ZOOTECHNICAL PERFORMANCE AND BLOOD BIOCHEMISTRY PROFILE OF ROSS 308 BROILER CHICKENS FED DIETS SUPPLEMENTED WITH DRIED *Opuntia ficus-indica* (prickly pear) FRUIT

A. Belghiti[1-2]*, O. Rafik[2], S. Bidah[3], L. Benidire[1-2], A. Mouslim[2], K. Mohtadi[4], I. El Ouahabi[4] and J. Mouslim[2]

[1]Sciences and Technology Research Team, Higher School of Technology of Laayoune, Ibn Zohr University – Agadir, Morocco.
[2]Department of biology, laboratory of ecology and environment, Microbiology unit, Faculty of Sciences Ben M'sik, Hassan II University - Casablanca, Morocco.
[3]Department of biology, laboratory of Analysis, Modeling and Simulation, Faculty of Sciences Ben M'sik, Hassan II University - Casablanca, Morocco.
[4]Department of biology, laboratory of Biology and Health-URAC 34, Faculty of Sciences Ben M'sik, Hassan II University - Casablanca, Morocco.
Corresponding author's Email: belghiti.ayoub@gmail.com

ABSTRACT

The use of antibiotic growth promoters in chicken broiler production has become increasingly questioned. The study evaluated the effect of including 10% dried *Opuntia ficus-indica* fruit on performance and serum biochemical parameters in Ross 308 broiler chicken. A total of 180 chicks were allocated to two experimental treatments: a control group and a treated group receiving the supplemented diet. Birds receiving the supplemented diet showed higher body weight (+9.84%) and average daily weight gain (+13.55%) than controls. Feed intake was only slightly increased (+2.73%), while feed conversion ratio decreased from 2.73 to 2.47, indicating improved feed efficiency in the OFI- supplemented group. Blood analysis showed increased total protein, albumin, and gamma-glutamyltransferase (GGT) ($p = 0.040$). Together with reduced AST and stable ALT, this pattern may reflect higher metabolic activity rather than liver damage, although this finding warrants cautious interpretation. No significant differences were observed for renal and lipid parameters ($p > 0.05$). Overall, these results indicate that dried *Opuntia ficus-indica* fruit could be used as a natural feed ingredient in broiler chicken production.

Keywords: *Opuntia ficus-indica*, phytobiotic, broiler chicken, growth performance, metabolic activity.

https://doi.org/10.36899/JAPS.2026.5.0119 Published first online July, 13, 2026

INTRODUCTION

Antibiotics have historically played a crucial role in modern broiler chickens production, serving as therapeutic agents, prophylactics and antimicrobial growth promoters (AGPs) to enhance growth performance and feed intake efficiency (El-Fateh et *al.,* 2024). Since their introduction in the 1960s, antibiotic growth promoters (AGPs) have significantly improved poultry production outcomes by reducing mortality rates and controlling subclinical infections (Abudabos et *al*., 2016; Chand et *al*., 2016; Alhidary et *al*., 2017). However, increasing concerns over antibiotic resistance and the presence of antimicrobial residues in animal-derived food products led regulatory restrictions. In this context, the European commission implemented a complete ban on the use of AGPs in animal feed from January 1, 2006 (European Parliament and Council of the European Union, 2003). The withdrawal of AGPs has since been associated with a resurgence of pathogenic infections and decline in overall poultry performance (Aljumaah et al., 2020). This situation has encouraged the search for safer alternatives. Among the proposed options, phytobiotics have received increasing attention in poultry feeding (Aljumaah et al., 2020; Krauze et al., 2021; Zaikina et al., 2022). Phytobiotics are plant-derived bioactive compounds recognized for their multifaceted properties, including appetite stimulation, enhancement of digestive enzymes activity, antimicrobial and antioxidant actions, and improved nutriment utilization (Lee et al., 2015; Shahid et al., 2015; Urban et al., 2025).

Opuntia ficus-indica (L.), commonly known as the prickly pear cactus is extensively distributed throughout the Mediterranean basin, Latin America and south of Africa. Renowned for its use in traditional medicine, this species has been extensively studied for its diverse bioactive properties, including antioxidant, anti-inflammatory, and antimicrobial activities. In addition, has shown neuroprotective, hepatoprotective, and cardioprotective effects, attributed to its rich content of phenolic compounds, flavonoids, betalains, and mucilaginous polysaccharides (Giraldo et al., 2023; Shoukat et al., 2023; Liu et al., 2025). Its fruit is particularly rich in bioactive compounds and has shown potential for enhancing animal productivity, including in poultry systems (Martins et *al*., 2023). Most studies have focused on specific extracts rather than the whole fruit, and data on its use in broiler diet remain limited. The whole fruit represents a complex matrix with potential synergistic interactions, and its inclusion in the diet may provide a practical and cost-effective alternative to purified extracts.

Nevertheless, data on the effect of whole OFI fruit supplementation on growth performance and blood biochemical parameters in broiler chicken remain scarce.

Therefore, this study aimed to assess the effect of dietary supplementation with dried *Opuntia ficus-indica* fruit on growth performance and blood biochemical parameters in Ross 308 broiler chicken.

