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Research Article

Effects of *Nigella Sativa* Seed on Rumen Parameters, Digestibility, and Nitrogen Balance of Nubian Goat Fed Aflatoxin Contaminated Diet

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ABSTRACT

Introduction: Animal health and productivity can be affected by Aflatoxin in feeds, which leads to reduced growth, decrease feed efficiency and potential contamination of animal-derived products. The current study investigated the influences of supplementing *Nigella sativa* (*NS*) seeds to an Aflatoxin-contaminated diet on rumen parameters, digestibility, and nitrogen balance of male Nubian goats.
Materials and methods: A total number of 20 healthy male Nubian goat kids aged 8-9 months and an average weight of 11 ± 0.5 kg were randomly assigned to 5 equal groups. The control group (T1) received a basal diet. The second treatment group (T2) received the same diet contaminated with 150 ppb Aflatoxin and other treatments received an aflatoxin-contaminated diet supplemented with different levels of crushed *NS* seeds including 2% (T3), 4% (T4), and 6% (T5) of the total dry matter. Through using naturally infected groundnut cake, Aflatoxin was mixed with other ingredients of ration in different proportions to formulate treated diets. High-performance liquid chromatography was used to determine Aflatoxin concentration. Rumen liquor samples were collected after 40 days feeding period to evaluate rumen pH, volatile fatty acids (VFAs), ammonia nitrogen (NH_3-N), rumen electrolytes, and protozoa count.
Results: Rumen pH, ammonia nitrogen, ruminal sodium, and potassium were not significantly affected by the treatment groups. Rumen volatile fatty acids, protozoa count, nutrient digestibility, and nitrogen retention were significantly reduced by an Aflatoxin-contaminated diet (T2) compared to other groups. Supplementation of *NS* seeds to an Aflatoxin-contaminated diet caused a significant increase in VFAs, decreased protozoa count, and improved digestibility and nitrogen retention in all treated groups compared to T2 and T1 groups.
Conclusion: Supplementing *NS* seeds to an Aflatoxin-contaminated diet positively affected rumen ecology and improved digestibility and nitrogen retention. The study recommended supplementing 6% of *NS* seeds to goat diets can reduce the negative effects of Aflatoxin in the diet.

1. Introduction

Inadequate storage conditions, including suboptimal temperature and humidity, can lead to the contamination of animal feed by the natural production of mycotoxins, specifically Aflatoxins. The mycotoxin as a secondary metabolite is produced by various fungal species, predominantly *Aspergillus spp*. Animal feeds commonly consist of Aflatoxins, that is considered powerful natural carcinogenic agents in mammals[1,2]. A high level of Aflatoxin at more than 20 ppb has been reported as toxic and as a primary causative agent of hepatic and extrahepatic carcinogenesis[3,4].

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Animal feeds samples contaminated with Aflatoxin have been observed in different regions of the world. High level of Aflatoxin has harmful and toxic effects which causes immunotoxicity, could negatively affect digestion, as well as reducing the reproductive function of animals[5,6].

Some strategies, including Physical, chemical, and biological methods have been developed to detoxify Aflatoxin-contaminated and reduce Aflatoxin biosynthesis or as an inhibitory growth factor of Aflatoxigenic molds. However, only some of these strategies have a practical application[7-9]. Several herbs were experimented to reduce the production of Aflatoxin and the growth of molds. Black cumin or black seeds which is also referred as Nigella sativa (*NS*) has been used for culinary and medical purposes for a very long time[10]. Crude oil extracts of *NS* showed antimicrobial, antipyretic, analgesic, and anti-inflammatory properties[11,12]. It has been investigated that *NS* supplementation inhibited fungus *Aspergillus* growth and thus reduced Aflatoxins synthesis[13].

Calves fed a supplemented diet with *NS* seed oil significantly gained more weight when they had been weaned and had a higher nutrient digestibility[14]. The seeds of *NS* have a dramatic effect on the digestive system, improving performance, and absorption. Adding *NS* to feed increases the bile flow rate, activates pancreatic lipases, and increases fat digestion as well as vitamin absorption[15].

The present study investigated the effects of supplementing *NS* seeds to an Aflatoxin-contaminated diet on Nubian goat kids' rumen parameters, digestibility, and nitrogen balance. The current study hypothesizes that *NS* seeds can positively detoxify Aflatoxin-contaminated animal feeds, affect rumen ecology and digestibility, as well as improve the performance of goat kids.

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 the current study (ethical number: 1/2017/3).

