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Original Article

The Effect of Nigella Sativa Meal and Enzymes Cocktail Supplementation on Broilers Raised Under High Ambient Temperature

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ABSTRACT

The utilization of *Nigella sativa* protein meal (NSM) was studied by replacing soybean meal protein (SBM) with NSM protein at 0%, 50% and 100%. This means that NSM was used instead of SBM in starter diets at 0, 24.7 and 50%, and in finisher diets at 0, 22 and 44%. Moreover, the different diets given to broiler chickens raised under high ambient temperature from 1 to 45 days of age were supplemented or not by an enzyme cocktail (0.5g/kg). A total of 210 broilers were divided into 6 treatments, each consisting of 7 replicates of 5 chicks each. The results of the interaction showed that complete replacement of SBM with NSM at 50 and 44% in the starter (1–30 d) and finisher (31–45 d) diets, respectively, did not negatively affect growth, feed intake, feed conversion ratio (FCR), carcass characteristics, inner organs, meat quality, and immune organs. However, the interaction between the level of NSM used and the supplementation with the enzyme cocktail supplementation had significant effect on white blood cells, revealing that enzyme effect is related to the diet composition. Therefore, it could be concluded that *Nigella sativa* meal can replace all soybean meal and can be included at 50 and 44% in starter and finisher diets, respectively, without any negative effects on the performance and carcass yields of broilers raised under high ambient temperatures from 1 to 45 days of age.

INTRODUCTION

The use of cereals and oilseeds for biofuel significantly increased the prices of traditional protein supplements for poultry diets, which in turn led to a general increase in feed prices (Al-Harthi *et al*., 2009; Al-Saffar *et al*., 2012). Therefore, finding alternative protein resources has become an economic necessity (Attia *et al*., 2008). *Nigella sativa* seed meal (Kalonji or black cumin) is a by-product of *Nigella sativa* L., which is a widely distributed herbaceous plant present in different areas of the world, particularly in Mediterranean countries and Saudi Arabia. It is utilized as a medicinal plant, herb and spice in Asia, the Middle East and Africa (Akhtar *et al*., 2003).

Several studies have been conducted on the use of *Nigella sativa* meal (NSM) in broiler diets, but complete replacement of soybean meal (SBM) protein with NSM protein has not yet been tested. Moreover, the effect of NSM on carcass characteristics, meat quality and lymphoid organs also needs further research. In addition, the impact of enzymes supplementation on the feeding value of NSM as a main protein in the diet has not been studied either. On the other hand, results on the use of NSM protein as a partial replacement for SBM protein are encouraging. For example, NSM could be successfully included up to 15% as a protein replacement in the diets of growing chickens (Attia *et al*., 2008; El-Deek *et al*., 2009). Moreover, the results of studies by Abdou

(2004) and Attia *et al*. (2008) indicated that enzyme supplementation improved growth performance and permitted an increasing level of NSM in chickens' diets. Similar results were reported by Abudabos (2012) and Kaczmarek *et al*. (2013).

Exogenous enzymes such as cellulase, xylanase, lipase, amylase, protease, pectinase, arabinase, α-galactosidase, and β-glucosidase, or their mixture, are widely used in the poultry industry. Their use improves feed utilization and even expands the use of raw materials, due to their similar approach to autoclaving/ pelletizing procedures, which helps reduce the effect of antinutritional factors and improves digestive processes and gut ecology, leading to improved utilization of nutrients, and thus improving the production and the health of poultry (Al-Harthi *et al*., 2018; Alqhtani *et al*., 2022; Al-Harthi *et al*., 2023). Previous studies have shown that the use of exogenous enzymes containing proteases and carbohydrases can improve protein, energy, calcium, and phosphorus utilization in broilers (Cowieson *et al*., 2006a, 2006b). Moreover, the utilization of exogenous enzymes targeting non-starch polysaccharides and other indigestible compounds may enhance endogenous digestion by breaking down the cell-wall-encapsulated nutrients (Masey-O'Neill *et al*., 2014; Mahmood *et al*., 2017; Al-Harthi *et al*., 2020).

In hot regions, the performance of broilers is lower due to low feed utilization and the consequently low growth rate. Moreover, *Nigella* seeds have several nutritional benefits and protective effects, and have ben recommended as a medicinal plant for decades (Akgul 1989; Al-Ghamdi, 2001; Akhtar *et al*., 2003; Al-Ghamdi, 2003). Thus, this study was designed to investigate the effects of *Nigella sativa* seed meal protein when entirely replacing soybean meal protein in the feed, with and without the supplementation of an enzyme cocktail, to broilers raised under high ambient temperature, on performance, carcass traits, meat quality, lymphoid organs and blood constituents.

