Beneficial Effects of Pinocembrin and Quercetin on the general conditions, immune parameters and gut lactobacilli in broiler chickens

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1. Abstract

There are various stressors that may contribute to commercial poultry production leading to economic losses due to decreased productivity and performance of growing chickens. Understanding the molecular pathways during the oxidative stress in birds is critical in the current immunological research. In this study, pinocembrin and quercetin are examined to explore their anti-inflammatory, antioxidant, hepatoprotective effects in broiler chickens along with their impact on gut health, and meat quality. A total of 300 one day old broiler chicks (Ross 308) were randomly allocated and divided into 2 groups. G1was kept as control non-treated and G2 received 2 supplements of Liba-Ton® as a pinocembrin natural source from propolis and Radical-Free®, a synthetic quercetin, for 8 hrs. daily for 3 days each week until the end of experiment at 35 days old. Parameters of growth (BWG, FI, and FCR), immune organ index (spleen, thymus, and bursa), anti-inflammatory signaling (IL-B1 and Phagocytic index), liver biochemistry (AST, ALT, AKP, and GT), antioxidant activity (SOD, CAT, TAC, and GSH in liver and muscle tissues) were assayed. The obtained results revealed positive impact of pinocembrin and quercetin supplementation in broilers. Carcass yield and muscle quality revealed that the dressing percentage was significantly increased in G2 to 73.63% as compared to 73.3% in G1. Also, the giblet percentage revealed significant increase of liver, heart, gizzard (2.35, 0.54 and 2.17 respectively), while breast muscle yield showed significant increase from 38.2% in chickens of G1 to 41.6% in G2 with 5.8 pH, 24hr and 71.55 water holding capacity.

Key words: Antioxidant, Anti-inflammatory, Broilers chickens, Pinocembrin, Quercetin.

2. Introduction

In recent decades, global demand for poultry meat has significantly increased [1]. This expansion has driven genetic selection in commercial broiler chickens toward rapid growth rates and increased breast muscle yield [1]. However, stress and inflammation can

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induce oxidative stress, characterized by lipid peroxidation, apoptosis, and DNA damage [1]. These effects result from an imbalance between the generation of reactive oxygen species (ROS) and the bird's innate antioxidant defenses, leading to negative consequences for overall health and performance [1].

The liver, a central metabolic organ, plays vital physiological roles including biosynthesis, detoxification, clearance, and immune defense [2]. Liver damage is increasingly common and may arise from exposure to xenobiotics, malnutrition, or other chronic conditions [2]. There is growing evidence suggesting a strong correlation between liver dysfunction and muscular disorders. Transcriptomic analyses have identified key transcriptional networks linking hepatic and muscle responses in broilers affected by myopathies, using tools such as Ingenuity Pathway Analysis [3]. ROS can originate within the cytosol and various cellular compartments, including the plasma membrane. Due to the high content of polyunsaturated fatty acids (PUFAs) in membranes, lipid peroxidation may occur, causing phospholipid damage and potentially signaling cell death [4].

Quercetin, a naturally occurring flavonoid found in the skins of citrus fruits, vegetables, seeds, flowers, nuts, leaves, and bark [5,6], has attracted interest for its health-promoting properties in poultry. Upon ingestion, quercetin is more effectively absorbed and retained in the body compared to many other flavonoids [7]. It exhibits potent antioxidant and antibacterial activities [8]. Recently, the strong antioxidant capacity of quercetin has prompted its evaluation as a dietary supplement in animal feed. It has been shown to reduce feed lipid peroxidation, enhancing the sensory quality and nutritional value of meat and eggs, and extending the shelf life of these products [9, 10]. In broilers, quercetin supplementation has also been linked to improved growth performance and enhanced oxidative stability and quality of meat [11]. The core mechanism underlying quercetin's antioxidant activity is its ability to scavenge free radicals. By neutralizing these reactive molecules, quercetin protects erythrocytes against damage from environmental stressors [12, 13].

