Effect of Marjoram (*Origanum majorana*) Leaf Powder as a Dietary Additive on Growth Performance and Blood Characteristics of Broiler Chickens

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Abstract

This study aimed to assess the effect of adding graduated levels of marjoram leaf powder) *Origanum majorana*) on the productive performance and some of the hematopoietic properties of the broiler strain (Ross 308). The trial involved 150 chickens at day 7, randomly distributed across four experimental treatments T1, T2, T3, and T4, at three repetitions per treatment, where marjoram ,(powder was added in percentages (1.0, 1.5, 2.0, and 2.5respectively, to the basal diets. The trial ly balanlasted for 35 days, during which nutritionalced prefix and finisher diets were provided. Results indicated that productive and physiological responses differed greatly; (treatment)T1at 1.0% was the best in terms of feed intake levels together with an observed decrease in feed conversion ratio (FCR), especially during the fourth week of the birds' age. Regarding the blood parameters, the higher concentration (2.5%) caused a significant increase) P≤0.05) in RBC count. The results indicated that the active compounds of marjoram have a protective action on oxidative hemolysis, while there was no significant difference in Hb concentration, WBC's count and PCV, indicating that this extract is physiologically safe. The best level of marjoram powder (1.0%) to support productive performance, while higher levels help in supporting the flock's general health status, was concluded this study. The study recommends adopting marjoram as a natural from sustainable alternative to chemical additives to improve biological and productive efficiency in poultry farms.

Keywords: Blood components, broiler chickens, marjoram (*Origanum majorana*), natural feed additives, productive performance.

Introduction

Poultry meat is a key pillar in securing animal protein of high biological value to humans, and is characterised by a balance of its nutritional content and high digestibility factors compared to other meat sources, which gives a vital role in enhancing global food security (Kashyap and Goswami, 2024). To achieve maximum production efficiency in poultry rearing projects, it is necessary to formulate to diets based on accurate scientific foundations that ensure that the physiological needs of the bird are met using optimal raw materials at a deliberate economic cost. These must also ensure the highest levels of absorption and metabolism, maintain the nutritional effectiveness of nutrients, and support the herd's health status to meet various environmental and pathological challenges (Gao, Zheng, and Xu, 2025).

As antibiotics have faced increasing limitations in their use as conventional growth promoters on a global scale , research has continued to focus on phytogenics as natural

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alternatives that can serve both the purpose of efficient growth promotion and health. In this context, the marjoram plant (*Origanum majorana*) emerges as one of the most promising alternatives ;it is an aromatic herbal plant belonging to the oralfamily .(Lamiaceae)This plant has a wide geographical spread that extends from the Mediterranean basin to the Arabian Peninsula and Asia, and is known in local popular heritage as "Al-Dosh" (Aminullah *et al.*, 2025).

This plant derives its value from its richness in biologically active compounds, particularly phenolics and volatile oils, which confer unique properties as a natural antibacterial, antifungal, and antioxidant agent. The scientific literature has confirmed the historical role of marjoram in folk medicine for treating digestive system disorders and inflammations, in addition to its contribution to regulating blood pressure and stimulating fluid excretion, and its possession of antioxidant enzymatic activity comparable to other natural sources (Bahadoran *et al.*, 2025; Abdel-Kader *et al.*, 2024).

Moreover, laboratory studies have demonstrated that marjoram extract exhibits broad inhibitory efficacy against Gram-positive and Gram-negative bacteria, with an efficiency approaching that of conventional antibiotics (Ellali *et al.*, 2025). Research has also revealed its cytotoxic effects on cancer cells and its high ability to protect genetic material (DNA) from oxidative damage resulting from external factors, which confirms that it possesses multi-functional biological properties (Yuan et al., 2022; Zhao et al., 2023; Lopez-Cantu *et al.*, 2022).

