Caraway (*Carum carvi* L.) in fast-growing and slow-growing broiler chickens' diets and its effect on performance, digestive tract morphology and blood biochemical profile

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ABSTRACT The aim of this study was to evaluate the addition of caraway (1%) in fast-growing and slow-growing broiler chickens' diet and its effect on performance parameters, blood biochemical profile, and relative organ sizes and ileum morphology in slow-growing broilers. Two separated experiments were performed. On the first day of age, the broilers were divided into 2 equal groups (Control and Caraway) with 6 replicates per treatment in both experiments. Experiment I: The total of 276 male fast-growing Ross 308 broiler chickens were used. The trial lasted from the first day to 35th day of chickens' age. Experiment II: The total of 216 male slow-growing (Hubbard JA 57) broilers were used. The trial lasted from the first to 50th day of chickens' age. Mean liveweight, weight gain, feed conversion ratio, blood biochemical parameters, and relative organ sizes were not significantly different in these trials. The group of slow-growing broilers supplemented with 1% of caraway in the diet showed longer villi and deeper crypt in the ileum after 50 d of life. Based on our results, it can be stated that the proportion of 1% caraway in fast-growing and slow-growing broiler chickens' diet did not influence performance parameters, blood biochemical profile and relative organ sizes. In case of the experiment with the slow-growing broilers supplemented with caraway, a significant difference in the height of the villi and the depth of the crypts was found. Caraway can be included in the broiler chickens' diets without negative effects, but further study of the effect on the intestinal morphology is necessary.

Key words: carvone, limonene, phytogenic feed, villus, ileum histomorphometry

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INTRODUCTION

Caraway (*Carum carvi* L.) is an annual herb in the *Apiaceae* family native to Northern America, western Asia, and Europe (Rasooli, 2016). The Czech Republic has suitable specific conditions for growing caraway, given by the soil composition and natural conditions based on tradition, as long-term experience in growing caraway is irreplaceable. With the introduction of nondeciduous caraway varieties, the Czech Republic, originally an importing country of this spice, became an exporting country of caraway (Jonák and Linhart, 2021). In the Czech Republic, the areas for caraway cultivation amounted to 2,755 ha in 2020 and the yield was 0.4 t/ha (Kozderová, 2020). Regarding this, caraway nonstandard grains and/or grain fragments could be a part of poultry diets with a potential health benefit due to the content of biologically active substances.

Carum carvi (L.) dried fruits (achenes) which are commonly called seeds contain 25 to 35% crude protein, 13 to 21% ether extract, 13 to 19% crude fiber, and 87 to 91% dry matter (Kocourkova et al., 1999; Ezz et al., 2010). Kozera and Majcherczak (2013) reported that caraway seeds from field without mineral fertilization contained 6.36 g/kg total phosphorus, 16.38 g/kg potassium, 6.55 g/kg calcium, 3.97 g/kg magnesium, 0.32 g/kg sodium. In addition to the nutrients content, the achenes contain 1 to 8% essential oils (**EO**) which give characteristic aroma and taste to it. The main constituents of these EOs are carvone and limonene with 95% proportion (Pank et al., 1996; Acimovic et al., 2015). Namely, carvone is present in approximately 73% and limonene is present approximately in 16.2 to 19.9% followed by linalool which ranged from 1.60 to 2.50% (Ezz et al., 2010).

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Limonene belongs to terpenes. It is one of the main bioactive substances found in aromatic plants and the most common terpene in nature. Limonene also oxidizes in contact with air and forms various oxidation products such as carvone, limonene oxide, carveol, and limonene hydroperoxides (Gupta et al., 2021). It was observed that limonene epoxide may reduce the lipid peroxidation level and may increase catalase and superoxide dismutase activities. In addition, limonene epoxide shows antioxidant and anxiolytic effects (de Almeida et al., 2014).

