

Article

Effect of *Nigella sativa* Seed Supplementation on Hematology, Acid-base Parameters, and Serum Biochemical Parameters in Nubian Goat Fed an Aflatoxin Contaminated Diet

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Abstract: This study aimed to investigate the effect of *Nigella sativa* (NS) seed supplementation on hematology, acid-base parameters, and serum biochemical parameters in Nubian goats fed an Aflatoxin-contaminated diet. In a completely randomized design, 20 growing male goats (aged 8-9 months; 11±0.5 kg) were allocated to five treatments (4 goats/treatment). The control group (G1) received a basal diet. The treatment groups received the same diet contaminated with 150 ppb Aflatoxin (G2), and other treatments received an Aflatoxin-contaminated diet supplemented with different levels of crushed NS seeds 2% (G3), 4% (G4), and 6% (G5). Blood samples were collected after 40 day feeding period to determine blood pH, glucose, hematological and biochemical parameters. Statistical analysis was performed to assess the significant differences among the treatments. Hemoglobin concentration (Hb), total erythrocytes count (TEC), mean corpuscular hemoglobin (MCH), serum total protein (TP), and globulins (GB) were significantly ($P \leq 0.05$) decreased by Aflatoxin-contaminated diet, whereas total leukocytes count (TLC) increased ($P \leq 0.05$). Supplementing NS seeds to an Aflatoxin-contaminated diet significantly ($P \leq 0.05$) increased Hb, TEC, TP, and GB. Lipid profile and serum liver enzymes were significantly ($P \leq 0.05$) increased by an Aflatoxin-contaminated diet. Supplementing NS seeds to an Aflatoxin-contaminated diet caused a decrease ($P \leq 0.05$) in lipid profile and serum liver enzymes. Supplementing NS seeds to an Aflatoxin-contaminated diet resulted in a good performance and improved physiological status, the superior effect to an Aflatoxin-contaminated diet supplemented with 6% NS seeds. The study recommended supplementing 6% NS seeds to goat diets to reduce suspected Aflatoxin contamination. Further investigations are needed to assess the protective effect of NS seeds in other animal species fed on Aflatoxin-contaminated diets.

Keywords: Aspergillus, Black seeds, Goat, Hemoglobin, Liver enzymes, Triglycerides.

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1. Introduction

Aflatoxin contamination is a global concern, affecting crops, livestock, and human health on a wide scale, especially in developing countries where inadequate storage and processing methods exacerbate the challenges of ensuring food safety and economic progress. Aflatoxin refers to a group of toxic and carcinogenic secondary metabolites produced by certain strains of *Aspergillus spp.* fungi that can grow on feeds and food products. Aflatoxins are common during the pre and post-harvest stages of feeds, causing adverse effects in different animals and negative economic impacts worldwide, especially in regions with hot and humid climates [1,2]. Many types of Aflatoxins commonly occur in animal feeds and have been considered powerful natural carcinogenic agents in mammals. The maximum limit of Aflatoxin in food and feeds for the consumption of humans and animals is set to 20 ppb by the U.S. Food and Drug Administration [3], and 15 ppb by the European Union [4]. Accurate values of the Aflatoxin concentration that causes Aflatoxicosis have not been confirmed; however, with the help of a few studies, it is estimated that generally, 50–300 ppb of Aflatoxin concentration in feeds can cause Aflatoxin toxicity in animals. Ruminants are more resistant to Aflatoxin than non-ruminant animals because the rumen microbiota can degrade or deactivate toxins [5,6].

Exposure of livestock to feed containing Aflatoxins leads to a broad spectrum of detrimental health impacts, causing notable changes in biochemical, hematological, and performance parameters. Changes in hematological and biochemical parameters occurred before clinical symptoms developed in chronic and subclinical Aflatoxicosis [7,8]. Significant changes in hematological and biochemical parameters have been observed in Aflatoxicosis cases, which can assist in the diagnosis of toxication [9,10].

Some strategies have been developed to detoxify Aflatoxin contaminated in animal feeds. Physical, chemical, and biological methods have been applied to the removal and biosynthesis of Aflatoxins or as an inhibitory growth factor of Aflatoxigenic molds. However, few of these strategies have practical applications. Therefore, several herbals were tested to reduce the production of Aflatoxin and the growth of molds; one of these herbal plants is *Nigella sativa* [11,12].