Nutritionally, marjoram leaves are a natural reservoir of vital elements. every 100 grams of dried leaves provides energy of 271 calories, a protein content of 12.66 grams, and a remarkable richness of fibre (40.3 grams). It also features an integrated metal profile topped by calcium (1990 mg) and potassium (1522 mg), as well as magnesium, phosphorus, and iron. As for its vitamin content, it is characterized by high levels of vitamin A 8068 IU and vitamin K 621.7 micrograms, in addition to being a source of vitamin C, folate, choline, and vitamin E (Abd-Rabbu *et al.*, 2024).

Based on the urgent need to develop sustainable nutritional strategies that ensure the efficiency of food conversion and enhance immunity, the inclusion of marjoram extract (Origanum majorana) emerges as a natural feed additive (Phytogenic additive) and a strategic option to improve productive and physiological indicators, while reducing reliance on chemical additives (Ibrahim *et al.*, 2025; Hassan *et al.*, 2024). From this standpoint, the current study aims to assess the impact of integrating gradual levels of marjoram into poultry diets and analyse their effects on productive performance and physiological status, in order to support modern trends towards safe and sustainable livestock production .

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MATERIALS AND METHODS

1- Preparation of the herb and the plant extract

Marjoram (*Origanum majorana*) samples were purchased from local markets in the Karbala governorate, Iraq as dried leaves. The samples were cleaned and subsequently ground mechanically in an electric mill to a powder form. The powder wasthen transferred into dark, airtight glass bottles and stored under the usual lab conditions to keep its bioactive compounds stable until use. The aqueous extract of the plant was prepared following the methodology of Sato et al. (1995) and Cetinkaya et al. (2025): a specific amount of the powder was mixed with distilled water, and the mixture was homogenised in an electric blender for 30 minutes. Thereafter, the mixture was filtered through double layers of sterile medical gauze to obtain the filtrate, which was subsequently transferred to a hot-air oven (Hot Air Oven) at 50°C for 24 hours to evaporate the aqueous solvent. In the final stage, the resulting dry extract was stored in sterilized containers under a refrigerated temperature (4°C) until its use in fortifying the experimental diets.

2- Experimental design and bird management

The field experiment was conducted at the Poultry Research Unit of the Department of Animal Production, College of Agriculture, University of Karbala, and continued until the birds reached marketing age (35 days). In the study, 150 (Ross 308) chicks were used at 7 days of age, with an average starting weight of 150 g/ bird.

Birds were moved into the house in a complete randomized design (CRD) and randomly assigned to four experimental –treatments with 3 replicates per treatment (12 n and birds/replicate) for statistical precisio 13 minimized bias as a result of environmental variation. Treatments consisted varying levels plant (*Origanum majorana*) of marjoram basal diet at 1.0, 1.5, 2.0 and powder in the Birds were raised .%2.5 in floorpens (Pens) with a single management system applied across all treatments. Environmental conditions, particularly temperature, were automatically regulated using gas brooders and an exhaust-fan ventilation system, following a gradual temperature reduction alprogram consistent with the physiologic requirements of the birds across different growth stages until the end of the rearing period.

Nutrition

An ad libitum feeding system was and used throughout the experimental period, and feed was offered to the birds in mash form. The feeding program was divided into two main stages. The first stage included the use of a starter diet unified for all treatments from 1 to 21 days of age, designed to meet the nutritional requirements of the Ross 308 strain, with a crude protein content of 23% and a metabolizable energy value of 3027 kcal/kg feed, and with an energy-to-protein ratio of 131:61.

The second stage included feeding the finisher diet from 22 to 35 days of age, and it contained 20% crude protein and a metabolizable energy value of 3195.3 kcal/kg feed, with an energy-to-protein ratio of 159.77.

Slaughter procedures and carcass measurements

At the end of the experiment, 6 birds were randomly selected from each treatment and subjected to a 4 hour a feed withdrawal period (with water remaining available) to reduce the content of the digestive tract. The birds were

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slaughtered, and then scalding was performed in a water bath at 54°C for two minutes. Thereafter, feathers and internal viscera were removed manually from the oesophagus to the cloaca according to the methodology described by Fleather (1999). The carcasses were washed well and dried, and then the eviscerated carcass weight (Eviscerated carcass weight) was obtained.