MATERIALS AND METHODS

Ethical approval

This study was conducted at the Hada Al-Sham Research Station, Faculty of Environmental Sciences, King Abdulaziz University, Jeddah, Saudi Arabia. The methodology of the feeding experiment was verified by the Committee of the Agriculture Department, King Abdulaziz University, regarding the regulations on scientific research ethics on living creatures that regulates the rights and welfare of animals according to the Royal Decree No. M/59 dated 24/8/2010. The study was approved under institutional approval code ACUC-22-1-2.

In the experiment, unsexed day-old Arbor Acres broiler chicks (n = 210) were randomly distributed among 6 dietary treatments, each consisting of 7 replicates of 5 chicks each. The groups were fed experimental diets (starter from 1 to 30 days of age, and finisher from 31 to 45 days of age, Table 1), in which SBM protein was replaced with NSM protein at 0%, 50% and 100%. This means that NSM was used instead of SBM in starter diets at 0%, S-NSM-

Table 1 – Calculated and determined composition of experimental diets.

	Starter diets (%)			Finisher diets (%)		
	Nigella seed meal protein, %					
	0	50	100	0	50	100
Ingredients						
Maize	48.50	48.18	42.00	54.00	44.00	44.00
Soybean meal (44%)	37.0	18.50	0.00	31.0	16.50	0.00
Nigella seed meal	0.00	24.7	50.00	0.00	22.00	44.00
Corn gluten meal (60%)	3.00	3.00	3.00	3.00	3.00	3.00
Vegetable oil blend (%)	5.30	1.50	1.00	6.80	7.00	3.50
Sodium chloride	0.30	0.30	0.30	0.30	0.30	0.30
Dl-methionine	0.25	0.18	0.10	0.18	0.23	0.28
L-lysine	0.16	0.39	0.60	0.19	0.39	0.62
Dicalcium phosphate	2.00	1.80	1.70	1.50	1.38	1.30
Limestone	0.90	1.00	1.00	0.90	0.90	0.90
Vit. and min. mixture*	0.30	0.30	0.30	0.30	0.30	0.30
Washed building sand	2.29	0.15	0.00	1.83	4.00	1.80
Calculated and determined composition (%)						
ME MJ/ kg diet‡	12.67	12.67	12.70	13.42	13.42	13.42
Dry matter†	90.22	89.56	89.92	89.71	89.71	89.71
CP†	21.89	21.89	21.89	18.71	18.71	18.71
Ca‡	0.92	0.93	0.93	0.83	0.83	0.83
Non-phytate phosphorus‡	0.51	0.50	0.51	0.41	0.41	0.41
Methionine‡	0.60	0.59	0.59	0.50	0.50	0.50
Sulphur amino acids‡	0.97	0.97	0.97	0.87	0.87	0.87
Lysine‡	1.27	1.27	1.27	1.15	1.15	1.15
Tryptophan‡	0.32	0.32	0.32	0.29	0.29	0.29
Threonine‡	0.80	0.80	0.79	0.69	0.69	0.69
Arginine‡	1.41	1.41	1.41	1.32	1.32	1.32
Crude fibre†	5.56	5.52	5.56	4.65	4.65	4.65
Crude fat†	8.83	8.76	8.45	13.91	13.91	13.91

[1]Vit+Min mix. provides per kilogram of the diet: vit. A 1.700 IU, vit. E 13 IU, vit. K1 mg, vit. D3 250 ICU, riboflavin 4 mg, pantothenic acid 12 mg, niacin 40 mg, choline 1.500 mg, vit. B_{12} 0.02 mg, vit. Pyridoxine 4 mg, thiamin 2 mg, folacin 1 mg, biotin 0.2 mg. Trace minerals (mg/kg of diet): Mn 70, Io 0.4, Zn 50, Fe 90, Cu 10, and Se 0.2 mg. †Determined values. ‡Calculated values.

free; 24.7%, S-intermediate-NSM; and 50%, S-high-NSM, and in finisher diets at 0%, F-NSM-free; 22%, F-intermediate-NSM; and 44%, F-high-NSM. Each experimental diet was fed to the groups alone or supplemented with an enzyme cocktail (Galzym is a product of Tex Biosciences (p) Ltd., India) at 0.5 g/kg diet, as recommended by the producer, making up a total of 6 dietary treatment groups. Experimental diets, were formulated to be isocaloric and isoproteic, and meet the nutrient requirements suggested by the National Research Council (NRC, 1994). Galzym is a multienzymes containing cellulase 100.000.000 U/kg, xylanase 1.500.000 U/kg, lipase 10.000 U/kg, amylase 125.000 U/kg, protease 15.000 U/kg, pectinase 30.000 U/kg, arabinase7.000 U/kg, α-galactosidase 10.000 U/kg, and β-glucosidase 10.000 U/kg.