Pinocembrin (S) - 5,7 – dihydroxy-flavanone is a naturally occurring flavonoid identified in various sources such as propolis, honey, and a range of plants including *Glycyrrhiza glabra*, ginger roots, and wild marjoram [14]. In addition to its natural extraction, pinocembrin can also be synthesized through chemical or biological methods [15]. Research has shown that pinocembrin possesses a broad spectrum of pharmacological properties, including antibacterial, anti-inflammatory, antiprotozoal, and antioxidant activities [16]. Thus, the current study aimed to assess the antioxidant and hepatoprotective effects of both quercetin and pinocembrin, with a particular focus on evaluating their impact on carcass yield and meat quality.

3. Materials and Methods

3.1. Ethical Approval

All experimental procedures were approved by the animal care committee of Beni-Sueif University, Egypt (approval number: 022-489).

3.2. Chicken Management and Treatment

A total of 120 one day old broiler chicks (Ross 308) with initial weight about 42 g were randomly allocated and divided into 2 equal groups. Feed and water were supplied *ad libitum*. The composition of starter, grower and finisher feed is indicated in Table 1. An immunization program against infectious bronchitis (IB) at hatchery coarse spray, avian influenza (AI) plus Newcastle disease (ND) at 7day old S/C, and infectious bursal disease (IBD) at 12 day old eye drop was carefully applied. The chickens under the experiment were divided into 2 groups. Group 1 (G1) was kept as control non-treated and G2 was treated with Liba-Ton® (pinocembrin natural source from propolis) and Radical-Free® (an apple origin quercetin) in drinking water with a dose of 0.5 mL/ liter from both products for 8 hrs. on the 7th, 8th and 9the days and repeated weekly at 16-18, 22-24 and 28-30 days of age. Both products are produced by MN Trade Industrial Inc., 10th of Ramadan city, Egypt.

3.3. Growth Indices and Gut Integrity

On days 10, 28 and 35 all chicks were weighted and the feed intake (FI), the average body weight gain (BWG) and the feed conversion ratios (FCR) were all determined. On the 35th day of age, five birds with weight near to the gathering average for every treatment were chosen to examine the final carcass characteristics [17].

3.4. Bacterial Counts

Intestinal samples (*n*=9) were collected at 10th, 20 th, and 30th days of age for counting of enteric lactic acid bacteria on De Man–Rogosa–Sharpe (MRS) agar and detecting the intestinal colonization of *C. perfringens* on tryptose sulfite-cycloserine (TSC) agar supplemented by D-cycloserine and egg yolk emulsion under anaerobic incubation at 37°C [18].

3.5. Anti-Inflammatory Cytokines and Phagocytic Index Determination

Interleukin-1β (IL-1β) and IL-6 concentrations were analyzed using ELISA kits, Chicken ELISA IL-1 (Range 15.6 - 1000 Pg / ml) (MyBioSource, CA, USA) and Chicken ELISA IL-6 (Range 0.85 - 20 ng / ml) (Invitrogen, Thermofisher, Massachusetts, USA) following the manufacturer's instructions according to [19]. CytoSelect™ 96-well phagocytosis assay, red blood cell substrate (Cell Biolabs, Inc., CA, USA) was carried out according to [20] on blood samples collected at 7, 15, 28 and 35 days of age from chickens of the two groups.

3.6. Blood and Tissue Samples

Blood samples (*n* = 9) were collected from birds at 10, 20, and 30 days of age both on EDTA-treated tubes for plasma and plain tubes for serum. Plasma-designated samples were immediately placed on ice, then centrifuged at 3000×g for 15 minutes at 4°C. The resulting plasma was stored at −80 °C for subsequent analysis. Serum was obtained by centrifuging the samples at 3500×g for 15 minutes at 20 °C and subsequently analyzed for total antioxidant status (TAS). Whole blood samples, used for measuring glutathione peroxidase (GSH-Px) activity, were stored at −20 °C. In addition, liver tissue and pectoralis superficialis muscle samples (approximately 4 cm from the distal part of the muscle) were collected immediately after death (n = 9). These tissues were sectioned into small fragments (10 × 5 mm), rapidly frozen in liquid nitrogen, and preserved at −80 °C for further analysis [21].

