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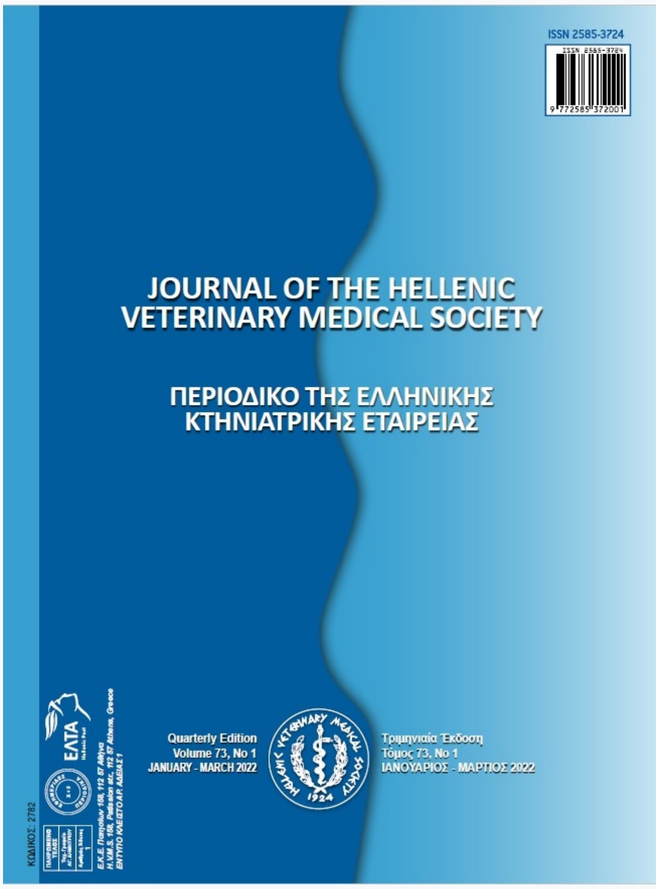

The effects of thyme essential oil and enzyme supplementation to corn or wheat based diets on performance, carcass traits and blood metabolites in broilers

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Research article
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Effect of thyme essential oil and enzyme supplements on performance, carcass and blood metabolites in broilers

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ABSTRACT: This experiment was conducted to evaluate the effects of thyme essential oil and enzyme supplementation to corn/wheat based diets on performance, carcass traits and blood metabolites in broilers. This experiment was carried out with 432 Ross-308 broilers (54.3±0.18) as a factorial arrangement with 36 units (12 birds per replicate) in a completely randomized design from 1 up to 42 days. Results showed that the basic ration and meal type significantly affected the performance (P<0.05). The use of wheat increased feed intake (FI) and feed conversion ratio (FCR), while soybean increased FI and weight gain (WG) and improved FCR (P<0.05). Meat and blood parameters and blood parameters were not affected by the experimental treatments (P>0.05). Use of enzyme supplement or thyme essential oil in treatments containing wheat is recommended, because it can improve broilers performance parameters.

Keywords: Broiler, Blood metabolites, Enzyme, Performance and Thyme essential oil

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INTRODUCTION

Thyme (*Thymus vulgaris*) was originally planted in the Mediterranean and currently cultivated in gardens and fields as an aromatic and medicinal plant (Çiftçi et al., 2018). Thyme active substances can stimulate the secretion of pancreatic enzymes, thereby improving digestion efficiency and absorption of protein and other nutrients from the bird's gastrointestinal tract (Hashemipour et al., 2016). The willing for the elimination of growth promoting antibiotics to poultry diets led to a gradual increase in the use of medicinal plants and their derivatives as safer alternatives (Yadav et al., 2016; Naghi Shokri et al., 2017; Devi et al., 2018; Huang et al., 2018; Cimrin et al., 2020; Sevim et al., 2020). These substances have the potential to promote, along with growth, other positive aspects such as improving health (Attia et al., 2017; Mehdi et al., 2018; Yesilbag et al., 2020) and improving the gut microbial population (Adeyemo et al., 2016; Rahman and Yang, 2018; Abd El-Ghany and Eraky, 2019).

Reducing the cost combined with the saving of the ration can play a crucial role in the final price of the poultry products, which may increase their consumption. Alongside these advantages, the use of wheat instead of corn in poultry diets also has some limitations, including the presence of inhibitors such as non-starch polysaccharides and phytates in wheat grain (Yaghobfar and Kalantar, 2017).