Husbandry of chickens

Chicks were kept in battery brooders (50×50×50cm) under similar managerial and hygienic conditions. Water and mash diets were provided *ad libitum* over the experimental period. The brooding temperature was respectively 34, 32 and 30ºC during the 1st, 2nd and 3rd weeks of age, with 45% RH. Subsequently, the ambient temperature and relative humidity were 28ºC and 45% RH, respectively, during the period between 22–45 days of age.

Collection of data

Body weight and feed intake were recorded at 14, 30 and 45 days of age to calculate body weight gain (BWG) and feed conversion ratio. At the end of the experiment (45 days of age), 7 chickens from each treatment were randomly selected and slaughtered to determine carcass characteristics. In addition, lymphoid organs including thymus, spleen and Fabricius bursa were removed and weighed. Seven blood samples from each treatment were collected to determine total white blood cells (WBCs) (Hepler, 1966).

A 50/50 (w/w) sample of skinless breast and thigh meat (n=7 per treatment) was weighed and kept in an electric drying oven at 70 °C until a constant weight was reached. The dried flesh was finely ground through a suitable mixer, passed through a sieve (0.5 mm), and then carefully mixed and stored in tightly sealed glass containers for subsequent chemical analysis. The chemical composition was done according to the following procedures determined by the Association of Official Analytical Chemists (AOAC) (2004): dry matter, method number 934.01; crude protein, method number 954.01; ether extract, method number 920.39; ash, method number 942.05. The physical characteristics of the meat, such as the ability to hold water (WHC), meat tenderness, pH, and color intensity were determined in accordance with Bovera *et al*. (2012).

Statistical analysis

Data were analyzed using the GLM procedure of SAS® (SAS, 2003) using two-ways factorial analyses according to the 2-factors randomized complete block design of the experiment (3 × 2). Means difference was significantly tested using Student Newman-Keuls test at p 0.05 (SAS, 2003). Before analysis, all percentages were subjected to logarithmic transformation (log10 x + 1) to normalize data distribution. Data were reported based on the main effects and the interactions. When significative results were found, the differences were discussed.

RESULTS

Growth performance

The body weight gain of heat-stressed broilers was affected by the different dietary treatments, as shown in Table 2. Body weight gain was significantly affected ($p < 0.05$) by NSM levels during all the experimental periods. Using S-intermediate-NSM had a negative effect ($p = 0.0001$) up to 30 days of age compared to the control diet. A converse response was obtained using F-intermediate-NSM, and no difference ($p > 0.05$) from the control group was recorded during the entire period. However, less growth was observed with S-high-NSM and F-high-NSM. This decrease was also evident throughout the experiment, with the group showing a lower BWG than the others. Enzyme supplementation significantly increased ($p = 0.017$) the growth rate of broilers during the period 15–30 d by 5.3%.

There was no significant interaction ($p < 0.05$) between NSM levels and enzyme supplementation regarding growth rate throughout the experimental periods, with the exception of the first period (1–14 d), when there was superior ($p = 0.0001$) weight gain in the group fed NSM-free diet without enzymes supplementation than the same diet with enzymes supplementation and the other groups fed S-intermediate-NSM and S-high-NSM.

Feed intake and FCR are shown in Table 3. The different levels of NSM had no significant effect ($p>0.05$) on feed intake and FCR during most of the

experimental periods, except for the period between 1–14 days of age, when using the S-intermediate-NSM diet significantly decreased (p = 0.003) feed intake by about 22% compared with the other groups, and improved (p = 0.001) FCR by 17.8% compared with the S-high-NSM diet. Enzyme supplementation did not affect (p > 0.05) feed intake and FCR during the different experimental periods. In addition, there was no significant interaction (p > 0.05) between NSM levels and enzyme supplementation in respect to the feed intake and FCR during the different experimental periods.

Table 2 – The effect of different ratios of *Nigella* seed protein that were used instead of the same ratios of soybean meal protein with enzyme cocktail supplementation on body weight gain of broiler chickens from 1 to 45 d of age†.

Treatments (diets)		Body weight gain (g)/period			
		1–14 d of age	15–-30 d of age	31–45 d of age	1–45 d of age
Effect of *Nigella* seed meal protein					
S-F-NSM-free		252[a]	563[a]	743[b]	1558[a]
S-F-intermediate-NSM		217[b]	533[b]	801[a]	1551[a]
S-F-high-NSM		228[b]	463[c]	694[c]	1385[b]
SE		4.84	9.99	13.96	21.00
Effect of enzyme cocktail					
-		236	506[b]	734	1476
+		228	533[a]	758	1519
SE		3.94	8.14	11.38	17.12
Interaction between *Nigella* seed meal protein and enzyme cocktail					
S-F-NSM-free	-	265[a]	564	726	1555
S-F-NSM-free	+	238[b]	561	760	1559
S-F-intermediate-NSM	-	212[c]	513	789	1514
S-F-intermediate-NSM	+	221[bc]	552	813	1586
S-F-high-NSM	-	231[bc]	440	687	1358
S-F-high-NSM	+	224[bc]	486	701	1411
SE		6.84	14.13	19.74	29.70
Analysis of variance					
Nigella		0.0001	0.0001	0.0001	0.0001
Enzyme		0.146	0.017	0.137	0.118
Interaction		0.028	0.165	0.865	0.356