Nigella sativa (NS), also known as black cumin or black seeds, belongs to the Ranunculaceae herbaceous family. It is primarily found in Mediterranean countries. The seeds of NS have been historically utilized for both culinary and medicinal purposes. *Nigella sativa* contains beneficial plant secondary metabolites in the principal component, thymoquinone, which shows antioxidant activities, including other valuable attributes. Dietary supplementation of NS may have favorable effects on nutrient intake, nutrient digestibility, growth and milk performance, and reproductive performances, along with improving immunity status and gut health of small ruminants. In addition, crude oil extracts of NS showed anti-cancer, antioxidant, antimicrobial, antipyretic, analgesic, and anti-inflammatory properties [13,14]. It has been investigated that NS supplementation inhibited fungus *Aspergillus* growth and thus reduced Aflatoxins synthesis. However, few studies are available on the protective effect of NS seeds against Aflatoxin and its effects on animals' performance and physiological status. Dietary supplementation with NS increased goats' hemoglobin concentration and total erythrocyte count [15]. Supplementation of NS increased serum total protein, albumin, and globulin while decreasing triglycerides and cholesterol in lambs [16]. Furthermore, the inclusion of NS in rabbit diets resulted in an increase in plasma total protein, albumin, and globulin [17]. *Nigella sativa* alleviates Aflatoxin-induced liver damage by its anti-inflammatory, antioxidant, and antiapoptotic effects. Therefore, NS can be used as a feed additive to alleviate any potential Aflatoxin toxicity from contaminated diets [18].

Nigella sativa supplemented diet may have a protective property against the development of aflatoxicosis in farm animals. Therefore, this study aimed to determine the effect of supplementing NS seeds to a diet contaminated with Aflatoxin on hematology, acid-base parameters, and serum biochemical parameters of growing Nubian goats.

2. Materials and Methods

2.1. Ethical approval: The Ethical Committee of the Faculty of Animal Production, University of Khartoum, Sudan, approved the animal experiment in this study (ethical number: 1/2017/3).

2.2. Experimental animals and management: Twenty healthy growing Nubian goats, aged between 8-9 months and with an average body weight of 11 ± 0.5 kg, were distributed randomly to five treatments (four goat/treatment). The goats were purchased from a local livestock market and allowed one week of the feed adaptation period. The growing goats received prophylactic treatment, including 20mg/kg of Oxytetracycline administered intramuscularly for five days, 20mg/kg of ivermectin administered subcutaneously to address ectoparasites, and 10mg/kg of Albendazole administered orally to treat endoparasites. The growing goats were housed individually in well-ventilated open-sided experimental pens (1.5 m \times 1.5 m \times 2 m). The pens were treated with cypermethrin spray as an anti-parasitic agent and disinfected with 40% formalin. Manual feeding and drinking equipment were used.

2.3. Experimental diets: Groundnut cake infected with Aflatoxin was combined in different ratios with other feed items to create treatment diets with around 150 ppb of Aflatoxins. Aflatoxin concentration was determined using high-performance liquid chromatography (HPLC) according to the method described by Sobolev [19]. *Nigella sativa* seeds were ground into medium size particles. The chemical composition of NS seeds used was dry matter (DM) 96.3%, ether extract (EE) 30.7%, crude protein (CP) 28.2%, Ash 4.7%, crude fiber (CF) 20.6%, nitrogen free extract (NFE) 12.1% and metabolizable energy (ME) 15.6 MJ/kg. Five isocaloric and isonitrogenous diets were formulated according to the standard nutrient requirements of goats published by the National Research Council, NRC [20]. The ingredients and the chemical composition of the experimental diets are shown in Table 1. The feed ingredients were manually mixed until final homogeneity was achieved in the mash mixture to formulate the experimental diets:

G1: Control diet (a basal diet without Aflatoxin and *NS* seeds), **G2:** diet contaminated with 150 ppb Aflatoxin, **G3:** diet contaminated with 150 ppb Aflatoxin + 2% *NS* seeds, **G4:** diet contaminated with 150 ppb Aflatoxin + 4% *NS* seeds, **G5:** diet contaminated with 150 ppb Aflatoxin + 6% *NS* seeds.

The chemical composition of experimental diets was determined on a dry matter basis. The experimental diets were analyzed for dry matter (DM), ether extract (EE), crude protein (CP), Ash, and crude fiber (CF) by the procedure described by the "Association of Official Analytical Chemists" [21]. Nitrogen Free Extract (NFE) was calculated as $NFE\% = \{DM - (EE\% + CP\% + CF\% + Ash\%)\}$. Metabolizable energy (ME) was calculated according to MAFF [22]: ME for Ruminants (MJ/Kg) = 0.12 CP + 0.31 EE + 0.05 CF + 0.14 NFE. The growing goats were fed experimental diets for 40 days and allowed unlimited water access.

