *et al.,* 2006; 2012). The poultry sector is continuously in look for new feed additives in order to improve the feed efficiency and health of poultry birds. One such substitute was the use of organic acids as a feed additive in the animal production. Organic acids stimulate the epithelial growth of the intestinal wall (Langhout and Sus, 2005). Dietary organic acids and their salts are able to inhibit the growth of detrimental microbes in the feed and subsequently maintains the level of beneficial bacteria in the gastrointestinal tract. In addition, they not only decrease the pH of the gastro-intestinal tract but also increases the solubility, digestion and absorption of the nutrients (Vogt *et al.,* 1981; Patten and Waldroup, 1988 and Skinner *et al.,* 1991). These studies would be related to the source, the amount of organic acids used, location, environmental condition and the composition of the diets (Gama *et al.,* 2000). Organic acids are either simple monocarboxylic acids (formic, acetic, propionic and butyric acid) or carboxylic acids with hydroxyl group (lactic, malic, tartaric and citric acid). In undissociated form, they penetrate the semi-permeable membrane of bacterial cell wall, enters the cytoplasm and decrease the internal pH affecting the enzyme system (e.g. decarboxylases and catalases) thus, inhibiting glycolysis, preventing active transport and interfering with signal transduction. Hence, the supplementation of organic acids in feed instead of sub-therapeutic dosage of antibiotics is being seriously considered, particularly in the context of reservations in using antibiotic as a feed additive. Considering the wide scope for the research of combination of single or blends of organic acids give optimum synergistic effect on blood biochemical performance and gut health of Japanese quail, the present study is planned.

Materials and Methods

An experimental study was conducted to study the effect of organic acids on blood biochemical and gut health of Japanese quail. A total 300 day old chicks were reared for five weeks with dietary treatments, T_0 (Standard broiler quail diet as per ICAR, (2013), T_1 (T_0 + 0.2% Acetic acid), T_2 (T_0 + 0. 2% Butyric acid), T_3 (T_0 + 0.2% Propionic acid), T_4 (T_0 + 0.2% Acetic acid + 0.2% Butyric acid + 0.2%) as shown in table 1. Each treatment group consisted of sixty birds with four replicates containing fifteen birds in each replication. The birds were reared on deep litter system and proper managemental practices were followed during the entire experimental period. Individual body weight of each bird was recorded at weekly interval and bodyweight gain was calculated as mean of each replicate. The birds from the experimental trial were assessed for the Biochemical parameters and gut health at 35 days of age. Blood samples from eight birds of each treatment (two from each replicate with average body weight) were collected toward the finish of test. The blood samples were collected via jugular vein from each bird and serum was isolated by centrifugation at 3000 RPM for 10 minutes and kept at – 20 ^{0}C till further investigation. Biochemical parameters included estimation of Total protein, Albumin, Triglyceride and Serum cholesterol utilizing biochemical kits produced by AGD Biomedicals PVT.LTD with the assistance of AGD Biochemistry Auto-analyzer whereas serum Globulin was determined by subtracting Albumin from total protein and represented in table 2. Gut health include intestinal weight, ileum pH, intestinal length, intestinal thickness and total microbial count. For Gut health parameter, the carcass of birds was subsequently opened and the entire gastrointestinal tract was removed aseptically and depicted in table 2.

The collected data during the study was analyzed statistically as per Snedecor and Cochran (1994) by utilizing Statistical Package for the Social Sciences (SPSS) Version 17.0.

Results and Discussion

The values of serum total protein (mg/dl) were T_0- 2.9 ± 0.007, T_1-3.48 ± 0.021, T_2-3.69 ± 0.016, T_3-3.54 ± 0.052and T_4 - 3.74 ± 0.008 with mean 3.47 ± .049. Among all the treatment group T_4 fed with mixture of organic acids significantly with highest value and among all the treatments however lowest serum total protein value was observed in control group. The values of serum albumin (mg/dl) for treatment group T_0 to T_4 with mean were 1.32 ± 0.011, 1.54±0.014, 1.6 ± 0.007, 1.65±0.005, 1.65 ± 0.004and 1.56 ± 0.021respectively. The data pertaining values of serum albumin subjected to analysis of variance found to be significant. The highest value for serum albumin was observed in T_3 group. Whereas lowest value was observed in treatment group T_0 fed normal diet as per ICAR, 2013. The data pertaining values of serum globulin (mg/dl) subjected to analysis of variance found to be significant. The values for treatment group T_0 to T_4 with mean (mg/dl) were 1.59 ± 0.013, 1.94 ± 0.033, 2.03 ± 0.016, 1.89 ± 0.06, 2.09 ± 0.029and 1.91 ± .032. It was observed that group T_4 fed with blends of acidifier showed numerically highest value among all the treatment group whereas control group showed numerically lowest value. The values for treatment groups of serum cholesterol (mg/dl) were T_0-232.87 ± 4.48, T_1-234.25 ± 1.96, T_2-195.87 ± 0.71, T_3-222 ± 2.02 and T_4-213.1 ± 2.06 with mean 219.62 ± 2.51. Significant differences were found among the treatments. Highest numerical value of serum cholesterol was observed in T_1 group (234.25 mg/dl) whereas lowest value of serum cholesterol was in group T_2 (195.87 mg/dl).