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3.7. Total Antioxidant Activity Determination

The antioxidant capacity of collected serum samples (n = 9) was assessed using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. Absorbance of the DPPH radical was measured at 516 nm using a double beam spectrophotometer. Each assay was conducted in triplicate, and the results were expressed as Trolox equivalent antioxidant capacity (TEAC), with values reported in millimoles per liter (mMol/L). Additionally, total antioxidant status (TAS) was determined using the Randox® Total Antioxidant Status Assay Kit (Randox Laboratories Ltd., County Antrim, United Kingdom) [22].

3.8. Glutathione Peroxidase (GSH-Px) Activity

Glutathione peroxidase (GSH-Px) was measured in the collected serum samples using a Randox® RANSEL Glutathione Peroxidase assay KIT (Randox Laboratories Ltd., County Antrim, United Kingdom). The results were expressed as activity of GSH-Px in log10 U/L or as mMol/L for TAS [22].

3.9. Antioxidant Activity in Pectoral Muscle and Liver Tissues

Catalase (CAT) and superoxide dismutase (SOD) activities were measured in liver and pectoralis muscle samples (n = 9) obtained from the investigated chickens. CAT activity was evaluated by monitoring the decomposition of hydrogen peroxide (H_2O_2) at 25 °C, with absorbance recorded at 240 nm using a double beam spectrophotometer. SOD activity was measured at 450 nm using a microplate reader (EPOCH2), based on the inhibition of xanthine/xanthine oxidase-induced cytochrome c oxidation. This assay employed a commercial kit (Sigma-Aldrich, St. Louis, MO, USA), with bovine erythrocyte-derived SOD serving as the reference standard. Enzyme activity levels were reported as units per liter (U/L) of supernatant for CAT and as units per well for SOD. All assays were conducted in triplicate to ensure accuracy and reproducibility [23].

3.10. Liver Enzymes Biochemistry

The activities of serum enzymes, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AKP), and gamma-glutamyl transferase (γ-GT), were measured using commercial assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's protocol [24].

3.11. Carcass Yield and Meat Quality

On the 35th day of age, the meat quality variables, carcass component yield, pH, drip loss, and water-holding capacity were measured. Also, cooked breast samples were evaluated for cooking yield and proximate chemical analysis was determined according to [25, 26].

3.12. Statistical Analysis

The results were displayed as SE using independent T- test to determine the significance of differences between chickens in G1 and G2 in all mentioned parameters. A probability (p values, $p \leq 0.05$) were considered statistically significant differences.

4. Results

The impact of pinocembrin and quercetin supplementation on chicken performance is recorded in table (2). The body weight gain in treated broiler

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chickens' G2 was 1920 g with FI of 2586.6 g and FCR of 1.35 which is significantly improved in comparison to the control non-treated G1 (1620 g, 2617 g and 1.62 g, respectively) in 35 days old chickens. Carcass yield and muscle quality revealed that the dressing percentage was significantly increased in G2 to 73.63% as compared to 73.3% in G1. Also, the giblet percentage revealed significant increase of liver, heart, gizzard (2.35, 0.54 and 2.17 respectively), while breast muscle yield showed significant increase from 38.2% in chickens of G1 to 41.6% in G2 with 5.8 pH, 24hr and 71.55 water holding capacity. All these parameters indicated good meat quality especially when confirmed with proximate chemical analysis, which showed high protein (21.97%), Fat (2.47%) and low ash (3.02%) and collagen (0.81%) with low drip loss (3.18%) in G2 in comparison to G1 (Table 3). A potent predicted effect of pinocembrin and quercetin on immunity of broiler chickens is evidenced as the immune organ index revealed significant regular and healthy growth of spleen, bursa of Fabricius and thymus (0.95, 1.51 and 1.62) compared to 0.97, 1.66 and 1.78 in G1, respectively (Table 3).