Table 1. Ingredients and chemical composition of the experimental diets

Ingredients %	Experimental diets groups				
	G1	G2	G3	G4	G5
Sorghum grain	43	43	42	43	42
Groundnut cake	10	10	10	9	8
Groundnut hull	25	25	27	27	27
Wheat bran	20	20	17	15	15
Limestone	1	1	1	1	1
NaCl (salt)	1	1	1	1	1
<i>Nigella sativa</i>	0	0	2	4	6
Aflatoxin (ppb)	0	150	150	150	150
Chemical composition, %DM					
Dry matter	94.17	94.16	94.31	94.8	94.8
Ether Extract	2.17	2.16	2.41	2.9	2.6
Crude protein	16.6	16.3	16.5	16.6	16.7
Crude fibre	9.6	9.3	9.7	9.4	9.8
Ash	12.6	12.2	12.3	12.7	12.6
Nitrogen free extract	53.2	54.2	53.4	53.2	53.1
ME (MJ/Kg)	10.5	10.1	10.7	10.8	10.7

G1: control diet. G2: diet with 150 ppb Aflatoxin. G3: diet with 150 ppb Aflatoxin + 2% *NS* seeds. G4: diet with 150 ppb Aflatoxin + 4% *NS* seeds. G5: diet with 150 ppb Aflatoxin + 6% *NS* seeds. ppb: part per billion. DM: dry matter. ME: Metabolizable energy.

2.4. Collection of blood samples: Blood samples were collected at the end of the feeding experimental period on day 40 at 9:00 am before feeding. The hair in a specific neck area was closely trimmed, and the region was disinfected with a 70% ethanol solution before performing a jugular vein puncture. Five milliliters of blood were drawn using disposable plastic syringes. Following the withdrawal, 1 milliliter of the blood sample was transferred into a capped test tube containing ethylene-diamine-tetra-acetate (EDTA) as an anticoagulant for hematological analysis. The remaining blood samples were left at room temperature for 1-2 hours and then subjected to centrifugation (Gallenkamp junior, Germany) at 3000 revolutions per minute (r.p.m.) for 15 minutes to separate the serum. The samples were pipetted into clean vials and promptly frozen at -20°C for subsequent serum analysis.

2.5. Hematological parameters: The hematological parameters (total erythrocyte count - TEC, total leukocyte count - TLC, mean corpuscular volume - MCV, mean corpuscular hemoglobin - MCH and mean corpuscular hemoglobin concentration - MCHC) were determined according to the methods described by Schalm's Veterinary Hematology [23]. Hemoglobin concentration was determined by the cyano-methemoglobin method using Drabkin's solution, as described by Van Kampen and Zijlstra [24]. Hematocrit (Hct) levels were determined using plain capillary tubes (Umedic, Germany), according to Weiss and Wardrop [25].

2.6. Blood and serum parameters: Blood glucose level was determined by the enzymatic method using a kit (Biosystem, Spain), according to Trinder [26]. Blood pH was promptly measured using a pH meter (HANNA instruments, Portugal) immediately after collection. Serum sodium [Na⁺] and serum potassium [K⁺] levels were determined using a flame photometer technique (PFP7 Jenway, E.U). Serum chloride [Cl⁻] concentration was determined using a commercial kit (Spinreact, Spain). Serum strong ion difference [SID₃] was calculated using the equation described by Constable et al. [27]: Serum [SID₃] (mmol/l) = ([Na⁺] + [K⁺] - [Cl⁻]).

Serum total protein was determined using a Biuret method according to Ohnishi and Barr [28] using kits (Spain react, Spain). Serum albumin was determined using the bromocresol green (BCG) method as described by Doumas et al. [29] using a commercial kit (Spain react, Spain). Serum globulins concentration was calculated by subtracting the serum albumin concentration from the serum total protein concentration. Serum urea concentration was determined by the colorimetric method [30]. Serum creatinine concentration was determined by a colorimetric method described by Henry [31]. Serum total lipids were measured using the method described by Frings and Dunn [32]. Serum triglycerides concentration was determined by the enzymatic method using a kit (Biosystem, Spain), according to Fossati and Prencipe [33]. Serum cholesterol concentration was determined using an enzymatic method with the assistance of a commercial kit (Biosystem, Spain), according to Svensson et al. [34]. The concentration of serum low-density lipoprotein (LDL) and high-density lipoprotein (HDL) were determined by a precipitating reagent using the method described by Friedman and Young [35] and Tietz et al. [36].