2.2. Experimental animals and management

The present experiment was conducted at the Physiology Department, Faculty of Veterinary Medicine and Animal Nutrition Department, Faculty of Animal Production, University of Khartoum (Sudan). A total number of 20 healthy male Nubian goat kids (aged 8-9 months, and average weight 11 ± 0.5 kg) were distributed randomly to five treatments. Kids were purchased from the Bahri livestock market in north Khartoum, Sudan, and were adapted to the feed for a period of one week. The goat kids were given Oxytetracycline as prophylactic. The dosage of 20mg/kg was injected intramuscularly based on the body weight for five days[16]. Ivermectin was given subcutaneously at a dosage of 20mg/kg body weight for ectoparasites[17], and Albendazole was used orally at a dose of 10mg/kg body weight to treat endoparasites[18].

The goat kids were housed individually in well-ventilated open-sided experimental pens with dimensions of 1.5 m × 1.5 m × 2 m height and consisted of a zinc roof, concrete floor, and wire net at the sides. Cypermethrin spray as an anti-parasitic agent and formalin 40% were applied to disinfect the pens. Manual feeding and drinking equipment were used.

2.3. Experimental diets

The seeds of *NS* were ground into medium size particles. The chemical composition of *NS* seeds included dry matter (DM) at 96.3%, ether extract (EE) at 30.7%, 28.2% of crude protein (CP), 4.7% of Ash, Crude fiber (CF), Nitrogen free extract (NFE), and Metabolizable energy (ME) at 20.6%, 12.1%, and 15.6 MJ/kg, respectively.

In the experimental diet, groundnut cake acted as the Aflatoxin carrier. The groundnut cake was obtained from a local feed market in Khartoum State. The spores of the fungus were found. In order to create treatment meals with around 150 ppb of Aflatoxins the infected groundnut cake with Aflatoxins was combined in different ratios with other feed items. High-performance liquid chromatography (HPLC) was used to determine Aflatoxin concentration[19].

Based on the standard nutrient requirements of goats published by the National Research Council (NRC), five isocaloric and isonitrogenous diets were formulated[20]. The ingredients and the chemical composition of the experimental diets are shown in Table 1. The feed ingredients were manually mixed to achieve homogeneity in the mash mixture and formulate the treatments. The control group (T1) had a basal diet. The treatment groups (T2) received the same diet contaminated with 150 ppb Aflatoxin and other treatments received an aflatoxin-contaminated diet supplemented with different levels of crushed *NS* seeds including 2% (T3), 4% (T4), and 6% (T5) of the total dry matter. The goat kids were fed with experimental diets for 40 days and accessed unlimited water supplies.

The chemical composition of experimental diets was estimated on a (DM) basis. The experimental diets were analyzed for (DM), (EE), (CP), (CF), and Ash through a procedure described by the Association of Official Analytical Chemists[21]. Formulas 1 and 2 was used to calculate Nitrogen-free extract (NFE),

$$NFE\% = \{DM - (EE\% + CP\% + CF\% + ASH\ \%)\} \quad \text{(Formula 1)}$$

Metabolizable energy (ME) was calculated by using the following equation,

$$ME \text{ for Ruminants } (MJ/Kg) = 0.12\ CP + 0.31\ EE + 0.05\ CF + 0.14\ NFE \quad \text{(Formula 2)}$$[22].

2.4. Collection of rumen fluid samples

On the 41st day of the experiment at 8:00 am, rumen fluid was collected every 2 hours with a stomach tube. Up

Table 1. Ingredients and chemical composition of the experimental diets

Ingredients	Treatments				
	T1	T2	T3	T4	T5
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
Nigella sativa (%)	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
Ash (%)	12.6	12.2	12.3	12.7	12.6
Crude fiber (%)	9.6	9.3	9.7	9.4	9.8
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

T1: Control diet. T2: Diet with 150 ppb Aflatoxin. T3: Diet with 150 ppb Aflatoxin + 2% *Nigella sativa*. T4: Diet with 150 ppb Aflatoxin + 4% *Nigella sativa*. T5: Diet with 150 ppb Aflatoxin + 6% *Nigella sativa*. ppb: Part per billion. *ME: Metabolizable energy[22].DM: Dry matter

to 6 hours after the morning meal the samples of rumen liquid were filtered through a mesh cloth, and 10 ml was pipetted into a clean dry test tube. The pH was determined immediately by using a standard laboratory pH meter (HANNA Instruments, Portugal). The liquid was then strained through two layers of cheesecloth and chilled to –20 °C for further examination.

