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Gas Chromatography-Mass Spectroscopy Analysis and Evaluate Cumin Seeds and Their Essential Oil as Growth Promoters of New Zeland White Rabbits

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Abstract: Present study was aimed to investigate the chemical composition of cumin seeds essential oil by using Gas Chromatography-Mass Spectroscopy (GC-MS), also evaluate the effect of cumin seeds at two levels (0.25 and 0.50%) and its essential oil at four levels (25, 50, 100 and 200 mg kg⁻¹ b.wt.) on growing New Zealand White (NZW) rabbits performance. Furthermore, a change in blood constituents was measured as indicators of metabolic enzymes. GC-MS data indicated that 28 constituents were identified, representing (91.37%) of the total amount of essential oil. Significant effects of cumin seeds and its essential oil on growing performance, digestibility and some metabolic enzymes functions were observed at some levels either from cumin seed or its essential oil.

Key words: Cumin, rabbits, promoters, feed efficiency, carcass, blood constituents

INTRODUCTION

Growth promoters are widely used nowadays in the commercial production of large and small animal flocks as well as poultry farms and antibiotics have been widely used in the poultry industry for several decades. Manipulation of gut functions and microbial habitat of domestic animals with feed additives has been recognized as an important tool for improving growth performance and feed efficiency (Collington et al., 1990). Up to 80% of domestic animals have been fed synthetic compounds for the purpose of either medication or growth promotion (Lee et al., 2001). The ban on the use of antibiotics as feed additives has accelerated and led to investigations of alternative feed additives in animal production. Herbal plants and their essential oils are already used as feed supplements to improve growth performance under intensive management systems (William and Losa, 2001; Gollnisch and Halle, 2001). In this respect, influence of some medicinal plants on performance; physiological and meat quality traits of broiler chicks has been investigated (Korczak and Grabowicz, 2003; Al-Harthi, 2004; Hassan et al., 2004). Also, Ibrahim et al. (2000), Rohilla and Bujarbaruah (2003), Das and Bora (2004) and Das and Khan (2004) applied some herbal and medicinal plants as growth promoters to broiler rabbits. On the other hand Meineri and Peiretti (2008) reported the influence of an blue-green alge on growth performance in growing rabbits and Williams and Lamprecht (2008) recorded the using of some commonly fed herbs in equine nutrition.

Cumin (*Cuminum cyminum* L.) is a small herbaceous annual plant of the Umbelliferae family and used as a condiment and an ingredient in many food industries. Cumin seeds yields 2.5-4.0% essential oil which has reputation as an important economic drug because of their stomach, diuretic, carminative, stimulant, astringent and their biological activities (antimicrobial, antifungal, antiviral, antitumor and

anti-inflammatory) (Floris *et al.*, 1996; Nalini *et al.*, 1998; Kalemba and Kunicka, 2003) and hypoglycemic agents (Ali, 2001). Cumin seed meal as an alternative to wheat bran in commercial laying hen diets have been evaluated by Behzad *et al.* (2006).

The present study aimed to analysis of essential oil in more details by using GC-MS, also evaluate the effect of cumin seeds at two levels (0.25 and 0.50%) and its essential oil at four levels (25, 50, 100 and 200 mg kg⁻¹ b.wt.) on growing New Zealand White (NZW) rabbits performance. Also, a change in blood constituents was measured as indicators of metabolic enzymes.

MATERIALS AND METHODS

The experiments was carried out at Poultry Farm; Central Laboratory and Biochemistry Department, Faculty of Agriculture, Cairo University, Giza, Egypt during academic year 2007-2008.

Plant Materials

Cumin seeds were purchased from Egyptian local market and the extraction of the essential oils was by distilled with water in glass apparatus. The volatile oil was separated and dried over anhydrous sodium sulphate (American Spice Trade Association, 1968).