Serum Aspartate transaminase (AST) and Alanine aminotransferase (ALT) activity were measured using the UV enzymatic method with the aid of a commercial kit (Liner chemical, Spain) according to the method described by Reitman and Frankel [37]. Alkaline phosphatase (ALP) activity was determined spectrophotometrically according to the method described by Moss et al. [38]. Serum Gamma-glutamyl-transferase (GGT) activity was determined spectrophotometrically according to the method described by Szasz [39].

2.7. Statistical analysis: Statistical analysis was performed using SPSS computer program (version 20). Analysis of variance (ANOVA) was used to assess the difference between the treatments. The significance of differences was examined by Duncan's multiple-range tests [40]. The mean difference was considered significant at $P \leq 0.05$.

3. Results

3.1. Hematological parameters: Hematological parameters of growing Nubian goats fed an Aflatoxin-contaminated diet supplemented with *NS* seeds are presented in Table 2. Hemoglobin concentration (Hb) displayed a significant variation among the experimental groups. The group fed an Aflatoxin-contaminated diet (G2) exhibited the lowest Hb value, which was significantly lower ($P \leq 0.05$) compared to the other groups. Supplemented %6 *NS* seeds to an Aflatoxin-contaminated diet (G5) caused a significant increase in Hb ($P \leq 0.05$) compared to the other *NS* supplemented groups.

Total erythrocyte count was significantly ($P \leq 0.05$) decreased in the Aflatoxin-contaminated diet group (G2). Supplementing different levels of *NS* seeds to an Aflatoxin-contaminated diet showed a significant increase in TEC; the higher value was observed in the group fed an Aflatoxin-contaminated diet supplemented with 2% *NS* seeds (G3). Aflatoxin-contaminated diet increased the total leukocyte count. Significantly ($P \leq 0.05$), the highest value of TLC was observed in the group fed an Aflatoxin-contaminated diet (G2), followed by the group fed an Aflatoxin-contaminated diet supplemented with 2% *NS* seeds (G3). On the other hand, the control group and the groups supplemented with 4% (G4) and 6% (G5) *NS* seeds exhibited a significant ($P \leq 0.05$) decrease in TLC.

There were no significant ($P > 0.05$) differences among the groups in Hct, MCV, and MCHC. The group fed an Aflatoxin-contaminated diet (G2) had a significantly ($P \leq 0.05$) lower mean MCH value than the other groups. Additionally, when *NS* seeds were supplemented at a level of 6% (G5), there was a significant increase ($P \leq 0.05$) in MCH compared to the other groups with different *NS* supplementation levels.

Table 2. Hematological parameters of growing Nubian goats (n=20) fed an Aflatoxin-contaminated diet supplemented with *Nigella sativa* seeds.

Parameters	Experimental groups					SEM	SL
	G1	G2	G3	G4	G5		
Hb (g/dl)	10.0 ^{ab}	8.4 ^b	9.7 ^{ab}	9.7 ^{ab}	11.3 ^a	0.32	*
TEC ($\times 10^6$ / μ l)	10.5 ^{ab}	8.7 ^b	12.0 ^a	10.0 ^{ab}	10.0 ^{ab}	0.40	*
TLC ($\times 10^3$ / μ l)	9.3 ^c	17.0 ^a	13.5 ^b	9.7 ^c	9.7 ^c	0.73	*
Hct (L/L)	0.23	0.26	0.27	0.29	0.29	0.01	N.S
MCV (fl)	22.5	31.1	23.2	29.1	29.3	1.31	N.S
MCH (pg)	7.9 ^b	6.7 ^c	8.2 ^b	7.9 ^b	11.2 ^a	0.48	*
MCHC (g/dl)	35.6	37.7	35.2	33.1	39.3	1.25	N.S

G1: control group. G2: group fed a diet with 150 ppb Aflatoxin. G3: group fed a diet with 150 ppb Aflatoxin + 2% NS seeds. G4: group fed a diet with 150 ppb Aflatoxin + 4% NS seeds. G5: group fed a diet with 150 ppb Aflatoxin + 6% NS seeds. Hb: Hemoglobin. TEC: Total erythrocytes count. TLC: Total leukocyte count. Hct: Blood hematocrit. MCV: Mean Corpuscular Volume. MCH: Mean Corpuscular Hemoglobin. MCHC: Mean Corpuscular Hemoglobin Concentration. SEM: Standard error of the means. SL: Significance Level. N.S: Non-significant. *: $P \leq 0.05$. abc: Means with different superscripts in the same row were significantly different.

