

Effect of Aqueous and Alcoholic Seeds Extracts of *Peganum harmala* on Hematological, Biochemical and Growth performance parameters in Broiler Chicken

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ABSTRACT

This study aimed to evaluate the effects of aqueous and alcoholic extracts of *Peganum harmala* L. seeds, on hematological, biochemical and growth parameters in broiler chicks. A total of 120 one-day-old Ross 308 broilers were randomly assigned to five groups: a control group and four extract-supplemented groups. The birds received either aqueous or alcoholic extracts at concentrations of 200 or 300 mg/L from day 7 to day 42. These findings support the potential of P. harmala seed extracts as natural feed additives in poultry production.

The birds, which were raised in the same climatic conditions, were given free access to water and feed. Blood was drawn during the different stages, and body weight was measured weekly, and the amount of feed consumed weekly was recorded. Statistical analysis was performed using SPSS V.22 software according to a completely randomized design. The results showed that the treatment with the aqueous extract at a concentration of 300 mg/L and the treatment with the alcoholic extract at a concentration of 200 mg/L were the most stable and improved immune and physiological WBC, H/L Ratio, and increased total protein albumin and stabilized liver enzymes, which positively reflects on the health of the birds and leads to increased growth rates and improved feed conversion efficiency, unlike the alcoholic extract 300 mg/L, which caused toxicity and hyper responsiveness. These findings support the potential of P. harmala seed extracts as natural feed additives in poultry production.

1. Introduction

Intensive broiler production systems, while economically advantageous and highly productive, are frequently associated with disturbances in oxidative homeostasis and increased disease susceptibility during early growth phases [1] As a result, research efforts have increasingly focused

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on plant-derived antioxidant extracts as natural sources of bioactive molecules capable of modulating oxidative processes and supporting physiological stability in broiler chickens [2,3].

Newly hatched broiler chicks are characterized by the incomplete development of their immune, antioxidant, and enzymatic defense systems, which makes them highly vulnerable to environmental stressors and nutritional challenges [4]. Hematological indices, such as white blood cell counts, alongside serum biochemical parameters including total protein, albumin, globulin, and liver enzyme activities, are widely used as sensitive indicators to assess health status, immune competence, and metabolic efficiency in poultry [5, 6].

The historical reliance on antibiotics as growth promoters in poultry diets has raised serious public health concerns due to the emergence of antimicrobial resistance and drug residues in animal products [7]. Consequently, the routine use of antibiotics in livestock farming is now strictly regulated or prohibited in many nations [8]. Due to this, extensive research has been conducted to find safe and efficient natural substitutes that can improve animal health and productivity [9][10].

Due to their abundance of bioactive substances, such as alkaloids, phenolics, flavonoids, and essential oils, which possess antimicrobial, immunomodulatory, anti-inflammatory, and antioxidant qualities, medicinal plants and their extracts have drawn more interest as phytogetic feed additives [11]. Among these plants, *Peganum harmala* L. is a medicinal species that has been used traditionally in various regions. It is known to be rich in β -carboline alkaloids, including harmine, harmaline, and harmalol, in addition to phenolic and flavonoid compounds with known biological activities [12][13]. The extraction solvent has a significant impact on the biological efficacy and safety of plant extracts since it determines the active components' qualitative and quantitative composition. While aqueous extracts typically contain higher levels of water-soluble phenolic compounds that may exhibit antioxidant activity with a relatively lower risk of toxicity [14][15], alcoholic extracts are typically more effective in extracting alkaloids with strong pharmacological effects [16].

Although several studies have evaluated the pharmacological properties of *P. harmala* seed extracts [17], comparative information regarding the hematological and biochemical effects of aqueous versus alcoholic seed extracts in broiler chicks, particularly during early developmental stages, remains limited and inconsistent [18]. Therefore, the present study was designed to evaluate and compare the effects of aqueous and alcoholic *P. harmala* seed extracts administered via drinking water on selected hematological and biochemical parameters in broiler chicks, aiming to provide scientifically validated evidence for the safe use of this medicinal plant as a natural alternative in poultry production systems.

2. Materials and Methods

2.1. Study Site and Ethical Approval

The study was conducted in a controlled poultry rearing unit from October to December 2025. Environmental conditions, including temperature, ventilation, and lighting, were managed according to standard Ross 308 management guidelines. The study adhered to the ethical standards of the College of Education and was approved by the Scientific Research Ethics Committee, Department of Biology, University of Basrah (ID no. 123, 2025).