2.5. Rumen parameters

2.5.1. Rumen pH

After collecting the rumen fluid, a pH meter (HANNA instruments, Portugal) was used to measure the rumen pH.

2.5.2. Volatile fatty acids

A steam distillation was used to determine the Volatile Fatty Acids (VFAs)[23]. An amount of five ml of rumen fluid was pipetted into a Kjeldhal flask with three ml of distilled water, 10 ml of $MgSO_4$, and 10 ml of H_2SO_4. Then, 150 ml of distilled NH_3-N was collected in a flask along with 1-2 drops of phenolphthalein as an indicator. The solution's concentration was titrated using 0.05N NaOH solutions. The following formula was used to determine VFA[23].

$$\text{VFAs (mmol/l)} = \frac{\text{Titration (ml} \times \text{ NaOH } \times \text{ 100)}}{\text{Volume of rumen fluid}}$$

(Formula 3)

2.5.3. Rumen ammonia nitrogen (NH_3-N)

Test tubes containing three ml of 20% trichloroacetic acid and 3 ml of rumen fluid were centrifuged for 10 minutes. An amount of 10 ml of sodium borate dehydrate was added when two ml of the previously described solution was put straight into the distillation setup. Then the mixture was distilled for around 5 minutes before titration with 0.05 N sulphuric acid[23]. The following formula was used to determine rumen ammonia nitrogen (NH_3-N).

$$\text{Rumen NH3N (mg/l)} = \frac{\text{Titration (ml } \times \text{ 0.0014)}}{\text{Amount of sample}}$$

(Formula 4)

2.5.4. Protozoa count

The amount of one volume of methyl-blue formalin saline (MFS) solution (100 ml formaldehyde 35%, 900 ml distilled water, 0.60 g of methyl-blue, and 0.80 g of sodium chloride) was used to fix and stain the rumen fluid. Then the mixture was stocked in a dark place until the time of examination. By adding one drop of the fixed rumen fluid sample on a hemocytometer slide, it was covered with a cover slip and examined using an Olympus Optical Co., LTD., Japan, light microscope with a low power (×10) objective. Formula 5 was used in this regard.

Number of protozoa/ml rumen fluid (RJ) = $N \times 10^4$
The dilution rate (1 ml rumen fluid (RJ) and 1 ml MFS) = 2
The number of protozoa in one large square corner of the Improved Neubauer hemocytometer = n
The number of protozoa / µl RJ = n× dilution × depth
The number of protozoa/ µl RJ = n× dilution × depth × 10^4 = $n \times 2 \times 10^4$
Where, n is the number of protozoa in the sample.
(Formula 5)

2.5.5. Rumen electrolytes

The ruminal Sodium, ruminal Potassium, and ruminal chloride concentrations were measured using a flame photometer technique (PFP7 Jenway, E.U) as described for serum [Na^+], serum [K^+], and serum [Cl^-], respectively.

2.6. Digestibility and nitrogen balance

The digestibility and nitrogen balance trial was performed after 40 days feeding period. The kids were placed in digestibility cages with free access to feed and water. After one week of the adaptation period, feces and urine samples were collected every morning for seven days. During the daily collecting period, the feces were collected into plastic bags and weight to around 50 g on 10 kg to loading balance. About 5% of the fecal sample was taken and stored at −20° C to determine nitrogen content. Then 10% of the feces were air-dried and stored in plastic buckets until the end of the collection period. The dried feces were measured daily. The urine voided by the kids was drained through a curved plastic plate fitted to the underside of the crate floor in a 2.5-liter wide-mouth bottle (Fitted with a plastic funnel) which contained 5ml sulphuric acid to prevent the loss of urine nitrogen. The collected urine was strained through a layer of glass wool to remove detached hair fragments or other solid contaminates, and the volume was recorded using a cylinder. After daily collection, the draining funnels were washed with tap water before placing the acid preservative for the next 24 hours. Then 20% volume of the urine samples were taken into a liter poly propylene container and stored at −20° C consecutive daily samples from each container were pooled together in one container. The dry matter digestibility (DMD) coefficient was calculated as follows (Formula 6 and 7).

$$\text{DMD coefficient} = \frac{\text{DM in feed} - \text{DM in feces}}{\text{DM in feed}} \times 100$$

(Formula 6)

The organic matter digestibility (OMD) coefficient was calculated by following model,

$$\text{OMD coefficient} = \frac{\text{OM in feed} - \text{OM in feces}}{\text{OM in feed}} \times 100$$