3.2. Blood glucose, serum protein parameters, urea, and creatinine: Blood glucose, serum protein parameters, urea, and creatinine of growing Nubian goats fed an Aflatoxin-contaminated diet supplemented with NS seeds presented in Table 3. Blood glucose levels showed non-significant ($P > 0.05$) differences among the experimental groups. There were significant ($P \leq 0.05$) variations among the experimental groups on serum total protein. Significantly ($P \leq 0.05$) lower mean value of TP was observed in the group fed an Aflatoxin-contaminated diet (G2) compared to other groups. Supplementation of NS seeds caused a significant increase ($P \leq 0.05$) in serum TP levels. Notably, the group fed an Aflatoxin-contaminated diet supplemented with 6% NS seeds (G5) exhibited a significantly higher ($P \leq 0.05$) value of TP compared to the other NS supplemented groups. No significant ($P > 0.05$) differences were recorded among the groups on serum albumin. However, a significantly ($P \leq 0.05$) lower value of serum globulins was observed in the group fed an Aflatoxin-contaminated diet (G2) compared to the other groups. Conversely, the group fed an Aflatoxin-contaminated diet supplemented with 6% NS seeds (G5) displayed a significantly higher ($P \leq 0.05$) value of serum globulins compared to the other NS supplemented groups. The results obtained in the present study indicate no significant ($P > 0.05$) differences among the groups on kidney function, as presented in creatinine and urea nitrogen concentrations, which were not affected by the supplementation of NS seeds to an Aflatoxin-contaminated diet. However, a slight increase in urea level was observed in NS supplemented groups.

Table 3. Blood glucose, serum protein parameters, urea, and creatinine of growing Nubian goats (n=20) fed an Aflatoxin-contaminated diet supplemented with *Nigella sativa* seeds.

Parameters	Experimental groups					SEM	SL
	G1	G2	G3	G4	G5		
Glucose (mg/dl)	42.5	42.2	42.5	42.7	43.2	0.74	N.S
Total protein (g/l)	45.0 ^c	36.0 ^d	45.0 ^c	67.0 ^b	80.0 ^a	0.38	*
Albumin (g/l)	25.0	22.0	21.0	24.0	26.0	0.10	N.S
Globulins (g/l)	20.0 ^c	13.0 ^d	24.0 ^c	43.0 ^b	54.0 ^a	0.37	*
Urea (mg/dl)	55.0	55.2	58.5	61.0	66.2	2.2	N.S
Creatinine (mg/dl)	0.85	0.97	0.82	0.85	0.87	0.42	N.S

G1: control group. G2: group fed a diet with 150 ppb Aflatoxin. G3: group fed a diet with 150 ppb Aflatoxin + 2% NS seeds. G4: group fed a diet with 150 ppb Aflatoxin + 4% NS seeds. G5: group fed a diet with 150 ppb Aflatoxin + 6% NS seeds. SEM: Standard error of the means. SL: Significance Level. N.S: Non-significant. *: $P \leq 0.05$. abc: Means with different superscripts in the same row were significantly different.

3.3. Blood pH, serum electrolytes, and strong ion difference: Blood pH, serum electrolytes, and strong ion difference of growing Nubian goats fed an Aflatoxin-contaminated diet supplemented with *NS* seeds are presented in Table 4. The results indicate that all groups showed no significant differences ($P>0.05$) in terms of blood pH, serum sodium [Na^+], serum potassium [K^+], serum chloride [Cl^-], and serum strong ion difference [SID_3].

Table 4. Blood pH, serum electrolytes, and strong ion difference (SID_3) of growing Nubian goats ($n=20$) fed an Aflatoxin-contaminated diet supplemented with *Nigella sativa* seeds (mmol/l).

Parameters	Experimental groups					SEM	SL
	G1	G2	G3	G4	G5		
Blood pH	7.35	7.35	7.30	7.35	7.30	0.01	N.S
Serum [Na^+]	141.0	142.2	142.7	142.5	141.0	0.45	N.S
Serum [K^+]	5.07	4.82	4.40	4.55	4.72	0.08	N.S
Serum [Cl^-]	103.7	107.7	108.5	108.5	102.7	1.11	N.S
Serum [SID_3]	42.3	39.3	38.6	38.5	42.9	1.17	N.S

G1: control group. G2: group fed a diet with 150 ppb Aflatoxin. G3: group fed a diet with 150 ppb Aflatoxin + 2% *NS* seeds. G4: group fed a diet with 150 ppb Aflatoxin + 4% *NS* seeds. G5: group fed a diet with 150 ppb Aflatoxin + 6% *NS* seeds. SEM: Standard error of the means. SL: Significance Level. N.S: Non-significant. abc: Means with different superscripts in the same row were significantly different.