2.2. Experimental diets

The chicken were fed on diets that was added according to their age periods

Diets	Crude Protein	Metabolizable energy
Starter diet (1-14) day	(22.70) %	(3023) kcal/kg
Growing diet(14-28) day	(20.47)%	(3137) kcal/ kg
Finisher diet (28-42) day	(18.46)%	(3220) kcal/kg

2.3. Preparation of Plant Extracts

Peganum harmala seeds were obtained from local markets, cleaned, air-dried, and finely ground. Alcoholic Extract: 30 g of seed powder was mixed with 300 mL of 99% ethanol and subjected to ultrasonic-assisted extraction as 76 c for 3 hours. The extract was concentrated using a rotary evaporator and stored at 4°C [19]. The Yield of the alcoholic extract was calculated as

$$\text{Yield (\%)} = \frac{\text{Weight of dried extract}}{\text{Weight of seed powder}} \times 100$$

Aqueous Extract: 30 g of powder was mixed with 300 mL of distilled water and boiled to extract water-soluble compounds. The mixture was filtered (medical gauze followed by Whatman No. 1 paper), centrifuged at 3000 rpm, freeze-dried, and stored in airtight containers [20], the yield was calculated in the same way as above

2.4. Diagnosis of alkaloids

The concentration of B-Caroline alkaloids was measured using GC-MS to determine the active chemical compound including, harmine, harmaline, harmalol, tetrahydroharmin

2.5. Experimental Animals and Design

A total of 120 one-day-old Ross 308 chicks (avg. weight 43 g) were randomly allocated into five treatments (3 replicates/treatment, 8 birds/replicate):

T1: Control

T2 & T3: Aqueous extract at 200 and 300 mg/L, respectively.

T4 & T5: Alcoholic extract at 200 and 300 mg/L, respectively.

Extracts were administered via drinking water from day 7 to 42 he required concentration in drinking water and the birds' average daily water intake were used to determine the dosage of the *Peganum harmala* extract. To achieve the desired concentrations (200 and 300 mg/L), the necessary quantity of the dried extract was dissolved in the proper volume of drinking water. To maintain stability and precise dosing throughout the trial, newly made drinking water containing the extract was changed every 24 hours.

2.6. Data Parameters

Blood samples were collected from the wing vein:

Hematology: Samples in EDTA tubes were used to determine WBC according to hematological methods use hemocytometer and use Natt and Herick Solution under a light microscope, Heterophils (H), Lymphocytes (L), and the H/L ratio. by blood smears were preparing on glass slides and, after drying for about 10 minutes, were stained with Wright –Giemsa and examined under a light microscope. [21]

Biochemistry: Samples in plain tubes were centrifuged (2000 rpm for 15 min at 4°C) to obtain serum. Parameters included total protein was determined using the Biuret method according to [22], albumin, globulin, and liver enzymes (AST, ALT) using the method according [23].

2.7. Weekly weight gain (g)

The weight gain in each replicate of the experimental units was calculated according to the following equation: Weight gain (g) = live body weight at the end of each week - live body weight at the beginning of each week [24]

2.8. The amount of feed per consumer (g)

The calculated amount of feed per consumer weekly for each experimental coefficient using the following equation.

Amount of feed consumed = Amount of feed provided at the beginning of the week - Amount of feed remaining at the end of the week [25].

2.9. Food conversion factor

Calculate the food conversion factor for each of the replicates that did not record deaths according to the following equation:

Weekly feed conversion factor = average feed consumed (g) per week

average weight gain (g) per week

2.10. Statistical Analysis

Data were analyzed using SPSS V.22, Normality was verified using the Shapiro–Wilk test. Differences among means were determined by one-way ANOVA followed by Turkey's post hoc test $p \leq 0.05$

3. Results and Discussion

Results indicate that *P. harmala* seed extracts significantly modulate hematological and biochemical parameters compared to the control group. Birds receiving aqueous and alcoholic extracts exhibited improved hematological profiles, indicating enhanced physiological and immune status. Serum biochemical analysis showed that 200 mg/L of the alcoholic extract had positive effects on protein metabolism without inducing hepatotoxicity, as evidenced by stable AST and ALT activities. These improvements are likely attributed to the bioactive components of *P. harmala*, particularly β -carboline alkaloids and phenolic compounds, which possess potent immunomodulatory and antioxidant properties.