MATERIALS AND METHODS

Plant material preparation: *Opuntia ficus-indica* fruits were harvested from the rural commune of “Sbouya” in the *Guelmim-Oued Noun* region of Morocco in August 2023. Only intact fruits free from external defects were selected, washed, peeled, sliced and air-dried at ambient temperature (25-30 °C). To ensure uniform drying and minimize the risk of fungal contamination, the sliced fruits were turned manually once daily until constant weight was achieved.

The dried fruits were ground into a fine powder using an attrition mill and passed through a 1-mm mesh sieve to ensure uniform particle size prior to diet formulation and incorporated into the basal diet at a fixed inclusion rate of 10% (w/w). This level was selected based on preliminary trials and previous reports indicating that 5-10% supplementation of OFI fruit can enhance growth performance without adverse effects on feed palatability or texture.

Experimental diets: Throughout the experimental period, birds were fed a basal diet composed of soybean meal, corn and a premix of vitamin and mineral supplements. The composition and nutrient profile of the diet are summarized in Table 1.

Table 1. Formulation and chemical composition of the experimental diets

Ingredient composition (%)

Ingredient	Control diet	Experimental diet
Maize	55.00	45.00
Soybean meal	30.00	30.00
Wheat bran	7.00	7.00
Vegetable oil	4.00	4.00
Opuntia ficus-indica fruit powder	0.00	10.00
Dicalcium phosphate	1.80	1.80
Limestone	1.20	1.20
Vitamin–mineral premix	0.50	0.50
DL-methionine	0.25	0.25
L-lysine	0.25	0.25
Total	**100**	**100**

Calculated chemical composition

Parameter	Control diet	Experimental diet
Metabolizable energy (MJ/kg)	12.60	12.60
Crude protein (%)	21.0	21.0
Crude fiber (%)	3.50	5.20
Calcium (%)	0.90	0.90
Available phosphorus (%)	0.45	0.45
Lysine (%)	1.20	1.20
Methionine (%)	0.50	0.50

Values of nutrient composition were calculated based in ingredient composition and standard feed composition tables (National Research Council, 1994; Aviagen, 2022). The diets were formulated to provide equal energy and nitrogen content.

ME: Metabolized energy; CP: Crude protein; CF Crude fiber; Ca: Calcium; AvP: Available phosphorus; Lys: Lysine; Met: Methionine.

The Vitamin–mineral premix provided per kg of diet: Vitamin A, 10,000 IU; Vitamin D_3, 2,000 IU; Vitamin E, 20 mg; vitamin K_3, 2 mg; Vitamin B_1, 2 mg; Vitamin B_2, 6 mg; Vitamin B_6, 4 mg; Vitamin B_{12}, 0.02 mg; Niacin, 40 mg; Pantothenic acid, 10 mg; Folic acid, 1 mg; Choline chloride, 500 mg; Iron, 60 mg; Zinc, 60 mg; Manganese, 80 mg; Copper, 8 mg; Iodine, 1 mg; Selenium, 0.3 mg.

Experimental design: For these experimental trials, a total of one-day-old male Ross 308 chicks were obtained from *Had soualem* commercial hatchery, located in the Casablanca region of Morocco. The birds were housed and fed in floor pens under standard commercial conditions. Stocking density was maintained at 10 birds/m^2 in accordance with recommended welfare guidelines for the Ross 308 breed. The poultry house was naturally ventilated and equipped with adequate air circulation to maintain appropriate temperature and humidity. Environmental conditions (temperature, ventilation and lighting programs) were managed uniformly for all experimental groups throughout the trial. During the first week, the ambient temperature was maintained at 32-34°C and then progressively lowered by about 2-3°C per week until it reached 22-24°C at the end of the experimental period. Lighting was provided continuously (23L: 1D) during the first week and then adjusted to a 20L: 4D photoperiod for the remainder of the trial.

The chicks were randomly assigned to two experimental groups of 90 birds each. The control group (C) received a standard basal diet; while the treated group (E) was fed the same basal diet supplemented with 10% crushed dried OFI fruit. The trial lasted 48 days and was divided into two phases: a starter phase (days 1-22) and grower-finisher phase (days 23-48). During the starter phase, all broiler chickens were fed the same basal diet. From day 23 to day 48 (grower-finisher phase), birds in the treated group received the basal diet supplemented with 10% dried *Opuntia ficus-indica* fruit, while the control group continued to receive the basal diet without supplementation. Grower-finisher diets were provided in pellet form.

Each treatment group (Control and OFI) was subdivided into three replicates (pens) of 30 birds each, making a total of 90 birds per group. All pens were managed under identical environmental and nutritional conditions. Feed intake and growth performance were recorded on a pen basis, which was considered to the experimental unit for statistical analysis. For Blood biochemical analysis, six birds were randomly selected from each replicate (n=18 per treatment) to represent the average physiological status of each group.

Mortality: Mortality was monitored daily throughout the experimental period.

Growth performances analysis: The parameters assessed in this study included body weight and weight gain progression, daily feed intake and Feed conversion efficiency.