GC-MS Analysis

Essential oil was analyzed by using a Gas chromatography- Mass Spectroscopy (HP 6890 series) equipped with Selective Detector Mass spectroscopy (HP 5973). This equipment interfaced via HP chemstation version A02.12 software (HP, Avondale, PA). The gas chromatography was equipped with carbowax 20 M capillary column, 50 m X 0.53 mm i.d, 1.5 m thickness (J and W Company, CA, USA). Operating condition for GC were: Injector temperature 250°C, carrier gas, Helium at 30 cm sec⁻¹, measured at 130°C. Oven temperature: 50°C for 5 min, 2°C min⁻¹ to 250°C and held at 250°C for 15 min, detector temperature: was 300°C. Mass spectroscopy operating parameters were: electron ionization at 70 eV, accelerating voltage 10 kV and scan M/Z range from 40-650. The identification of oil constituents was carried out by comparing retention times with those of authentic reference compounds, or peak-matching library research using the standard mass library (NIST Standard Mass library, version 2.0) and available literature (Adams, 2007).

Determination of LD₅₀ in Mice

LD₅₀ in mice was determined to detect the dose to be given to rabbits. Graded doses of the essential oil of cumin seed in tween as emulsifier was administered intraperitonealy to six groups of six non fasted male albino mice (25-30 g each). Mortality was start noted after 1 h.

Animal Experiment

Eighty-four growing male New Zealand White (NZW) rabbits at 6 weeks of age (720-790 g) were used. Animals were randomly divided into 7 groups 9 rabbits for each in 3 replicates (4 rabbits for each), control group and six experimental groups. All groups were kept under the same managerial and hygienic conditions. These groups were fed on the diets as shown in Table 1. Dried cumin seeds were grounded and used at two levels 0.25 and 50%. Experimental diets were formulated to be iso-caloric and iso-nitrogenous according to National Research Council (1977). The experimental rabbits were housed in individual batteries provided with feeders and drinkers. Experimental groups 2 and 3 were fed on experimental diets. Groups 1, 4, 5, 6 and 7 were fed on control diet. Groups 4, 5, 6 and 7 were received cumin oil suspend in saline solution and tween as emulsifier by interaperitoneal injection at four doses 25, 50, 100 and 200 mg kg⁻¹ b.wt. once every week for 8 weeks. Rabbits were weighed weekly intervals during the experimental period. At the same time, feed consumption and feed conversion were recorded. At the last week of experiment, digestion trails were carried out by using

Table 1: Composition and chemical analysis of the experimental diets

	Cumin seed			
Ingredients	Control	0.25%	0.50%	
Alfalfa hay	30.50	30.20	29.90	
Wheat bran	25.10	25.10	25.10	
Barley	23.00	23.00	23.00	
Soybean meal (44% CP)	15.00	15.00	15.00	
Molasses	3.00	3.00	3.00	
Vit /min mix ⁻¹	0.30	0.30	0.30	
Salt	0.45	0.45	0.45	
Di-cal phosphate	1.77	1.77	1.77	
lime stone	0.53	0.53	0.53	
DL-methionine	0.35	0.35	0.35	
Cumin seeds		0.25	0.50	
Total	100	100	100	
CP	17.62	17.60	17.59	
DE ² (kcal kg ⁻¹)	2517	2523	2529	
CF	9.67	9.67	9.67	
EE	4.10	4.10	4.10	
Ca	1.17	1.17	1.16	
T. phos.	0.80	0.80	0.80	
Lysine	0.86	0.86	0.86	
Methionine	0.60	0.60	0.60	

 $^1\text{Each}$ kg of premix contains: Vit. $A_2.000.000\text{IU};$ Vit. $D_31500\text{IU};$ Vit. E 8.339 g; Vit. K0.33 g; Vit. B $_10.33$ g; Vit. B $_2$, 1.0 g; Vit. B $_6$ 0.33 g, Vit. B $_9$ 8.33 g; Vit. B $_{12}$ 107 mg; Pantotheanic acid 3.33 g; Biotine 33 mg; Mg 66.7 mg; Folic acid 0.83; Choline Chloride 200 g; Zn 11.7 g; Fe 12.5 g; Cu 0.5 g; Se 16.6 mg and Mn 59 mg. $^2\text{Digestible energy}$ (DE) kcal kg $^{-1}$ was calculated according to Cheeke (1987). DE (kcal g $^{-1}$) = 4.36 - 0.0491 (% NDF) NDF = 28.294+0.657 (% CF)

3 male rabbits from each group to determine the digestible coefficient and feeding values of cumin essential oil (Radwan et al., 1997). At the end of experimental period, three representative male rabbits from each group were randomly chosen and fasted for 12 h before slaughter according to Blasco et al. (1993) for determine the carcass traits and blood samples were collected. Serum was separated and used for determination of total protein (Peters, 1968), albumin (Dumas et al., 1971), total lipid (Zollner and Kirsch, 1962), total cholesterol (Wgbenga and Inkpen, 1974), triglyceride (Fossati and Prencipe, 1982), transaminase (AST, aspartate aminotransferase and ALT alanine aminotransferase) (Reitman and Frankel, 1957) and creatinine (Husdan and Rapopart, 1968) using commercial kits. Proximate analysis was carried out on representative samples using the conventional methods of Association of Official Analytical Chemists (1990).