The total antioxidant activity (TAA) of pinocembrin and quercetin was proven through a significant increase of liver glutathione, catalase, superoxide dismutase and muscular catalase and superoxide dismutase achieving their maximum level at 35 days old in G2 compared to G1 (Figure 1). Also, normal levels of liver enzymes (ALT, AST, AKP and GT) appeared in chickens of G2 compared to significantly higher levels in G1 (Figure 2).

Significantly higher levels of gut lactobacillus counts (10^9 CFU/g), associated with a significantly lower count of *C. perfringens* (10^4 CFU/g) were detecetd in G2. In contrast, G1 chickens had only 10^5 CFU/g lactobacilli and high count of *C. perfringens* (10^8 CFU/g) at the 35^{th} days of age (Figure 3).

The anti-inflammatory effects of quercetin and pinocembrin revealed a significant increase in phagocytic index combined with moderate increase of IL6 (pro-inflammatory cytokines) and low level of IL-1 (inflammatory cytokines) in G2 in contrast with G1 which had significant lower phagocytic index, and IL6 and significant higher IL1 levels (Figure 4).

5. Discussion

In recent years, polyphenols, and phytochemicals such as pinocembrin and quercetin have garnered significant research interest due to their well-documented health-promoting effects. Due to the different molecular mechanisms proposed for their effects, the antioxidant activity of polyphenols and flavonoids has significant scientific interest [27]. However, recent investigations suggest that these compounds may not exert their benefits solely through direct antioxidant activity [28, 29]. Instead, it is proposed that their effects may involve hormetic mechanisms, wherein low-level stress induced by polyphenols primes cells to better manage subsequent oxidative challenges. This preconditioning response may play a crucial role in the regulation of the vitagene network, a key system involved in cellular defense and stress adaptation [30].

The excessive free radical production and the resulting damage to biomolecules are key contributors to the adverse effects of stress in commercial animal production, and therefore understanding the molecular basis of antioxidant defenses is essential. Research indicates that the antioxidant defense system functions at both cellular

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and subcellular levels, involving a diverse array of protective mechanisms within the body [31].

Flavonols, a subclass of flavonoids, are known to influence feed intake, support intestinal microbial balance (eubiosis), and exhibit a range of bioactivities including antimicrobial, immunomodulatory, anti-inflammatory, and antioxidant effects in monogastric animals [32]. Quercetin (3,3′,4′,5,7-pentahydroxyflavone), a representative flavonol, is naturally found in fruits such as apples, berries, and grapes, as well as in herbs and vegetables like onions and broccoli [33]. It is recognized for its potent antioxidant, anti-inflammatory, antimicrobial, and anti-obesity properties [34]. Similarly, pinocembrin, a bioactive compound extracted from propolis, has also demonstrated significant antioxidant effects, comparable to those of quercetin [35].

In the present study, the liver TAA glutathione, catalase and superoxide dismutase and the muscular catalase and superoxide dismutase increased in G2 chickens by age achieving their maximum levels at 35 days old when compared to G1. Hydroperoxides are harmful to cells and must be neutralized and eliminated. Enzymes such as glutathione peroxidases (GSH-Px), catalase (CAT), play essential roles in maintaining cellular homeostasis, and their contribution to antioxidant defense systems warrants greater attention [36]. Also, the levels of liver enzymes (ALT, AST, AKP and GT) in G2 chickens were within the normal level, compared to higher levels in G1 indicating the hepatic and muscular oxidation and probability of tissue destruction and this was reflected on significantly better carcass yield and muscle quality (dressing %) in treated chickens of G2 as compared to G1.

