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Effect of Citric Acid and Saccharomyces Administration on the Growth Performance, some Biochemical and Histopathological Parameters on Broiler Chicks.

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Abstract

The present study was carried out to assess the efficacy of yeast probiotics and citric acid supplementation on the growth performance, some biochemical profiles, and histopathological alterations of broiler chicks. A total of 240 one-day-old Cobb 500 broiler chicks were purchased and randomly assigned into four groups (n=60) for the experimental trial. The four groups are, the control group (C-gr), the citric acid group (CA-gr), the probiotics group (PB-gr), and the combination group (CO-gr). The control group Fed on a standard diet and tap water. The CA group was given a standard diet and acidified water (at pH 4.5) using citric acid monohydrate (0.8 g /liter) in drinking water. Probiotic (Saccharomyces) was given to the PB group at a rate of $2x10^9$ CFU/liter, while the combination group received combination of PB $2x10^9$ CFU/liter and CA in their drinking water 0.8 g /liter. The trial was lasted for 5 weeks. Results revealed that live body weight (LBW) of the combination group was significantly higher than other groups. Comparing CA gr to other groups, the gizzard weight was significantly (P<.05) high. Liver weight revealed significant increasing in all treated groups. Likewise, heart weight showed a significant increase in the CA and PB groups. MDA level showed significant decrease in all treated groups. GSH level and Serum T3 concentration were significantly (p<0.05) increased only in PB and combination groups, while serumT4 concentration was obviously elevated in all treated groups. The CA, PB, and CO groups showed a significant (p<.05) decrease in TC and TG. In all experimental groups, HDL-C was significantly (p<.05) increased, but LDL-C showed a significant reduction especially in the PB group compared to C-gr. CA and PB groups showed long villi than the C-gr and CO-gr at 3rd and 5th weeks. In general, a combination of PB and CA has a promising effect and may boost the growth performance and biochemical profiles of broiler chicks after short-term supplementation.

Keywords: Broilers, Probiotics, Citric acid, Biochemical, Histopathological parameters

1. Introduction

Poultry meat and their products are widely consumed in Egypt. Large-scale consumption of poultry meat is due to their competitive pricing in comparison with other animal meat products. One goal of every poultry producer is to maintain the balance between low-cost diets and maximizing output. Poultry production is a company that, like other businesses, aims to make a profit [1]. Thus, the industry works toward obtaining the greatest amount of weight gain for the lowest production cost [2]. The production of poultry still experiences significant losses because of pathogenic bacterial invasion and the harmful effects of these organisms on the birds includes decrease weight

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gain or higher mortality, poor feed efficiency, and weak chicken performance [3]. All of these effects, including the load in the environment and gastrointestinal tract, are tightly correlated with the host bird's qualitative and quantitative microbial load [4].

Antibiotics as antimicrobial feed additives are usually used to eliminate or suppress the hazardous intestinal microorganisms and to hasten growth and feed efficiency [5]. Recently, confidence has diminished about the use of antibiotics due to fears over bacterial resistance and possible transmission of these antibiotic residues into the human food chain [6]. These residues may have a potential health hazard to humans.

The Aim of work is to study the effect of citric acid and saccharomyces cerevisiae on body weight, Edible organs weight, hepatic malondialdehyde (MDA), reduced glutathione (GSH), thyroid hormones (triiodothyronine T3 and thyroxine T4), Lipid profile (TC, TG, HDL-C and LDL-C) and histomorphological and morphometrical changes in different segments of intestine.

2. Materials and methods:

2.1. Birds and management

A total of 240 one-day-old apparent healthy Cobb 500 broiler chicks of both sexes, weighing 50±5 g has been purchased from Ismailia-Miser for Poultry Company, Egypt. Chicks were raised in a well-ventilated, clean environment with a range of temperatures (32–35 °C). Electric heaters were employed to change the temperature, and electric lamps had been used as a source of light. Chicks received light for the entirety of the day (24 h). Vaccinations were supplied to chicks, against Newcastle disease (ND) and Infectious Bursal Disease (Gumboro) at some point of the experimental period as ordinary.