Carvone is a terpenoid ketone commonly occurring in nature in the form of the enantiomers (S)-(+)-carvone (S-carvone) and (R)-(−)-carvone (R-carvone). S-carvone is the major constituent (50−70%) of the *Carum carvi* (L.) oil, while R-carvone is a constituent of mint oil (60−70%) obtained mainly from *Mentha spicata* (Younis and Beshir, 2004). Carvone is a colorless or yellow oil, insoluble in water and miscible with ethanol which has potential uses for inhibiting the growth of bacteria (Naigre et al., 1996; Oosterhaven et al., 1996; Helander et al., 1998), fungi (Smid et al., 1995) and has antitumoral effects (Aydın et al., 2015; Moro et al., 2018). Caraway EOs also contain acetaldehyde, furfural, carveol, pinene, thujone, camphene, phelandrene etc. In caraway, the EOs are protected in oil ducts inside a hard peel and they are not easily accessible there (Bailer et al., 2001).

Generally, studies have shown that *Carum carvi* have antidyspeptic, antispasmodic, antiulcerogenic, antibacterial, anticancerogenic, antiproliferative, antioxidant, antihyperglycemic, antihyperlipidemic, and in addition diuretic effects (Crowell, 1999; Sedláková et al., 2003; Alhaider et al., 2006; Rasooli, 2016).

Nevertheless, it seems that addition of *Carum carvi* into poultry diets may induce differences in the relative weight of the digestive tract (Khajeali et al., 2012), for example, increase in the relative weight of the crop and increase in body weight and feed conversion ratio (Mansoori et al., 2006; Al-Kassi, 2009; Khajeali et al., 2012).

Caraway is a widely grown crop in the Czech Republic (from the category of medicinal, aromatic, and spicy plants) which by-products (nonstandard grains and/or grain fragments) could be a part of poultry diets with a potential health benefit due to the content of biologically active substances. Our work is the primary study to verify the suitability and possible positive or negative impact on the performance and health of chickens. A limited number of publications examining the effect of caraway in poultry diets have been published so far. A ban on use of antibiotics in animal nutrition is a promising direction because there is a possibility of use of alternative feedstuffs containing bioactive compounds or use of phyto additives or plant extracts or by-products. These natural origin substances may have benefits in the form of positive effects on the metabolism, health and thus animal performance (Stastnik et al., 2020).

Our hypotheses were − 1% of caraway in the broiler diets will have an effect on performance, biochemical blood parameters, individual sections of the digestive tract and in slow-growing chickens on the morphology of the ileum.

The aim of this study was to evaluate the effects of 1% addition of caraway in fast-growing and slow-growing broiler chickens' diets on performance parameters, blood biochemical profile, and digestive tract morphology.

MATERIALS AND METHODS

Experimental Conditions

The animal procedures were reviewed and approved by the Animal Care Committee of Mendel University in Brno and by the Ministry of Education, Youth and Sports (**MSMT**-21593/2020-2) of the Czech Republic. The experiments were performed at the experimental stables of Mendel University in Brno. During the experiments, the microclimatic conditions and the light regime was controlled according to the Technological procedure for Ross 308 (Aviagen, 2018) or Husbandry Guidelines Premium Chickens (Hubbard, 2021). A conventional system of deep litters with wood shavings as the bedding material was used.

Animals and Diets

Experiment I: The total of 276 male fast-growing (**FG**) Ross 308 broiler chickens were used. The trial lasted from the first day to 35th day of chickens' age. On the first day of age, broilers were weighed and divided by body mass into 2 equal groups with 6 replicates per treatment, that is, there were 23 broilers in one experimental pen. The control group (Control, n = 138) was fed with a diet without caraway addition. The second experimental group (Caraway; n = 138) was fed with a diet containing 1% *Carum carvi*. Diets were formulated according to the broiler nutrition specifications (Aviagen, 2019). Broilers were fed with experimental starter diets until 11th day of age. Chickens were fed with experimental grower diets from 12th day to 35th day of age.

Experiment II: The total of 216 male slow-growing (**SG**) Hubbard JA57 broiler chickens were used. The trial lasted from the first day to 50th day of chickens' age. On the first day of age broilers were weighed and divided by body mass into 2 equal groups with six replicates per treatment, that is, there were 18 broilers in one experimental pen. The control group (Control; n = 108) was fed with a diet without addition of caraway (*Carum carvi* L.). The second experimental group (Caraway; n = 108) was fed with a diet with addition of 1% caraway. Broilers were fed with experimental starter diets from 1st to 21st day of age. Chickens were fed with experimental grower diets from 22nd to 35th day of age. Broilers were fed with experimental finisher diets from 36th day of age until the end of the experiment.