Body weight and daily weight gain: Individual body weights were recorded every three days. At each interval, 6 birds were selected randomly from each experimental group for measurement. Growth performance was evaluated during the grower phase, spanning from day 22 to day 48 of the trial. At the end of this period, weight gain for each group was determined by calculating the average final body weight of the birds. The percentage of weight gain was then calculated using the following equation:

$$\text{Weight gain (\%)} = [(\text{Final weight} - \text{Initial weight})/\text{Initial weight}] \times 100$$

Where the initial weight corresponds to the average body weight recorded at the beginning of the grower-finisher period (day 22).

Average daily gain was calculated at the pen level using the following formula:

$$\text{ADG} = (\text{final body weight} - \text{initial body weight}) / \text{number of experimental days}$$

where the number of experimental days corresponded to the grower-finisher period from day 22 to day 48.

Feed intake (FI)

Daily feed intake was calculated at the pen level using the following formula:

$$\text{Daily feed intake} = \text{Total feed consumed per pen during the experimental period} / \text{number of live birds} / \text{number of experimental days.}$$

Feed Conversion Ratio (FCR): The feed conversion ratio, also referred to as the Consumption Index (CI), is a critical parameter in poultry production used to evaluate the efficiency with which animals convert feed into body weight. A lower FCR indicates better feed efficiency and improved growth performances. FCR is calculated as follows:

$$\text{FCR} = \text{Total feed consumed per pen}/\text{total body weight gain per pen}$$

Blood sampling and biochemical profile analysis: At the end of the experimental period (day 48), blood samples were collected from 6 birds per replicate. Blood samples were collected during the morning hours to minimize circadian variation. Birds were sampled under normal feeding conditions without prior fasting, as commonly practiced in broiler production studies. Blood was obtained from live birds via the brachial (wing) vein using sterile needles, and no euthanasia was performed for blood collection. For serum biochemical analyses, blood was drawn into a plain BD Vacutainer® tube and then centrifuged at 3000 rpm for 15 minutes to separate the serum. The serum was stored at -20°C until further analysis. Biochemical indices measured included glucose, urea, creatinine, triglycerides, total cholesterol, high-density lipoproteins (HDL), low-density lipoproteins (LDL), total protein (TP), albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma-glutamyltransferase (GGT). These were analyzed using commercial kits (Roche Diagnostics “Elecsys BRAHMS, Germany) with an automated chemistry analyzer (Roche Hitachi, Cobas c 501 Chemistry Analyzer, Germany).

Ethical approval

All procedures involving animals were conducted in accordance with internationally recognized guidelines for animal research and reporting, including the ARRIVE 2.0 guidelines and the Guide for the Care and Use of Laboratory Animals (8^{th} edition). The experiment involved routine poultry husbandry practices and non-invasive blood sampling performed by trained personnel, with efforts made to minimize stress and ensure animal welfare throughout the study.

Statistical analysis: The study was conducted as a completely randomized design with two dietary treatments and three replicate pens per treatment. Since dietary treatments were applied at the pen level, the pen was considered the experimental unit for all statistical analyses.

Growth performance data were analyzed using pen-level means. Individual bird measurements were used only to calculate the mean value for each pen and were not considered independent experimental units. For serum biochemical parameters, six birds were sampled per pen, and individual values were averaged to obtain one representative value per pen. Thus, biochemical analyses were performed using replicate pen means, with $n = 3$ pens per treatment.

Data were analyzed using SPSS (version 29, IBM). Treatment effects were evaluated by one-way ANOVA, and body weight over time was analyzed using repeated-leasures ANOVA. Results are presented as mean ± SD of pen means, and differences were considered significant at $p \leq 0.05$. Given the limited number of replicate pens, the reported p-values should be interpreted with caution.

RESULTS AND DISCUSSION

Growth performance indicator analysis: Table 2 shows the values of body weight and daily weight gain, as daily feed consumption and efficiency indices at the end of experimental trials.

Table 2: Initial body weight, Final body weight, average daily gain, daily feed intake, and feed conversion ratio of broiler chickens during the grower-finisher period (mean ± SD)

Zootechnical parameters	Group (C)	Experimental (OFI 10%)	P-value
Initial body weight (g)	582.20 ± 15.50	580.80 ± 8.72	0.898
Final body weight (g)	2166.05 ± 23.44	2379.25 ± 25.14	≤ 0.001
Average daily gain (g/bird/day)	60.92± 0.88	69.17± 1.01	0.014
Daily Feed intake (g/bird/day)	166.31± 2.24	170.85± 1.98	0.008
Feed conversion ratio (feed/gain)	2.73± 0.06	2.47± 0.04	0.021

Significant differences in average body weight related to diet were observed around day 27. Although all chicks exhibited steady growth throughout the study, the growth rate differed significantly between the treated and control groups. By the end of breeding period, the final body weight of treated group was higher than that of the control’s, with an increase of 9.84%. Furthermore, a significant difference in average daily weight gain between the two groups became apparent from day 30 onwards. The treated chicks showed a higher average daily gain of 69.17 g/birds/day, corresponding to a 13.55% increase compared with 60.92 g/birds/day in the control group. Similar to growth patterns, FI increased progressively during experimental trials.

Average daily feed intake was slightly higher in the OFI-supplemented group than in the control group. Birds fed the OFI-supplemented diet consumed 170.85 g/birds/day in the control group, corresponding to a 2.73% increase.