†n=7 replicates of 5 chicks per treatment; SE=standard error; means within a column not sharing similar superscripts are significantly different (p < 0.05).
S-F-NSM-free=starter (1-30 d, 0 % NSM)-finisher (31-45 d, 0 % NSM) diets.
S-F-intermediate-NSM=starter (1-30 d, 24.7 % NSM)-finisher (31-45 d, 22 % NSM) diets.
S-F-high-NSM=starter (1-30 d, 50 % NSM)-finisher (31-45 d, 44 % NSM) diets.

Carcass characteristics and inner organs

Carcass characteristics and inner organs are presented in Table 4. NSM level had no significant influence (p > 0.05) on heart, liver, proventriculus and gizzard percentages. However, the dressing percentage was significantly increased (p = 0.030) in broilers fed S-F-intermediate-NSM diets compared to the control group. On the other hand, intestine percentage was increased (p = 0.0001) when broilers were fed S-F-high-NSM diets (complete replacement of SBM by NSM) compared to the other groups. Using the previous diets induced a similar result with pancreas percentage, which was higher (p = 0.001) than the control. Relative (p = 0.0001) and absolute (p = 0.0001) weights of abdominal fat were progressively decreased with increasing NSM levels. Enzymes or their interaction (NSM levels x enzymes) had no significant effects (p > 0.05) on carcass characteristics.

Meat quality

The physical traits and chemical composition of the meat are shown in Table 5. Neither NSM level, nor enzyme supplementation, nor their interaction affected (p > 0.05) meat quality traits.

Lymphoid organs

The relative weights of lymphoid organs (Fabricius bursa, thymus and spleen) and white blood cells are presented in Table 6. No significant effects (p > 0.05) due to NSM inclusion level, enzymes supplementation, or their interaction were detected in lymphoid organs.

Also, no significant effects (p > 0.05) of different NSM levels or enzymes addition on the WBCs were recorded. However, there was a significant interaction (p = 0.002) between NSM levels and enzymes supplementation in WBCs, which indicated that enzymes supplementation to NSM-free diets induced an increase in WBCs compared with the group on the same diets but without enzymes addition, and there was no difference in respect to groups fed with S-F-intermediate-NSM diets with or without enzymes, while a significant reduction occurred when S-F-high-NSM diets with enzymes were used compared with a similar group without the addition of enzymes.

Table 3 – The effect of different ratios of *Nigella* seed protein that were used instead of the same ratios of soybean meal protein with enzyme cocktail supplementation on feed intake and feed conversion ratio of broiler chickens from 1 to 45 d of age†.

Treatments (diets)		Body weight gain (g)/period				Feed conversion ratio (g feed/g gain)			
		1–14 d of age	15—30 d of age	31–45 d of age	1–45 d of age	1–14 d of age	15—30 d of age	31–45 d of age	1–45 d of age
Effect of *Nigella* seed meal protein									
S-F-NSM-free		516[a]	909	1957	3382	2.05[b]	1.62	2.64	2.18
S-F-intermediate-NSM		400[b]	837	2015	3252	1.85[b]	1.57	2.52	2.10
S-F-high-NSM		513[a]	764	1972	3249	2.25[a]	1.66	2.84	2.35
SE		29.2	82.9	130	217	0.136	0.155	0.203	0.140
Effect of enzyme cocktail									
-		503	830	1994	3327	2.13	1.65	2.72	2.26
+		448	843	1969	3260	1.97	1.58	2.60	2.15
SE		75.4	213	336	561	0.351	0.400	0.525	0.361
Interaction between *Nigella* seed meal protein and enzyme cocktail									
S-F-NSM-free	-	554	919	1956	3429	2.09	1.63	2.69	2.21
S-F-NSM-free	+	477	899	1958	3334	2.00	1.60	2.58	2.14
S-F-intermediate-NSM	-	395	784	2003	3182	1.86	1.53	2.54	2.10
S-F-intermediate-NSM	+	404	890	2027	3321	1.83	1.61	2.49	2.09
S-F-high-NSM	-	561	788	2022	3371	2.43	1.79	2.94	2.48
S-F-high-NSM	+	464	739	1922	3125	2.07	1.52	2.74	2.21
SE		130	371	583	997	0.607	0.693	0.909	0.626
Analysis of variance									
Nigella		0.003	0.322	0.156	0.221	0.001	0.188	0.623	0.072
Enzyme		0.120	0.658	0.293	0.312	0.077	0.793	0.425	0.328
Interaction		0.541	0.847	0.620	0.714	0.612	0.582	0.532	0.430

†n=7 replicates of 5 chicks per treatment; SE=standard error; means within a column not sharing similar superscripts are significantly different (p < 0.05).
S-F-NSM-free=starter (1-30 d, 0 % NSM)-finisher (31-45 d, 0 % NSM) diets. S-F-intermediate-NSM=starter (1-30 d, 24.7 % NSM)-finisher (31-45 d, 22 % NSM) diets. S-F-high-NSM=starter (1-30 d, 50 % NSM)-finisher (31-45 d, 44 % NSM) diets.

