



## Effect of Dietary Supplementation of *Nigella sativa* and *Thymus vulgaris* on Growth Performance, Hematological, and Biochemical Parameters of Broiler Chickens

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تأثير إضافة حبة البركة والزعتر إلى النظام الغذائي على أداء النمو والمعايير الدموية والكيميائية الحيوية لدجاج التسمين

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### Abstract:

This investigation was conducted during the 2024 season in Bani Walid, Libya, to evaluate the influence of *Nigella sativa* (black seed) and *Thymus vulgaris* (thyme) as natural feed additives on the growth performance, hematological profiles, and biochemical parameters of broiler chickens. A total of 240 one-day-old Ross 308 broiler chicks were randomly distributed into five experimental groups, each consisting of four replicates with 12 birds per replicate. The dietary treatments included a basal control diet and four supplementation levels: *Nigella sativa* at 1.5%, 3%, and 4.5%, or thyme at 1%, 2%, and 3%. The study spanned a 42-day production cycle, during which performance metrics and blood samples were meticulously analyzed. Results revealed that birds supplemented with 3% *Nigella sativa* and 2% thyme exhibited significantly superior weight gain and improved body weight compared to other groups. Interestingly, the control group recorded the highest feed intake and the poorest feed conversion ratio (FCR), suggesting that the herbal additives enhanced nutrient utilization efficiency. Hematological analysis indicated that 4.5% *Nigella sativa* significantly elevated hemoglobin levels, packed cell volume (PCV), and red and white blood cell counts, reflecting an enhanced immune response and oxygen-carrying capacity. Furthermore, biochemical analysis demonstrated a significant reduction in serum total cholesterol, triglycerides, and glucose levels in all treated groups compared to the control. In conclusion, incorporating moderate levels of *Nigella sativa* and thyme into broiler diets serves as an effective strategy to boost productivity, optimize lipid profiles, and maintain overall physiological health in poultry production.

**Keywords:** Broiler chickens, *Nigella sativa*, thyme, Growth performance, Hematological and biochemical parameters.

## الملخص:

أجريت هذه الدراسة خلال موسم 2024 في مدينة بني وليد بليبيا، لتقييم تأثير استخدام حبة البركة (*Nigella sativa*) والزعتر (*Thymus vulgaris*) كمضافات علفية طبيعية على أداء النمو، وصورة الدم، والمعايير الكيموحيوية لدجاج التسمين. تم توزيع ما مجموعه 240 كتاكيت تسمين من سلالة (Ross 308) عمر يوم واحد عشوائياً على خمس مجموعات تجريبية، تتكون كل مجموعة من أربع مكررات بواقع 12 طائراً لكل مكرر. تضمنت المعاملات الغذائية عليقة مقارنة (كنترول) وأربعة مستويات من الإضافات: حبة البركة بنسب 1.5%، 3%، و4.5%، أو الزعتر بنسب 1%، 2%، و3%. استمرت الدراسة لمدة 42 يوماً، تم خلالها تحليل مقاييس الأداء وجمع عينات الدم بدقة. أظهرت النتائج أن الطيور التي تغذت على 3% من حبة البركة و2% من الزعتر حققت زيادة معنوية متفوقة في وزن الجسم مقارنة بالمجموعات الأخرى. ومن المثير للاهتمام أن مجموعة المقارنة سجلت أعلى معدل لاستهلاك العلف وأسوأ معامل تحويل غذائي (FCR)، مما يشير إلى أن المضافات العشبية حسنت من كفاءة الاستفادة من العناصر الغذائية. وأشار التحليل الدموي إلى أن إضافة 4.5% من حبة البركة رفعت بشكل معنوي مستويات الهيموجلوبين، وحجم الخلايا المكسدة (PCV)، وعدد خلايا الدم الحمراء والبيضاء، مما يعكس تحسناً في الاستجابة المناعية وقدرة حمل الأكسجين. علاوة على ذلك، أظهر التحليل الكيموحيوي انخفاضاً معنوياً في مستويات الكوليسترول الكلي، والدهون الثلاثية، والجلوكوز في مصل الدم لجميع المجموعات المعاملة مقارنة بمجموعة المقارنة. تخلص الدراسة إلى أن دمج مستويات معتدلة من حبة البركة والزعتر في علائق الدواجن يعد استراتيجية فعالة لتعزيز الإنتاجية، وتحسين مستويات الدهون، والحفاظ على الحالة الفسيولوجية العامة في إنتاج الدواجن.