The FCR values increased during starter phase (days 1-22), indicating lower feed efficiency in young birds, and then stabilized during the grower-finisher phase (days 23-45). After day 39, FCR values showed resurgence, particularly in the control chicks; whose FCR increased substantially until the end of the experiment. This increase in FCR among was attributed to a reduction in weight gain while FI remained constant. In contrast, the FCR in experimental chicks exhibited only a slight increase, reflecting a gradual decline in weight gain and resulting in lower FCR values by the study’s conclusion.

From a practical standpoint, the lower feed conversion ratio observed in the OFI-supplemented group, decreasing from 2.73 in the control group to 2.47, indicates improved feed efficiency. This improvement may have economic relevance, as it could contribute to reducing feed cost per kilogram of live weight produced. The improvement in growth performance observed in the present study appears to be mainly associated with increased body weight gain and improved feed conversion efficiency, rather than a marked increase in feed intake.

Mortality during the experimental period was 2.22 % (2/90 birds) in the control group and 3.33 % in the OFI-supplemented group (3/90 birds). The observed difference was minimal; however, mortality data were recorded descriptively only and were not subjected to statistical analysis.

Growth performance is further influenced by nutrient digestibility and feed intake, both of which are closely linked to the physicochemical properties of the gastrointestinal tract (Alyileili et *al*., 2020). Several studies have explored relationship between phytobiotics and gut microbiota modulation, seeking to determine whether growth-promoting effects are mediated through microbial alterations (Windisch et *al*., 2008; Valenzuela et *al*., 2017; Ceylan et *al*., 2023). Although the effects of phytobiotics depend on their chemical nature, mode of administration, and dosage, numerous studies have demonstrated their ability to modulate gut microbiota composition, which often correlates with enhanced growth performance, even in cases where overall growth metrics are not significantly improved (Stanley et *al*., 2016; Liu et *al*., 2021; Chen et *al*., 2023; Liu et *al*., 2023). Discrepancies between *in vivo* and *in vitro* finding may be explained by multiple factors, including differences in phytobiotic concentrations and the complex biological environment of the digestive tract (Huyghebaert et *al*., 2011; Chodkowska et *al*., 2022). Moreover, certain dietary components, such as high level of lipids and proteins, may reduce the bioavailability of efficacy of bioactive compounds by forming protective barriers around bacterial cells (Alyileili et *al*., 2020).

Although, the experimental diet contained a higher crude fiber level than the control diet (5.20 % vs 3.50 %), growth performance was improved in the OFI-supplemented group. This suggests that the fiber-rich fraction of *Opuntia ficus-indica* fruit, particularly its soluble polysaccharides and mucilaginous components, may have exerted a prebiotic-like effect that supported intestinal function and nutrient utilization.

Regarding performance, the use of certain essential oils such as garlic or rosemary has been associated with up to a 10% increase in body weight gain (Salaheen et *al*., 2017; Y. Liu et *al*., 2021). It has also been demonstrated that phytobiotic influence feed intake: rosemary essential oil has been reported to reduce feed intake by 16%, whereas thyme may increase it by 11 % (Cross et *al*., 2007; Stanley et *al*., 2016). Variability in feed conversion ratio has also been documented; turmeric supplementation has been shown to reduce feed conversion by 16% while thyme and *Zataria multiflora* essential oil have led increase of 7% and 4%, respectively (Al-Sultan, 2003; Cross et *al*., 2007).

The efficacy of phytobiotic remains inconsistent across studies, largely due to differences in experimental designs, animal species or strains, administration methods, and particularly the preparation and standardization of phytobiotic products (Salaheen et *al*., 2017; Stanley et *al*., 2016). Animal age and genetic background also contribute significantly to the observed effects.

Blood biochemical profile analysis: Table 3 summarized the values of blood parameters in chicken broilers at the end of experimental period.

Table 3. Serum biochemical parameters of broiler chickens fed a control of OFI-supplemented diet (48 days of age, mean ± SD, n = 3 replicates per treatment)

Biochemical parameter	Control	Experimental (OFI 10%)	*p-value*
Total protein (g/dL)	4.52 ± 0.19	5.14 ± 0.22	0.041
Albumin (g/dL)	2.15 ± 0.08	2.42 ± 0.07	0.029
AST (U/L)	256.43 ± 12.8	242.50 ± 1.50	0.035
ALT (U/L)	45.82 ± 3.10	44.17 ± 2.88	0.055
GGT (U/L)	12.11 ± 1.02	15.84 ± 0.97	0.040
HDL (g/L)	0.62 ± 0.04	0.68 ± 0.02	0.015

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: Gamma-glutamyltransferase; HDL: High-density lipoprotein.