Statistical Analysis

All data were subjected to analysis of variance using the general linear models (SAS, GLM), main effect differences obtained upon statistical analysis were compared using Duncan's multiple range test (Hoppe and Dunnett, 1993).

RESULTS AND DISCUSSION

GC-MS Result

Twenty eight constituents were identified, representing (91.37%) of the total amounts. Nine hydrocarbon monoterpens identified (19.84%), were α -terpinene (4.54%), β -pinene (3.60%), p-cymene (2.73%), γ -terpinene(2.45%), α - pinene (1.88%), γ -careen (1.87%), α -phellandrene (1.22%), myrcene (0.91%) and Sabinene (0.64%). Five oxygenated monoterpens identified (50.43%) were cumin aldehyde (43.32%) as the major components, terpinene-4-ol (2.76%), thymol (2.26%), cumin alcohol (1.88%), α -terpineol (0.21%). The results showed that 19.93% of oil was sesquiterpenes (Table 2). Five sesquiterpenes hydrocarbons (15.49%) identified were β -caryophyllene (5.50%),

Table 2: GC-MS analysis of cumin seeds essential oil

Name of compound	Retention time (Rt)	Percentage
Monoterpene hydrocarbons		
α-Pinene	4.54	1.88
Sabinene	5.76	0.64
β-Pinene	6.22	3.60
Myrcene	9.56	0.91
α-Phellandrene	9.87	1.22
γ-Carene	10.34	1.87
α-Terpinene	10.67	4.54
p-Cymene	10.80	2.73
γ-Terpinene	11.76	2.45
Oxygenated monoterpene		
Terpinene-4-ol	12.34	2.76
α -terpineol	14.54	0.21
Cumin aldehyde	16.11	43.32
Thymol	17.04	2.26
Cumin alcohol	17.98	1.88
Sesquiterpene hydrocarbons		
cis bergamotene	19.54	1.11
β-Caryophyllene	20.63	5.50
β- Farnesene	22.77	0.87
Acoradiene	24.21	5.09
Cuparene	14.87	2.92
Oxygenated sesquiterpenes		
Caryophyllene oxide	15.22	0.22
Carotol	15.81	0.13
Daucol	17.21	0.09
Acids and esters		
Propyl tiglate	20.65	1.65
Hydrocinnamyl acetate	22.87	0.22
Benzoic acid	26.11	0.08
Anisyl acetate	28.91	0.06
Menth-8-ene-3-ol, acetate	30.42	0.17
Hexadecanoic acid	36.76	4.87
Total		91.37
Unknown		8.63

acoradiene (5.09%), cuparene (2.92%), *cis* bergamotene (1.11%) and β-Farnesene (0.87%). Three oxygenated sesquiterpenes (0.44%) were caryophyllene oxide (0.22%), carotol (0.13%) and daucol (0.09%). Among acids and esters two acids hexadecanoic acid (4.87%) and benzoic acid(0.08%), and four esters Propyl tiglate (1.85%), Hydrocinnamyl acetate (0.22%), Menth-8-ene-3-ol, acetate (0.17%) and Anisyl acetate (0.06%) were identified. Anon (1993), Baser *et al.* (1992), Shaath and Azzo (1993), Ali, (2001) and Salma *et al.* (2002) reported that the major component of cumin seeds oil was cuminaldehyde. It has been emphasized by Baser *et al.* (1993) that cumin oil contained low percentage of cuminaldehyde (20%) and high percentage of terpens (up 70%), this not in line with our results. Generally, chemical composition of essential oil is different in the same plant during season and affect by storage period, so analysis of each used source of essential oil is important.