Table 4 – The effect of different ratios of *Nigella* seed protein that were used instead of the same ratios of soybean meal protein with enzyme cocktail supplementation on carcass characteristics and inner organs of broiler chickens from 1 to 45 d of age†.

Treatments (diets)		Dressing (%)	Heart (%)	Liver (%)	Proventriculus (%)	Gizzard (%)	Intestine (%)	Pancreas (%)	Abdominal fat (%)	Abdominal fat (g)
Effect of *Nigella* seed meal protein										
S-F-NSM-free		66.07[b]	0.498	1.95	0.487	1.751	4.10[b]	0.226[b]	2.095[a]	29.53[a]
S-F-intermediate-NSM		72.72[a]	0.503	1.99	0.512	1.933	4.33[b]	0.262[ab]	0.772[b]	10.14[b]
S-F-high-NSM		70.08[ab]	0.488	1.97	0.538	1.690	5.85[a]	0.298[a]	0.128[c]	1.88[c]
SE		1.464	0.020	0.064	0.022	0.070	0.223	0.016	0.126	1.819
Effect of enzyme cocktail										
-		70.21	0.481	2.02	0.490	1.777	4.62	0.273	0.979	14.16
+		69.02	0.512	1.92	0.534	1.805	4.89	0.250	1.017	13.53
SE		1.195	0.016	0.052	0.018	0.057	0.182	0.013	0.103	1.485
Interaction between *Nigella* seed meal protein and enzyme cocktail										
S-F-NSM-free	-	66.00	0.446	2.08	0.443	1.798	4.33	0.231	1.989	29.13
S-F-NSM-free	+	66.13	0.550	1.82	0.531	1.704	3.86	0.220	2.201	29.92
S-F-intermediate-NSM	-	75.10	0.528	2.00	0.550	1.988	4.03	0.286	0.829	11.57
S-F-intermediate-NSM	+	70.33	0.479	1.98	0.473	1.878	4.63	0.238	0.715	8.70
S-F-high-NSM	-	69.54	0.468	1.97	0.477	1.545	5.50	0.302	0.120	1.77
S-F-high-NSM	+	70.61	0.508	1.96	0.598	1.834	6.19	0.293	0.136	1.98
SE		2.070	0.028	0.090	0.031	0.099	0.316	0.022	0.178	2.573
Analysis of variance										
Nigella		0.030	0.955	0.900	0.104	0.226	0.0001	0.001	0.0001	0.0001
Enzyme		0.338	0.213	0.087	0.303	0.429	0.334	0.219	0.997	0.793
Interaction		0.146	0.070	0.139	0.063	0.117	0.071	0.921	0.922	0.907

†n=7 replicates of 5 chicks per treatment; SE=standard error; means within a column not sharing similar superscripts are significantly different (p < 0.05).
S-F-NSM-free=starter (1-30 d, 0 % NSM)-finisher (31-45 d, 0 % NSM) diets. S-F-intermediate-NSM=starter (1-30 d, 24.7 % NSM)-finisher (31-45 d, 22 % NSM) diets. S-F-high-NSM=starter (1-30 d, 50 % NSM)-finisher (31-45 d, 44 % NSM) diets.

Table 5 – The effect of different ratios of *Nigella* seed protein that were used instead of the same ratios of soybean meal protein with enzyme cocktail supplementation on physical traits and chemical composition of broiler chickens from 1 to 45 d of age†.