**الكلمات المفتاحية:** دجاج التسمين، حبة البركة، الزعتر، أداء النمو، المعايير الدموية والكيموحيوية.

## Introduction

With the global population projected to exceed 9.7 billion by 2050, a significant surge in the demand for meat, milk, and eggs is anticipated. Consequently, animal farming systems are under substantial pressure to enhance efficiency and sustainability while ensuring product safety (Mills & Driscoll, 2022). To address these challenges, farmers have traditionally utilized antibiotic growth promoters (AGPs) in animal feed to accelerate growth rates, improve feed efficiency, and maintain livestock health (Bilen et al., 2020, Taştan, & Salem, 2021, Wythe et al., 2023).

Poultry farming is a rapidly expanding industry that provides high-quality protein at affordable prices. Genetic advancements and optimized dietary formulations have led to significant improvements in broiler performance. In poultry nutrition, feed additives such as antibiotics, prebiotics, and probiotics are extensively used as growth promoters to enhance nutrient utilization (Mohamed et al., 2024). Traditionally, antibiotics have been the most widely used promoters. Chickens represent a vital animal protein source for humans, with feed accounting for approximately 60–70% of total production costs (Lakwani, & Salem, 2024). Recently, natural feed additives have gained prominence as alternatives to synthetic boosters and antibiotics, enhancing immunity and productivity by modulating gut microbiota (Abdel Baset et al., 2022; Elsherbeni et al., 2024). Due to their non-toxicity and accessibility, medicinal herbs have demonstrated significant potential as antibiotic substitutes in the poultry market (Arif et al., 2019; Vase-Khavari et al., 2019).

The rapid growth of the global population has increased the demand for poultry products, necessitating that the industry meets this demand while ensuring food safety and quality. Dietary costs significantly impact overall production, constituting roughly 70–75% of variable expenses. Thus, improving diet quality and consumption is crucial for various poultry species (Saleh et al., 2023). Phytogetic feed additives play a vital role in contemporary agriculture, particularly in influencing growth and immune responses (Salem, & Lakwani, 2024, Ntsongota et al., 2025). Natural herbal products offer a safe alternative to antibiotics and can improve

growth performance (Asghar et al., 2023; Yousaf et al., 2024). Plant-derived by-products are viewed as economical alternatives in animal rations, reported to improve performance and carcass characteristics (Abdallah et al., 2012). Among these is black cumin seed meal (BCSM), the primary by-product of *Nigella sativa* L., accounting for 70–75% of the fruit's weight (Tekeli, 2014). The processing of black cumin generates substantial BCSM waste, which can be utilized as supplemental feed for broilers (El Kashef, 2020; Zaazaa et al., 2023).

Medicinal plants are regarded as safe and effective alternatives in animal production systems due to their beneficial impacts on physiological responses, offering a broad spectrum of actions for the immune, endocrine, and digestive systems (El Kashef, 2020). Black cumin (*Nigella sativa*) shows therapeutic promise in managing diabetes and cancer, possesses diuretic properties, prevents hypertension, and provides analgesic, antimicrobial, and anti-inflammatory benefits (Yıldırım & Çınar, 2017). In Islamic literature (Hadith), Prophet Mohammed (Peace Be Upon Him) described its healing power, stating: “Continue to use this black seed, for it holds a cure for every disease except death” (Bukhari, 2018). *Nigella sativa* has been employed for various health issues for approximately two millennia (Darakhshan et al., 2015). Its seeds contain bioactive constituents, primarily in the essential oil, such as thymoquinone (27.8–57.0%), p-cymene, and carvacrol (Adam et al., 2016). Furthermore, its oil is rich in unsaturated fatty acids, improving the fatty acid profile of breast meat (Demirci et al., 2019). It also enhances protein content (Kumar et al., 2017), sensory traits (Fath et al., 2023), and lowers lipid oxidation (Fath et al., 2023).