Growth Performance

Results in Table 3 represented the effect of diets contained cumin or cumin essential oil on growing rabbits. Compared with control group average live body weight and weight gain were significantly (p<0.05) increased at levels 0.25% cumin seeds and 100 mg kg⁻¹ b.wt. of oil. While, insignificant increase was observed at levels 0.50% cumin seeds, 50, 150 and 200 mg kg⁻¹ b.wt. cumin essential oil. It is interesting to notice that rabbits fed the 0.25% cumin seeds and 100 mg kg⁻¹ b.wt. cumin oil had the highest values of weight gain may be due to the biological functions of the main

Table 3: Growth performance parameters of growing NZW rabbits

		Cumin s	` '	Cumin essential oil (mg kg ⁻¹ b.wt.)						
Body weight (g)	Control	0.25	0.50	50	100	150	200	Sig.	Pooled SEM	
Initial	754ª	780ª	765°	790°	770ª	750°	720ª	NS	0.43	
at 4 weeks	1437ª	1320a	1350°	1383ª	1371ª	1316ª	1386ª	NS	62.19	
at 7 weeks	1767°	1960ª	$1800^{\rm b}$	1765c	1765°	1951 ^b	1818 ^b	*	54.35	
Weight gain (g)										
1-4 weeks	683ª	540°	585ª	593⁴	601ª	566ª	666ª	NS	65.31	
4-7 weeks	330°	640°	450 ^b	382⁰	394ª	635 ^b	432 ^b	*	57.13	
1-7 weeks	1013°	1180^{a}	1035^{b}	975°	995ª	$1201^{\rm b}$	1098⁰	*	64.21	
Feed intake (g)										
1-4 weeks	2150a	2040^{ab}	1910^{b}	2039^{ab}	2018^{ab}	2038ab	1944 ^b	*	90.00	
4-7 weeks	1595 ^b	2000^{ab}	2015ab	1892^{ab}	1981ab	1893ab	2103a	*	111.00	
1-7 weeks	3745^{b}	4040a	3925ab	3931ab	3998^{ab}	3931ab	4046a	*	154.00	
Feed conversion										
1-4 weeks	3.15^a	3.60°	3.20^{a}	3.30^{a}	3.59 ^a	3.30^{a}	3.20^{a}	NS	0.25	
4-7 weeks	4.83ª	3.13^{b}	4.48a	4.80^{a}	3.12^{b}	4.23ª	4.45a	*	0.31	
1-7 weeks	3.70a	3.35a	3.75a	3.89⁴	3.34ª	3.69a	3.75a	NS	0.27	

Mean values in the same row differently superscripted letter(s) are significantly different (p≤0.05)

Table 4: Digestion coefficients and nutritive values of the experimental diets

		Cumin s	eed (%)	Cumin e	Cumin essential oil (mg kg ⁻¹ b.wt.)				
Diets	Control	0.25	0.50	50	100	150	200	Sig.	Pooled SEM
DM	74.8⁴	75.1ab	75.9ª	71.9°	70. 8 ⁶	67.8°	81.1ª	*	4.62
OM	76.04 ^b	77.3 ^b	$79.4^{\rm ab}$	73.3^{bc}	72.2°	70.2°	83.1ª	*	3.14
CP	77.3^{bc}	80.4^{b}	85.1ª	83.0^{ab}	78.7 ^{bc}	77.1°	87.2ª	*	2.56
EE	62.1^{bc}	64.70	70.5ª	65.8°	60.5°	60.1ª	70.9ª	*	4.36
CF	41.2^{b}	41.2^{b}	43.4^{a}	37.6 ^{bc}	44.1ª	35.0°	44.1ª	*	4.13
NFE	86.1ª	86.4^{ab}	85.7ª	85.1ª	86.2^{a}	85.2ª	86.2ª	*	5.17
ADF	47.2ª	47.1ª	49.1ª	43.9ª	49.5ª	41.4^{a}	49.7ª	NS	1.54
NDF	55.2ª	55.4ª	56.8ª	53.1ª	57.1ª	51.3a	57.3ª	NS	4.56

Mean values in the same row differently superscripted letter(s) are significantly different (p \leq 0.05)

component of essential oil. However, feed intake was significantly increased at levels 0.25% cumin seeds and 200 mg kg $^{-1}$ b.wt. cumin essential oil, while, insignificant increase was noticed at levels 0.50% cumin seeds, 50, 100 and 150 mg $^{-1}$ b.wt. cumin essential oil. The results concerning to feed conversion values, it revealed that rabbits fed on diet contained 0.25% cumin seeds and treated with cumin essential oil at level 100 mg kg $^{-1}$ b.wt. were significantly better than control group. On other hand, there was a slight improvement in feed conversion values was observed at levels 0.50% cumin seeds, 50, 150 and 150 mg kg $^{-1}$ b.wt. cumin essential oil.