Treatments (diets)	Physical traits				Chemical composition			
	pH	Color	Tenderness (cm^2/g)	WHC (cm^2/g)	DM (%)	CP (%)	Lipid(%)	Ash (%)
Effect of *Nigella* seed meal protein								
S-F-NSM-free	5.71	0.24	9.00	15.89	24.99	19.18	4.66	1.06
S-F-intermediate-NSM	5.68	0.24	9.05	15.95	25.01	19.15	4.69	1.07
S-F-high-NSM	5.71	0.24	9.27	15.84	25.00	19.19	4.68	1.06
SE	0.023	0.006	0.069	0.046	0.038	0.040	0.033	0.008
Effect of enzyme cocktail								
-	5.70	0.24	9.09	15.87	24.99	19.16	4.68	1.06
+	5.69	0.23	9.12	15.91	25.00	19.19	4.66	1.07
SE	0.019	0.005	0.056	0.038	0.031	0.033	0.027	0.007
Interaction between *Nigella* seed meal protein and enzyme cocktail								
S-F-NSM-free -	5.68	0.24	9.09	15.90	25.02	19.18	4.68	1.06
S-F-NSM-free +	5.73	0.24	8.91	15.87	24.95	19.18	4.63	1.06
S-F-intermediate-NSM -	5.70	0.24	9.12	15.87	24.99	19.14	4.69	1.06
S-F-intermediate-NSM +	5.65	0.23	8.97	16.02	25.02	19.16	4.68	1.08
S-F-high-NSM -	5.73	0.25	9.06	15.84	24.97	19.16	4.67	1.06
S-F-high-NSM +	5.68	0.23	9.48	15.83	25.03	19.22	4.68	1.06
SE	0.032	0.008	0.284	0.195	0.053	0.057	0.046	0.012
Analysis of variance								
Nigella	0.620	0.713	0.628	0.881	0.918	0.763	0.793	0.423
Enzyme	0.520	0.126	0.874	0.844	0.879	0.581	0.632	0.609
Interaction	0.287	0.517	0.527	0.875	0.422	0.870	0.748	0.479

†n=7 replicates of 5 chicks per treatment; SE=standard error. S-F-NSM-free=starter (1-30 d, 0 % NSM)-finisher (31-45 d, 0 % NSM) diets. S-F-intermediate-NSM=starter (1-30 d, 24.7 % NSM)-finisher (31-45 d, 22 % NSM) diets. S-F-high-NSM=starter (1-30 d, 50 % NSM)-finisher (31-45 d, 44 % NSM) diets. pH=hydrogen power. WHC=water-holding capacity. DM=dry matter. CP=crude protein.

Table 6 – The effect of different ratios of *Nigella* seed protein that were used instead of the same ratios of soybean meal protein with enzyme cocktail supplementation on lymphoid organs and white blood cells of broiler chickens from 1- to 45 d of age†.

Treatments (diets)	Fabricius Bursa (%)	Thymus (%)	Spleen (%)	WBCs 1X 10^3/ mm
Effect of *Nigella* seed meal protein				
S-F-NSM-free	0.201	0.376	0.093	24.00
S-F-intermediate-NSM	0.218	0.319	0.086	24.17
S-F-high-NSM	0.216	0.285	0.081	23.58
SE	0.013	0.025	0.008	0.226
Effect of enzyme cocktail				
-	0.199	0.301	0.089	23.91
+	0.224	0.352	0.083	23.93
SE	0.011	0.021	0.006	0.184
Interaction between *Nigella* seed meal protein and enzyme cocktail				
S-F-NSM-free -	0.175	0.358	0.079	23.22^{bc}
S-F-NSM-free +	0.227	0.394	0.106	24.78^{a}
S-F-intermediate-NSM -	0.237	0.284	0.106	24.28^{a}
S-F-intermediate-NSM +	0.199	0.353	0.065	24.06^{ab}
S-F-high-NSM -	0.186	0.261	0.082	24.22^{a}
S-F-high-NSM +	0.246	0.309	0.079	22.94^{c}
SE	0.019	0.036	0.011	0.319
Analysis of variance				
Nigella	0.565	0.063	0.702	0.288
Enzyme	0.121	0.148	0.538	0.726
Interaction	0.094	0.854	0.133	0.002

†n=7 replicates of 5 chicks per treatment; SE=standard error; means within a column not sharing similar superscripts are significantly different ($p < 0.05$). S-F-NSM-free=starter (1-30 d, 0 % NSM)-finisher (31-45 d, 0 % NSM) diets. S-F-intermediate-NSM=starter (1-30 d, 24.7 % NSM)-finisher (31-45 d, 22 % NSM) diets. S-F-high-NSM=starter (1-30 d, 50 % NSM)-finisher (31-45 d, 44 % NSM) diets. WBCs=white blood cells.

DISCUSSION

Based on the results found here for the varying inclusion of NSM at different levels and enzymes, *Nigella* seed meal can be considered a potential vegetable protein source capable of replacing all soybean meal, and could be included up to respectively 50 and 44 % in the starter and finisher diets (S-F-high-NSM) of broilers raised at high ambient temperature from 1 to 45 days of age without any enzyme supplementation. This conclusion is evidenced by the lack of negative effects on growth performance, carcass characteristics, meat quality, and immune organs.

However, using S-intermediate-NSM diet during the period 1–30 d of age caused depression in growth rate. This effect was age-dependent, as growth was higher than the control at 45 days and was similar to those fed soybean meal diet (the control) when calculated for the entire period.

