Effect of different levels of alcoholic extract of oak fruit added to drinking water on growth performance and carcass traits of Ross 308 broiler chickens

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Abstract

This experiment was conducted at the College of Agriculture, Department of Animal Production, Al-Qasim Green University, Iraq, from November 27 to December 31, 2025, to evaluate the effects of adding an alcoholic extract of oak fruit to drinking water on the growth performance of broiler chickens for a period of 35 days. A total of 300 one-day-old broiler chicks were randomly allocated to five treatment groups with five replicates and 12 birds per each replicate and fed a standard corn–soybean meal diet. Treatments included a control group without any additive and four groups receiving drinking water supplemented with the alcoholic extract of oak fruit at levels of 1, 2, 3, and 4 ml/L. Broilers receiving different levels of the alcoholic extract indicated no significant differences in body weight (BW), body weight gain (BWG), feed intake (FI), or feed conversion ratio (FCR) compared to the control group during the whole of experimental period ($P > 0.05$). The treatment groups indicated a significant decrease in thigh, liver and bursa of fabricius weights at 35 days of age ($P < 0.05$). "Based on the results obtained, the supplementation of drinking water with an oak fruit alcoholic extract at concentrations up to 4 mL/L did not adversely affect feed intake or feed conversion ratio (FCR) in broiler

Keywords: alcoholic, additive, extract and performance

Introduction

The poultry industry, Besides profitability, the poultry industry faces numerous challenges that limit productivity and bird health. Factors like insufficient feeding practices, suboptimal housing systems, environmental stressors, and high input costs significantly affect growth performance and immunity in poultry [1,2]. In recent years, increasing attention has been given to nutritional interventions and feed additives designed to optimize metabolism, improve overall efficiency, and specifically enhance the immune response to ensure robust bird health "Plant-derived additives are receiving increasing attention in recent scientific studies due to their natural origin and diverse biological functions. Previous research has indicated that these additives are rich in active compounds that enhance overall gut health and improve metabolic efficiency in broiler . Consequently, oak fruits have emerged as one of the most significant sources, owing to their substantial levels of proteins, carbohydrates, and lipids, as well as their high content of bioactive compounds. aegilops) have considerable values of carbohydrates, lipids, proteins and bioactive compounds, so they are nutritionally rich. Metabolites in oak make acorns a valuable functional feed ingredient for poultry and livestock because they exhibit strong antiviral, antibacterial, antifungal, anti-inflammatory, and antioxidant activities [3,4]. Oak-derived metabolites, especially tannins, are considered promising phytobiotics, herb-based bioactive compounds capable of enhancing metabolism, boosting immunity, and supporting sustainable production in poultry systems. Compounds of oak help maintain gut health, all of which positively affect growth performance and feed efficiency [5,5] by stabilizing intestinal microbiota, improving nutrient digestibility, reducing oxidative stress, and supporting mucosal integrity. Extracts from related tree species, in addition to oak, such as Alnus, have also been investigated, demonstrating similar antioxidant, antimicrobial, and anti-inflammatory properties [6,7]. All of the favorable factors are primarily attributed to high levels of polyphenols, flavonoids, terpenoids, and steroids. Improving the separation of these compounds is demanding, as their structural diversity complicates the development of a universal method. Several studies have indicated that mixed solvents, especially combinations of water and alcohol, are more effective than single solvents for separating phenolic compounds "Because water acts as a carrier medium that improves the solubility of the active compounds in the extract, thereby enhancing their bioavailability [8,9,10,11]. Thus, proper separation procedures are essential to exaggerate the crop and

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biological activity of acorn phytochemicals. Plant-derived compounds such as flavonoids and polyphenols exhibit diverse mechanisms of action, including disruption of microbial membranes, inhibition of nucleic acid synthesis, neutralization of toxins, reduction of virulence, prevention of biofilm formation, and restriction of pathogen entry into host cells [12,13]. These factors not only improve disease resistance but also increase overall health and productivity in poultry and other livestock, supporting the use of acorn-derived additives as natural growth promoters. The aim of this study is to evaluate the biological changes and this experiment seeks to determine the efficiency of phenolic compounds and tannins present in the alcoholic extract of oak plant as natural growth promoters, as well as to study their effect on the anatomical response of internal organs such as the bursa of Fabricius and liver development to reveal the physiological safety as a result of these additives.