2.2. Experimental Diet

A starter between the ages of 1 and 17 days and a finisher between the ages of 18- and 35-days' meals were offered to the experimental birds meanwhile the trial as provided in table 1. According to [7], both diets have been designed to meet the nutritional needs of broilers.

2.3. Experimental Design:

Experimental chicks were divided into 4 equal groups, each contributing 60 chicks. Control group (C-gr) provided with a normal diet and tap water. While citric acid (CA-gr) was produced using citric acid monohydrate (Sigma, Australia) with a normal diet and drinking water acidified to (pH 4.5) [8]. Probiotic group (PB-gr) received a basal diet and probiotics 2x10^9 CFU / liter in their drinking water. A combination group (CO-gr) received mixture of probiotics 2x10^9 CFU / liter and citric acid in their drinking water 0.8 g /liter. The treatment continued for 35 days. Body weight gain of each chick were measured at 28 and 35 days.

Table 1: Compositions of the basal diets

Ingredients (%)	Starter (1-17 days)	Finisher (18-35 days)
Yellow corn	61.77	73.00
Soybean meal (44 % CP)	24.50	13.60
Corn gluten meal	10.00	10.00
Bone meal	2.60	2.0
Limestone	0.30	0.60
Vit. & Min Premix	0.30	0.30
NaCl	0.25	0.25
Lysine	0.18	0.20
Dl- Methionine	0.10	0.05
Total	100	100

2.4. Blood sampling:

Wing vein blood samples were obtained at the age of 28 days and at the end of the experimental period (at 35 days) after an overnight fast, serum samples were drawn into simple tubes and kept at -20°C till lipid and hormonal profile analysis.

2.5. Edible organs weight:

The edible visceral organs (heart, liver, and gizzard) of the slaughtered chickens were removed, cleaned, and weighed at the end of the trial.

2.6. Thyroid Hormones:

Using Taytec Incorporation's (Canada) commercial enzyme-linked immunoassay kits, serum levels of T3 and T4 have been measured in accordance with the manufacturer's protocol.

2.7. Lipid profile:

Using commercial kits (Diamond, Egypt), the levels of high-density lipoprotein cholesterol (HDL-c), triglycerides (TGs) and total cholesterol (TC) were calorimetrically determined. Enzymatic calorimetric kits (QCA Co., Spain) were used to measure serum low-density lipoprotein cholesterol (LDL-c) via accordance with [9].

2.8. Histopathological Examination:

Small intestine samples were obtained shortly after the chicks were slaughtered and preserved in 10% formalin saline for 24 h. Hematoxylin and eosin staining of 5-μm thick sections was done using standard histological laboratory methods [10]. Using Image J software, a morphometric analysis of intestinal villus height (VH, m) and crypt depth (CD, m) was performed. [11]. The CD was calculated as the depth of the invagination between two adjacent villi whereas the VH was measured from the villus top to the lamina propria [12].

2.9. Statistical analysis:

Data collected from treated groups were statistically analyzed in comparison to control group for the mean and standard error using statistical software program (SPSS for Windows, version 16, USA) according to [13]. Differences between means of different groups were carried out using one-way ANOVA followed by Duncan Multiple Range tests [14]. Differences were considered significant at (P<0.05) and highly significant at (P<0.01).

3. Results

3.1. Effects of dietary Citric acid and/or yeast probiotic supplement on body weight and internal organs of broilers

Table (2) demonstrated that LBW, gizzard weight showed a significant (P<0.05) improvement in citric acid, yeast probiotic and combination group than control after 28 days, Combination group showed significant (P<0.05) higher body weights than yeast probiotic and citric acid group. Liver and heart weights resulted in non-significant (P>0.05) alternation between all groups at 28 days. At 35 days, citric acid, yeast probiotic and citric acid and yeast probiotic groups exhibited significant (P<0.05) higher body weights, liver weight than control but no significant variations were observed between them. Heart weight exhibited significant (P<0.05) higher values in citric acid and yeast probiotic groups than control and combination groups while the gizzard weight exhibited non-significant (P>0.05) alternation at 35 days among all groups.