As shown in table 3, dietary supplementation significantly ($p \leq 0.05$) influenced several serum biochemical parameters in broiler chickens at the end of the experimental period. Birds receiving the dietary additive exhibited higher levels of total protein (TP) and albumin compared to the control group ($p \leq 0.05$), indicating improved protein metabolism. Similarly, serum concentration of high density lipoproteins (HDL) were significantly higher in the treated group (p = 0.015), suggesting a beneficial modulation of lipid metabolism. Conversely, the activity of aspartate aminotransferase (AST) was significantly reduced ($p \leq 0.05$), potentially reflecting hepatoprotective effects of the additive. No significant differences ($p > 0.05$) were found for glucose ([2.32 ± 0.07 *vs* 2.44 ± 0.01], p = 0.12), urea ([0.39 ± 0.03 *vs* 0.39 ± 0.01], p = 0.802), creatinine ([27.97 ± 1.11 *vs* 28.51 ± 1.01], p = 0.665), triglycerides ([1.56 ± 0.08 *vs* 1.29 ± 0.11], p = 0.073), total cholesterol ([1.21 ± 0.04 *vs* 1.07 ± 0.06], p = 0.063), alkaline phosphatase ([1790.37 ±

20.16 *vs* 1750.33 ± 39.82], p = 0.332), or alanine aminotransferase (45.82 ± 3.10 *vs* 44.17 ± 2.88], p = 0.055), indicating that renal and general lipid metabolism remained unaffected by supplementation.

Liver marker enzymes are reliable indicators for evaluating hepatic integrity and functionality in poultry, with serum concentrations typically increasing under stress or hepatotoxic conditions (Tang et *al*., 2022). In the present study, the notably reduced serum AST levels in broilers receiving the supplemented diet suggest a hepatoprotective effect, aligning with finding by Odetola et *al*. (2019), who reported decreased AST activity following dietary inclusion of *Petiveria alliacea* root meal. Although serum AST activity was significantly reduced in broiler fed the OFI-supplemented diet, ALT values were not significantly affected (p = 0.055). This discrepancy should be interpreted cautiously, as the diagnostic relevance of these enzymes differs between mammals and birds. In avian species, AST is considered a more sensitive and early marker of hepatic stress, whereas ALT activity remains low and less specific to hepatocellular injury (Tang et *al*., 2022). Therefore, the observed reduction in AST, together with stable ALT and elevated GGT levels, may indicate improved hepatic metabolic efficiency rather than structural liver repair. Similar interpretations have been proposed by Odetola et *al*. (2019) and Bueno et *al*. (2017), who reported reduced AST but unchanged ALT following dietary supplementation with phytogenic additives exhibiting antioxidant and hepatoprotective effects. These patterns suggest that OFI fruit bioactives could enhance hepatic enzyme homeostasis and support liver function under normal physiological conditions.

Serum albumin, synthesized exclusively by the liver, is the principal plasma protein with multiple physiological functions, including transport and antioxidant roles. An increase in serum albumin in birds fed rosemary leaves, rich in phenolic acids and flavonoids, had been previously documented (Ghozlan et *al*., 2017). Given that polyphenolic compounds bind extensively to serum albumin, this interaction is believed to influence their systemic distribution and metabolic clearance (Oso et *al*., 2023). The improved protein profile observed in broilers fed phytobiotic-supplemented diets may be partly attributed to increase body mass, which is associated with higher total protein content. Moreover, phytobiotics are known to enhance intestinal health by stimulating digestive enzyme secretion and improving nutrient absorption (Abudabos et *al*., 2016), contributing further to protein assimilation. Considering the rapid growth and protein accretion during the short broiler chickens production cycle, changes in serum protein levels are expected (Abdul Basit et *al*., 2020).

These effects may be partly attributed to the high content of bioactive compounds in OFI fruit, particularly, betalains and polysaccharides. Betalains are known for their antioxidant and hepatoprotective properties, which may support hepatic protein synthesis, while polysaccharides can improve intestinal integrity and nutrient absorption, thereby contributing to enhanced systemic protein metabolism. Gamma glutamyltransferase (GGT) is present in several tissues but is mainly of hepatic origin in serum due to renal excretion (Bueno et *al*., 2017). It is involved in glutathione metabolism and serves as a marker of cholestasis and bile duct proliferation.

However, it should be noted that an increase in serum GGT activity may reflect either enhanced hepatic metabolic and antioxidant activity or a mild adaptive response to metabolic stress. Therefore, this finding should be interpreted with caution. The increase in serum GGT observed in the OFI-fed group should be interpreted carefully, as GGT may reflect both enhanced metabolic activity and potential hepatic stress. In mammals and birds, this enzyme participates in glutathione metabolism and cellular antioxidant defense, and moderate elevations can therefore indicate increased hepatic metabolic turnover or adaptation to oxidative challenges (Bueno et *al*., 2017) However, markedly elevated GGT levels are also considered biomarkers of cholestasis or hepatobiliary dysfunction. In the present study, the concurrent decrease in AST and the lack of change in ALT suggest that the GGT increase is more likely associated with enhanced glutathione activity and improved hepatic metabolic function rather than liver injury. A similar pattern was reported by Oso et *al*. (2023), who observed mild GGT elevation alongside improved protein metabolism in broiler supplemented with phytogenic feed additives. Collectively, these findings imply that OFI fruit may stimulate hepatic detoxification and antioxidant systems within physiological limits.

The present findings are in agreement with previous studies investigating OFI in poultry and related experimental models. Martins et *al*. (2023) reported that dietary inclusion of OFI-derived products improve nutrient digestibility and growth performance in broiler chickens, which was associated with enhanced protein utilization and metabolic efficiency. Similarly, Giraldo et *al*. (2023) highlighted the hepatoprotective and antioxidant properties of OFI, attributed mainly to its betalains and polysaccharide content, supporting the observed reduction in AST activity and improvement in serum protein profile in the present study.