(Formula 7)

To calculate the nutrient digestibility the following formula was used,

$$\text{Nutrient digestibility} = \frac{\text{Nutrient in feed} - \text{Nutrient in feces}}{\text{Nutrient in feed}} \times 100$$

(Formula 8)

Feed, urine, fecal, and refusal samples taken from the digestibility trial were used to determine nitrogen content as described by the Kjeldhal method[21]. Nitrogen balance was calculated using the following equation (Formula 9)[24],

Nitrogen retention = Nitrogen Intake - (Fecal nitrogen + Urinary Nitrogen) (Formula 9)

2.7. Statistical analysis

Statistical analysis was performed using the SPSS computer program (USA, version 20). Analysis of variance (ANOVA) was used to assess the difference between the treatments. The significance differences were determined by Duncan's multiple-range tests[25]. Linear regression was performed to explain the relationship between *NS* levels and the parameters investigated (ruminal parameters). The mean difference was considered significant at $p \leq 0.05$.

3. Results

3.1. Rumen parameters

Rumen fermentation parameters (pH, VFAs, NH_3-N, and protozoa count) curves of male Nubian goat kids fed with an Aflatoxin-contaminated diet supplemented with *NS* seeds are presented in Figure 1.

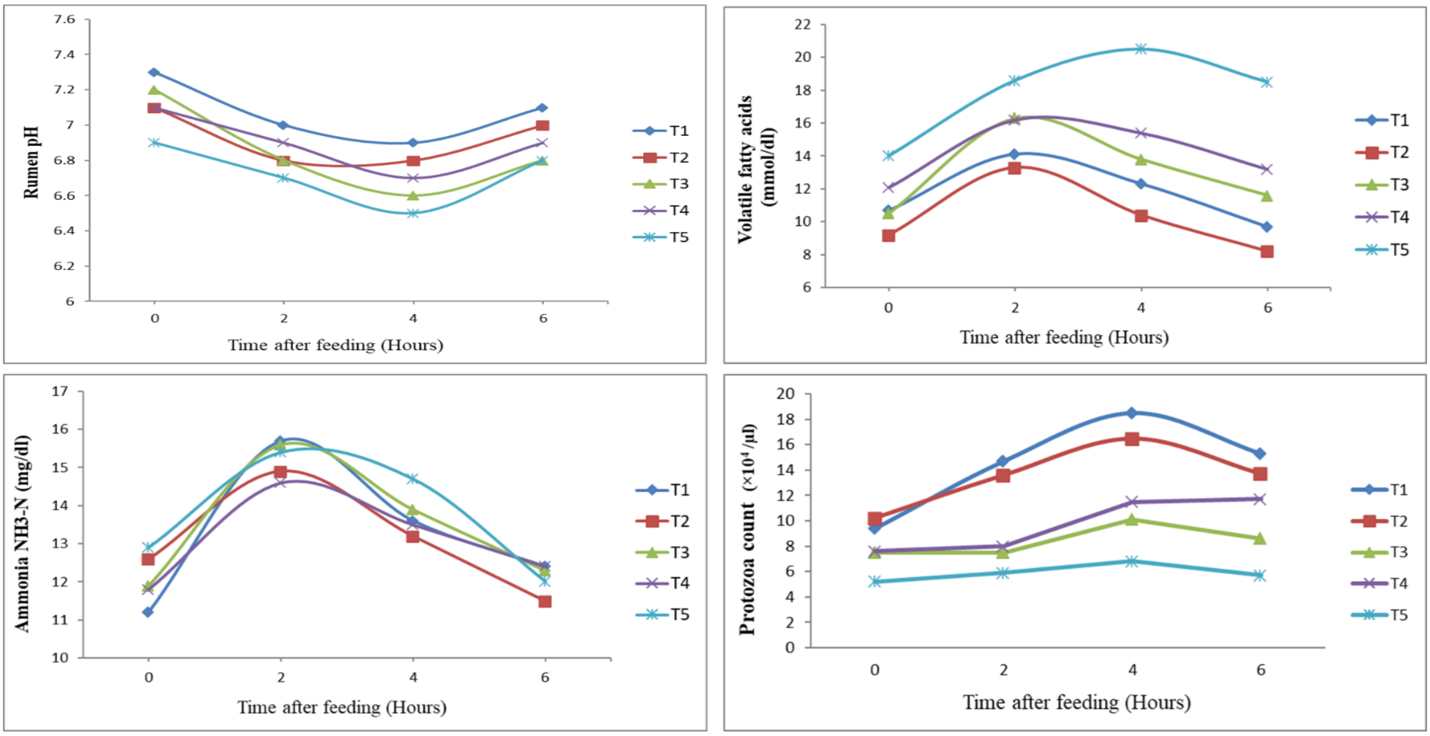

Figure 1. Rumen fermentation parameters (pH, VFAs, NH_3-N, and protozoa count) curves of male Nubian goat kids (n=20) fed an Aflatoxin-contaminated diet supplemented with *Nigella sativa* seeds