The impact of pinocembrin and quercetin supplementation on chicken performance revealed significant higher body weight gain in the treated broiler chickens' G2 in comparison to the control non-treated G1 within 35 days old. These results of improved weight gain, FCR, and uniformity agreed with [37], who recorded that quercetin improved performance parameters, such as BW, BWG, and FCR. The observed improvements in performance parameters in our study may be linked to the metabolic prebiotic effects of quercetin and pinocembrin. These compounds appear to influence gut microbiota composition by promoting the proliferation of beneficial bacteria, such as *Lactobacillus* spp., while reducing populations of *Clostridium perfringens* and total coliforms. This selective modulation of microbial populations likely contributes to enhanced health and performance in broiler chickens [38]. This was confirmed here through the significantly higher levels of lactobacillus counts (10^9 CFU/g) associated with a significantly lower count of *C. perfringens* (10^4 CFU/g) appeared in G2 chickens in comparison to those of G1 at 35 days of age.

The immune response to vaccination serves as a critical indicator of immune system functionality in broiler chickens [39]. In this study, we examined the effects of dietary supplementation with quercetin and pinocembrin on immune health, with particular attention to their influence on the birds' anti-inflammatory responses. A potent effect of pinocembrin and quercetin on immunity of broiler chickens as immune organ index in Table (3) is revealed by the regular and healthy growth of spleen, bursa, and thymus (0.95, 1.51 and 1.62) in chickens of G2 compared to those of G1. The anti-inflammatory effects of quercetin and pinocembrin revealed a significant increase in phagocytic index combined with moderate increase of IL6 (pro-

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inflammatory cytokines) and low level of IL-1 (inflammatory cytokines) in G2 in contrast with G1 which had significant lower phagocytic index, and IL6 and significant higher IL1 levels.

6. Conclusions

The current study clearly indicated that both quercetin and pinocembrin have strong antioxidant and anti-inflammatory effects and can modulate immune response and performance parameters in broiler chickens. However, additional research is necessary to elucidate the molecular mechanisms underlying the interactions between gut microbiota, redox balance, and various intracellular signaling pathways and transcription factors. A deeper understanding of these processes is essential for developing effective adaptive responses and mitigating the harmful effects of stress in avian species.

Conflict of interest: Nothing to declare

7. References

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Table (1): Experiment dietary supplementation protocol for broiler chickens' groups

Type	Treatment	Age of treatment
Treated gp. (G2)	½ cm LIB-TON ½ cm RADICAL FREE	7 -8-9 day old 16-17-18 day old 22-23-24 day old 28-29-30 day old
Control gp. (G1)	Nil	-

Table 2: Impact of dietary Pinocembrin and Quercetin supplementation on performance of broiler chickens

Item	Pinocembrin and Quercetin supplementation		*p-value*
	Control	Treated	
Starter (0-10 d)			
Body weight gain, g	304±0.58^{b}	414.33±1.2^{a}	0.001
Feed intake, g	386.67±1.2^{a}	367.67±0.88^{b}	0.001
Feed conversion ratio	1.27±0.003^{a}	0.89±0.004^{b}	0.001
Grower (11-28 d)			
Body weight gain, g	822.33±1.86^{b}	891.67±0.88^{a}	0.001
Feed intake, g	1249.33±0.88^{b}	1256±0.58^{a}	0.003
Feed conversion ratio	1.52±0.004^{a}	1.41±0.001^{b}	0.001
Finisher (29-35 d)			
Body weight gain, g	494±0.58^{b}	614.33±0.58^{a}	0.001
Feed intake, g	981.33±0.88^{a}	963±1.15^{b}	0.001
Feed conversion ratio	1.99±0.004^{a}	1.57±0.003^{b}	0.001
Total (0-35 d)			
Body weight gain, g	1620.33±1.45^{b}	1920±1.15^{a}	0.001
Feed intake, g	2617.33±0.88^{a}	2586.67±0.33^{b}	0.001
Feed conversion ratio	1.62±0.002^{a}	1.35±0.001^{b}	0.001

Any two means for a performance parameter bearing different superscript letters in a row are significantly ($P < 0.05$) different from each other. European production efficiency factor = [(viability % × body weight Kg / age (d) × FCR)] ×100.