Material and Methods

Ethical Approval and Animal Welfare

When conducting this study, all bird administration and experimental instructions were conducted in accord with the regulations and recommendations established by Iraqi law and the “Guide for the Care and Use of Laboratory Animals ensuring proper animal welfare throughout the study.

Extraction of Plant Material

For this study, acorns were randomly collected from selected trees. After collection, the acorn fruits were air-dried and broken. The moisture content of the acorn fruits was analyzed, and homogeneous particles were achieved by crumbling the dried fruits using a closed lab mill to avoid hydration. The resulting powder particles were stored in a refrigerator at -80°C until further analysis [15]. For extract preparation, the method of Nawirska-Olszańska et al. [15] was used. Identification and measurement of phenolic compounds were performed using the UPLC-PDA–MS method

Table 1. Results of the examination of acorn samples are listed.

Test	Percentage
Protein	4.5
Fat	7.73
Moisture	2.09
Ash	2.7
Fiber	2.3
Carbohydrate	85.68

Table 2. Qualitative chemical analysis of some active groups in the alcoholic extract of oak plant

Active ingredients	Reagent type	Hot alcoholic extract
Glycosides	Benedict's Reagent	+++Red precipitate
Alkaloids	Meyer's Reagent	++White precipitate
Tannins	Lead acetate 1%	+++Appearance of white precipitate
Resins	Ethyl alcohol in boiled distilled water + acid HCl	+++Turbidity
Saponins	1. Shake the extract. 2. Mercuric chloride.	++ Foam White precipitate
Coumarins	Sodium hydroxide + filter paper + UV source	-
Flavonoids	Ethyl alcohol + potassium hydroxide	++ Yellow color
Phenols	Ferric chloride 1%	+++ Bluish-green color
Terpenes	Chlorofoam + acetic acid	+ Brown color appears
Steroids	Anhydrous + concentrated sulfuric acid	-

+ Available ++ Widely Available +++ Highly Available
- Not Available

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Animals and Experimental Design

The investigation was conducted at the Department of Animal Production, College of Agriculture, Al-Qasim Green University, testing Ross 308 broiler (average weight nearly 41–43 g) acquired from local hatcheries. The selected chicks were randomly appointed to five treatment groups, each with five replicates and twelve birds per replicate (pen dimensions of 1.2 × 1.5 m), following a completely randomized design. The treatments included different concentrations of alcoholic extract of oak fruit in drinking water (0, 1, 2, 3, and 4 mL/L), while control birds received plain water. Throughout the 35-day trial, birds were provided ad libitum access to feed and water, with diets formulated according to the nutrient requirements specified in the Ross Broiler Manual [16] and NRC [17]. Housing included Inverted Drinkers, Cylindrical Feeders , and bedding of wood shaving

Table 3. The formulation and determined analysis of standard diets.

	Starter (0-10 d)	**Grower (11-24 d)**	**Finisher (25-35 d)**
Corn	39.43	43.00	47.00
Soya meal (44%)	44.34	40.10	34.45
Wheat	10	10	10
Concentrate[1]	2.5	2.5	2.5
Calcium carbonate	1.28	1.07	1.27
Sunflower oil	2.27	3.15	4.6
Salt (NaCl)	0.18	0.18	0.18
Sum	100	100	100
Composition analysis			
ME (kcal/Kg)	3000	3.100	3.200
Crude protein%	23	21.5	19.5
Ca%	0.96	0.87	0.79
Available P%	0.48	0.43	0.39
Na%	0.16	0.16	0.16
Lysine%	1.44	1.29	1.15