T1: Control diet, T2: Diet with 150 ppb Aflatoxin, T3: Diet with 150 ppb Aflatoxin + 2% *Nigella sativa*, T4: Diet with 150 ppb Aflatoxin + 4% *Nigella sativa*, T5: Diet with 150 ppb Aflatoxin + 6% *Nigella sativa*

Table 2. Average rumen fermentation parameters of male Nubian goat kids (n=20) fed an Aflatoxin-contaminated diet supplemented with *Nigella sativa* seeds

Parameters	Treatments					± SE	LS
	T1	T2	T3	T4	T5		
Rumen pH	6.98	6.93	6.85	6.90	6.73	0.06	NS
VFAs (mmol/l)	11.70 ^{d}	10.28 ^{d}	13.05 ^{c}	14.23 ^{b}	17.90 ^{a}	1.30	*
NH_3-N (mg/l)	13.23	13.05	13.43	13.08	13.75	0.13	NS
Protozoa count (×10⁴ /µl)	14.5 ^{a}	13.5 ^{a}	9.5 ^{b}	8.5 ^{b}	5.0 ^{c}	0.98	*

^{abc} Means with different superscripts in the same row were significantly different (p ≤ 0.05).
T1: Control diet, T2: Diet with 150 ppb Aflatoxin, T3: Diet with 150 ppb Aflatoxin + 2% *Nigella sativa*, T4: Diet with 150 ppb Aflatoxin + 4% *Nigella sativa*, T5: Diet with 150 ppb Aflatoxin + 6% *Nigella sativa*, SE: Standard error of the means, LS: Level of significance, NS: Non-significant. *: (p ≤ 0.05)

Non-significant (p > 0.05) variation was observed among the treatment groups in rumen pH. Rumen pH for all treatment groups slightly decreased in 2 to 4 hours after feeding. The lowest pH values were recorded after 4 hours then the values increased again. At all different periods (0, 2, 4, and 6 hours), the control group (T1) showed the highest value of rumen pH. In contrast, the kids in group T5 showed the lowest values. The mean values of rumen pH for all the collection times were within the normal range for goats.

Rumen VFAs showed a significant (p ≤ 0.05) variation among the treatment groups in different time periods. The initial mean value of VFAs was low. After two hours, the mean values increased, and the curve slowed down. At all different periods (0, 2, 4, and 6 hours), the treatment group (T5) showed the highest values of VFAs. In contrast, the treatment group (T2) showed the lowest values. All mean values of VFAs were within the normal range for goats.

Among the treatment groups, the difference in (NH_3-N) variation was Non-significant (p > 0.05). The numerical lower values of NH_3-N were recorded at zero time, the values for all treatment groups slightly increased in the second hour, and then the values decreased. At zero time, the treatment group (T5) showed the highest value, while (T1) showed the lowest value. After two hours, (T1) showed the highest value, whereas (T4) had the lowest value. The treatment group (T2) showed a decrease in NH_3-N while time increased. All mean values of rumen NH_3-N were within the normal range for goats.

The curve of rumen protozoa count showed a significant (p ≤ 0.05) variation among the treatment groups in different time periods. At zero time, the treatment group (T2) showed the highest value of protozoa count. In contrast, the treatment group (T5) had the lowest value. From 2 to 6 hours after feeding, the rumen protozoa count was increased in the control group (T1) and showed the highest values, while (T5) had the lowest values. The protozoa count decreased with increasing time.

3.2. Average rumen fermentation parameters

Average rumen fermentation parameters of male Nubian goat kids fed with Aflatoxin-contaminated diet supplemented *NS* seeds are presented in Table 2.

There was no significant (p > 0.05) difference among treatment groups on rumen pH. The concentrations of VFAs significantly (p ≤ 0.05) increased by supplemented *NS* seeds to an Aflatoxin-contaminated diet. The lowest concentration of VFAs was obtained in the treatment group (T2). In contrast, the highest values were recorded by the treatment group (T5). There were no significant (p > 0.05) differences among treatment groups on NH_3-N concentration.