The lack of adverse impact on the feed intake and FCR of broilers confirms the safety of utilizing NSM instead of SBM in broilers' diets. The positive effects of using NSM could be attributed to its ability to enhance the tolerance of broilers to high ambient temperature due to its active substances (nigellone and melanthin). These substances have a diversifying power and act synergistically to improve digestibility, and help with the eliminating and cleansing actions (El-Dakhakhny *et al*., 2000, 2002).

In addition, nigellone has both anti-spasmodic and anti-bronchitis properties that can improve respiratory tract health, a major problem under heat stress conditions. Thymoquinone, however, helps the body to get rid of toxins due to its anti-inflammatory, analgesic, antioxidant, and cleansing properties (Al-Ghamdi, 2001, Meral *et al*., 2001; Al-Ghamdi, 2003; Kanter *et al*., 2003). NSM also has an antimicrobial effect that could help chickens to stand up to hot climate conditions (Akgul, 1989; Abdel-Aal & Attia, 1993; Badary *et al*., 2000). These results are similar to those reported by Al-Homidan *et al*. (2002), Abdou (2004), Attia *et al*. (2008) and El-Deek *et al*. (2009), who observed that NSM could be utilized as a plant protein source in the diets of broilers and laying hens without a negative impact on the utilization of feed.

With respect to the entire period and to the interaction, enzyme supplementation to broilers fed S-F-high-NSM diets improved growth (1411 g) by 3.9% and FCR (2.21) by 10.9% compared with groups on the same diets but without enzyme supplementation. This resulted in complete recovery for FCR when compared with the control groups fed diets with or without enzymes supplementation; however, this improvement was not significant. The positive effect of enzymes was confirmed by Attia *et al*. (2008), Abdou (2004), Abudabos (2012) and Kaczmarek *et al*. (2013).

By looking at the interaction effect on growth rate (Table 2), it is interesting to see that the weight gain of broilers (1-14 days of age) fed on the control diet with enzymes addition was significantly lower than that of their counterpart group, fed on the same diet but without enzymes addition (10%, 238 g vs. 265 g, respectively). Also, it is interesting that the weights of all groups fed on NSM diets were not affected by the addition of enzymes. However, responses regarding the use of external enzymes in animal's diets are considered somewhat complex issues. This is due to the many factors that impact the interaction between enzymes and the animal. These factors include, but are not limited to, the type of animal, age, production purpose, the type of enzymes used, whether they are product from bacteria or fungi, whether it is a single or several enzymes, whether the enzymes are added with other additives such as yeast and/or citric acid, the dose, the period of use, and whether they are added to the diet or the water. Another set of potentially important aspects are the circumstances of the experiment, such as the type of feed (in terms of its chemical and physical properties), the proportion of each component in the diet, environmental factors (e.g. temperature and humidity). All these factors can affect the results of using enzymes among the different studies. The literature shows that the matching between enzyme activity and substrate is essential to obtain the desired effects (Bedford 2000, 2003; Al-Harthi, 2006; Choct, 2006; Attia *et al*., 2008).

In this regard, some studies have found positive effects of using enzymes on broilers and/or laying hens (Torki *et al*., 2016; Abd El-Hack *et al*., 2017; Turgay *et al*., 2018), others noted no effect (Roberts & choct, 2006; Khajali *et al*., 2007; Cufadar *et al*., 2010; Al-Saffar *et al*., 2012, Al-Harthi, 2017; Al-Harthi *et al*., 2023), while others reported negative effects (Roberts *et al*., 1999; Yaghobfar *et al*., 2011; Al-Harthi *et al*., 2018, Olgun *et al*., 2018). Many scientists have tried to explain, as much as possible, the reasons for these different responses when using enzymes in animals' diets. For example, some of them explained the positive effect by their role in helping animal to digest diets, as well as improving the microbial environment in their intestinal tract. Some of them explained the absence of any results, whether positive or negative,

to the ineffectiveness of the enzymes used, or to the weakness of the dose given. Usually, the dose used is dependent on the recommendation of the companies that produce these enzymes. These doses are determined by using traditional feedstuffs that are most common in such companies' experiments. However, this does not represent the reality in many researchers' experiments, where enzymes are used with unconventional ingredients that are included in the diet composition to be tested as feed components.