Thyme (*Thymus vulgaris*), a fragrant herb of the Lamiaceae family, possesses notable medicinal and culinary applications (Majedi et al., 2024). Its antioxidant and antibacterial strengths are well-documented, largely attributed to thymol and carvacrol (Eldeeb et al., 2024). Incorporating thyme yields antimicrobial effects without cytotoxicity (Kishawy et al., 2019; Vlaicu et al., 2023). Thyme in broiler diets has been shown to enhance weight gain, feed intake (FI), and feed conversion ratio (FCR), while lowering mortality (Zaazaa et al., 2022; Cufadar et al., 2024). It also stimulates digestive enzymes and fortifies immune status (Naderiboroojerdi et al., 2022). While some studies reported improved performance indices (Adam et al., 2020), others indicated no significant effect (Popović et al., 2016). Therefore, the present study aims to assess the impact of *Nigella sativa* and Thyme on the growth performance and hematological parameters of broiler chickens.

## Materials and Methods

This investigation was conducted during the 2024 season on a private farm in Bani Walid, Libya. A total of 240 one-day-old broiler chicks (Ross 308) were sourced from a local hatchery. On arrival, birds were weighed and randomly assigned to one of five treatments, with four replicates of 12 birds each, following a completely randomized design (CRD). The dietary treatments included: a basal control diet, and the basal diet supplemented with 1.5%, 3%, and 4.5% black seed, or 1%, 2%, and 3% thyme.

### Data Recorded:

- **Growth Performance:** Body weight gain and feed intake were measured at 15, 30, and 42 days of age. The feed conversion ratio ( $FCR = \text{feed intake} / \text{weight gain}$ ) was then computed.
- **Blood Samples Collection:** At 42 days, six chicks per group were randomly selected between 08:00 and 09:00 AM. Approximately 3 ml of blood was drawn from the wing vein into vacuum tubes containing K3-EDTA (1 mg/ml). One portion was used for immediate hematological assessment, while the second was centrifuged at 4000 rpm for 15 minutes to separate plasma, which was stored at -20°C for biochemical analysis.
- **Hematological Parameters:** RBC count was determined following Feldman et al. (2000). Hemoglobin (Hb) and packed cell volume (PCV%) were assessed according to

Drew et al. (2004). Wintrobe indices (MCV, MCH, and MCHC) were calculated using standard formulas. Blood smears were prepared and stained with Giemsa stain.

- **Blood Biochemical Parameters:** Plasma total protein (g/dl) was measured at 35 days using a spectrophotometer (Beckman DU-530) following Armstrong and Carr (1964). Total cholesterol (mg/dl) was measured according to Bogin and Keller (1987), and alkaline phosphatase (ALP, U/L) was determined via the colorimetric method of Bauer (1982).
- **Statistical Analysis:** Data were subjected to analysis of variance (ANOVA) for a completely randomized design using the General Linear Model (GLM) procedures of SAS (1997).

## Results and Discussion

**A) Growth Performance** Table (1) and Figure (1) illustrate the effects of varying dietary levels of *Nigella sativa* (1.5%, 3%, and 4.5%) and thyme (1%, 2%, and 3%) on the growth performance of broiler chickens. The results indicate that supplementing the diet with 3% *Nigella sativa* produced the highest weight gain at 15, 30, and 42 days (50.72, 114.43, and 55.94 g, respectively), followed by the 4.5% inclusion level. Interestingly, the control group exhibited the highest feed intake (FI) throughout the experimental periods, followed by the 1.5% *Nigella sativa* group. Consequently, the control group recorded the highest (least efficient) feed conversion ratio (FCR) at 15, 30, and 42 days (1.64, 2.07, and 1.98, respectively). Regarding thyme supplementation, the 2% level yielded the most significant weight gain at all intervals, followed by the 3% level.