The means for protozoa count were significant (p ≤ 0.05). The highest value was seen in the control group (T1) followed by (T2). The protozoa count significantly (p ≤ 0.05) decreased by supplemented *NS* seeds to an Aflatoxin-contaminated diet. The lowest (p ≤ 0.05) value of protozoa count was observed in (T5).

3.3. Relationship between Nigella sativa levels and rumen parameters

The relationship between *NS* levels and their corresponding values of VFAs and rumen protozoa count was determined using linear regression (Figure 2). The

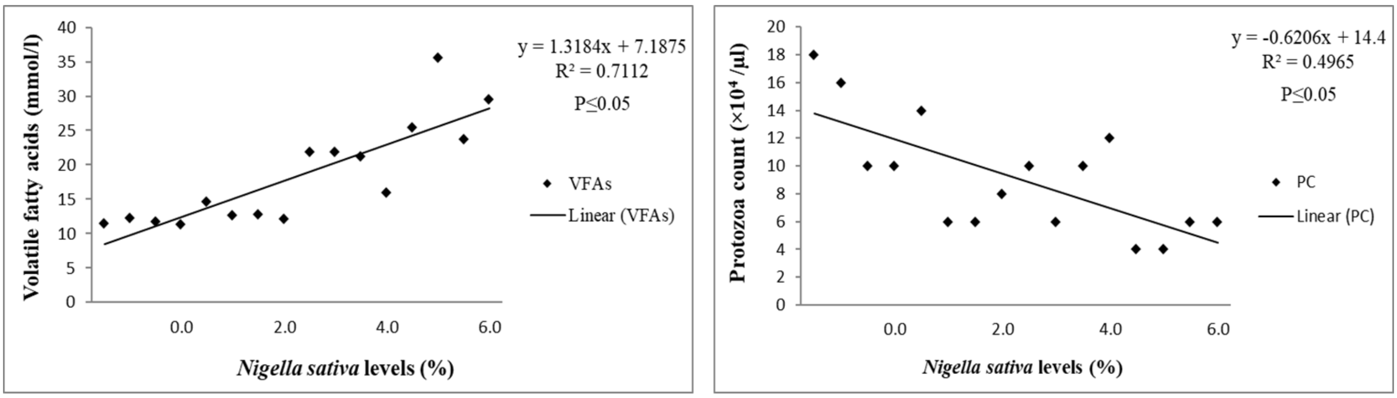

Figure 2. Relationship between Nigella sativa levels and rumen fermentation parameters (VFAs and protozoa count) of male Nubian goat kids (n=20). (Linear regression equation y=ax+b; R2= Coefficient of determination).

Table 3. Rumen electrolytes (mmol/l) of male Nubian goat kids (n=20) fed an Aflatoxin-contaminated diet supplemented with *Nigella sativa* seeds

Parameters	Treatments					± SE	LS
	T1	T2	T3	T4	T5		
Ruminal $[Na^+]$	144.2	143.5	143.6	142	143.1	0.41	NS
Ruminal $[K^+]$	6.7	7.5	8.5	8.2	7.7	0.61	NS
Ruminal $[Cl^-]$	110.7 ^{ab}	115.7 ^{a}	111.2 ^{ab}	110.3 ^{ab}	107.5 ^{b}	0.96	*

^{abc} Means with different superscripts in the same row were significantly different ($p \leq 0.05$).
T1: Control diet, T2: Diet with 150 ppb Aflatoxin, T3: Diet with 150 ppb Aflatoxin + 2% *Nigella sativa*, T4: Diet with 150 ppb Aflatoxin + 4% *Nigella sativa*, T5: Diet with 150 ppb Aflatoxin + 6% *Nigella sativa*, SE: Standard error of the means, LS: Level of significance, NS: Non-significant. *: ($p \leq 0.05$)

relationship between *NS* levels and VFAs showed a rising regression line. The coefficient indicated VFAs increased when *NS* seeds were supplemented to an Aflatoxin-contaminated diet. All individual values of the coefficient of determination (R^2) lay close to the regression line. The relationship between *NS* levels and protozoa count showed an inverse relationship. On the fitted line plot, the values generally follow the regression line in the reverse direction, which means that an increase in *NS* levels, decreased the protozoa count.

There was an association between *NS* levels and rumen fermentation parameters. In all mentioned relationships (R^2) was generally higher.