3.4. Lipid profile: Significant ($P\leq 0.05$) differences were observed among the groups in serum lipid profile parameters, as presented in Table 5. A higher ($P\leq 0.05$) total lipids concentration was observed in the group fed an Aflatoxin-contaminated diet (G2) compared to the other groups. However, supplemented *NS* seeds to an Aflatoxin-contaminated diet led to a significant ($P\leq 0.05$) decrease in serum total lipids. Specifically, supplementing 2% *NS* seeds to the Aflatoxin-contaminated diet (G3) resulted in a significantly ($P\leq 0.05$) lower value of total lipids compared to the other groups with different supplementation levels.

Table 5. Serum lipid profile of growing Nubian goats ($n=20$) fed an Aflatoxin-contaminated diet supplemented with *Nigella sativa* seeds.

Parameters	Experimental groups					SEM	SL
	G1	G2	G3	G4	G5		
Total lipids (g/dl)	17.3 ^{ab}	21.3 ^a	9.2 ^b	15.2 ^{ab}	15.1 ^{ab}	1.34	*
Triglycerides (mg/dl)	14.0 ^{ab}	19.2 ^a	6.7 ^{cd}	12.0 ^{bc}	5.7 ^d	1.36	*
Cholesterol (mg/dl)	38.5 ^b	49.2 ^a	34.7 ^c	36.5 ^c	29.0 ^d	2.70	*
HDL (mg/dl)	26.5 ^b	30.7 ^a	22.5 ^c	25.0 ^b	19.5 ^a	1.90	*
LDL (mg/dl)	6.0 ^b	12.4 ^a	6.2 ^b	5.5 ^b	3.5 ^c	0.39	*

G1: control group. G2: group fed a diet with 150 ppb Aflatoxin. G3: group fed a diet with 150 ppb Aflatoxin + 2% *NS* seeds. G4: group fed a diet with 150 ppb Aflatoxin + 4% *NS* seeds. G5: group fed a diet with 150 ppb Aflatoxin + 6% *NS* seeds. HDL: High-density lipoprotein. LDL: Low-density lipoprotein. SEM: Standard error of the means. SL: Significance Level. *: $P\leq 0.05$. abc: Means with different superscripts in the same row were significantly different.

Serum triglyceride level was significantly ($P\leq 0.05$) highest in the group fed an Aflatoxin-contaminated diet (G2) compared to the other groups. Notably, supplementing *NS* seeds to an Aflatoxin-contaminated diet at a level of 6% (G5) resulted in a significantly ($P\leq 0.05$) lowest concentration of serum triglycerides. The experimental group fed an Aflatoxin-contaminated diet (G2) exhibited a significantly ($P\leq 0.05$) highest mean value of total cholesterol. Supplementing *NS* seeds to an Aflatoxin-contaminated diet led to a significant ($P\leq 0.05$) decrease in serum total cholesterol. Specifically, supplementation of *NS* seeds at a level of 6% (G5) resulted in a significantly ($P\leq 0.05$) lower value of serum cholesterol compared to the

other groups. The highest value ($P \leq 0.05$) of high-density lipoprotein (HDL) was observed in the group fed an Aflatoxin-contaminated diet (G2). However, supplementing *NS* seeds to an Aflatoxin-contaminated diet caused a decrease ($P \leq 0.05$) in HDL, with the lowest mean value observed in the group fed an Aflatoxin-contaminated diet supplemented with 6% *NS* seeds (G5). A significantly higher ($P \leq 0.05$) mean value of LDL was observed in the group fed an Aflatoxin-contaminated diet (G2) compared to the other groups. In contrast, the *NS* supplemented groups and the control group showed a significant ($P \leq 0.05$) decrease in serum LDL, with the lowest ($P \leq 0.05$) value observed in the group fed an Aflatoxin-contaminated diet supplemented with 6% *NS* seeds (G5).