3.1. Cellular Parameter Analysis

The cellular parameters (Table 1) revealed that both extracts significantly improved immune indicators across different age groups ($P \leq 0.05$).

Aqueous Extract (T2 & T3): Treatment T2 (200 mg/L) showed a graded immune response, achieving stability by day 42. However, T3 (300 mg/L) exhibited the most significant improvement in WBC (37.40 to $36.72 \times 10^3/\text{ml}$) counts and a reduction in the Heterophil to Lymphocyte (H/L) ratio. This is attributed to the synergistic effect of flavonoids and phenolic compounds, which mitigate oxidative stress and stimulate lymphocyte proliferation [26].

Alcoholic Extract (T4 & T5): T4 (200 mg/L) significantly boosted the immune response compared to the control, increasing WBC counts from (30.38 to $32.9 \times 10^3/\text{ml}$). In contrast, the high dosage in T5 (300 mg/L) caused fluctuations in leukocyte counts. This may be due to the potent concentration of alkaloids which, at high levels, can exert a restrictive effect on bone marrow function [27].

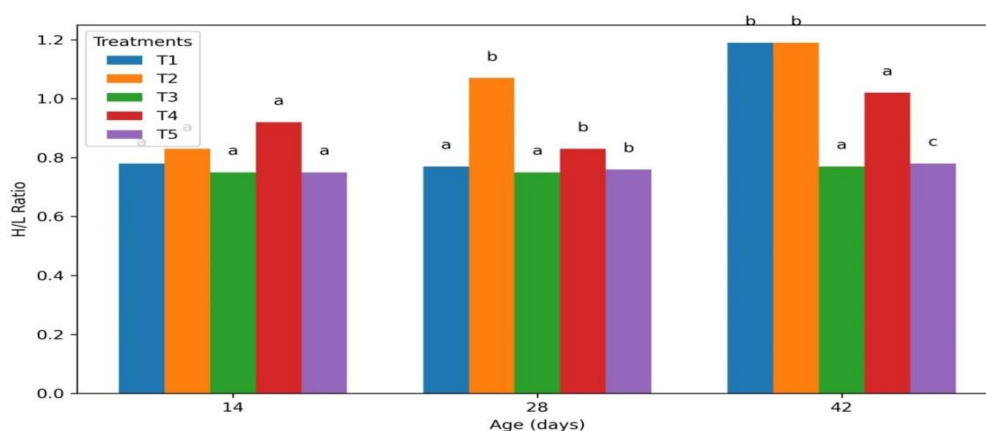
The activation of T and B lymphocytes observed in treated groups characterizes a robust adaptive immune response. The findings demonstrate a functional integration between innate and adaptive immunity, where a balance between heterophils and lymphocytes was established. The H/L ratio is a critical indicator of physiological stress in poultry [28]; the decrease in this ratio in treated groups confirms that *P. harmala* extracts effectively alleviate environmental and physiological stress.

The lowest immunological response was observed in the control group, which lacked the stimulatory effects of the plant's bioactive molecules [29]. These results support the role of *P. harmala* as an organic immunity booster in broiler production. These results are consistent with previous studies that will address the role of medicinal plants in addition to poultry as feedstuffs, which improved immune immunity and lymphocytes, and this is attributed to the role of biologically active compounds in plant extracts that enhanced humoral and cellular immunity.

Table 1. Effect of Harmal Extracts on Some Cellular Parameters in Poultry blood at Different Age Stages (Mean $\pm$ Standard Deviation)

Treatment	Age	WBCX10 ⁶ /ml	H/L Ratio
T1	14	32.40 $\pm$ 1.10 ^a	0.030 $\pm$ 0.780 ^a
	28	35.31 $\pm$ 1.57 ^a	0.040 $\pm$ 0.770 ^a
	42	45.40 $\pm$ 0.58 ^b	0.290 $\pm$ 1.190 ^b
T2	14	29.84 $\pm$ 2.60 ^a	0.040 $\pm$ 0.830 ^a
	28	26.77 $\pm$ 1.98 ^b	0.040 $\pm$ 1.070 ^b
	42	43.07 $\pm$ 2.02 ^c	0.100 $\pm$ 1.190 ^b
T3	14	37.40 $\pm$ 0.48 ^a	0.010 $\pm$ 0.750 ^a
	28	34.57 $\pm$ 1.48 ^a	0.030 $\pm$ 0.750 ^a
	42	36.72 $\pm$ 3.02 ^a	0.020 $\pm$ 0.770 ^a
T4	14	32.09 $\pm$ 0.58 ^a	0.040 $\pm$ 0.920 ^a
	28	44.24 $\pm$ 1.15 ^b	0.040 $\pm$ 0.830 ^a
	42	30.38 $\pm$ 1.48 ^a	0.060 $\pm$ 1.020 ^b
T5	14	42.67 $\pm$ 1.88 ^a	0.030 $\pm$ 0.750 ^a
	28	22.74 $\pm$ 0.94 ^b	0.030 $\pm$ 0.760 ^a
	42	31.36 $\pm$ 3.02 ^c	0.030 $\pm$ 0.780 ^a
P -Value		*	*