Nutrients Digestibility and Nutritive Value

Data revealed that the dry matter (DM), organic matter (OM), crude protein (CP) ether extract (EE) and crude fiber (CF) digestibility values were significantly (p<0.05) decreased at level 150 mg kg⁻¹ b.wt. of cumin essential oil as compared with those of control group (Table 4). OM and CF digestibility values were significantly (p<0.05) decreased at level 100 mg kg⁻¹ b.wt. of cumin essential oil compared with control group. CP, EE and CF digestibility values were significantly (p<0.05) decreased at 0.50% of cumin seeds compared with control group. OM, CP, EE and CF digestibility values were significantly (p<0.05) increased at level 200 mg kg⁻¹ b.wt. of cumin essential oil, while DM value was slightly increased as compared with those of control group. Nitrogen Free Extract (NFE), ADF and NDF digestibility values were not affected by any treatment compared with those of control group.

Carcass Characteristics

The average pre-slaughter weight and empty caracass traits including dress, head, kidney, liver and heart percentage were not significantly differed among tested groups compared with control group (Table 5). The same results were found with edible part and Non edible part.

Meat Chemical Composition

The data presented in Table 6 showed that, no significant were detected for carcass protein fat and moisture content of rabbits meat among all treatments compared with the control group.

Blood Constituents

The values of plasma total protein, albumin, cholesterol, total lipid, triglyceride, creatine, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) of treatment groups and control group are shown in Table 7. It has been observed that total protein values were significantly higher in rabbits were treated with cumin essential oil at levels 100, 150 and 200 mg $\,\mathrm{kg^{-1}}$ b.wt. than those in the other treatments. The same results were found with albumin values while globulin values were slightly different as compared with those of control group except in the rabbits were treated with cumin essential oil at dose 150 mg $\,\mathrm{kg^{-1}}$ b.wt. were significantly higher than control group. As a matter

Table 5: Effects of additives on carcass characteristics

Characteristics		Cumin seed (%)		Cumin essential oil (mg kg ⁻¹ b.wt.)					
	Control	0.25	0.50	50	100	150	200	Sig.	Pooled SEM
Weight (g)									
Pre-slaughter	1770°	1950a	1810^{bc}	1780°	2000a	1870^{ab}	1840^{b}	*	45.37
Empty carcass	870ª	970ª	890ª	870ª	980ª	910a	900ª	NS	90.21
Dress	49.2ª	49.7ª	49.1ª	48.9ª	49.1ª	48.6^{a}	48.9ª	NS	0.86
Head	104ª	110^{a}	109ª	106ª	115ª	110^{a}	109ª	NS	5.33
Liver	51.91^{ab}	52.1ª	49.8°	52.3ª	52.2ª	$49.0^{\rm b}$	50.1^{ab}	**	0.67
Kidney	11.18⁴	11.23ª	11.1a	11.8°	10.5ª	12.0^{a}	11.7ª	NS	0.85
Heart	3.75a	3.80^{a}	3.90^{a}	4.12	3.74^{a}	4.30^{a}	4.10^{a}	NS	0.31
Total edible part	1041a	1147ª	1064ª	1043a	1161ª	1085a	1075ª	NS	75.40

a,b: Means in the same row differently superscripted are significantly different (p≤0.05)

Table 6: Effect of additives on chemical analysis of rabbits meat

		Cumin s	eed (%)	Cumin essential oil (mg kg ⁻¹ b.wt.)						
Rabbit's meat	Control	0.25	0.50	50	100	150	200	Sig.	Pooled SEM	
Protein	20.67^{a}	20.74ª	20.14ª	20.74ª	21.59 ^a	20.54ª	20.61ª	*	0.96	
Fat	5.64ª	5.86°	5.51ª	5.53°	6.01ª	5.05 ^a	5.83ª	*	1.06	
Moisture	74.25ª	74.97ª	73.67ª	73.87ª	75.46ª	74.21ª	74.10^{7a}	*	1.32	