There are various methods for reducing antinutrients in feeds, such as using enzyme supplements and essential oils of medicinal plants. These enzymes degrade antinutritional factors and break down their complex, thus increase protein digestibility and improve poultry performance.

Wade et al. (2018) reported that broilers which received 100 mg / kg of thyme had higher weight gain (WG). Cross et al. (2007) studied the effects of thyme essence on growth, digestibility, and intestinal microflora of broilers, stating that using thyme essence had positive effects on poultry performance. Cao et al. (2010) found that supplementation of thyme essential oil in the poultry diet significantly improved feed intake (FI) ($P<0.05$).

Pournazari et al. (2017) reported that the use of thyme extract increased live weight (LW) and FI. In the study by Çiftçi et al. (2018) thyme-containing treatments reduced serum glucose, triglyceride, total cholesterol, uric acid and total protein in Japanese quail *(Coturnix coturnix Japonica)*. Hedayati and Manafi (2018) reported that probiotics and herbal composition (including thyme extract) increased daily WG and decreased feed conversion ratio (FCR).

Hosseini and Mimandipour (2018) reported that thyme extract increased daily WG and improved FCR. Witkowska et al. (2019) reported that thyme extract had no adverse effect on poultry health and increased the immune response of poultry. Sugout and Mohammed (2019) suggested that thyme powder could be used as a growth promoter in the poultry diet of 1% without adverse effects on poultry. In the study of Abo-Eid et al. (2019), thyme extract showed the best anticholesteryl activity and reduced crude protein in poultry. Therefore, the aim of this study was to determine the effects of thyme essential oil and enzyme supplementation on performance and blood metabolites.

MATERIALS AND METHODS

In this experiment, 432 Ross-308 broilers (54.3±0.18) were divided into three factorial trials with 36 units. The tested factors were the carbohydrate (corn and wheat), the meal type (soybean and rapeseed) and the additive type (thyme essential oil and enzyme). The experiment was conducted in a completely randomized design in two periods, including growing (11 to 24 days) and finisher (24 to 42 days). Diets conformed to the advised levels of nutrients, as established by the Ross-308 broiler nutrition specification, and using the UFFDA software program (Hamidi et al., 2021).

In the first three days, the saloon lighting was continuous, and from the fourth day it changed to 23 hours light and 1 hour dark. In the first day, the saloon temperature was 34 °C. From the first week onwards and every two weeks, the temperature was dropping by 2 degrees. In the sixth week, the temperature was set at 20 °C and remained until the end of the experiment.

Feed conversion ratio was calculated considering the wastage and specifying the age of chickens.

The chemical compounds of enzyme and thyme extract, used in the experiments, are listed below (Tables 1 and 2).

Table 1. Chemical composition of thyme essential oil (GC-MS analysis)

Chemical composition	amount
α - Pinene	1.47
Camphene	0.03
β - Pinene	0.04
Myrcene	0.05
ρ - Cymene	14.32
Limonene	0.24
Ocimene	0.09
Terpinene	22.46
3, 8 - ρ - Menthadine	0.05
Linalool Oxide	0.1
Fenchone	0.02
Linalool	4.96
4- Terpineol	1.39
Thymol	43.84
Cavacrol	10.94

Table 2. Chemical composition of COMBO enzyme Bland 10X

Chemical composition	amount
Cellulase	>750.000 CU Unite/Kg
Fungal Amylase	>300.000 SKB Unite/Kg
Fungal Protease	>10.000.000 HUT Unite/Kg
Nutral Protease	>1.000.000 PC Unite/Kg
Alkaline Protease	>12 Anion Unite/Kg
Xylanase	>200.000 XU Unite/Kg
Beta Glucanase	>200.000 BG Unite/Kg
Hemicellulase	>200.000 HCU Unite/Kg
Lipaze	>750.000 FIP Unite/Kg
Pectinase	Activities Present
Mananase	Activities Present

In this study, the body weight (BW) of the broilers and the daily feed intake (FI) were recorded. Health status and mortality rates were recorded daily during the experimental period. The resulting data was used to calculate the feed conversion ratio (FCR).