The negative response from enzymes addition was usually explained as being caused by a prevention of the secretion of the animals' endogenous enzymes as an adaptive response, in other words as a physiological reaction of the animal. Others attributed this negative effect to the presence of anti-nutritional factors in the diet that hindered the secretion and/or actions of these internal enzymes, and consequently will act similarly on the external enzymes. The two control diets in this experiment (NSM-free dies) had similar conditions, only differing in terms of enzyme addition. The negative effect of enzyme addition on growth during the period between 1-14 days of age can be explained as an adaptive response, a physiological reaction, which decreases the secretion of endogenous enzymes, that was accompanied by the weakness of the action of the external enzymes. In fact, this hypothesis is supported by the lower feed intake in this group when compared with its counterpart group without enzyme supplementation (14%, 477 g vs. 554 g, respectively; Table 3). Despite the difference in feed intake not being significant, this can indicate the weakness of the digestive tract to deal with the feed consumed, leading to a reduction in the total amount of feed intake. Moreover, the lack of effect observed for these added enzymes on weight gain between these two control groups during the other periods, and also among the NSM groups during all periods, can also assure their ineffectiveness, at least at the dose used in these diets' composition. Regarding the adaptive response, there was a noted reduction in the relative weight of the pancreas to the live body weight when α-amylase enzyme was used in the broilers' diet, which indicated internal adaptation of the enzymes' secretion comparable to the quantity of external enzyme intake (Gracia *et al*., 2003). Similar findings have been previously recorded, whereby the secretion of pancreatic amylase and proteases (trypsin and chymotrypsin) were reduced when broilers were given diets that included amylase and protease (Mahagna *et al*., 1995). On the other hand, adaptation was found in heat-stressed broilers when they were given diets containing triiodothyronine hormone (T3), while this could not help compensating for its reduction in the broilers' blood resulting from heat stress (Al-Harthi *et al*., 2002).

Regarding the effect of NSM level, it is worth mentioning that broilers fed S-F-intermediate-NSM diets had a greater dressing percentage than those fed NSM-free diets (the control), although they had similar weight gains at 45 days of age. This could be explained by the greater amount of abdominal fat in the control group, so when this large amount was removed, a decreased dressing percentage was obtained.

With regard to the NSM level effect under the environmental heat stress, it is difficult for the animal to get rid of the heat produced by its body as a result of metabolism, and thus this energy will be stored in the body as fat, which in turn leads to an increase in the quantity of body fat deposition. However, in this study, using NSM in the broilers' diet led to a decrease in the amount of stored body fat as NSM levels increased compared to those fed on the control diets (NSM-free diets). This indicates that using NSM in broilers' diets played a very important role in the metabolism of these fats, which finally led to a decrease in the amount of fat stored (29.53 g, 10.14 g, 1.88 g, S-F-NSM-free, S-F-intermediate-NSM, S-F-high-NSM, respectively; Table 4). This positive effect can be attributed to the previously mentioned active substances in NSM. Also, these results agree with those reported by Al-Harthi (1997), Badary *et al*. (2000), Meral *et al*. (2001), Kanter *et al*. (2003), and Jahan *et al*. (2015).

Dietary NSM has arguably helped broilers to increase their ability to withstand heat stress conditions, which should be considered a remarkable result. This encourages conducting in-depth studies on the use of NSM in the diets of heat-stressed broilers and laying hens to confirm these results. Such a confirmation will contribute significantly and effectively to the use of NSM in poultry' diets that are exposed to heat stress, a major problem in the poultry industry. Confirming such results would mean reducing heat-stress-related diseases and mortality in poultry.

Enzyme supplementation and the interaction between NSM levels and enzymes did not significantly affect carcass characteristics and inner organs. These results are similar to those found by Abdou (2004), Attia *et al*. (2008), El-Deek *et al*. (2009) and Abudabos (2012). Also, the lack of significant effect among dietary treatments on meat quality traits is in line with those reported by Attia *et al*. (2008).

It was clear from the interaction in respect to diets without enzyme supplementation that using S-F-intermediate-NSM and S-F-high-NSM diets led to an increase in WBCs compared to the NSM-free diet. Akgul (1989) and Akhtar *et al*. (2003) indicated that *Nigella* seeds have antimicrobial, anti-inflammatory, analgesic, antioxidant and cleansing properties (Al-Ghamdi, 2001; Meral *et al*., 2001; Al-Ghamdi, 2003; Kanter *et al*., 2003;).

The present results also suggest that the effect of enzymes depend on the NSM level, whereby enzymes supplementation increased WBCs with broilers fed a NSM-free diet, but decreased WBCs in S-F-high-NSM diets, and did not affect WBCs with the S-F-intermediate-NSM diets. These results are similar to those reported by Bedford (2000, 2003) and Choct (2006).

CONCLUSION

According to the results found here, and by focusing on the interaction among the different treatments, *Nigella sativa* meal could replace all soybean meal, and could be included at 50 and 44 % in starter (1–30 d) and finisher (31–45 d) diets (S-F-high-NSM), respectively. This use will not induce negative effects on the growth performance, carcass characteristics, inner organs, meat quality and immune organs of broilers raised under high ambient temperature from 1 to 45 d of age. In addition, the interaction between the level of NSM used and the supplementation with the enzyme cocktail supplementation had significant effect on white blood cells, revealing that enzyme effect is related to the diet composition

DATA AVAILABILITY STATEMENT

Data of the current study is available from the authors upon request.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

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