Table 3: Impact of dietary Pinocembrin and Quercetin supplementation on carcass characteristics of broiler chickens

Item	Pinocembrin and Quercetin supplementation		*p-value*
	Control	Treated	
Carcass weight, g	1721±0.58b	2118±0.58a	0.001
Dressing, %	73.30±0.06b	73.63±0.09a	0.034
Giblets, %			
Liver	2.13±0.01b	2.35±0.01a	0.001
Heart	0.63±0.01a	0.54±0.01b	0.004
Gizzard	2.11±0.01b	2.17±0.01a	0.006
Immune organs index			
Spleen	0.97±0.01a	0.95±0.003a	0.184
Bursa	1.66±0.006a	1.51±0.007b	0.001
Thymus	1.78±0.006a	1.62±0.003a	0.001
Breast muscle			
Yield, %	38.23±0.09b	41.63±0.09a	0.001
pH, 24 h	5.94±0.01a	5.81±0.01b	0.001
Water holding capacity	67.33±0.08b	71.55±0.03a	0.001
Liver yield	2.09±0.02b	3.32±0.20a	0.004
Drip loss	3.79±0.12a	3.18±0.03b	0.009
Cooking loss	29.57±0.09a	27.40±0.06b	0.001
Breast muscle analysis, %			
Protein	20.50±0.21b	21.97±0.12a	0.004
Fat	1.67±0.09b	2.47±0.07a	0.002
Ash	3.09±0.01a	3.02±0.01b	0.015
Moisture	75.23±0.09b	76.47±0.18a	0.003
Collagen	0.86±0.01a	0.81±0.01b	0.042

Any two means for a performance parameter bearing different superscript letters in a row are significant ($P < 0.05$)