At the end of the experimental period, on the 42nd day, two birds per replicate (one male and one female) were selected and were slaughtered to determine carcass traits. The percentage of the carcass was determined comparing heart weight, abdominal fat, gizzard, liver, breast, and thigh weights to the corresponding carcass weights. At the day 42, blood samples from 3 birds per treatment were randomly taken via the wing vein. The samples were centrifuged (3000 rpm × 10 min) after clotting (2h) to obtain serum and stored at -20ºC before analysis. Glucose, cholesterol, triglyceride, albumin and high-density lipoprotein-cholesterol (HDL) were measured using commercial kits (Pars Azmoon, Tehran, Iran) (Sigolo et al., 2019).

Data were subjected to statistical analysis according to a completely randomized design as a factorial arrangement of 2 × 3 using the general linear model procedure of SAS 9.4 (2018). Means were compared using Tukey's tests at 5% probability, according to the following model:

$$Y_{ijkl}=\mu+A_i+B_j+C_k+AB_{ij}+AC_{ik}+BC_{jk}+ABC_{ijk}+\varepsilon_{ijkl}$$

Where Y_{ijkl} = dependent variable, μ = overall mean, A_i = the effect of Factor A, Bj = the effect of Factor B, C_k = the effect of Factor C, ABij = Interaction of Factors A×B, AC_{ik} = Interaction of Factors A×C, BC_{jk} = Interactivity of Factors B×C ABC_{ijk} = Interaction of Factors A×B×C and ε_{ijkl} = the random error.

RESULTS

Feed Intake

As can be seen in Table 3, the main effects of the basal diet, meal, and the interactions between the basal diet × type of meal, the basal diet × additive and the type of meal and additive had a significant effect on FI in the grower period (P <0.05). In the finisher periods, the main effects of the basal diet, and the interaction effects of the basal diet and the type of the meal had a significant effect on FI (P <0.05). However, other experimental treatments were not statistically significant (P >0.05).

Weight Gain

The main effects of meal type as well as the interaction effects of dietary meal × meal type, basal diet × additive and meal type × additive had a significant effect on weight gain (P <0.05). In the finisher periods, the main effects of the basal diet (corn or wheat) as well as the interactions of the basal diet × meal type had a significant effect on weight gain (P <0.05).

Feed Conversion Ratio

As shown in the Table 3, the main effects of the basal diet, meal, and the interactions between the basal diet × type of meal, the base diet × additive and the type of meal and additive had a significant effect on the FCR (P <0.05).

Table 3. Effect of supplementation thyme essential oil and enzyme on daily weight gain (WG; g/chick/day), feed intake (FI, g/chick/duration), and feed conversion ratio (FCR)

	Growing period (12-21 day)			Finisher period (22-42 day)		
Treatments	FI	WG	FCR	FI	WG	FCR
Ration type						
Corn	54.36b	40.34	1.35b	159.12b	77.23b	2.06
Wheat	56.97a	39.42	1.44a	162.2a	80.01a	2.02
SEM	0.083	1.031	0.003	0.032	0.284	0.19
Meal type						
Soybean	58.74a	40.03a	1.47b	157.02	79.84	1.97
Rapeseed	55.12b	35.27b	1.56a	156.94	78.03	2.01
SEM	0.083	1.031	0.003	0.032	0.284	0.19
Supplementation type						
Essential oil	55.93	36.05	1.55	156.89	76.25	2.05
Enzyme	56.01	36.42	1.54	157.01	76.94	2.04
SEM	0.083	1.031	0.003	0.032	0.284	0.19
Ration × Meal						
Corn × Soybean	59.4a	40.19a	1.48d	159.35ab	78.04b	2.04
Corn × Rapeseed	57.39ab	37.95b	1.51c	158.79b	77.43b	2.05
Wheat × Soybean	56.24bc	36.49c	1.54b	160.35ab	79.32a	2.02
Wheat × Rapeseed	54.73c	34.78d	1.57a	161.01a	79.95a	2.01
SEM	0.842	0.019	0.002	0.812	0.472	0.009
Ration × Supplementation						
Corn × Essential oil	59.12a	40.53a	1.46b	159.72	79.54	2.01
Corn × Enzyme	59.33a	40.64a	1.46b	159.91	79.65	2.01
Wheat × Essential oil	55.18b	36.12b	1.53a	160.01	79.78	2.00
Wheat × Enzyme	55.39b	36.25b	1.53a	161.20	80.01	2.01
SEM	1.032	1.074	0.005	0.947	0.663	0.004
Meal × Supplementation						
Soybean × Essential oil	57.83a	38.72a	1.49a	157.32	77.01	2.04
Soybean × Enzyme	58.02a	39.37a	1.47b	158.12	77.13	2.05
Rapeseed × Essential oil	54.94b	35.29b	1.56b	155.73	76.63	2.03
Rapeseed × Enzyme	54.98b	35.78b	1.54c	156.49	76.95	2.03
SEM	0.642	0.532	0.004	1.735	0.812	0.012
Ration × Meal × Supplementation						
Corn × Soybean × Essential oil	59.85	40.89	1.46	158.25	78.32	2.02
Corn × Soybean × Enzyme	59.97	40.93	1.46	159.12	79.17	2.01
Corn × Rapeseed × Essential oil	57.72	39.76	1.45	157.02	77.64	2.02
Corn × Rapeseed × Enzyme	58.03	40.12	1.45	157.34	77.83	2.02
Wheat × Soybean × Essential oil	56.89	39.00	1.46	159.26	79.97	1.99
Wheat × Soybean × Enzyme	56.95	39.12	1.46	160.24	80.12	2.00
Wheat × Rapeseed × Essential oil	55.78	38.74	1.44	159.87	79.22	2.02
Wheat × Rapeseed × Enzyme	56.01	38.92	1.44	160.32	79.49	2.02
SEM	1.825	1.027	0.009	2.021	1.324	0.027