To evaluate the physical properties of meat, 3 carcasses from each treatment were allocated to conduct the following measurements:

Drip loss (Drip Loss): The percentage loss was estimated according to the protocol of Alvarado and Sams (2000) as updated by Al-Hijazeen (2022); the carcasses were weighed fresh (hot weight), then placed in tightly sealed polyethylene bags and stored refrigerated at 5°C for 24 hours. After that, the carcasses were removed and surface moisture was dried using filter paper, then they were reweighed (cold weight) to calculate the percentage of exudative loss.

Thawing loss (Thawing Loss): The methodology of Nam *et al.* (2000), followed by Zhang *et al.* (2022), was adopted to estimate the loss resulting from freezing and thawing. The carcasses were frozen at -18°C for 3 days. To perform thawing, the carcasses were transferred to the refrigerator (5°C) for 24 hours. After thawing, the separated fluid was removed, the samples were dried, and they were reweighed to calculate the percentage weight loss relative to their weight before freezing.

5- Blood sample collection and analysis

Blood samples were collected from the brachial vein (Brachial vein) of the birds and were transferred directly to test tubes containing an anticoagulant substance (Ethylene Diamine Tetra-acetic Acid - EDTA), then they were sent chilled to the laboratory to conduct the following examinations:

• Plasma separation and estimation of packed cell volume (PCV): A quantity of blood was transferred to micro-hematocrit capillary tubes (Micro-hematocrit capillary tubes), the ends were sealed with clay, then it was processed with a micro-hematocrit centrifuge (Micro-hematocrit centrifuge) to separate plasma from cellular components and to estimate the cell volume percentage.

• White blood cell count (WBC): The direct counting technique was used; 10 µL (0.01 mL) of blood was withdrawn and diluted using normal saline (Normal Saline) to reach a volume of 1 mL (dilution ratio 1:100). Then the sample was transferred to a haemocytometer (Haemocytometer) to estimate the total number of cells under a light microscope.

• Examination of red blood cells (or estimation of haemoglobin): 10 µL of blood was mixed with 2 mL of Parka reagent. After ensuring complete homogenization of the mixture, the optical density of the sample was estimated using a spectrophotometer (Spectrophotometer) at a wavelength of 520 nm according to the following methodology.

Statistical analysis

The resulting data were statistically analysed using a Completely Randomised Design (CRD) to evaluate the effects of graded levels of marjoram leaf powder on productive performance and the studied blood traits of broiler chickens. Significant differences between treatment means were compared using Duncan's Multiple Range Test at a significant probability level ($P \leq 0.05$)

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(Duncan, 1955). The statistical program SPSS (version 26) was used.

RESULTS AND DISCUSSION

Effect of marjoram (*O. majorana*) extract on weekly feed intake

The data in Table (1) show changes in weekly feed intake rates (g/bird) for broiler chickens, when graded levels of marjoram extract are added during the five-week rearing period. The statistical readings indicate that there were no significant differences ($P > 0.05$) feed consumption among all experimental treatments during the first, second, third, and fifth weeks. This stability in intake indicated that adding marjoram extract at different levels did not negatively affect the bird's feeding pattern in the early and late stages of the experimental period.

In contrast, the statistical analysis revealed significant variation ($P \leq 0.05$) during the fourth week of the birds' age; the low-concentration treatment (1.0%) was numerically and significantly superior by recording the highest feed intake rate of (610.70 ± 0.60 g/bird) compared with the other treatments, whereas a decrease in intake was observed in the (1.5%) concentration treatment, which recorded (465.70 ± 30.50 g/bird).

This significant superiority at the 1.0% addition level can be interpreted from a physiological and nutritional perspective, as marjoram possesses appetite-stimulating (Stomachic properties) properties due to its high content of volatile oils and hydrocarbon compounds, which improve feed palatability (Palatability) and stimulate the digestive system. As for the relative decline in intake with increased concentrations, it is attributed to the sensory effect of concentrated phenolic compounds and aromatic oils with a pungent odour and sharp taste, which may, when exceeding a certain limit, lead to a type of "sensory aversion" (Sensory aversion) in birds, thereby reducing the frequency of approaching the feed. As for the stability (non-significance) in the remaining weeks, it is most likely due to the incomplete functional maturity of the digestive and enzymatic system in the early weeks, followed by the occurrence of physiological adaptation (Physiological adaptation) with the plant additives in the advanced stages.