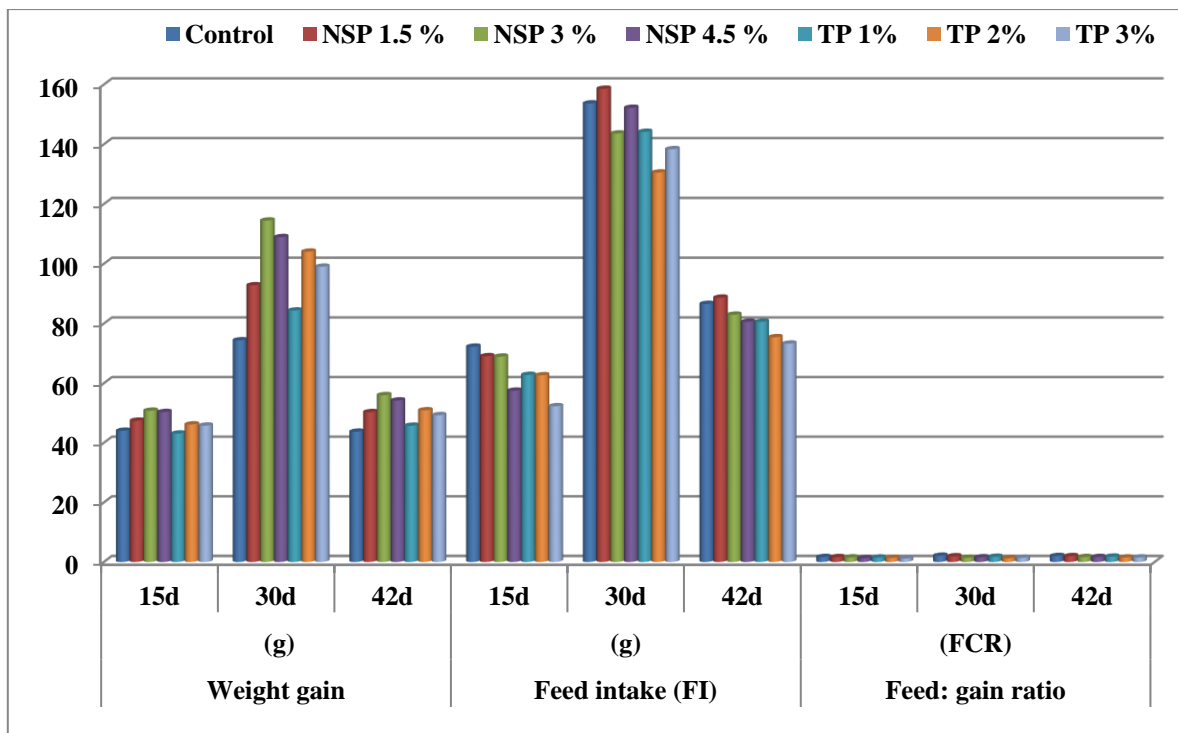
Phytogenic feed additives have gained substantial interest as potential growth enhancers in livestock production (Gálik & Rolinec, 2011). The observed improvements in poultry performance due to dietary thyme are consistent with Youssef et al. (2024). These enhancements are partially attributed to the bioactive compounds in thyme, which stimulate the activity of digestive enzymes such as protease, amylase, and lipase, leading to more efficient nutrient utilization (Abdel-Wareth et al., 2012). Furthermore, Hristakieva et al. (2023) reported that a 2% inclusion of thyme powder significantly optimized FCR. The primary active constituents, carvacrol and thymol, not only provide a palatable stimulus that encourages feed intake but also act as potent antimicrobial agents. Thymol, in particular, has been shown to reduce coliform bacteria populations in the intestinal tract, thereby improving gut health and nutrient absorption (Cross et al., 2004).