Data are given as Mean $\pm$ SD; Different small letters(a,b,c) indicate statistically significant differences ($P\leq 0.05$) between groups within the same column; Similar small letters indicate statistically no significant differences between groups

**Fig 1.** Effect of different concentrations of ethanolic aqueous extract of harmala seeds on some studied parameters on ages 14, 28, 42 days.(H/L Ratio)

3.2. Effect of *P. harmala* Extracts on Growth Performance and Feed Conversion

The results presented in Table (2) illustrate the impact of aqueous and alcoholic *P. harmala* extracts on growth indicators, including final live body weight, feed conversion ratio (FCR), and mortality rate. The highest final body weight was observed in treatment T4 (1506.67 ± 40.41 g), which received 200 mg/L of alcoholic extract, significantly outperforming the control group (1380.00 ± 25.00 g) ($P < 0.001$).

Regarding feed efficiency, the lowest (best) Feed Conversion Ratio (FCR) was recorded in treatment T3, reaching 1.50 ± 0.04 . This indicates a superior efficiency in converting ingested feed into body mass. Despite the known potency of *P. harmala* alkaloids, no mortality was recorded across all experimental groups, suggesting that the concentrations used (200-300 mg/L) are safe for broiler consumption.

The significant improvement in live body weight and FCR in extract-treated groups ($P \leq 0.001$) can be attributed to enhanced digestive health and a reduction in pathogenic intestinal microbiota [30]. The alcoholic extract at 200 mg/L (T4) exhibited positive effects on weight gain. This growth-promoting effect is likely due to the presence of beta -carboline alkaloids, which act as natural antioxidants and antimicrobials against intestinal microorganisms [31,32].

Furthermore, these extracts appear to improve nutrient utilization by stabilizing the intestinal environment and boosting the immune system's efficiency [33]. While higher concentrations of alcoholic extracts can sometimes induce physiological stress, the 200 mg/L dose (T4) provided an optimal balance, promoting growth without reaching toxic thresholds. These findings confirm the potential of *P. harmala* as a safe, natural alternative to synthetic growth promoters in the poultry industry.

Table 2. Effect of aqueous and alcoholic harmal extracts compared with control group on growth indicators, including final live weight, total food conversion, and mortality rate during different age stages.

Treatment	Final live weight (g)	Total food conversion	Perishing rate
T1	1380.00 ± 25.00^c	1.45 ± 0.08^a	0.00 ± 0.00
T2	1405.00 ± 40.41^{bc}	1.45 ± 0.39^a	0.00 ± 0.00
T3	1470.00 ± 5.00^{ab}	1.50 ± 0.04^b	0.00 ± 0.00
T4	1506.67 ± 40.41^a	1.44 ± 0.03^a	0.00 ± 0.00
T5	1496.67 ± 5.77^a	1.47 ± 0.02^{ab}	0.00 ± 0.00
P -Value	**	*	NS

Data are given as Mean $\pm$ SD; Different small letters(a,b,c) indicate statistically significant differences ($P \leq 0.05$) between groups within the same column; Similar small letters indicate no statistically significant differences between groups. T1:Control group, T2:aqueous extracts group 200 mg/l, T3:aqueous extracts group 300 mg/l, T4:alcoholic extracts group 200 mg/l, T5:alcoholic group 300 mg /l.