The data pertaining values of Triglyceride (mg/dl), subjected to analysis of variance found to be non-significant. The values of triglyceride for different treatment groups were T_0 -164.26 ± 1.08, T_1-162.45 ± 1.82, T_2-162.67 ± 1.94, T_3 - 163.80 ± 0.78 and T_4 - 161.29 ± 1.30with mean 162.90 ± 0.63. Group T_4 significantly lower triglyceride as compared to control.

The results obtained in the present study are with respect to total protein and Albumin in accordance with Elnesr *et al.,* (2018) who reported linear increase in serum total protein values when Japanese quails were supplemented with sodium butyrate. Non-significant differences for serum total protein were recorded by Naveenkumar *et al.,* (2018) when broilers were fed with diet containing organic acids. Similar results were reported by Abdel-Mageed (2012) in Japanese quails (*Coturnix coturnix japonica*) when supplemented diet with butyric acid glycerides as a growth promoter. Seifi *et al.,* (2015) observed non-significant differences for serum globulin values of broiler chickens when supplemented with acetic acid. Fouladi *et al.,* (2017) reported significant decrease in serum cholesterol values of Japanese quails when supplemented diet with organic acids. Brzoska *et al.,* (2013) reported non-significant differences for values of serum cholesterol concentration of broilers reared on different dietary treatments when supplemented with organic acids.

Table.1 Percent ingredient composition of starter diet (Starter phase and Finisher Phase)

Ingredient (%)	Starter						Finisher			
	T_0	T_0	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4
Maize	42	50.4	50.4	50.4	50.4	50.4	42	42	42	42
Soybean (DOC)	48.07	38.8	38.8	38.8	38.8	38.8	48.07	48.07	48.07	48.07
Palm oil	4	4	4	4	4	4	4	4	4	4
L-Lysine	0.12	0.13	0.13	0.13	0.13	0.13	0.12	0.12	0.12	0.12
DL-Methionine	0.14	0.25	0.25	0.25	0.25	0.25	0.14	0.14	0.14	0.14
Arginine	1.20	1.42	1.42	1.42	1.42	1.42	1.20	1.20	1.20	1.20
Threonine	1.00	0.70	0.70	0.70	0.70	0.70	1.00	1.00	1.00	1.00
Limestone powder	1	1.12	1.12	1.12	1.12	1.12	1	1	1	1
Dicalcium Phosphate	1.65	1.9	1.35	1.35	1.35	1.35	1.65	1.65	1.65	1.65
Trace-mineral mix	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin mixture	0.05	0.1	0.1	0.1	0.1	0.1	0.05	0.05	0.05	0.05
Salt	0.3	0.4	0.4	0.4	0.4	0.4	0.3	0.3	0.3	0.3
Choline chloride	0.12	0.13	0.13	0.13	0.13	0.13	0.12	0.12	0.12	0.12
Coccidiostat*	0.05	0.45	0.45	0.45	0.45	0.45	0.05	0.05	0.05	0.05
Toxin binder*	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Acetic acid	-	-	0.2	-	-	0.2	0.2	-	-	0.2
Butyric acid	-	-	-	0.2	-	0.2	-	0.2	-	0.2
Propionic acid	-	-	-	-	0.2	0.2	-	-	0.2	0.2
Total	100	100	100	100	100	100	100	100	100	100
CP(%)	25.0	21.5	21.5	21.5	21.5	21.5	25.0	25.0	25.0	25.0
ME (Kcal/kg)	2900	2950	2950	2950	2950	2950	2900	2900	2900	2900