Investigations into *Nigella sativa* (NS) have also shown favorable influences on broiler body weight and feed intake (Erener et al., 2010; Toghyani et al., 2010). Talebi et al. (2021) observed that 1% NS inclusion boosted performance, although some findings vary; for instance, Durrani et al. (2007) noted higher gains at 4%, while Shewita and Taha (2011) found 2% to be more effective than 10%. Conversely, extremely high levels, such as 17%, have been reported to impair performance (El-Nattat & El Kady, 2007). In our study, the improved weight gain in treated groups despite lower feed intake compared to the control suggests a superior nutrient metabolism. This aligns with the findings of Khan et al. (2012), where 2.5% and 5.0% NS inclusion significantly increased body weight gain compared to lower doses.

*Nigella sativa* serves as a digestive stimulant, improving diet palatability and appetite, which often leads to better growth metrics (Gilani et al., 2004). While some researchers found no significant effect on FCR at low doses (Majeed et al., 2010; Saeid et al., 2013), our results support the consensus that moderate levels of these medicinal plants (3% NS and 2% Thyme) serve as effective natural alternatives to synthetic growth promoters, enhancing both growth efficiency and physiological health (Al-Beitawi & El-Ghousein, 2008).

**Table (1):** Impact of varying levels of *Nigella sativa* powder (NSP) and thyme powder (TP) on weight gain, feed consumption, and feed: gain ratio in broiler growth performance during the 2024 season.

Treatments	Weight gain (g)			Feed intake (FI) (g)			Feed: gain ratio (FCR)		
	15d	30d	42d	15d	30d	42d	15d	30d	42d
Control	43.99	74.34	43.68	72.19	153.62	86.53	1.64	2.07	1.98
NSP 1.5 %	47.37	92.76	50.25	69.01	158.53	88.62	1.61	1.88	1.94
NSP 3 %	50.72	114.43	55.94	68.86	143.56	82.92	1.50	1.38	1.63
NSP 4.5 %	50.28	108.89	54.15	57.44	152.15	80.55	1.25	1.54	1.64
TP 1%	43.06	84.33	45.68	62.74	144.12	80.56	1.46	1.71	1.76
TP 2%	46.11	104.03	50.85	62.60	130.51	75.38	1.36	1.25	1.48
TP 3%	45.71	98.99	49.23	52.22	138.32	73.23	1.14	1.40	1.49



**Fig. (1):** Impact of varying levels of *Nigella sativa* powder (NSP) and thyme powder (TP) on weight gain, feed consumption, and feed: gain ratio in broiler growth performance during the 2024 season.

## B) Blood Constituents (Hematological and Biochemical Profiles)

**1. Hematological Parameters** The results regarding the supplementary effects of *Nigella sativa* (NS) and thyme powder on hematological values are presented in Table (2) and Figure (2). Findings indicate that increasing *Nigella sativa* inclusion to 4.5% yielded the highest values for Hemoglobin (Hb: 9.85 g/dL), Packed Cell Volume (PCV: 43.49%), Mean Corpuscular Volume (MCV: 231.29  $\mu\text{m}^3$ ), Mean Corpuscular Hemoglobin (MCH: 74.67 pg), Mean Corpuscular Hemoglobin Concentration (MCHC: 43.34%), Red Blood Cell count (RBC: 1.67

mil/mm<sup>3</sup>), and White Blood Cell count (WBC: 86.16 thous/mm<sup>3</sup>). This was closely followed by the 3% thyme powder group. In contrast, the control group recorded the lowest values across all hematological indices (Hb: 8.14 g/dL, PCV: 23.10%, RBC: 1.35 mil/mm<sup>3</sup>, and WBC: 52.97 thous/mm<sup>3</sup>).

The hematological data suggest that black seed exerts a favorable effect on hematopoiesis. The elevation in RBC count and Hb concentration aligns with Justine and Oluwatosin (2008), who reported significant increases in PCV, Hb, and RBC counts in rats treated with black seed oil. Similarly, Meral et al. (2004) observed that NS treatment improved hematological profiles in diabetic rabbits. The beneficial impact of *Nigella sativa* can be ascribed to its highly active constituents, notably thymoquinone and thymohydroquinone, which possess potent antioxidant properties (Arslan et al., 2005). These compounds likely enhance RBC stability by lowering lipid peroxide levels in the erythrocyte membrane, thereby reducing susceptibility to hemolysis.