3.3. Effect of *P. harmala* Extracts on Serum Biochemical Parameters

Data presented in Table 3 indicate that both aqueous and alcoholic *P. harmala* seed extracts (200 and 300 mg/L) exerted significant effects on biochemical pathways in the blood serum of broiler chickens. Particular focus was placed on the hepatic enzymes Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT). Alterations in these enzymes are widely recognized as sensitive indicators of hepatic function, as elevated levels typically signal cellular membrane disruption or tissue damage.[34]

Aqueous Extract (T3): The 300 mg/L aqueous extract led to a decrease in AST and ALT levels at 14, 28, and 42 days of age compared to the control group. This reduction reflects improved liver health and cellular integrity.

Alcoholic Extract (T4 & T5): In T4 (200 mg/L), enzyme levels remained stable and within physiological ranges, implying that this concentration is non-toxic. However, in T5 (300 mg/L), early

signs of physiological stress were observed. This is likely due to the high concentration of alkaloids which, at excessive levels, can exert cytotoxic effects on hepatic tissues.

Serum total protein, albumin, and globulin levels serve as vital indicators of metabolic efficiency and immune system health.

Treatment T3 (Aqueous, 300 mg/L): This group showed significantly higher values of total protein and globulin across in age at day 42. These elevated levels indicate a robust immune response and efficient protein synthesis, reinforcing the immunomodulatory role of the extract at this concentration.[35]

Control Group: Recorded the lowest protein profile in age 42 compared to the third group, highlighting the importance of plant-derived bioactive compounds in stimulating the birds' immune systems.

The stability of these biochemical markers, particularly in the aqueous treatments, confirms that *P. harmala* can be safely integrated into poultry diets to enhance health without inducing systemic toxicity

Table 3. Effect of harmal extract on some biochemical parameters in poultry blood serum at different age stages (arithmetic mean $\pm$ standard deviation) compared with the control group .

Treatment	Age	T. Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	AST(U/L)	ALT(U/L)
T1	14	2.83 $\pm$ 0.01 ^a	1.37 $\pm$ 0.05 ^a	1.46 $\pm$ 0.05 ^a	241.4 $\pm$ 11.0 ^c	8.60 $\pm$ 0.53 ^c
	28	2.54 $\pm$ 0.11 ^b	1.36 $\pm$ 0.10 ^c	1.10 $\pm$ 0.02 ^c	212.7 $\pm$ 23.0 ^a	9.10 $\pm$ 0.44 ^d
	42	2.86 $\pm$ 0.58 ^b	1.35 $\pm$ 0.18 ^b	1.51 $\pm$ 0.39 ^a	265.4 $\pm$ 23.0 ^d	6.83 $\pm$ 1.13 ^a
T2	14	2.62 $\pm$ 0.03 ^b	1.34 $\pm$ 0.08 ^a	1.27 $\pm$ 0.11 ^{bc}	217.5 $\pm$ 8.5 ^b	6.27 $\pm$ 0.06 ^b
	28	2.74 $\pm$ 0.22 ^a	1.44 $\pm$ 0.12 ^b	1.30 $\pm$ 0.10 ^b	213.6 $\pm$ 3.2 ^a	5.87 $\pm$ 0.47 ^a
	42	2.54 $\pm$ 0.04 ^c	1.30 $\pm$ 0.06 ^c	1.25 $\pm$ 0.05 ^c	225.2 $\pm$ 2.8 ^a	8.00 $\pm$ 0.99 ^d
T3	14	2.16 $\pm$ 0.03 ^c	1.05 $\pm$ 0.02 ^c	1.12 $\pm$ 0.01 ^c	201.3 $\pm$ 1.8 ^a	5.04 $\pm$ 2.29 ^a
	28	2.59 $\pm$ 0.42 ^b	1.50 $\pm$ 0.26 ^a	1.10 $\pm$ 0.15 ^c	221.1 $\pm$ 28.8 ^b	6.73 $\pm$ 1.06 ^b
	42	3.10 $\pm$ 0.48 ^a	1.53 $\pm$ 0.24 ^a	1.37 $\pm$ 0.20 ^b	244.1 $\pm$ 19.4 ^c	7.22 $\pm$ 1.77 ^b
T4	14	2.63 $\pm$ 0.04 ^b	1.24 $\pm$ 0.01 ^b	1.39 $\pm$ 0.09 ^a	215.9 $\pm$ 19.9 ^a	8.50 $\pm$ 0.70 ^a
	28	2.55 $\pm$ 0.04 ^b	1.23 $\pm$ 0.01 ^d	1.32 $\pm$ 0.09 ^a	222.2 $\pm$ 16.0 ^c	6.53 $\pm$ 0.79 ^c
	42	2.36 $\pm$ 0.09 ^d	1.32 $\pm$ 0.01 ^b	1.04 $\pm$ 0.08 ^d	224.7 $\pm$ 27.7 ^a	7.60 $\pm$ 0.47 ^a
T5	14	2.58 $\pm$ 0.05 ^b	1.27 $\pm$ 0.03 ^b	1.31 $\pm$ 0.06 ^b	241.7 $\pm$ 3.9 ^c	7.07 $\pm$ 1.45 ^c
	28	2.70 $\pm$ 0.35 ^a	1.23 $\pm$ 0.01 ^d	1.32 $\pm$ 0.09 ^a	237.2 $\pm$ 16.0 ^b	8.53 $\pm$ 0.79 ^b
	42	2.47 $\pm$ 0.23 ^c	1.21 $\pm$ 0.09 ^c	1.26 $\pm$ 0.21 ^d	227.8 $\pm$ 22.9 ^b	7.07 $\pm$ 0.85 ^a