Notably, while most previous studies focused on OFI extracts or cladodes, the use of whole fruit in the current work suggests that similar biological effects can be achieved through a more practical and nutritionally integrated form, potentially allowing synergetic interactions among bioactive compounds.

Variations reported in the literature concerning biochemical responses to dietary additives may stem from differences in birds genetics, diet composition, age and experimental conditions.

Overall, our results support the hypothesis that *Opuntia ficus-indica* fruit can serve as an effective natural feed additive in broiler chickens production, combining growth-promoting and hepatoprotective effects similar to other well-characterized cactus product. The use of the fruit fraction, however, represents a novel approach compared to most previous studies, which primarily focused on cladode or extract forms.

Despite the promising results observed in the present study, certain limitations should be acknowledged. The evaluation was limited to zootechnical performance and serum biochemical parameters, without direct assessment of gut microbiota composition or intestinal and hepatic histological features. In addition, the use of three replicate pens per treatment provided adequate but limited statistical power; therefore, the present findings should be interpreted with caution and confirmed in larger-scale studies. Consequently, the underlying mechanisms by which *Opuntia ficus-indica* fruit exerts its metabolic and hepatoprotective effects cannot be fully elucidated. Future studies integrating microbiota profiling, oxidative stress markers, and tissue histopathology would provide deeper insight into biological pathways involved.

Conclusion: The inclusion of *Opuntia ficus-indica* fruit in broiler chickens diets improved body weight gain and feed conversion efficiency, with only a slight increase in feed intake. Moreover, this dietary incorporation induced changes in blood chemical parameters, particularly by improving serum protein levels, suggesting a potential hepatoprotective effects. This finding support the use of OFI fruit as functional feed additive in poultry nutrition. Future research should further investigate the effects of *Opuntia ficus-indica* fruit supplementation on gut health and microbiota composition, meat quality attributed, and optimal inclusion levels to maximize its benefits in broiler chickens production.

Authors' Contributions: Belghiti Ayoub and Mouslim Jamal designed experiments; Belghiti Ayoub and Rafik Oussama collected samples; Belghiti Ayoub, Rafik Oussama, Benidire Loubna and Mouslim Assia performed experiments; Bidah Sara conducted statistical analysis; El ouahabi Imane and Mohtadi Karima performed the biochemical tests; Belghiti Ayoub wrote the manuscript. All authors agreed to publish this article.

Conflicts of interest: The authors did not declare any conflict of interest in this research.

REFERENCES

Abdul Basit, M., A. Abdul Kadir, T. C. Loh, S. Abdul Aziz, A. Salleh, U. Kaka, and S. Banke Idris (2020). Effects of Inclusion of Different Doses of *Persicaria odorata* Leaf Meal (POLM) in Broiler Chicken Feed on Biochemical and Haematological Blood Indicators and Liver Histomorphological Changes . *Animals (Basel)* 10(7): 1209. https://doi.org/10.3390/ani10071209

Abudabos, A. M., A. H. Alyemni, Y. M. Dafalla, and R. U. Khan (2016). The Effect of Phytogenic Feed Additives to Substitute In-Feed Antibiotics on Growth Traits and Blood Biochemical Parameters in Broiler Chicks Challenged with *Salmonella Typhimurium*. *Environ. Sci. Pollut. Res*. 23(23): 24151-24157. https://doi.org/10.1007/s11356-016-7665-2

Alhidary, I. A., Z. Rehman, R. U. Khan, and M. Tahir (2017). Anti-Aflatoxin Activities of Milk Thistle (*Silybum marianum*) in Broiler. *World's Poult. Sci. J*. 73(3): 559-566. https://doi.org/10.1017/S0043933917000514

Aljumaah, M. R., G. M. Suliman, A. Alabdullatif, and A. M. Abudabos (2020). Effects of Phytobiotic Feed Additives on Growth Traits, Blood Biochemistry, and Meat Characteristics of Broiler Chickens Exposed to *Salmonella Typhimurium*. *Poult. Sci*. 99(11): 5744-5751. https://doi.org/10.1016/j.psj.2020.07.033

Al-Sultan, S. I. (2003). The effect of *Curcuma longa* (Turmeric) on overall performance of broiler chickens. *Int. J. Poult. Sci*. 2(5): 351-353. https://doi.org/10.3923/ijps.2003.351.353

Alyileili, S. R., I. E. H. Belal, A. S. Hussein, and K. A. El-Tarabily (2020). Effect of Inclusion of Degraded and Non-Degraded Date Pits in Broilers' Diet on Their Intestinal Microbiota and Growth Performance. *Animals (Basel)* 10(11): 2041. https://doi.org/10.3390/ani10112041

Aviagen (2022). Ross 308/Ross 308 FF Broiler Nutrition Specifications. Aviagen Group, Huntsville, AL, USA.