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Methionine%	0.77	0.70	0.64
Threonine%	0.97	0.88	0.78
Met + Cys%	1.08	0.99	0.90
DCAB[2] (mEq kg^{-1})	236	220	200
Crude fiber %	5.05	4.84	4.55
Fat%	3.10	3.08	3.04
Linoleic acid%	1.02	1.09	1.17

[1]Provime concentrate produced by Provime / Dutch: Jordanian origin Each kg contains: 17% crude protein, 1.5% crude fat, 1% fiber, 14% calcium, 6.7% available phosphorus, 4.8% sodium, 5.8% chloride, 13.7% total phosphorus, 9.3% non-lysine, 7.8% methionine + cysteine, 0.4% tryptophan, 2100kcal/kg energy representative, 480000 IU vitamin A, 220000 IU vitamin D3, 3000 mg Vitamin E, 138 mg Vitamin K3, 138 mg Vitamin B1, 280 mg Vitamin B2, 600 mg Vitamin B5, 48 mg Vitamin B6, 1000 mg Vitamin B9, 1800 mg Niacin, 6900 mg Biotin, 2400 mg Iron, 3500 mg Zinc, 3200 mg Manganese, 480 mg Copper, 10 mg Selenium, 48 mg Iodine, 250 mg Antioxidant. (2) According to each of the energy represented, crude protein, crude fiber, fat, lysine, and methionine + cysteine calcium, and bio phosphorus. Note. ME: metabolizable energy. [2]DCAB = Na + K – Cl.

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Studied traits

Body weight was recorded at 7, 14, 21, 28, and 35 days of age, while feed consumption and body weight gain were measured periodically to calculate the feed conversion ratio (FCR) Mortality was monitored daily as it occurred, and the body weight of dead birds was used to adjust the FCR

Carcass Traits

On day 35, a predetermined number of birds from each treatment group (six per treatment), after an overnight fasting period, were killed for carcass assessment [14,17]. Then, carcass weights were registered after evisceration, and internal organs—including the heart, liver, gizzard, pancreas, and abdominal fat—along with the breast, thigh, wings, back, and neck were removed and weighed [14]. Additionally, lymphoid organs such as the bursa of Fabricius and spleen were collected, and their

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weights were expressed as a percentage of live body weight [17]. The yield percentage of each carcass part and internal organ was calculated relative to live weight [14,17].

Statistical Analysis

Statistical analysis data were conducted using a completely randomized design. Total data, counting feed intake (FI), feed conversion ratio (FCR), body weight (BW), body weight gain (BWG), and carcass traits, were subjected to analysis of variance (ANOVA) using the General Linear Model (GLM) procedures of SAS software (versions 9.1 and 2016; SAS Institute, Cary, NC, USA). When significant differences among means were detected, post hoc comparisons were performed using Duncan's multiple range test and statistical significance was declared at $P < 0.05$ [22]

Results and Discussion
Productive Performance

The results of experimental treatments on BW values are showed in Table 4. The data showed that there was no significant difference in BW between the group treated with the alcoholic extract of oak fruit and the control group throughout the experiment Chemical treatments of acorns or tannin-rich feedstuffs have been reported to reduce tannin content and improve nutritive value and growth performance [13, 14, 17, 18]. For example, treating acorns with sodium hydrogen carbonate reduced the amounts of all phenolic compounds — total phenols, condensed tannins, and entire tannins [18]. Kyarisiima et al. [12] reported that treatment of oak or sorghum with wood ash, as an alkaline material, decreases tannin content and improves nutritive value and growth rate. Positive effects of chemical treatments on tannin-rich feedstuffs have also been shown by Mahmood et al. [13, 14] and Ochanda et al. [18]

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Table 4. Effect of alcoholic extract of oak fruit added to drinking water on body weight of broiler chickens (Mean ± SEM)