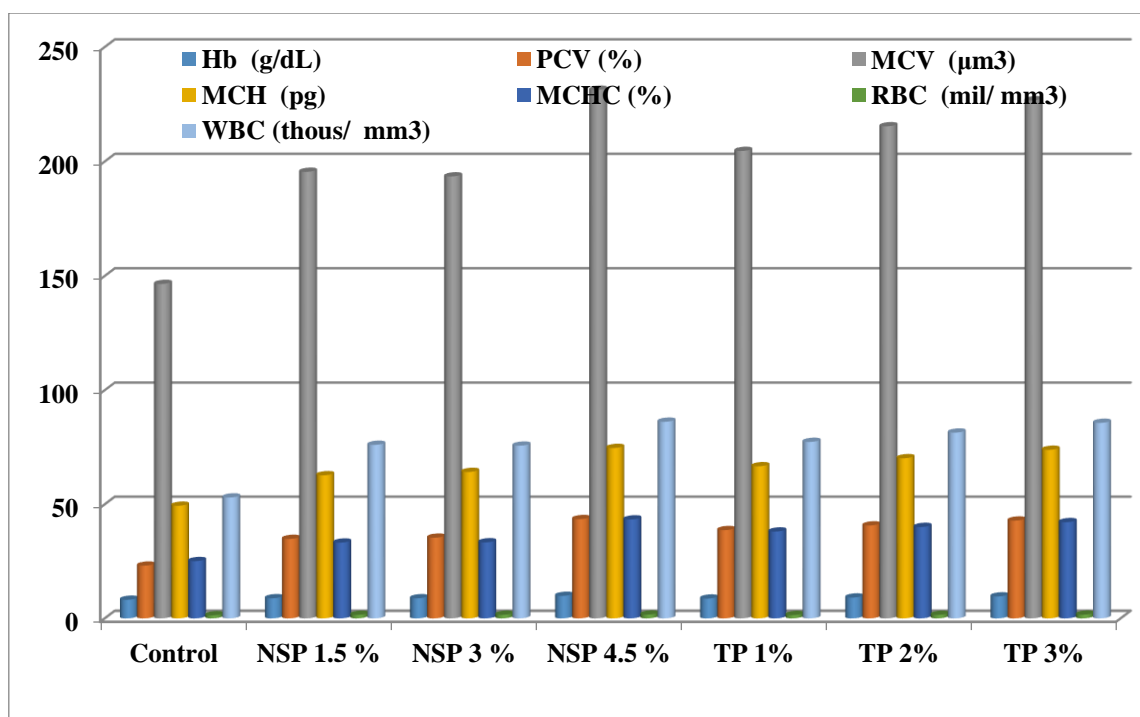
Blood parameters serve as reliable indicators of the physiological, pathological, and nutritional status of poultry (Adekunle & Omoh, 2014). The improvements observed in the thyme-supplemented groups are consistent with Al-Kassie (2009), who found that oil extracted from thyme significantly improved RBC and WBC counts in broilers. However, Toghyani et al. (2010) reported that thyme powder did not exert any significant influence on these parameters, suggesting that the form of the additive (powder vs. oil) or the dosage may influence the outcome.

**2. Biochemical Parameters** The study further confirms the hypocholesterolemia and antilipidemic effects of thyme and black seed. Thyme's active compounds are thought to inhibit  $\beta$ -hydroxy- $\beta$ -methylglutaryl-CoA reductase, the rate-limiting enzyme in cholesterol synthesis (El-Ghousein & Al-Beitawi, 2009). Our findings align with Rostami et al. (2012) and Khaksar et al. (2012), who observed significant reductions in serum triglycerides, glucose, and cholesterol in birds fed thyme-enriched diets.

While some studies, such as Sengül et al. (2008), found no significant effect of thyme on lipid profiles, our data support the role of these phytogetic additives in optimizing blood biochemistry. This reduction in lipids, coupled with increased HDL-C levels as noted by Raya et al. (2014), underscores the potential of *Nigella sativa* and thyme to improve the overall health and meat quality of broiler chickens.