These results agreed with what was reached by Biswas and Kim (2025) and Shahbakht *et al*. (2024), who indicated that the optimal levels of phytogenic feed additives (Phytogenic Feed Additives) act as biological stimulants to enhance the secretion of digestive enzymes and improve the intestinal environment, which is reflected positively on the desire to feed. This study also agrees with the conclusions of Erdaw (2023) and Kazemi and Ariapour (2025) that the efficacy of medicinal plants fundamentally depends on the "dose-response effect" (Dose-response effect); moderate concentrations achieve the required balance between enhancing productive performance and maintaining normal feed intake, whereas high doses may show anti-nutritional effects (Anti-nutritional effects) resulting from interference with absorption efficiency or affecting taste. Based on the above, the 1.0% concentration of marjoram extract is considered the most appropriate strategic level to enhance nutritional efficiency during the critical (middle) growth stage, thereby raising the economic feasibility of the project without causing disturbances in the general feeding behaviour of the flock.

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Table 1. Weekly feed intake (g/bird) as affected by graded levels of marjoram extract.

Significance	Concentration of marjoram extract				Week
	2.5%	2.0%	1.5%	1.0%	
N.S.	0	0	0	0	Week 1
N.S.	182.60 ± 10.60	170.44 ± 10.51	170.44 ± 7.64	182.60 ± 3.50	Week 2
N.S.	290.44 ± 7.44	288.70 ± 9.02	288.70 ± 18.61	273.00 ± 6.29	Week 3
*	485.00 ±	475.00 ±	465.70 ±	610.70 ±	Week 4
N.S.	460.70 ± 45.00	467.22 ± 33.03	495.00 ± 70.00	420.70 ± 39.22	Week 5

Note: Values are mean ± standard error. Different superscript letters within the same week indicate significant differences ($P \leq 0.05$). N.S. = not significant.

Effect of marjoram (O. majorana) extract on weekly feed conversion efficiency

Table 2 shows the effect of the gradual addition levels of marjoram extract on the nutritional conversion efficiency (Feed Conversion Ratio - FCR) of broiler chickens during the different stages of growth.

The statistical results indicate stability in feed conversion values and the absence of significant differences ($P>0.05$) among all experimental treatments during the first, second, third, and fifth weeks of the experimental age.

The absence of a significant effect in the first three weeks is attributed to the state of incomplete functional maturity of the digestive tract and enzymatic system of the birds at this early stage, which makes their response to phytogenic feed additives (Phytogenic Feed Additives) limited, due to the similarity of the basic nutritional requirements for tissue growth in the starter phase. The continuation of this statistical pattern through the third week also indicates that the active compounds in marjoram require a "cumulative period" or a certain physiological maturity of the digestive system to activate their stimulatory mechanisms in a tangible manner, improving nutrient metabolism efficiency.

In contrast, the statistical analysis revealed clear significant variation ($P \leq 0.05$) during the fourth week (the critical growth stage), where the birds whose diets were supplemented with marjoram extract at a concentration of 1.0% recorded the best (lowest) feed conversion ratio of (1.54 ± 0.02), thereby outperforming the other treatments, particularly the high-concentration treatment of 2.5%, which recorded the lowest conversion efficiency (1.59 ± 0.02).

The qualitative superiority of 1.0% concentration can be explained by the catalytic role of hydrocarbons and aromatic oils (such as Terpinenes and Phenols) present in marjoram, which promote the secretion of

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endogenous enzymes and improve the motility of intestinal villi, increasing nutrient absorption efficiency and reducing energy loss. As for the decline in conversion efficiency when the concentration is raised to 2.5%, it is physiologically attributed to the reverse effect of the increase in pungent phenolic compounds, which may cause a disturbance in the intestinal microflora balance (Intestinal Microflora) or adversely affect the palatability of the feed, leading to nutritional waste that increases the value of the FCR.