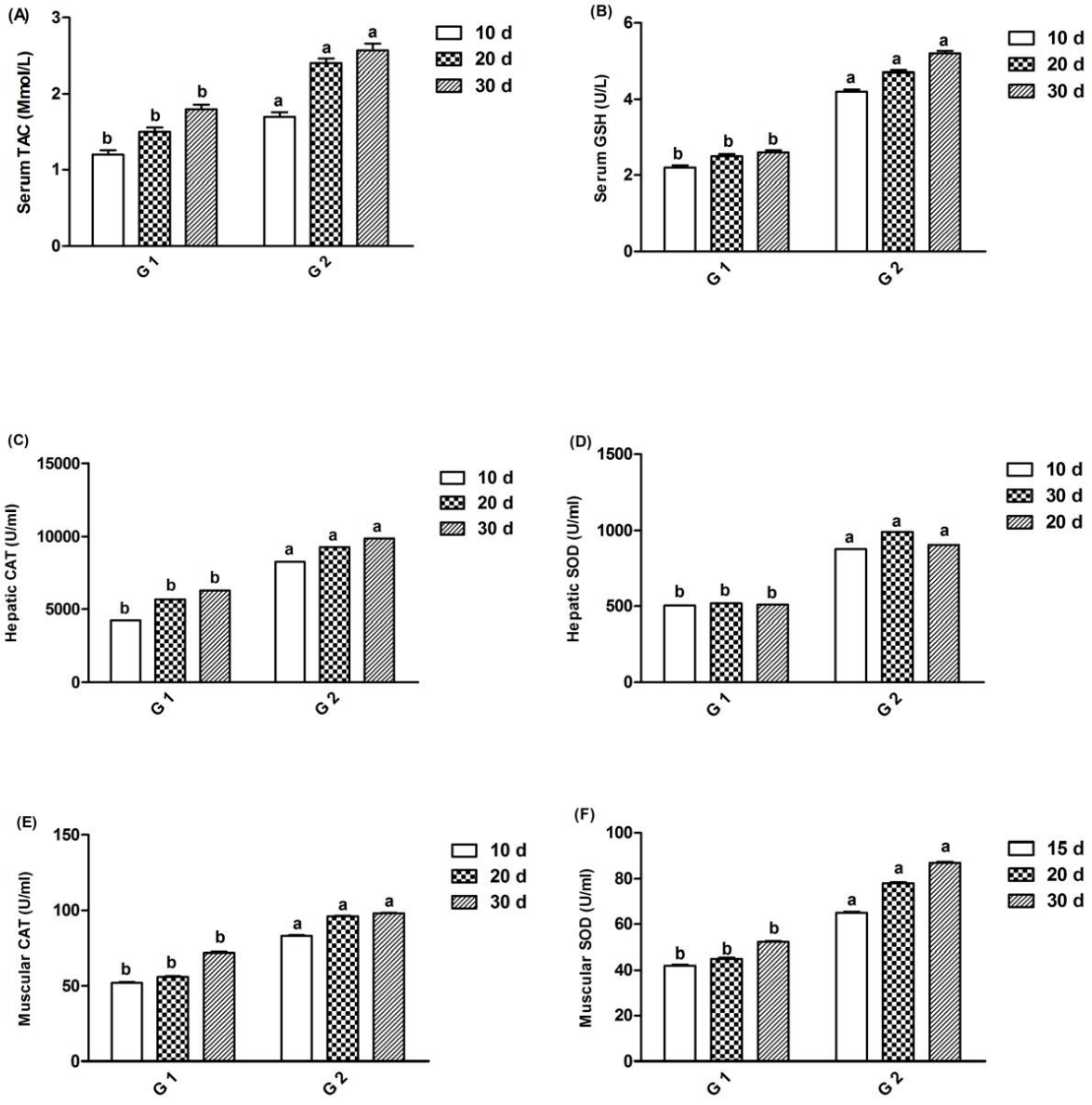

Fig (1): Impact of dietary Pinocembrin and Quercetin supplementation on antioxidant enzymes of broilers