**Table (3):** Impact of varying doses of *Nigella sativa* powder (NSP) and thyme powder (TP) on blood hematological parameters across the 2024 season.

Treatments	Hb (g/dL)	PCV (%)	MCV ( $\mu\text{m}^3$ )	MCH (pg)	MCHC (%)	RBC (mil/mm <sup>3</sup> )	WBC (thous/mm <sup>3</sup> )
Control	8.14	23.10	146.48	49.35	25.08	1.35	52.97
NSP 1.5 %	8.79	34.81	195.46	62.70	33.20	1.55	76.04
NSP 3 %	8.78	35.34	193.45	64.16	33.31	1.57	75.68
NSP 4.5 %	9.85	43.49	231.29	74.67	43.34	1.67	86.16
TP 1%	8.66	38.66	204.58	66.66	38.05	1.46	77.33
TP 2%	9.12	40.70	215.35	70.17	40.05	1.54	81.40
TP 3%	9.60	42.84	226.68	73.86	42.16	1.62	85.68



**Fig. (3):** Impact of varying doses of *Nigella sativa* powder (NSP) and thyme powder (TP) on blood hematological parameters across the 2024 season.

### Biochemical Parameters (Continued)

Regarding the protein profile, Ghammry et al. (2002) found that Hubbard broiler chicks fed 4 g/kg black seeds-maintained plasma total protein, albumin, and globulin levels comparable to the control group. In contrast, Al-Beitawi and El-Ghousein (2008) observed that total plasma protein values rose significantly in broilers consuming diets containing 2.0% *Nigella sativa* (NS) seeds. The rise in total serum protein and albumin observed in the present study aligns with Nasser et al. (1998), who reported significant increases when broiler chicks were fed rations containing 2% and 3% NS, especially in hot climates. Since serum protein levels often reflect dietary protein availability and metabolic efficiency, these results suggest that the proteins in NS-supplemented diets were more accessible and better utilized by the birds (Khan et al., 2012; Tóthová et al., 2019).

Furthermore, the activities of liver enzymes such as SGPT (ALT) and SGOT (AST) are critical markers of physiological health. Any abnormal elevation in these enzymes typically signals hepatocellular degeneration or liver damage. Therefore, the observed reduction in SGPT concentration in this study provides evidence for the hepatoprotective effects of black seed. Most of these pharmacological properties are attributed to the antioxidant activity of its bioactive compounds, primarily by scavenging free radicals and blocking lipid peroxidation (Badari et al., 2003).

The impact of NS on glucose levels in this study showed a marked influence, which contrasts with the non-significant effects reported by Toghyani et al. (2010), but conflicts with the hypoglycemic effects documented by El-Dakhakhny et al. (2002) and Meral et al. (2004). Such inconsistencies in literature regarding glucose and body weight gain (Majeed et al., 2010) may stem from variations in supplementation levels, preparation methods, environmental conditions, or the chemical composition of the seeds based on their cultivation region.