When these results are linked to feed consumption data (Table 1), we find that the concentration of 1.0% achieved the "Optimal Threshold", where the highest feed consumption was recorded in conjunction with the best conversion efficiency, reflecting the ability of this concentration to convert food into biomass with high efficiency, which is a fundamental economic and productive indicator in the poultry industry.

These findings are consistent with those of Chen *et al.,* (2023) and Alem (2024), who confirmed that herbal additives in moderate doses act as natural growth promoters by improving intestinal microbial communities and stimulating digestive enzymes. This study also reinforces the argument of Biswas *et al.* (2024) and Madesh *et al.* (2025) that the effectiveness of plant additives is governed by the "dose-response relationship", as unstudied high concentrations may lead to negative results that hinder the maximum utilization of nutrients

From this, it is concluded that the integration of marjoram extract at a concentration of 1.0% represents a nutritional strategy to improve the performance of broilers, especially at the peak of the growth stage, ensuring better productivity and economic returns without compromising the physiological stability of birds.

Table 2. Effect of different levels of marjoram extract on weekly feed conversion ratio of broiler chickens (mean ± standard error).

Significance level	Treatment T4 (2.5%)	Treatment T3 (2.0%)	Treatment T2 (1.5%)	Treatment T1 (1.0%)	Week
-	0	0	0	0	First
N.S	1.69 ± 0.11	1.68 ± 0.90	1.69 ± 0.10	1.50 ± 0.02	Second
N.S	1.33 ± 0.01	1.30 ± 0.08	1.24 ± 0.10	1.34 ± 0.06	Third
*	1.59 ± 0.02 a	1.62 ± 0.04 a	1.64 ± 0.17 a	1.54 ± 0.02 b	Fourth
N.S	1.86 ± 0.29	1.86 ± 0.07	1.67 ± 0.33	1.97 ± 0.31	Fifth

*Means with different letters within the same row differ significantly at ($P \leq 0.05$).
N.S: no significant differences.

Effect of marjoram (O. majorana) extract on blood characteristics of broiler chickens

Table (3) shows the physiological responses to the blood variables in the affected broiler

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chickens by adding graded concentrations of marjoram extract. Statistical results indicate the stability of haemoglobin concentration (Hb), white blood cell count (WBCs) , and packed cell volume percentage (PCV) , as no significant differences (P>0.05) were ~~recorded~~ observed between the experimental treatments and the control group.

The stability of haemoglobin concentration and PCV within normal physiological limits reflects the biosafety of the plant extract used, and confirms the absence of haemolytic or toxic effects that may impede the ability of blood to transport oxygen to tissues. The absence of a significant change in white blood cell (WBC) count is also considered a positive indicator that the herbal additions did not stimulate a hostile immune response or inflammatory conditions, thereby negating the presence of physiological or immune stress resulting from the interference of active substances with the birds' defence system.

On the other hand, the statistical analysis revealed significant variation ($P \leq 0.05$) in red blood cell count (RBCs); treatment T4 (2.5% concentration) showed significant superiority by recording the highest value of (33.01 ± 2.00 million/mm³), whereas treatment T1 (1.0% concentration) recorded the lowest statistical value among the treatments.

This significant improvement in red blood cell counts at high concentrations is physiologically attributed to the stimulatory role of phenolic compounds and flavonoids abundantly present in marjoram; these compounds act as potent antioxidants protecting the red cell membrane from oxidative hemolysis by neutralizing free radicals, which enhances the cell's lifespan and survival in the circulatory system. This mechanism is consistent with what Zhang et al. (2022) reported regarding the role of flavonoids in protecting cell membrane integrity. The extract may also stimulate haematopoiesis by improving the bone marrow environment and providing the protective medium required for cell division (Paul-Chima and Ugo, 2025). These findings are consistent with the conclusions of Papatsiros *et al.* (2024) and da Silva *et al.* (2022), who confirmed that phytogenic additives can improve blood parameters without causing functional disorders. These readings also support the views of Kalogerakou and Antoniadou, (2024) and Sunet *et al.* (2023) that the physiological response depends directly on the level of addition, as higher concentrations achieve tangible preventive health support.