3.2. Effects of dietary Citric acid and/or yeast probiotic supplement on malondialdehyde content (MDA) and glutathione content(GSH)

Table (3) demonstrated that the levels of hepatic MDA, as an indicator for lipid peroxidation, was significantly (P<0.05) reduced in citric acid, yeast probiotic and combination groups than control at 28 days and 35 days. There were non-significant (P>0.05) differences observed among treated groups at 28 days or 35 days. Nonetheless, at 28 days of experiment, the activity of hepatic GSH revealed a significant promotion (P<0.05) in PB-gr and CO-gr than control and citric acid groups. At 35 days, the activity of GSH in serum showed significant (P<0.05) elevation in yeast probiotic and combination groups than citric acid treated group and control group. No significant (P>0.05) differences observed between citric acid and control groups as well as yeast probiotic and combination groups.

3.3. Effects of dietary Citric acid and/or yeast probiotic supplement on serum thyroid hormones (T3 and T4)

As indicated in Table 4, the level of T3 was significantly (P<0.05) elevated in yeast probiotic and combination groups than control and citric acid groups at 28 days and 35 days. The combination group showed superior T3 level than yeast probiotic group at 28 days and 35 days. The T4 levels were significantly (P<0.05) improved in citric acid,

Table 2: Effects of dietary citric acid and yeast probiotic supplement on body weight and internal organs weight of broilers

Tested parameters / days	After 28 days				After 35 days			
	C-gr	CA-gr	PB-gr	CO-gr	C-gr	CA-gr	PB-gr	CO-gr
Body weight (g)	$920.00^{d} \pm 30.55$	$1153.33^{c} \pm 17.63$	$1286.66^{b} \pm 6.66$	$1516.00^{a} \pm 23.43$	$1536.25^{b} \pm 27.65$	$2104.50^{a} \pm 61.16$	$2098.25^{a} \pm 99.69$	$2270.00^{a} \pm 88.53$
Gizzard (g)	$19.00^{b} \pm 2.08$	$22.33^{a} \pm 2.18$	$23.34^{a} \pm 1.66$	$26.66^{a} \pm 0.88$	$23.41^{a} \pm 0.93$	$29.51^{a} \pm 0.51$	$28.90^{a} \pm 1.00$	$28.34^{a} \pm 1.23$
Liver (g)	$21.67^{a} \pm 1.66$	$20.00^{a} \pm 2.88$	$21.66^{a} \pm 1.66$	$16.67^{a} \pm 1.65$	$42.93^{b} \pm 2.32$	$50.51^{a} \pm 1.78$	$52.61^{a} \pm 1.24$	$49.98^{a} \pm 2.42$
Heart (g)	$5.37^{a} \pm 0.26$	$5.26^{a} \pm 0.18$	$5.73^{a} \pm 0.27$	$6.11^{a} \pm 0.40$	$7.55^{b} \pm 0.37$	$10.43^{a} \pm 0.48$	$11.49^{a} \pm 0.65$	$7.96^{b} \pm 0.49$

* Values are significant at $p < 0.05$

Table 3: Effects of dietary citric acid and yeastprobiotic supplement on malondialdehyde (MDA) and glutathione content (GSH) ofbroilers

Groups	After 28 days				After 35 days			
	C-gr	CA-gr	PB-gr	CO-gr	C-gr	CA-gr	PB-gr	CO-gr
MDA (nmol/mg)	$0.82^{a} \pm 0.02$	$0.54^{b} \pm 0.06$	$0.66^{b} \pm 0.03$	$0.55^{b} \pm 0.03$	$0.80^{a} \pm 0.01$	$0.48^{b} \pm 0.05$	$0.56^{b} \pm 0.03$	$0.53^{b} \pm 0.02$
GSH (nmol/g)	$6.17^{b} \pm 0.27$	$7.63^{b} \pm 0.43$	$8.81^{a} \pm 0.61$	$8.83^{a} \pm 0.71$	$8.87^{b} \pm 0.61$	$10.70^{b} \pm 0.62$	$12.77^{a} \pm 0.22$	$14.17^{a} \pm 0.58$

* Values are significant at $p < 0.05$

yeast probiotic and combinations group than control group at 28 days and 35 days.

3.4. *Effects of dietary Citric acid and/or yeast probiotic supplement on lipid profile parameters*

At 28 days, the yeast probiotic group had significantly lower total cholesterol (TC) and triglycerides (TG) than the control group (P<.05). All experimental groups revealed non-significant changes in High-density lipoprotein cholesterol (HDL-c) and Low-density lipoprotein cholesterol (LDL) compared to the control.