3.4. Rumen electrolytes

Rumen electrolytes of male Nubian goat kids fed an Aflatoxin-contaminated diet supplemented with *NS* seeds is presented in Table 3. No significant ($p > 0.05$) differences were observed among treatment groups in rumen [Na+] and [K+] concentrations. The rumen [Cl-] concentration was significantly ($p \leq 0.05$) different among groups. The group (T2) showed the highest value ($p \leq 0.05$) of rumen [Cl-]. The concentrations of rumen [Cl-] were decreased ($p \leq 0.05$) by supplementing *NS* seeds to an Aflatoxin-contaminated diet. The lowest value of rumen [Cl-] was observed in the treatment group (T5).

3.5. Digestibility

The digestibility of male Nubian goat kids fed an Aflatoxin-contaminated diet supplemented with *NS* seeds is presented in Table 4. Nutrient digestibility was influenced by DM except for CF, and EE, which were similar among the treatments. There was a significant ($p \leq 0.05$) difference among treatments on DMD and organic matter digestibility (OMD). The DMD and OMD efficiency were increased by supplementing *NS* seeds with an Aflatoxin-contaminated diet. The highest value ($p \leq 0.05$) was recorded in the treatment group (T5). There was no significant ($p > 0.05$) difference between (T1) and (T2) on DMD, which represented the lowest values.

Digestible crude protein (DCP) showed significant ($p \leq 0.05$) variation among the treatment groups. Significantly ($p \leq 0.05$) lower value was observed in (T2) compared to the other groups. Supplemented *NS* seeds to an Aflatoxin-contaminated diet improved digestible protein. Supplemented 6% *NS* seeds to Aflatoxin-contaminated diet showed higher ($p \leq 0.05$) digestible protein compared to the other supplemented groups.

There were significant differences ($p \leq 0.05$) among treatment groups in digestible nitrogen-free extract (DNFE). The kid's group (T2) had a lower value ($p \leq 0.05$) compared to the other groups. There were no significant ($p > 0.05$) differences between the control group (T1) and the treatment group (T3). The DNFE was improved by supplementing *NS* seeds with Aflatoxin contaminated diet.

3.6. Nitrogen balance

Table 5 presents the nitrogen balance of male Nubian goat kids fed an Aflatoxin-contaminated diet supplemented with *NS* seeds. The nitrogen intake and nitrogen retention were significantly ($p \leq 0.05$) improved by supplemented *NS* seeds to an Aflatoxin-contaminated diet. Higher nitrogen intake and retention were observed in the treatment group (T5). Significantly ($p \leq 0.05$) the lowest value of nitrogen intake and retention was observed in the treatment group (T2). Nitrogen excretion was decreased by supplemented *NS* seeds to an Aflatoxin-contaminated diet. However, urine nitrogen was not influenced by dietary treatments ($p > 0.05$).

Table 4. Digestibility of male Nubian goat kids (n=20) fed an Aflatoxin-contaminated diet supplemented with *Nigella sativa* seeds (%)

Parameters	Treatments					± SE	LS
	T1	T2	T3	T4	T5		
DMD	73.7^{d}	72.3^{d}	75.9^{c}	78.1^{b}	80.3^{a}	3.24	*
OMD	73.2^{c}	71.8^{d}	75.1^{b}	78.4^{a}	79.2^{a}	1.36	*
DCP	71.6^{c}	67.7^{d}	75.5^{b}	77.8^{a}	78.9^{a}	4.63	*
DCF	47.1	47.7	43.7	47.4	47.3	1.66	NS
DEE	62.5	63.9	61.4	61.1	62.8	1.13	NS
DNFE	85.9^{b}	83.7^{c}	85.2^{b}	87.5^{a}	88.6^{a}	1.92	*

^{abc} Means with different superscripts in the same row were significantly different ($p \leq 0.05$).
T1: Control diet. T2: Diet with 150 ppb Aflatoxin. T3: Diet with 150 ppb Aflatoxin + 2% *Nigella sativa*. T4: Diet with 150 ppb Aflatoxin + 4% *Nigella sativa*. T5: Diet with 150 ppb Aflatoxin + 6% *Nigella sativa*. DMD: Dry matter digestibility. DCP: Digestible crude protein. DCF: Digestible crude fiber. DEE: Digestible ether extract. DNFE: Digestible nitrogen-free extract. SE: Standard error of the means. LS: Level of significance. NS: Non-significant. *: ($p \leq 0.05$)