Item (ml/100 ml)	1-week	2-week	3-week	4-week	5-week
0	76.56 ± 4.45	453.97 ± 12.14	979.50 ± 11.93	1707.28 ± 23.05	2500.02 ± 12.90
1	172.85 ± 4.45	443.65 ± 12.14	977.92 ± 11.93	1676.93 ± 23.05	2500.05 ± 12.90
2	176.90 ± 4.45	454.60 ± 12.14	983.83 ± 11.93	1696.40 ± 23.05	2483.39 ± 12.90
3	168.38 ± 4.45	441.05 ± 12.14	964.75 ± 11.93	1683.28 ± 23.05	2483.37 ± 12.90
4	168.45 ± 4.45	459.87 ± 12.14	957.17 ± 11.93	1669.07 ± 23.05	2516.71 ± 12.90
SEM	4.45	12.14	11.93	23.05	12.90
P-value	0.49	0.78	0.49	0.77	0.35

The effects of the experimental treatments on BWG are presented in Table 5. Inclusion of the alcoholic extract of oak fruit in drinking water had no effect on BWG Furthermore, Varmaghani et al. [24] showed that the daily body weight gain of the broilers was not significantly affected by the inclusion of oak kernel at levels of 0, 5, 10, 15, and 20%. Sinaei and Houshmand [19] found that there was no significant difference in body weight gain among broiler treatment groups (diet containing 20 to 25% raw and treated oak acorn)

Table 5. Effects of alcoholic extract of oak fruit in drinking water on body weight gain in broiler chicks

Item (ml/100 ml)	BWG – 1-week	BWG -2-week	BWG -3-week	BWG -4-week	BWG -5-week	BWG 1-5-week
0	137.73	277.40	525.53	727.78	792.74	2461.19
1	134.71	277.70	534.27	699.02	823.12	2461.92
2	137.93	270.80	529.23	712.57	786.99	2444.43
3	128.58	272.67	523.70	718.53	800.08	2443.57
4	129.54	291.42	497.30	711.90	847.64	2477.81
SEM	4.78	12.28	10.03	19.32	22.21	12.45
P-value	0.50	0.78	0.12	0.87	0.31	0.30

Feed intake (FI) value is introduced in Table 6. All groups fed diets containing the alcoholic extract of oak fruit in water showed similar FI with the control group until the starter, grower and finisher steps and throughout the experimental period (P> 0.05).

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Table 6. Effects of alcoholic extract of oak fruit in drinking water on feed intake in broiler chicks

Item (ml/100 ml)	AFI-1-week	AFI-2-week	AFI-3-week	AFI-4-week	AFI-5-week	AFI 1-5-week
0	147.61	329.35	646.33	963.70	1192.98	3279.98
1	152.80	324.91	644.67	980.37	1198.72	3301.47
2	153.80	326.56	647.17	953.80	1194.70	3272.43
3	142.20	322.96	638.83	971.50	1204.22	3283.32
4	151.58	326.51	634.80	934.63	1194.67	3242.20
SEM	3.93	4.02	10.65	14.16	20.07	33.99
P-value	0.25	0.84	0.90	0.22	0.99	0.80

The results of experimental treatments on feed conversion ratio (FCR) value is offered in Table 7. The inclusion of the alcoholic extract of oak fruit in the drinking water had no effect on the FCR when compared to the control group throughout the experiment. In line with this study, "The results obtained from the experiment indicated no significant effect of the alcoholic extract of oak fruit on the feed conversion ratio (FCR). This can be attributed to the ability of broiler to adapt to the levels of phenols and tannins added according to the studied concentrations, which did not significantly hinder the process of digestion and absorption of nutrients. Furthermore, the balanced chemical content of oak fruits played an important role in stabilizing feed intake balance and production performance.