At 35 days, the CA, PB, and combination groups had significantly lower levels of TC and TG than the control group. In comparison to control, HDL-C revealed a significant increasing (p<0.05) in all treated groups. Compared to the control, LDL-C significantly decreased in all experimental groups (CA, PB, and combination groups), with highly significant reduction in PB as compared with CA and combination group (Table 5).

3.5. *Effects of dietary Citric acid and/or yeast probiotic supplement on histology of intestine at 3rd and 5th week*

Control group showed normal healthy intestinal villi of all segments (duodenum, jejunum and ilium. Citric acid fed group showed normal healthy long intestinal villi with increase in number of goblet cells lining the intestinal villi. The probiotic fed group showed normal histological architecture, including long villi with normal epithelial lining and normal intestinal glands. The combination group had normal histological intestinal villi. Figures (1-6).

4. Discussion

In many countries throughout the world, the use of antibiotics as a growth enhancer in poultry feed has been prohibited.

In order to improve growth and prevent diseases in poultry, it is important to look for al-

Table 4: Effects of dietary citricacid and yeast probiotic supplement on triiodothyronine (T3) and thyroxine (T4)levels of broilers

Groups	After 28 days				After 35 days			
	C-gr	CA-gr	PB-gr	CO-gr	C-gr	CA-gr	PB-gr	CO-gr
T3 (ng/ml)	3.98^{c} ±0.09	4.91^{a} ±0.11	4.73^{a} ±0.18	4.45^{b} ±0.15	4.13^{c} ±0.09	5.37^{a} ±0.01	5.16^{a} ±0.02	4.84^{b} ±0.08
T4 (ng/ml)	0.49^{b} ±0.01	0.59^{a} ±0.03	0.54^{a} ±0.03	0.60^{a} ±0.03	0.47^{b} ±0.01	0.58^{a} ±0.02	0.64^{a} ±0.03	0.61^{a} ±0.03

* Values are significant at $p < 0.05$

Table 5: Effects of dietary citricacid and yeast probiotic supplement on Lipid profile parameters of broilers

Groups	After 28 days				After 35 days			
	C-gr	CA-gr	PB-gr	CO-gr	C-gr	CA-gr	PB-gr	CO-gr
TC	184.00^{a} ±4.50	164.00^{a} ±8.50	148.30^{b}±5.50	167.00^{a} ±2.65	140.00^{a} ±2.04	100.00^{b} ±8.17	85.00^{b} ±6.12	90.00^{b} ±4.08
TG	120.00^{a} ±5.60	113.33^{a} ±2.18	98.30^{b}±2.33	113.00^{a} ±1.0	120.80^{a} ±2.04	73.10^{b} ±1.22	63.00^{b} ±1.23	63.20^{b} ±1.23
HDL-C	120.30^{a} ±6.90	108.30^{a} ±4.88	100.00^{a} ±7.50	103.00^{a} ±1.20	40.00^{c} ±1.29	65.00^{a} ±2.06	63.00^{a} ±1.23	55.00^{b} ±2.39
LDL-C	39.70^{a} ±4.30	33.00^{a} ±3.20	28.70^{a} ±2.40	41.30^{a} ±1.70	76.00^{a} ±1.03	20.00^{b} ±0.70	9.00^{c} ±1.05	22.00^{b} ±0.89