Carcass traits

The effect of using different feeds on carcass traits of broilers at the end of the experimental period is shown in Table 4. Experimental treatments had no significant effect on carcass traits ($p > 0.05$).

Table 4. Effect of supplementation thyme essential oil and enzyme on carcass traits (%)

Treatments	Carcass	Breast	Tights	Abdominal fat	Gizzard	Liver	Heart
Ration type							
Corn	71.22	34.19	25.14	2.53	2.35	3.67	0.743
Wheat	70.37	34.58	25.76	2.45	2.42	3.45	0.735
SEM	0.714	0.526	0.326	0.166	0.078	0.088	0.029
Meal type							
Soybean	70.79	34.26	25.59	2.08	2.42	3.60	0.778
Rapeseed	70.71	34.02	25.02	2.84	2.60	3.65	0.685
SEM	0.714	0.526	0.326	0.166	0.078	0.088	0.029
Supplementation type							
Essential oil	72.16	34.29	26.52	2.66	2.60	2.76	0.765
Enzyme	70.88	34.38	25.33	2.60	2.15	3.43	0.687
SEM	0.714	0.526	0.326	0.166	0.078	0.088	0.029
Ration × Meal							
Corn × Soybean	71.11	34.65	25.33	2.76	2.51	3.42	0.733
Corn × Rapeseed	69.13	34.74	25.42	1.98	2.46	3.49	0.775
Wheat × Soybean	70.31	34.53	25.59	2.22	2.44	3.53	0.784
Wheat × Rapeseed	70.38	34.34	25.08	2.86	2.63	3.48	0.755
SEM	0.683	0.702	0.249	0.178	0.095	0.101	0.048
Ration × Supplementation							
Corn × Essential oil	69.66	34.63	24.94	1.98	2.57	3.46	0.836
Corn × Enzyme	69.95	34.52	24.39	2.26	2.52	3.49	0.827
Wheat × Essential oil	70.15	34.22	25.18	2.53	2.60	3.55	0.779
Wheat × Enzyme	71.71	34.55	25.38	2.61	2.49	3.77	0.758
SEM	0.683	0.702	0.249	0.178	0.095	0.101	0.048
Meal × Supplementation							
Soybean × Essential oil	69.36	33.74	24.10	2.03	2.55	3.44	0.875
Soybean × Enzyme	71.21	33.79	24.86	2.75	2.62	3.78	0.706
Rapeseed × Essential oil	72.18	34.41	24.97	2.73	2.71	3.81	0.663
Rapeseed × Enzyme	73.28	34.71	25.22	2.79	2.59	3.65	0.753
SEM	0.683	0.702	0.249	0.178	0.095	0.101	0.048
Ration × Meal × Supplementation							
Corn × Soybean × Essential oil	71.92	33.90	26.23	2.19	2.64	3.74	0.720
Corn × Soybean × Enzyme	70.21	34.31	24.82	2.92	2.58	3.52	0.663
Corn × Rapeseed × Essential oil	71.04	34.79	25.54	2.60	2.50	3.71	0.866
Corn × Rapeseed × Enzyme	72.75	35.77	25.11	2.55	2.08	3.23	0.723
Wheat × Soybean × Essential oil	70.76	34.38	24.76	2.67	2.55	3.34	0.740
Wheat × Soybean × Enzyme	66.69	35.19	26.23	2.11	2.42	3.63	0.736
Wheat × Rapeseed × Essential oil	69.01	32.98	27.93	2.65	2.22	3.63	0.650
Wheat × Rapeseed × Enzyme	71.46	34.91	25.91	2.85	2.47	3.51	0.726
SEM	1.278	0.996	0.647	0.374	0.187	0.155	0.052