***Indicates over and above the hundred

Table.2 Biochemical and gut health parametars

Treatments	Total Protein (mg/dl)	Albumin (mg/dl)	Globulin (mg/dl)	Cholesterol (mg/dl)	Triglyceride (mg/dl)
T0	2.91±0.007 [a]	1.32± 0.011 [a]	1.59±0.013 [a]	232.87±4.48 [d]	164.26±1.08 [a]
T1	3.48±0.021 [b]	1.54±0.014 [b]	1.94±0.033[bc]	234.25±1.96 [d]	162.45 ±1.82 [a]
T2	3.69±0.016 [c]	1.66±0.007 [c]	2.03±0.016 [cd]	195.87±0.71 [a]	162.67 ±1.94 [a]
T3	3.54±0.052 [b]	1.65±0.005 [c]	1.89±0.06 [b]	222±2.02 [c]	163.80 ±0.78 [a]
T4	3.74±0.008 [c]	1.65±0.004 [c]	2.09±0.029 [d]	213.12±2.06 [b]	161.29 ±1.30 [a]
Pooled Mean	**3.47±.049**	**1.56±0.021**	**1.91±.032**	**219.62±2.51**	**162.90 [a]±0.63**

Treatment Particular	T0	T1	T2	T3	T4	Pooled Mean
Ileal pH	6.41 ± 0.00 [e]	6.34 ± 0.00 [d]	5.96 ± 0.00 [c]	5.63 ± 0.00[b]	5.52 ± 0.00[a]	**5.97 ±0.05**
Intestinal Length	59.43 ± 2.44 [a]	55.30±1.03 [a]	52.49 ±2.13 [a]	54.32 ±3.12 [a]	58.19 ± 1.69 [a]	**55.94 ±1.01**
Int. wt	6.00 ± 0.30[bc]	5.98 ± 0.10[bc]	6.14 ± 0.08 [c]	5.66 ± 0.06[ab]	5.51 ± 0.07 [a]	**5.86 ±0.07**
Int. Thickness	0.38 ± 0.01 [c]	0.41 ± 0.00 [d]	0.27 ± 0.00 [a]	0.33 ± 0.00 [b]	0.27 ± 0.00 [a]	**0.33 ±0.00**
***E.Coli*(*10^7 CFU/g)**	7.24 ± 0.00 [b]	7.15 ± 0.06 [b]	6.84 ± 0.014 [b]	5.79 ± 0.38 [a]	5.72 ± 0.35 [a]	**6.55 ±0.14**
Salmonella (*10^7 CFU/g)	4.50 ± 0.32 [b]	2.84 ± 0.02 [a]	2.74 ± 0.02 [a]	2.86 ± 0.02 [a]	2.67 ± 0.01[a]	**3.12 ±0.12**

Treatments in column bearing common superscripts doesn't differ significantly (P<0.05)

The results of present study with respect to triglyceride was found to be similar with biochemical profile of hens given by Brzoska *et al.*, (2013) and Yesilbag and Colpan (2006) in chicken. On the other hand, Fouladi *et al.*, (2017) recorded significant difference in terms of triglyceride in Japanese quail.

This results obtained in present study are in agreement with Ishfaq *et al.*, (2015) who reported significant difference in terms of intestinal pH in broiler chickens. However, Kral *et al.*. (2011) and Abdel-Fattah *et al.*, (2008) found non-significant differences in GI-tract segments. Huff *et al.*, (1994) who performed a study to determine the effects of propionic acid-based mold inhibitor (Mycocurb®), calcium propionate and propionic acid on intestinal pH. Cobb x Cobb male broiler chicks and revealed the non-significant result among all treatment groups. In accordance with results obtained in present study Rehman *et al.*, (2016) worked on the influence of dietary acetic acid found higher intestinal weight. results corroborates with Kum *et al.*, (2010) and Kaya *et al.*, (2004) who found significant difference among treatment groups when broiler birds were feddiet supplemented with organic acids supplemented diet. Rodjan *et al.*, (2017) who evaluated the effect of organic acids or probiotics alone or in combination on gut health of broiler chickens and reported significant result. However, Gul *et al.*, (2014) and Paul *et al.*, (2007) found non-significant differences for *E. coli* count among treatments and control. The results of the present study are in accordance with Kazempour and Jahanian (2017) and Abdel *et al.*, (2012) who found significant results.

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