* Values are significant at $p < 0.05$

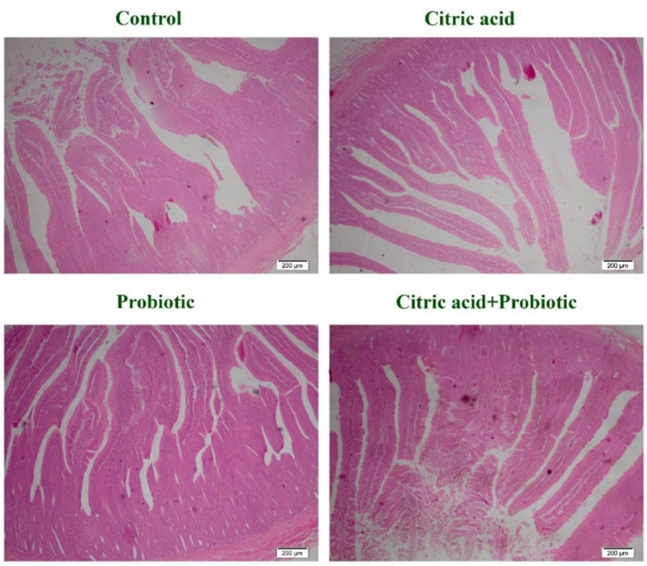

Figure 1: Duodenum at 3rd week showing elongation of villus heights in all treated groups especially probiotic fed group. H&E. Bar=200 μm.

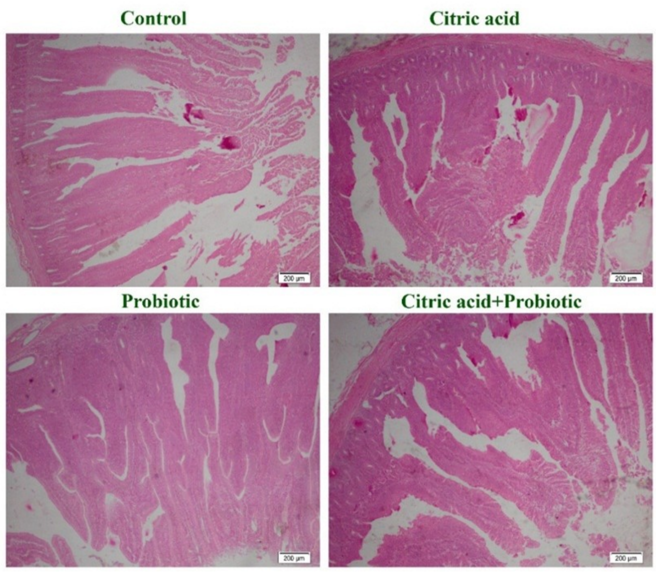

Figure 2: Duodenum at 5th week showing elongation of villus heights in all treated groups especially probiotic fed group. H&E. Bar=200 μm.

ternatives. Probiotics are considered as alternative feed additives to antibiotics and microbial food supplements that have a beneficial effect on the host by improving its intestinal microbial balance [15]. To increase production or stop the spread of diseases, organic acids have been introduced to broiler chick's food or drinking water. OAs have been applied for antimicrobial, disinfecting and hygienic purposes in animal feed industry. Supplementation of a combination of probiotic and organic acids shows a significant improvement in the performance of poultry [16]. Thyroid hormones, especially T3, which have a physiological impact on all major metabolic pathways and can, lead to improved body weight. The potential impact of this study of PB and CA as feed additives on the body weight, weight of their edible organs, feed intake, lipid peroxidation (MDA), glutathione ratio (GSH), serum T3 and T4 and Histopathological examination of intestine.

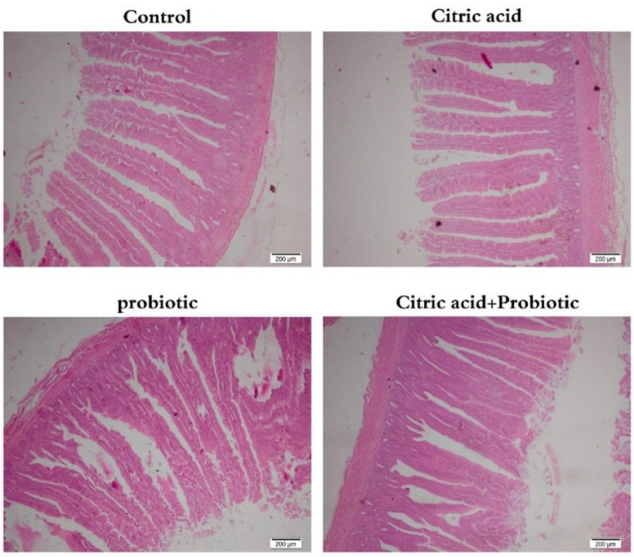

Figure 3: Duodenum at 5th week showing elongation of villus heights in all treated groups especially probiotic fed group. H&E. Bar=200 μm.

The results of body weight were in accordance with those of [17–21], [23].