Bueno, J. P. R., M. R. B. de M. Nascimento, J. M. da S. Martins, C. F. P. Marchini, L. R. M. Gotardo, G. M. R. de Sousa, A. V. Mundim, E. C. Guimarães, and F. P. Rinaldi (2017). Effect of Age and Cyclical Heat Stress on the Serum Biochemical Profile of Broiler Chickens. Semina: *Rev. Ciênc. Agrár*. 38(3): 1383-1392. https://doi.org/10.5433/1679-0359.2017v38n3p1383

Ceylan, N., E. Yenice, İ. Yavaş, A. A. Çenesiz, N. N. Toprak, and İ. Çiftçi (2023). Comparative Effects of Medium chain Fatty Acids or Phytobiotics based Feed Additives on Performance, Caecum Microbiota, Volatile Fatty Acid Production and Intestinal Morphology of Broilers. *Vet. Med. Sci*. 9(6): 2719-2730. https://doi.org/10.1002/vms3.1249

Chand, N., H. Faheem, R. U. Khan, M. S. Qureshi, I. A. Alhidary, and A. M. Abudabos (2016). Anticoccidial effect of mananoligosacharide against experimentally induced coccidiosis in broiler. *Environ. Sci. Pollut. Res*. 23(14): 14414-14421. https://doi.org/10.1007/s11356-016-6600-x

Chen, F., Y. Wang, K. Wang, J. Chen, K. Jin, K. Peng, X. Chen, Z. Liu, J. Ouyang, Y. Wang, X. Zhang, H. Zou, J. Zhou, B. He, and Q. Lin (2023). Effects of *Litsea cubeba* essential oil on growth performance, blood antioxidation, immune function, apparent digestibility of nutrients, and fecal microflora of pigs. *Front. Pharmacol*. 14: 1166022. https://doi.org/10.3389/fphar.2023.1166022

Chodkowska, K. A., H. Iwiński, K. Wódz, T. Nowak, and H. Różański (2022). *In Vitro* Assessment of Antimicrobial Activity of Phytobiotics Composition towards Avian Pathogenic *Escherichia Coli* (APEC) and Other *E. coli* Strains Isolated from Broiler Chickens. *Antibiotics* 11(12): 1818. https://doi.org/10.3390/antibiotics11121818

Cross, D. E., R. M. McDevitt, K. Hillman, and T. Acamovic (2007). The Effect of Herbs and Their Associated Essential Oils on Performance, Dietary Digestibility and Gut Microflora in Chickens from 7 to 28 Days of Age. *Br. Poult. Sci*. 48(4): 496-506. https://doi.org/10.1080/00071660701463221

El-Fateh, M., M. Bilal, and X. Zhao (2024). Effect of Antibiotic Growth Promoters (AGPs) on Feed Conversion Ratio (FCR) of Broiler Chickens: A Meta-Analysis. *Poult. Sci.* 103(12): 104472. https://doi.org/10.1016/j.psj.2024.104472

European Parliament and Council of the European Union (2003). Regulation (EC) No. 1831/2003 of 22 September 2003 on additives for use in animal nutrition. *Off. J. Eur. Union L* 268: 29-43.

Ghozlan, S. A., A. H. El-Far, K. M. Sadek, A. A. Abourawash, and M. A. Abdel-Latif (2017). Effect of Rosemary (*Rosmarinus officinalis*) Dietary Supplementation in Broiler Chickens Concerning Immunity, Antioxidant Status, and Performance. *Alexandria J. Vet. Sci*. 55(1): 152-161. https://doi.org/10.5455/ajvs.275350

Giraldo-Silva, L., B. Ferreira, E. Rosa, and A. C. P. Dias (2023). *Opuntia ficus-indica* Fruit: A Systematic Review of Its Phytochemicals and Pharmacological Activities. *Plants (Basel)* 12(3): 543. https://doi.org/10.3390/plants12030543

Huyghebaert, G., R. Ducatelle, and F. Van Immerseel (2011). An update on alternatives to antimicrobial growth promoters for broilers. *Vet. J*. 187(2): 182-188. https://doi.org/10.1016/j.tvjl.2010.03.003

Krauze, M., M. Cendrowska-Pinkosz, P. Matusevičius, A. Stępniowska, P. Jurczak, and K. Ognik (2021). The Effect of Administration of a Phytobiotic Containing Cinnamon Oil and Citric Acid on the Metabolism, Immunity, and Growth Performance of Broiler Chickens. *Animals* 11(2): 399. https://doi.org/10.3390/ani11020399

Lee, K. W., J. S. Kim, S. T. Oh, C. W. Kang, and B. K. An (2015). Effects of Dietary Sanguinarine on Growth Performance, Relative Organ Weight, Caecal Microflora, Serum Cholesterol Level and Meat Quality in Broiler Chickens. *J. Poult. Sci*. 52(1): 15-22. https://doi.org/10.2141/jpsa.0140073

Liu, S., K. Wang, S. Lin, Z. Zhang, M. Cheng, S. Hu, H. Hu, J. Xiang, F. Chen, G. Li, and H. Si (2023). Comparison of the Effects between Tannins Extracted from Different Natural Plants on Growth Performance, Antioxidant Capacity, Immunity, and Intestinal Flora of Broiler Chickens. *Antioxidants (Basel)* 12(2): 441. https://doi.org/10.3390/antiox12020441