Table 5. Nitrogen balance of male Nubian goat kids (n=20) fed an Aflatoxin-contaminated diet supplemented with *Nigella sativa* seeds

Parameters	Treatments					± SE	LS
	T1	T2	T3	T4	T5		
N-intake (g/d)	14.7[b]	7.8[c]	14.5[b]	15.1[a]	15.7[a]	1.45	*
N-excretion (g/d)	4.1[a]	3.5[b]	3.4[b]	3.1[b]	2.4[c]	0.28	*
Fecal-N (g/d)	2.5[a]	2.2[b]	2.1[b]	1.9[b]	1.5[c]	0.17	*
Urinary-N (g/d)	1.6	1.3	1.3	1.2	0.9	0.11	NS
N-retention (g/d)	10.6[b]	4.3[c]	11.1[b]	12[a]	13.3[a]	1.56	*
N-retention (%)	72.1[c]	55.1[d]	76.5[b]	79.5[a]	84.7[a]	5.05	*

[abc] Means with different superscripts in the same row were significantly different ($p \leq 0.05$).
T1: Control diet, T2: Diet with 150 ppb Aflatoxin, T3: Diet with 150 ppb Aflatoxin + 2% *Nigella sativa*, T4: Diet with 150 ppb Aflatoxin + 4% *Nigella sativa*, T5: Diet with 150 ppb Aflatoxin + 6% *Nigella sativa*, N: Nitrogen, SE: Standard error of the means, LS: Level of significance, NS: Non-significant. *: ($p \leq 0.05$)

4. Discussion

The results of rumen ecology in the current study showed no significant differences among treatment groups on rumen pH. Supplementing *NS* seeds to an Aflatoxin-contaminated diet increased the concentration of VFAs due to highly digestible carbohydrates and volatile fat. There were no significant differences among the treatment groups on rumen NH_3-N concentration, which indicated that *NS* may be stimulating the rumen's proper functions and digestion.

The results of the present study indicated that the nutrient digestibility of kids in group (T2) was lower in comparison to the other groups. It was indicated by previous studies that the reduced nutrient utilization in animals might be due to the effect of Aflatoxin on the metabolic system rather than digestibility action[26,27]. It has been believed Aflatoxin caused the malabsorption of macronutrients and reduced activity of digestive enzymes[28].

The present study showed improvement in DM digestibility by supplemented *NS* seeds to an Aflatoxin-contaminated diet. The active ingredients and phenolic compounds present in *NS* might positively affect enzymes and microflora of the digestive tract that may help to improve digestibility and thus, utilization of nutrients[29]. The highest digestibility result recorded by the kid's groups fed with an Aflatoxin-contaminated diet, supplemented with *NS* seeds may increase nutrient absorption from the small intestine and consequently increase body weight gain. Therefore, *NS* may modulate rumen microorganisms and their rumen digestion capabilities.

The obtained results in the present study indicated that nitrogen balance decreased significantly in the Aflatoxin-contaminated diet group, however, it increased significantly by supplementing *NS* seeds. Higher nitrogen intake and nitrogen retention were observed in the kid's group fed with an Aflatoxin-contaminated diet supplemented with different levels of *NS* seeds. Fecal nitrogen is an important aspect of ruminant nutrition as an indicator of the degree of protein degradability in the rumen[30]. The Kid's group (T5) relatively retained more nitrogen than the other groups, possibly due to the positive association effects of nitrogen intake.

5. Conclusion

The *NS* seed supplementation positively affected rumen pH, total volatile fatty acids, rumen ammonia nitrogen, protozoa count, rumen electrolytes, nutrient digestibility, and nitrogen balance. Moreover, supplemented *NS* seeds, especially at 6% of the diet improved VFAs production, reduced protozoa count, enhanced nutrient utilization, and increased nitrogen intake and retention. The obtained findings suggest that *NS* seeds can mitigate the adverse effects of aflatoxin contamination on rumen ecology and nutrient metabolism in goats. Further research is needed to explore the underlying mechanisms and determine optimal inclusion levels for maximizing goat performance and health.

Declarations

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Authors' contributions

This study is a part of the M.Sc. thesis of the first author. Mahmoud O. A. Elfaki designed the study, collected and analyzed the data, and prepared the manuscript; Nawal M. Elkhair supervised the study. The final draft of the manuscript was reviewed by the authors and approved for publication.

Availability of data and materials

The authors will provide all necessary data to the editor upon request.

Competing interests

There is no conflict of interest.

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