3.5. Serum liver enzymes: Significant ($P \leq 0.05$) differences were observed among the groups in all serum liver enzyme values, as shown in Table 6. The results indicate that the AST, ALT, ALP, and GGT values significantly ($P \leq 0.05$) decreased by supplementing *NS* seeds to an Aflatoxin-contaminated diet. The experimental group fed an Aflatoxin-contaminated diet (G2), and the group fed an Aflatoxin-contaminated diet supplemented with 2% *NS* seeds (G3) showed significant ($P \leq 0.05$) highest values of AST, ALT, and ALP. However, the group fed an Aflatoxin-contaminated diet supplemented with 6% *NS* seeds (G5) had significantly lower ($P \leq 0.05$) values compared to the other groups. The Aflatoxin-contaminated diet (G2) significantly ($P \leq 0.05$) increased the level of GGT. The addition of 6% *NS* seeds to an Aflatoxin-contaminated diet resulted in a significant ($P \leq 0.05$) decrease in GGT levels compared to the other groups.

Table 6. Serum liver enzymes of growing Nubian goats (n=20) fed an Aflatoxin-contaminated diet supplemented with *Nigella sativa* seeds (μl).

Parameters	Experimental groups					SEM	SL
	G1	G2	G3	G4	G5		
AST	167.0 ^b	178.5 ^a	177.5 ^a	161.0 ^b	147.0 ^c	8.04	*
ALT	37.2 ^b	68.7 ^a	68.2 ^a	35.7 ^b	29.2 ^c	4.90	*
ALP	15.5 ^b	21.7 ^a	19.7 ^a	16.7 ^b	13.1 ^c	1.06	*
GGT	16.2 ^c	23.0 ^a	19.0 ^b	14.0 ^c	9.7 ^d	1.42	*

G1: control group. G2: group fed a diet with 150 ppb Aflatoxin. G3: group fed a diet with 150 ppb Aflatoxin + 2% *NS* seeds. G4: group fed a diet with 150 ppb Aflatoxin + 4% *NS* seeds. G5: group fed a diet with 150 ppb Aflatoxin + 6% *NS* seeds. AST: Serum Aspartate Transaminase. ALT: Serum Alanine Aminotransferase. ALP: Serum Alkaline Phosphatase. GGT: Serum Gamma-Glutamyltransferase. SEM: Standard error of the means. SL: Significance Level. *: $P \leq 0.05$. abc: Means with different superscripts in the same row were significantly different.

4. Discussion

Aflatoxin contamination is a known risk in feed ingredients such as groundnuts, corn, and cottonseed. The findings of this study provide valuable insights into the impact of Aflatoxin exposure and the potential benefits of *Nigella sativa* seed supplementation on the physiological performance of growing Nubian goats.

The present study findings on hematological parameters, specifically Hb concentration and TEC, agree with previous research conducted by Oguz et al. [41], Abdel-Wahhab et al. [42], and Yousef et al. [43], who reported similar decreases in Hb concentration and TEC as a result of consuming an Aflatoxin-contaminated diet. These findings align with the current study and provide additional support for the adverse effects of Aflatoxin exposure on hematological health. Dietary supplementation with different levels of *NS* seeds significantly increased the Hb concentration and TEC. These findings are consistent with the studies conducted by El-Saadany et al. [15] and Habeeb and El Tarabany [44], which reported similar results of increased Hb and TEC in lactating goats when supplemented with *NS* seeds. The results obtained in the present study for TLC showed a significantly highest value in the group fed an Aflatoxin-contaminated diet (G2), which attributed to the cytotoxic effects of Aflatoxin on a variety of cells, including hematopoietic precursor cells and lymphocytes [45]. The TLC was significantly decreased by supplementing different levels of *NS* seeds to an Aflatoxin-contaminated diet due to the anti-inflammatory properties of *NS* and its active principle, thymoquinone [46].

The results obtained in the present study demonstrate that supplemented *NS* seeds to an Aflatoxin-contaminated diet had no significant effect on blood glucose levels. This result agrees with the findings of Awadallah [47], who reported that the supplementation of *NS* does not significantly impact blood glucose levels in Friesian calves. Furthermore, the results are consistent with the findings of Omer [48], who observed that the supplementation *NS* to the rabbit ration did not affect blood glucose levels.