Liu, X., Y. Xing, G. Liu, D. Bao, W. Hu, H. Bi, and M. Wang (2025). Extraction, Purification, Structural Features, Biological Activities, and Applications of Polysaccharides from *Opuntia ficus-indica* (L.) Mill. (Cactus): A Review. *Front. Pharmacol*. 16: 1566000. https://doi.org/10.3389/fphar.2025.1566000

Liu, Y., T. Yan, Z. Ren, and X. Yang (2021). Age-Associated Changes in Caecal Microbiome and Their Apparent Correlations with Growth Performances of Layer Pullets. *Anim. Nutr*. 7(3): 841-848. https://doi.org/10.1016/j.aninu.2020.11.019

Martins, M., M. H. Ribeiro, and C. M. M. Almeida (2023). Physicochemical, Nutritional, and Medicinal Properties of *Opuntia ficus-indica* (L.) Mill. and Its main agro-industrial use: A review. *Plants* (Basel) 12(7): 1512. https://doi.org/10.3390/plants12071512

National Research Council (1994). Nutrient Requirements of Poultry: Ninth Revised Edition. National Academy Press, Washington, DC. https://doi.org/10.17226/2114

Odetola, M. O., O. O. Adejinmi, O. A. Owosibo, O. T. Banjo, and O. O. Awodola-Peters (2019). Growth Response, Serum Biochemistry and Organ Histopathology of Broilers Fed Diets supplemented with Graded levels of *Petiveria alliacea* Root Meal. *Int. J. Poult. Sci*. 18(1): 45-50. https://doi.org/10.3923/ijps.2019.45.50

Oso, O. A., R. Umaya Suganthi, G. B. Manjunatha Reddy, P. K. Malik, A. P. Heartwin, and V. B. Awachat (2023). Serum Biochemistry, Meat Quality and Oxidative Stability in Broiler Chicken Supplemented with a Novel Phytogenic Feed Additive Formulated from *P. Betle*, *P. Nigrum*, *A. Lanata and C. Dactylon*. *Indian J. Anim. Sci*. 93(6): 617-625. https://doi.org/10.56093/ijans.v93i6.130689

Salaheen, S., S. W. Kim, B. J. Haley, J. A. S. Van Kessel, and D. Biswas (2017). Alternative Growth Promoters Modulate Broiler Gut Microbiome and Enhance Body Weight Gain. *Front. Microbiol*. 8(10): 2088. https://doi.org/10.3389/fmicb.2017.02088

Shahid, S., N. Chand, R. U. Khan, S. M. Suhail, and N. A. Khan (2015). Alternations in Cholesterol and Fatty Acids Composition in Egg Yolk of Rhode Island Red x Fyoumi Hens Fed with Hemp Seeds (*Cannabis Sativa* L.). *J. Chem*. 2015: 362936. https://doi.org/10.1155/2015/362936

Shoukat, R., M. Cappai, G. Pia, and L. Pilia (2023). An Updated Review: *Opuntia ficus-indica* (OFI) Chemistry and Its Diverse Applications. *Appl. Sci*. 13(13): 7724. https://doi.org/10.3390/app13137724

Stanley, D., R. J. Hughes, M. S. Geier, and R. J. Moore (2016). Bacteria within the Gastrointestinal Tract Microbiota Correlated with Improved Growth and Feed Conversion: Challenges Presented for the Identification of Performance Enhancing Probiotic Bacteria. *Front. Microbiol.* 7: 187. https://doi.org/10.3389/fmicb.2016.00187

Tang, L. P., Y. L. Liu, J. X. Zhang, K. N. Ding, M. H. Lu, and Y. M. He (2022). Heat Stress in Broilers of Liver Injury Effects of Heat Stress on Oxidative Stress and Autophagy in Liver of Broilers. *Poult. Sci.* 101(10): 102085. https://doi.org/10.1016/j.psj.2022.102085

Urban, J., K. Y. Kareem, A. Matuszewski, D. Bień, P. Ciborowska, K. Lutostański, and M. Michalczuk (2025). Enhancing Broiler Chicken Health and Performance: The Impact of Phytobiotics on Growth, Gut Microbiota, Antioxidants, and Immunity. *Phytochem. Rev*. 24(2): 2131-2145. https://doi.org/10.1007/s11101-024-09994-0

Valenzuela-Grijalva, N. V., A. Pinelli-Saavedra, A. Muhlia-Almazan, D. Domínguez-Díaz, and H. González-Ríos (2017). Dietary Inclusion Effects of Phytochemicals as Growth Promoters in Animal Production. *J. Anim. Sci. and Technol*. 59: 8. https://doi.org/10.1186/s40781-017-0133-9

Windisch, W., K. Schedle, C. Plitzner, and A. Kroismayr (2008). Use of Phytogenic Products as Feed Additives for Swine and Poultry. *J. Anim. Sci*. 86 (Suppl 14): E140-E148. https://doi.org/10.2527/jas.2007-0459

Zaikina, A. S., N. P. Buryakov, M. A. Buryakova, A. Y. Zagarin, A. A. Razhev, and D. E. Aleshin (2022). Impact of Supplementing Phytobiotics as a Substitute for Antibiotics in Broiler Chicken Feed on Growth Performance, Nutrient Digestibility, and Biochemical Parameters. *Vet. Sci*. 9(12): 672. https://doi.org/10.3390/vetsci9120672