Data are given as Mean $\pm$ SD; Different small letters(a,b,c) indicate statistically significant differences ($P\leq 0.05$) between groups within the same column ; Similar small letters indicate no statistically significant differences between groups.T1:Control group ,T2-T5 :Treatment group

4.Conclusion

The results of the present study demonstrate that supplementing broiler drinking water with aqueous or alcoholic *Peganum harmala* seed extracts significantly improves hematological and biochemical parameters. The findings suggest that 300 mg/L for aqueous extracts and 200 mg/L for alcoholic extracts are the optimal concentrations to enhance immune response and physiological stability without adverse effects on liver function. These results support the potential of *P. harmala* seed extracts as safe, natural, plant-based additives to improve health and productivity in poultry production systems.

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تأثير المستخلصات المائية والكحولية لبذور الحرمل على المعايير الدموية والبيوكيميائية والاداء النمو في دجاج التسمين

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معلومات البحث	الملخص
الاستلام 15 شباط 2026 المراجعة 03 نيسان 2026 القبول 03 نيسان 2026 النشر 30 حزيران 2026	هدفت هذه الدراسة إلى تقييم تأثير المستخلصات المائية والكحولية لبذور الحرمل (<i>Peganum harmala</i> L) على المؤشرات الدموية والكيميائية الحيوية ومؤشرات النمو في فراخ التسمين. تم توزيع 120 فرخاً من سلالة روس 308، عمر يوم واحد، عشوائياً على خمس مجموعات: مجموعة ضابطة وأربع مجموعات مغذاه بالمستخلصات. تلقت الطيور إما مستخلصات مائية أو كحولية بتركيز 200 أو 300 ملغم/لتر من اليوم السابع إلى اليوم الثاني والأربعين. تدعم هذه النتائج إمكانية استخدام مستخلصات بذور الحرمل كمضافات علفية طبيعية في إنتاج الدواجن. رُبيت الطيور في نفس الظروف المناخية، ووُفّر لها الماء والعلف بشكل دائم. سُحبت عينات دم خلال المراحل المختلفة، وُقيس وزن الجسم أسبوعياً، وسُجّلت كمية العلف المستهلكة أسبوعياً. أُجري التحليل الإحصائي باستخدام برنامج SPSS الإصدار 22 وفقاً لتصميم عشوائي كامل. أظهرت النتائج أن المعالجة بالمستخلص المائي بتركيز 300 ملغم/لتر والمعالجة بالمستخلص الكحولي بتركيز 200 ملغم/لتر كانت الأكثر استقراراً، وحسّنت من وظائف المناعة والوظائف الفسيولوجية، بما في ذلك تعداد خلايا الدم البيضاء، ونسبة الخلايا الليمفاوية إلى الخلايا الليمفاوية، وزيادة إجمالي بروتين الألبومين، واستقرار إنزيمات الكبد، مما ينعكس إيجاباً على صحة الطيور ويؤدي إلى زيادة معدلات النمو وتحسين كفاءة تحويل العلف، على عكس المستخلص الكحولي بتركيز 300 ملغم/لتر، الذي تسبب في التسمم وفقر الاستجابة. تدعم هذه النتائج إمكانية استخدام مستخلصات بذور الحرمل كمضافات علفية طبيعية في إنتاج الدواجن.
الكلمات المفتاحية	
دجاج التسمين؛ الحرمل؛ مستخلص البذور؛ المؤشرات البيوكيميائية والدموية.	

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