When these results are linked to productivity parameters (Tables 1 and 2), a fundamental conclusion emerges. Although the high concentration (2.5%) was the most effective in supporting the health condition (number of RBCs), the low concentration (1.0%) achieved the highest production efficiency (best FCR and the highest feed consumption). This variation indicates that productive performance depends mainly on the efficiency of digestive enzymes and nutrient absorption, while high concentrations are related to the general "protective status ". Based on the above, it can be concluded that marjoram extract is a safe physiological addition that enhances the efficiency of the circulatory system. Strategically, a concentration of 1.0% remains the optimal level for balancing economic efficiency and productivity on the one hand, and the birds' stable physiological state on the other.

Table 3. Effect of graded levels of marjoram extract addition on some blood parameters of broiler chickens (mean ± standard error).

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Significance level	Treatment T4 (2.5%)	Treatment T3 (2.0%)	Treatment T2 (1.5%)	Treatment T1 (1.0%)	Parameters
N.S	12.91 ± 0.20	12.50 ± 0.45	12.48 ± 0.60	11.44 ± 0.61	Hemoglobin (Hb) g/dL
*	33.01 ± 2.00^{a}	31.57 ± 1.63^{bc}	32.01 ± 3.00^{ab}	30.23 ± 2.22^{c}	Red blood cells (RBCs) 10^{6} /mm³
N.S	8.52 ± 0.22	8.50 ± 0.33	8.21 ± 0.66	8.16 ± 0.22	White blood cells (WBCs) 10^{3}/mm³
N.S	8.01 ± 0.31	7.88 ± 0.44	6.97 ± 0.12	7.40 ± 0.33	Packed cell volume (PCV) %

Means with different letters within the same row differ significantly at ($P \leq 0.05$). N.S: no significant differences.

CONCLUSIONS

The results of this study conclude with a set of scientific indicators confirming that the use of marjoram extract represents a high-physiological-and-biological-safety feed additive. The responses of the blood parameters (Hb, PCV, and WBCs) indicated the absence of any negative effects or immunological or toxic stresses throughout the duration of the experiment, which enhances the possibility of adopting it as a natural and sustainable alternative to traditional chemical additives.

With regard to the efficiency of productive performance, the research proved that the concentration of 1.0% is the optimal strategic level to balance between feed consumption rates and food conversion ratio (FCR), especially during the critical growth stages in the fourth week, which is reflected directly and positively on the indicators of the final weights of birds. Moreover, the protective role of the extract became evident in high concentrations, specifically at the level of 2.5%, which brought about a significant improvement in the numbers of red blood cells (RBCs), indicating the biological effectiveness of phenolic compounds and flavonoids in protecting the cell membrane from oxidative hemolysis and enhancing the efficiency of the circulatory system.

On the other hand, the results revealed that the effectiveness of this addition is governed by the phenomenon of the "dose effect", as the very high levels led to a relative decrease in the amount of feed consumed as a result of the sensory aversion to the pungent odors of volatile oils, which requires adherence to moderate concentrations to ensure the continuity of the nutritional demand of the flock. In conclusion, the birds demonstrated a clear potential for physiological adaptation to herbal additives

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in ,relation to ageas evidenced by the stability of productive and blood parameters at the final stage of the experiment, which may reflect a balanced physiological response to the bioactive compounds present .in marjoram

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Ethics statement:

The study involved broiler chickens, and all experimental procedures were conducted in accordance with institutional guidelines for the care and use of animals, and were approved by the relevant ethics committee at the College of Agriculture, University of Karbala.

Originality and plagiarism:

The authors confirm that this manuscript is original, has not been published previously, and is not under consideration elsewhere. The manuscript contains no plagiarized material, and all sources are appropriately cited.

Consent for publication:

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Author contributions: Hind F. Al-Shammari: Conceptualization, methodology, investigation, data curation, formal analysis, writing-original draft, and writing-review and editing.

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