Mean values in the same row differently superscripted letter(s) are significantly different (p $\!\leq\! 0.05)$

Table 7: Effect of additives on blood parameters

		Cumin seed (%)		Cumin essential oil (mg kg ⁻¹ b.wt.)						
Parameters	Control	0.25	0.50	50	100	150	200	Sig.	Pooled SEM	
Total protein	8.12^{b}	11.02^{ab}	11.5a	7.31 ^b	12.06a	14.11 ^a	12.54ª	*	2.31	
Albumin	4.13^{b}	6.11^{ab}	6.8^{ab}	4.23^{b}	7.42ª	8.0°	7.04ª	oje	3.74	
Globulin	3.99^{b}	4.33ª	4.7ab	3.28^{b}	4.64^{ab}	6.11ª	5.04ª	oje	54.35	
Total lipid	250^{b}	300^{ab}	350^{a}	310^{ab}	315ª	320^{a}	360°	*	6.31	
Triglyceride	260°	310^{ab}	345a	280°	300^{ab}	330^a	376ª	oje	6.48	
Cholesterol	50.17 ^a	30.63^{b}	28.72^{b}	45.15°	29.22ª	25.04 ^b	32.1^{b}	*	4.85	
Creatine	0.91ª	0.73ª	0.96a	0.85a	0.79⁴	0.71ª	0.81ª	NS	0.32	
AST	20.12^{b}	24.51a	25.76°	18.66°	22.34ª	24.07°	26.31a	*	2.54	
ALT	17.58°	19.21ª	18.09ª	15.76 ^b	18.2ª	17.07ª	18.44ª	NS	2.54	

Mean values in the same row differently superscripted letter(s) are significantly different (p≤0.05)

of fact, blood proteins have considerable importance owing to their function. It is capable to binding with toxic compounds rendering their harmless. Moreover, proteins reserve as nutritional materials for developing cells (Murray et al., 2003) albumin and globulin have importance for immunity. Results showed that the plasma total lipids values of rabbits fed diets contain cumin seeds 0.25 and those were treated at dose 50 mg kg⁻¹ b.wt. were not affected compared with control group. While the plasma total lipids values of rabbits fed diets contain cumin seeds 0.50% and those were treated with cumin essential oil at doses 100, 150 and 200 mg kg⁻¹ b.wt. were significantly increased compared with control group. On the other hand the plasma triglycerides values of rabbits fed diets contain cumin seeds 0.25% and at doses 50 and 100 mg kg⁻¹ b.wt. were not affected compared with control group, while at 0.50% and 150 and 200 mg kg⁻¹ b.wt. were significantly increased compared with control group. It was clearly noted that the plasma cholesterol values of rabbits fed diets contain cumin seeds 0.25 and 0.50% and those was treated at dose 50 mg kg⁻¹ b.wt. were not affected compared with control group. While at doses 100, 150 and 200 mg kg⁻¹ b.wt. were significantly decreased compared with control group. These results agreed with EL-Manylawi et al. (2003) who reported that addition of certain aromatic plants cumin fruits, thyme leaves and babul pods individually or mixed to control diet at two levels (0.10 and 0.20%) on the performance of growing rabbits significantly decreased plasma cholesterol levels.

In terms of AST and ALT activities it was clearly noted that AST values increased significantly of rabbits were fed on diets contained 0.25 and 0.50% of cumin seeds and those treated with cumin essential oil at doses 100, 150 and 200 mg kg⁻¹ b.wt. compared with control group. While AST values of rabbits treated with cumin essential oil at dose 50 mg kg⁻¹ b.wt. were not affected compared with control group. ALT values were not affected by any treatment compared with those of control group.

CONCLUSION

There is evidence to suggest that cumin seed and their essential oil has digestion-stimulating properties and significant effect on growth performance of rabbits under study without adverse effect. This conclusion is valid only for the experiments period observed in this study, particularly considering at treatment with dose 50 and 100 mg kg⁻¹ b.wt. of cumin essential oil. Although there is still a lack of evidence of the underlying mechanisms by which dietary essential oil affect growth performance. Cumin seeds and its essential oil contain different molecules may be have intrinsic bioactivities on animal physiology and metabolism.

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