Concerning the internal organs of broilers results of the current study revealed that, for the gizzard weight, the results agree with [18], [22], [23–27], and disagree with [19], [30]. For liver weight, the results agree with [19], [27], and disagree with [31–33]. And for the heart weight, the results were in accordance with those of [19], [34] and disagree with [27].

The improvement in body weight and edible organs weight of birds supplemented with CA may be due to improved utilization of minerals, protein proteolysis and amino acids digestibility [35]. The more powerful effect of CA could be attributed to citrate, the conjugate base of CA as one of a series of compounds involved in the physiological oxidation of acetate from fats, proteins, and carbohydrates. The acetate from these macronutrients is converted into the intracellular energy of ATP, as well as the common by products carbon dioxide, and water. This series of chemical reactions is central to nearly all metabolic reactions and is the source of two-thirds of the food-derived energy in higher organisms [36].

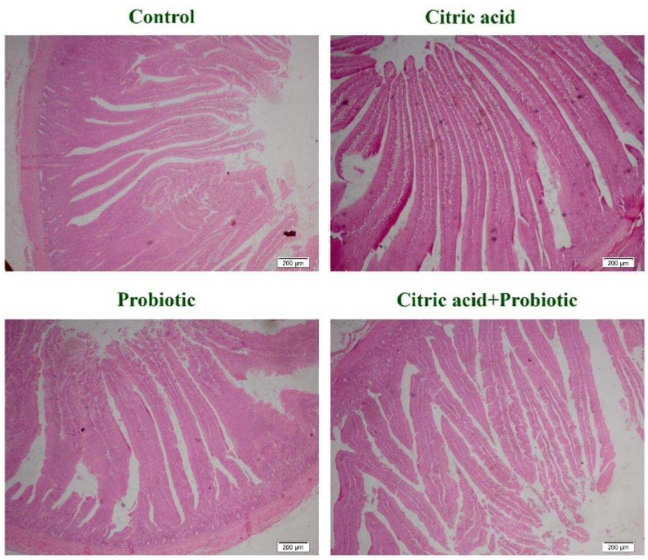

Figure 4: Jejunum at 5th week, showing pronounced elongation of villi in all supplemented groups. H&E. Bar=200 μm.

The results related to hepatic MDA level were similar to those obtained by [37- 43] and disagree with [44].

The typical shelf life of fresh meat is only a few days due to the quick oxidation of the lipid and protein contents [45]. MDA has been recognized as the primary byproduct of lipid oxidation by reactive oxygen species (ROS), which is typically used to measure the level of lipid peroxidation in poultry flesh [46]. In this study, dietary supplementation with OA might reduce lipid peroxidation, as seen by decreased serum MDA, which could enhance the quality of the meat and increase its shelf life.

The results showing the activity of hepatic GSH are in agreement with [37], [40- 44].

The antioxidant enzyme GSH acts as the first line of defense against oxidative damage, relieving it by removing the excessively produced free radicals. It has been observed that dietary probiotics in diets were valuable to the antioxidant capacity of broilers, which is consistent with our results [45]. The peroxides end up turning out to be nonoxidative, hazardous, or more stable metabolites in the metabolic pathway of the GSH process, which involves GSH oxidation. It has been discovered that including dietary probiotics in diets may increase the body's capacity for antioxidants [47].

Concerning the concentration of the T3 serum, the results of the current study are in agreement with [35], [48-49].

Concerning the concentration of the T4 serum, the results are in agreement with [50- 53] and disagree with [19].