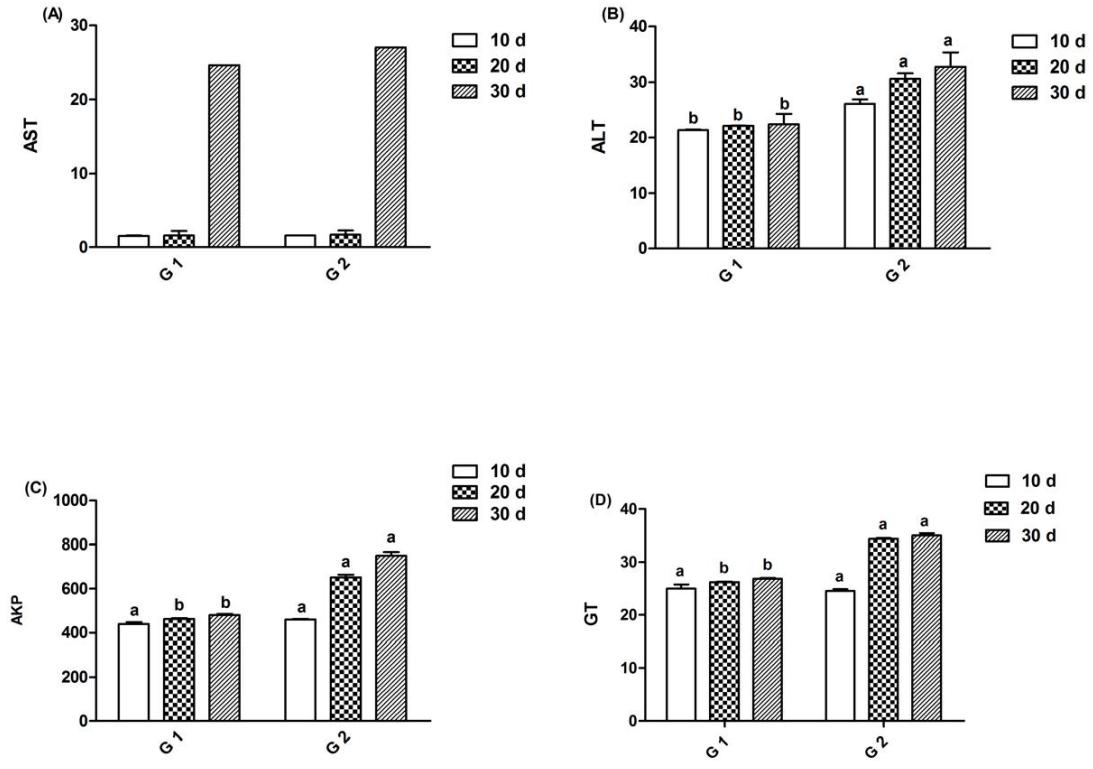

Fig (2): Impact of dietary Pinocembrin and Quercetin supplementation on liver enzymes of broilers

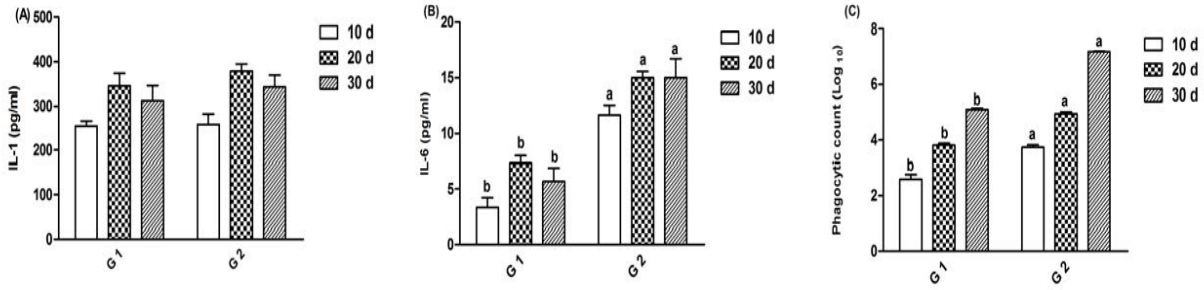

Fig (3): Impact of dietary Pinocembrin and Quercetin supplementation on anti-inflammatory parameters of broilers

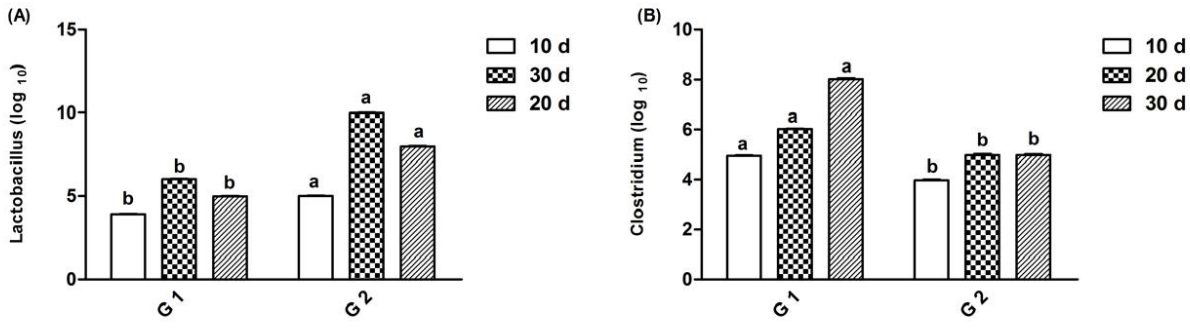

Fig (4): Impact of dietary Pinocembrin and Quercetin supplementation on gut microflora of broilers