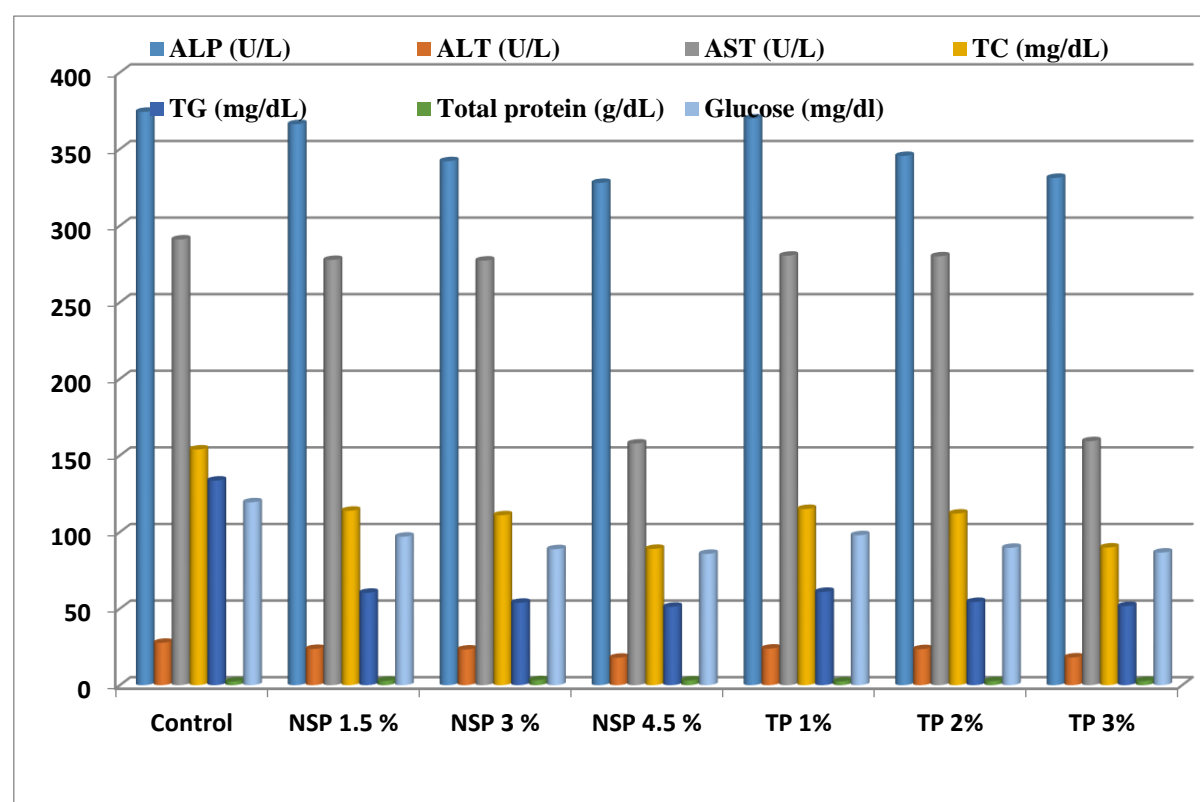
In terms of lipid metabolism, incorporating NS into the diet significantly reduced serum cholesterol compared to the control. These findings align with Tollba and Hassan (2003) and Al-Beitawi et al. (2009). This cholesterol-lowering effect may be due to the high content of unsaturated fatty acids in black cumin, which promotes cholesterol oxidation and its excretion

into the intestine (Khodary et al., 1996). Similarly, thyme exerts hypocholesterolemic effects by inhibiting  $\beta$ -hydroxy- $\beta$ -methylglutaryl-CoA reductase, the rate-limiting enzyme in cholesterol synthesis (El-Ghousein & Al-Beitawi, 2009).

In conclusion, providing moderate levels of *Nigella sativa* and thyme enhances performance traits and physiological status. However, additional studies are warranted to further elucidate the precise molecular mechanisms of their active constituents.

**Table (4):** Impact of supplying varying levels of *Nigella sativa* powder (NSP) and thyme powder (TP) on the biochemical blood parameters observed in the 2024 season.

Treatments	ALP (U/L)	ALT (U/L)	AST (U/L)	TC (mg/dL)	TG (mg/dL)	Total protein (g/dL)	Glucose (mg/dl)
Control	374.24	27.63	290.90	153.82	133.52	2.40	119.34
NSP 1.5 %	366.18	23.61	277.54	113.83	60.28	2.79	96.90
NSP 3 %	342.01	23.24	277.13	110.87	53.75	3.24	88.74
NSP 4.5 %	327.83	17.88	157.69	88.94	51.10	3.06	85.68
TP 1%	369.84	23.85	280.32	114.97	60.88	2.43	97.87
TP 2%	345.43	23.47	279.90	111.98	54.29	2.60	89.63
TP 3%	331.11	18.06	159.27	89.83	51.61	2.70	86.54



**Fig. (4):** Effect of feeding different levels of *Nigella sativa* powder (NSP) and thyme powder (TP) on biochemical constituents of blood values during 2024 season.



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