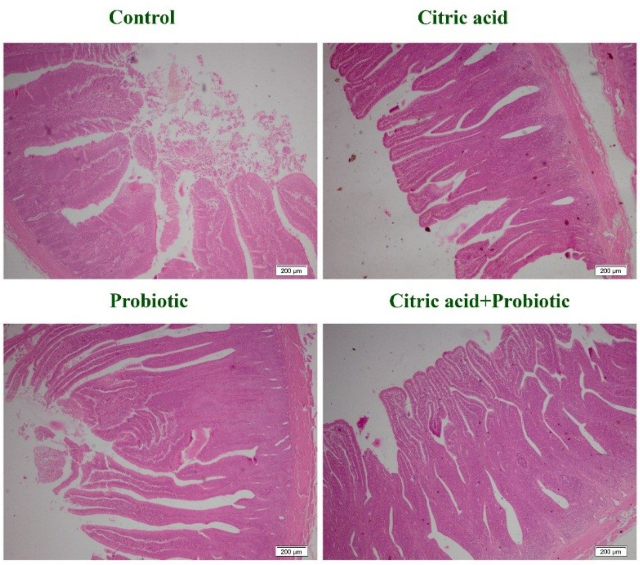

Figure 5: ileum at 3rd week showing elongated villi along with hyperplastic lymphoid follicles. H&E. Bar=200 μm.

Similar to mammals, birds' thyroid glands generate hormones that effectively regulate the metabolism and growth of body tissues [54]. Previous investigations suggested that the indirect effects of normal thyroid hormone levels on bird

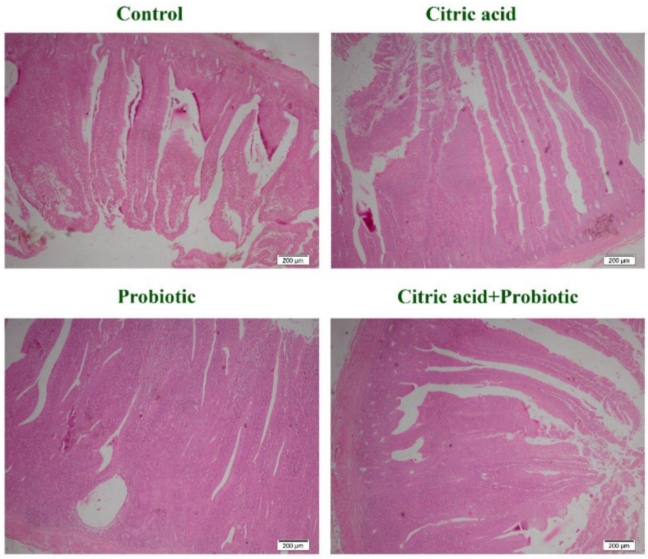

Figure 6: ileum at 5rd week showing pronounced elongated villi along with distinct hyperplastic lymphoid follicles. H&E. Bar=200 μm

growth were caused by the stimulatory effects of growth factors like insulin-like growth factor [55]. According to the results of various research, including thyme in the diet of poultry improved feed conversion and increased broiler weight [56-58]. In a similar way, numerous studies revealed that the use of probiotics increased the growth performance of poultry [59], [60].

Over 90% of the thyroid hormones produced by chicken's thyroid gland are T4 while the majority of T3 hormones are produced by the pituitary gland's conversion of T4 to T3 [61]. Probiotic use, however, significantly raised blood T4 levels. The probiotics utilized in this study appeared to have a greater impact on the creation and secretion of T4 because of their positive effects on the digestive tract enhanced digestibility, nutrient absorption, and nitrogen retention materials [62]. Previous studies showed that the thyroid gland is more successful at producing T4 than T3 when digestion and absorption of nutrients for the synthesis of thyroid hormones are optimal in the diet of poultry. [63], [64].

The findings of lipid profile parameters are in agreement with [34], [65- 70].

The reduction in lipid profile parameters may be explained as the observed lower feed consumption during the period of growth and consequently lower fat intake resulted in fat depletion may also contribute in reducing blood lipid content. Moreover, the observed hyperthyroidism associated with water acidification could also explain the observed reduction in serum lipid profile [71] where thyroid hormones regulate lipid synthesis, mobilization and degradation, even though degradation is influenced more than synthesis [72]. T3, T4 favor lipolysis in adipose tissue resulting in a decrease in plasma cholesterol content and they may have an indirect effect on lipogenesis [73]. They favor lipolysis in adipose tissue resulting in a decrease in plasma cholesterol content and they may have an indirect effect on lipogenesis [74]. They also can stimulate liver gluconeogenesis through gene activation [75] and [76].

5. Conclusion

By incorporating CA and PB to the broilers' water, researchers were able to influence the health of their liver, intestinal mucosa, and thyroid hormones (T3 and T4), were improved and promoted. That illustrates how CA and PB have a stronger impact on balancing body internal homeostasis.

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