Serum total proteins and globulins concentrations increased significantly by supplemented *NS* seeds to an Aflatoxin-contaminated diet. The increased concentration of total protein and its fractions can be attributed to the enhanced activity of hepatic functions as a result of *NS* seed supplementation. The significant increase in globulins concentration observed indicates a good immune status of the growing goats. The results obtained from the study indicate that supplementing the diet of the growing goats with *NS* seeds improved immune function and restored physiologically relevant levels of protein function. The results of the present study agreed with Awadalla and Gehad [49], who found that supplementing the rations of growing sheep with 2% *NS* seeds significantly increased total protein and globulin concentrations, while albumin concentration was not significantly affected. Tousson et al. [50] reported increased blood total protein and albumin concentrations in rabbits fed diet containing *NS* seeds. Moreover, El-Saadany et al. [15] reported that supplementation with *NS* improved animal immune function and increased total protein and globulins concentrations. Zeweil et al. [17] found that the addition of *NS* to rabbit diets resulted in increased levels of plasma total protein, albumin, and globulins. Similarly, Habeeb and El Tarabany [44] found that dietary supplementation with *NS* in Zaraibi goats significantly increased serum total protein and globulins. The previous studies agree with the findings in the present study.

Blood pH, serum electrolytes (Na^+ , K^+ , and Cl^-), and strong ion difference (SID_3) were not affected by an Aflatoxin-contaminated diet or supplementation with different levels of *NS* seeds. These findings disagree with the study conducted by El-Saadany et al. [15], which reported that dietary supplementation with *NS* significantly increased the concentrations of Na^+ , K^+ , Ca^+ , Pi , and Zn in lactating goats. Additionally, the present study's results are inconsistent with the findings of Habeeb and El Tarabany [44], who reported that serum electrolyte concentrations significantly increased with dietary supplementation of *NS* during the hot summer season.

The study's Serum lipid profile results reveal significant differences among the treatment groups. The group fed an Aflatoxin-contaminated diet showed a significant increase in serum cholesterol and LDL levels. However, the supplementation of *NS* seeds resulted in a significant decrease in serum cholesterol and LDL levels. The present study's findings agree with the results reported by Al-Beitawi et al. [51], who observed that feeding with *NS* seeds reduced plasma cholesterol and triglyceride concentrations and increased plasma HDL concentrations. Previous studies showed that *NS* has a promising effect similar to drugs that reduce serum cholesterol and decrease its atherogenic pathological impact [52,53]. The decrease in triglycerides and cholesterol levels observed in this study may be attributed to active ingredients such as thymoquinone and compounds like monounsaturated fatty acids. These components have been shown to reduce cholesterol synthesis by hepatocytes and decrease cholesterol absorption from the small intestine [54]. El-Dakhkhny et al. [55] concluded that reduced serum cholesterol levels may be attributed to enhanced bile production. The decrease in serum cholesterol levels could be due to the high content of unsaturated fatty acids in *NS* seeds, which stimulate cholesterol uptake by the intestine and can be converted to bile acids through oxidation [56].

Liver enzymes profile (AST, ALT, ALP, and GGT) obtained in the present study show significant differences among the groups. Serum liver enzymes increased significantly in the Aflatoxin-contaminated diet group. However, liver enzyme levels were significantly decreased by supplementing *NS* seeds to an Aflatoxin-contaminated diet. This result disagreed with Awadalla and Gehad [49], who found that supplementing growing sheep rations with *NS* did not affect serum liver enzyme activities and indicated that supplementation *NS* had no adverse effects on liver function. The findings of studies conducted by Rastogi et al. [57] and Karakilcik et al. [58] are consistent with the present study, as they also observed an increase in the activity of ALP with Aflatoxin exposure. Similarly, the present study observed a significant increase in the activities of AST and ALT with an Aflatoxin-contaminated diet. These results agreed with Oguz et al. [59] and Madheswaran et al. [60], who reported that an Aflatoxin-contaminated diet significantly increased AST and ALT activities. The observed increases in AST and ALT levels were directly related to the doses of Aflatoxin.

5. Conclusion

The current study indicates that supplemented NS seeds to an Aflatoxin-contaminated diet positively affected blood and serum parameters. *Nigella sativa* supplementation improved hematological parameters such as Hb and TEC, suggesting potential benefits for blood health. It also demonstrated immune-enhancing properties, as evidenced by increased serum total proteins and globulins concentrations. Furthermore, NS seed supplementation was associated with reduced cholesterol and LDL levels, indicating a potentially beneficial impact on lipid profile. However, no significant effects on kidney function, blood glucose levels, or electrolyte balance were observed with NS supplementation. The results suggest that NS seeds may hold promise as a dietary supplement to counteract the adverse effects of Aflatoxin-contaminated diets. Further research is needed to explore the underlying mechanisms and confirm these findings in diverse animals. Supplementing NS seeds may successfully replace synthetic detoxifying agents, providing an alternative method to reduce suspected Aflatoxin contamination.

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Data Availability Statement: All the relevant data is available in the manuscript.

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