Blood Parameters

The effect of the different treatments on serum metabolites is presented in Table 5. Experimental treatments had no significant effect on serum metabolites (p >0.05).

Table 5. Effect of supplementation thyme essential oil and enzyme on blood biochemical parameters (mg/dL)

Treatments	Glucose	Cholesterol	Triglyceride	Abumin	HDL
Ration type					
Corn	117.49	149.23	32.95	1.61	88.96
Wheat	159.73	172.58	40.90	1.71	96.32
SEM	29	32	12	0.165	5.57
Meal type					
Soybean	140.92	135.02	47.72	1.94	78.40
Rapeseed	176.23	130.96	39.77	1.92	71.36
SEM	29	32	12	0.165	5.57
Supplementation type					
Essential oil	131.12	100.50	64.77	1.40	61.76
Enzyme	144.22	95.43	82.95	1.42	62.05
SEM	29	32	12	0.165	5.57
Ration × Meal					
Corn × Soybean	153.46	144.16	61.37	1.63	89.60
Corn × Rapeseed	145.54	158.37	64.24	1.68	91.52
Wheat × Soybean	141.58	153.29	64.77	1.74	95.04
Wheat × Rapeseed	150.16	157.92	65.28	1.81	91.84
SEM	23	25	14	0.213	4.39
Ration × Supplementation					
Corn × Essential oil	154.13	95.43	81.81	1.74	71.36
Corn × Enzyme	154.45	124.87	87.50	1.76	74.24
Wheat × Essential oil	157.09	108.62	88.11	1.71	88.96
Wheat × Enzyme	156.76	145.17	115.90	1.79	89.60
SEM	23	25	18	0.501	4.87
Meal × Supplementation					
Soybean × Essential oil	160.72	121.82	37.17	1.79	58.24
Soybean × Enzyme	169.96	125.13	34.10	1.68	57.14
Rapeseed × Essential oil	168.12	130.95	40.95	1.11	61.06
Rapeseed × Enzyme	170.87	132.66	44.32	1.50	62.19
SEM	23	25	15	0.612	5.03
Ration × Meal × Supplementation					
Corn × Soybean × Essential oil	157.57	153.29	45.45	1.88	95.07
Corn × Soybean × Enzyme	160.72	166.49	87.60	1.93	96.32
Corn × Rapeseed × Essential oil	169.96	157.36	32.95	1.97	100.01
Corn × Rapeseed × Enzyme	118.48	150.32	34.09	2.05	99.53
Wheat × Soybean × Essential oil	172.60	174.49	64.27	1.89	104.00
Wheat × Soybean × Enzyme	185.80	168.22	59.09	2.21	102.54
Wheat × Rapeseed × Essential oil	177.23	169.37	62.35	1.92	99.98
Wheat × Rapeseed × Enzyme	184.00	176.08	61.08	2.64	89.33
SEM	32	27	17	0.812	5.76

DISCUSSION

Feed Intake

On the one hand, the present data showed that wheat diets contained more than the double concentration of soluble NSP relative to corn diets, which may be implicated in the higher jejunal digestion viscosity observed in birds fed wheat diets, and this may have increased their feed intake due to the lack of energy supply. On the other hand, the relative increase in FI in experimental groups containing wheat can be due to various causes, including fiber and stimulation of the secretory and digestive tracts in the stomach, intestine, liver, pancreas and bile. Cross et al. (2007) stated that the use of thyme oil improves the FCR in broilers but has no significant effect on WG and FI. Shakouri and Kermanshahi (2007) reported that using enzyme leads to a significant increase in daily FI of broilers and also Zakeri and Kafashi (2011) reported that using enzyme had a significant effect on daily FI of broilers. Non-starch polysaccharides such as pectin, cellulose and amyloid in canola in the intestine absorb a large amount of water, increasing the concentration and viscosity of the digestive contents. Increased viscosity decreases the speed of digestion and reduces FI.