Table 7. Effects of alcoholic extract of oak fruit in drinking water on feed conversion ratio in broiler chicks

Item (ml/100 ml)	FCR -1- week	FCR -2- week	FCR -3- week	FCR -4- week	FCR -5- week	FCR 1-5- week
0	1.07	1.19	1.23	1.32	1.50	1.33
1	1.13	1.21	1.20	1.40	1.45	1.34
2	1.12	1.17	1.22	1.34	1.53	1.33
3	1.10	1.20	1.22	1.36	1.50	1.34
4	1.17	1.13	1.27	1.31	1.41	1.30
SEM	0.04	0.06	0.02	0.03	0.05	0.01
P-value	0.59	0.90	0.45	0.51	0.52	0.31

The results indicated the weights of the thigh, neck, abdominal fat pad, liver, and Bursa of Fabricius (Table 8) were significantly influenced by treatments ($P < 0.05$). Specifically, the inclusion of 1 ml/100 ml drinking water containing the oak fruit extract increased thigh weight and decreased neck weight in broiler chicks. The weights of the Bursa of Fabricius were significantly decreased by the inclusion of 2 and 3 ml/100 ml of drinking water containing the alcoholic extract of oak fruit ($P < 0.05$). In addition, the liver weights were significantly decreased by the inclusion of 3 and 4 ml/100 ml of drinking water containing the alcoholic extract of oak fruit ($P < 0.05$). Varmaghani et al. [24] also reported that carcass characteristics—including breast, legs, and total internal organs—were not significantly affected by the different levels of oak kernel inclusion at the 6th and 7th weeks of age, with the exception of abdominal fat weight at the 6th week. Feeding broilers with high levels of tannin may inhibit pancreatic enzymes, consequently increasing the need for enzyme production, which can cause

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pancreatic hypertrophy [13]. Nevertheless, in this experiment, pancreas weight was not affected. Similar to the current study, Nyachoti et al. [18] showed that tannins did not significantly affect pancreas weight in broilers. Rezaei and Semnaninejad [25] reported that the relative weight of the pancreas and proventriculus, when compared to the control group, increased in broiler chicks fed raw or water-soaked acorn seeds at levels of 10% and 20%. In contrast to our study, the inclusion of raw and treated oak acorn in the diet had no significant influence on liver weight and abdominal fat in broilers at 42 days of age [19].

Table 8. Effects of alcoholic extract of oak fruit in drinking water on carecass in broiler chicks

Item (ml/100 ml)	Carcass (%)	**Thigh %**	breast	back	neck	wings	liver	Gizzard%	Fabricia	heart	Pancreas %	Abdominal Fat	Spleen
0	75.87	30.73^{a}	17.76	14.65	5.18^{b}	7.27	2.32^{abc}	1.33	0.13^{a}	0.47	0.21	1.32^{ab}	0.09
1	72.12	24.93^{b}	19.29	11.82	7.66^{a}	7.60	2.64^{a}	1.51	0.11^{ab}	0.47	0.22	1.62^{ab}	0.09
2	74.14	28.77^{ab}	20.07	10.84	6.24^{b}	8.21	2.43^{ab}	1.38	0.06^{b}	0.38	0.20	1.92^{a}	0.11
3	75.92	30.74^{a}	20.37	11.27	5.50^{b}	8.04	2.00^{c}	1.20	0.07^{b}	0.45	0.25	0.92^{ab}	0.11
4	73.69	28.16^{ab}	20.04	11.14	6.17^{b}	8.16	2.20^{bc}	1.20	0.10^{ab}	0.46	0.21	0.73^{b}	0.10
SEM	1.91	1.12	1.19	1.55	0.44	0.52	0.12	0.13	0.01	0.03	0.03	0.29	0.01
P-value	0.61	0.03	0.51	0.45	0.02	0.68	0.04	0.51	0.03	0.44	0.91	0.09	0.72

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Conclusion

"It can be concluded from this study that the addition of the alcoholic extract of oak fruit did not lead to any negative effects on the production performance of broiler chickens, even at the level of(4 ml/L.)This can be explained by the fact that these concentrations were within the safe limit; they did not affect water palatability or significantly change its taste, nor did they reduce feed intake. Furthermore, the active compounds present in this extract functioned in a balanced manner without causing any disturbance in metabolic processes throughout the rearing period (35 days), which indicates that this extract can be used at the mentioned concentrations as a safe natural additive without concern for a decline in the production performance of broiler

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