Weight Gain (WG)

It was predictable that non-viscous feed ingredients such as corn result in better weight gain than viscous feed ingredients such as wheat which have high levels of soluble and viscous NSP, but it turned out quite differently. However, the interactions of enzyme and grain type clearly showed the role of enzyme addition in this matter. On the other hand, probably due to the high levels of NSP in this feed, it has led to an increased viscosity in the poultry intestine. Viscosity increases the length of the small intestine by increasing absorption, and consequently, it increases weight gain. Another possibility is that using wheat may have led to increased WG due to the digestive system's palatability or stimulation (due to high fiber), leading to increased WG, which is advisable at this stage of the report. Rapeseed meal could be used as an economically viable alternative to soybean meal in poultry diets. The significant reduction in performance parameters of broiler chicks during the growing period could be the result of the high glucosinolate content of the experimental diets. The tolerance to glucosinolates in younger birds is less, which impairs thyroid functions. As the birds grow, the thyroid develops, and mature birds can tolerate a relatively high amount of glucosinolates (Mnisi and Mlambo, 2018). Nobakht et al. (2010) reported that using different levels of nettle, mackerel, and cactus herbs in early and growing periods had significant effects on broiler performance. The results of this experiment were in agreement with the results of Nasiri et al. (2011). Shakouri and Kermanshahi (2007) reported that the use of the enzyme resulted in a significant increase in the DWG of broilers that did not match the results obtained in this study.

Feed Conversion Ratio (FCR)

Starch is the dominant energy source in corn. The significant improvement in ileal starch digestibility could have contributed to the increase in available energy content of the corn diet, and higher energy efficiency with less feed intake also leads to improved FCR. The higher WG and reduced FCR in canola treatments could be attributed to humic acid and enzymes, which may have improved the feed efficiency given the reduced feed intake (Disetlhe et al., 2018). Similarly, to our results, Al-Harthi (2017) reported that supplementation of poultry diet with phytase enzyme improves FI, BW and FCR of broilers. Improvement of digestion and absorption of nutrients in poultry intestine, by medicinal plants, is related to the increased secretion of digestive enzymes such as lipase, amylase and protease, theincreased digestibility, and the increased intestinal length and depth and number of villi. Nutrient retention in the gut is higher, which gives more opportunities for them to be absorbed. (Yadav and Jha, 2019).

Carcass traits

Due to the increasing poultry age, the digestive system's development led to improving the secretion of enzymes required for digestion. It resulted that experimental diets did not significantly affect carcass traits (Bautil et al., 2019; David et al., 2020). Improving digestion and absorption of nutrients in poultry intestines during the use of medicinal plants is related to the effect of medicinal plants on increasing the secretion of digestive enzymes, such as lipase, amylase and protease and increasing digestive function, increasing intestinal length and depth and number of villi (Thapa et al., 2019; Sharma et al., 2019). Since the use of essential oils of medicinal plants reduced the harmful microbial population of the gastrointestinal tract, the rate of proteins and amino acids degradation was decreased, and more of them were absorbed and stored in the body.

Blood Parameters

Experimental treatments had no effect on serum glucose, cholesterol, triglyceride, albumin and high-density lipoprotein-cholesterol (HDL) concentrations. Blood biochemical parameters are closely associated with health status and play their role as important indicators of health conditions in birds. Together with other serum metabolites, albumin concentration indicates the adequacy of protein in the diet and the efficiency with which the broilers are utilizing it; therefore, both protein and energy sources have been able to meet the needs of poultry equally (Yang et al., 2018; Hatab et al., 2020). Saki et al. (2014) reported that dietary inclusion of phytogenic feed additives did not change the serum cholesterol and triglyceride levels in laying hens. Soltan et al. (2008) demonstrated that aniseed supplementation in the broiler diet did not affect the broiler serum cholesterol levels. Our results were in accordance with Saki et al. (2014) and Soltan et al. (2008) reports.

CONCLUSION

Even though increased viscosity, wheat in the diets led to an increase in the length of the gastrointestinal tract and improved performance parameters. This study recommends using enzymes or essential oils to reduce antinutrients' negative aspects and increase performance.

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CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationship that could influence the work reported in this paper.

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