Table 1. Composition of experimental diets.

	Fast-growing				Slow-growing					
	Control		Caraway		Control			Caraway		
	Starter	Grower	Starter	Grower	Starter	Grower	Finisher	Starter	Grower	Finisher
Maize (g/kg)	300.0	370.0	300.0	370.0	330.0	357.6	399.1	354.1	372.0	393.0
Soybean meal (g/kg)	436.0	395.5	436.0	391.8	341.0	295.0	252.0	339.0	292.0	247.8
Wheat (g/kg)	176.7	151.7	168.5	145.4	252.2	272.0	272.5	220.0	251.2	272.5
Rapeseed oil (g/kg)	40.0	40.0	40.0	40.0	33.6	37.6	40.0	33.6	37.0	40.3
Premix[1] (g/kg)	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Limestone milled (g/kg)	5.5	3.3	5.5	3.3	0.8	-	-	0.8	-	-
Monocalcium phosphate (g/kg)	8.0	8.0	8.0	8.0	10.0	5.6	4.9	10.0	5.6	4.9
DL-Methionine (g/kg)	2.2	1.5	2.0	1.5	2.4	2.2	1.5	2.5	2.3	1.6
Wheat gluten (g/kg)	1.8	-	-	-	-	-	-	-	-	-
Caraway (g/kg)	-	-	10.0	10.0	-	-	-	10.0	10.0	10.0

[1]Premix contains (per kg of **starter** diet): L-lysine 2.34 g; DL-methionine 2.4 g; L-threonine 0.99 g; calcium 5.25 g; phosphorus 1.95 g; sodium 1.44 g; copper 15 mg; iron 84 mg; zinc 99 mg; manganese 99 mg; iodine 0.99 mg; selenium 0.18 mg; retinol 13,500 IU (retinyl acetate); cholecalciferol 5,001 IU; tocopherol 45 mg (d-a-tocopherol); phylloquinone 1.5 mg; thiamine 4.2 mg; riboflavin 8.4 mg; pyridoxin 6 mg; cobalamin 30 μg; biotin 0.21 mg; niacinamid 36 mg; folic acid 1.8 mg; calcium pantothenate 13.5 mg; cholin chloride 180 mg. Premix contains (per kg of **grower** and **finisher** diet): L-lysine 2.58 g; DL-Methionine 2.52 g; Threonine 1.47 g; calcium 5.04 g; phosphorus 1.65 g; sodium 1.38 g; copper 15 mg; iron 75 mg; zinc 99 mg; manganese 99 mg; iodine 0.9 mg; selenium 0.36 mg; retinol 9,900 IU (retinyl acetate); cholecalciferol 5,001 IU; tocopherol 45 mg (d-a-tocopherol); phylloquinone 1.5 mg; thiamine 4.2 mg; riboflavin 8.4 mg; pyridoxin 6 mg; cobalamin 28.8 μg; biotin 0.18 mg; niacinamid 36 mg; folic acid 1.71 mg; calcium pantothenate 13.35 mg; cholin chloride 180 mg.

Table 2. Chemical composition of experimental diets (as fed).

	Phase		ME_N (MJ/kg)*	Crude protein (g/kg)	Ether extract (g/kg)	Crude fibre (g/kg)	Crude ash (g/kg)
Fast-growing	Starter	Control	12.1	233.9	58.9	35.7	65.9
		Caraway	12.1	243.4	63.6	36.3	66.2
	Grower	Control	12.3	222.4	61.1	36.4	61.0
		Caraway	12.3	221.3	61.2	33.6	62.5
Slow-growing	Starter	Control	12.3	202.6	55.3	21.5	56.9
		Caraway	12.3	207.2	53.1	15.9	57.0
	Grower	Control	12.6	196.2	61.0	16.8	53.2
		Caraway	12.6	192.0	60.9	21.8	51.9
	Finisher	Control	12.9	174.9	63.3	14.5	48.9
		Caraway	12.8	180.3	65.7	23.8	49.7

*ME_N, Apparent metabolizable energy, calculated value.

All broilers were individually weighed in each week of life. The animals had free access to water and feed. Composition of used diets is shown in Table 1. The chemical compositions of all diets (Table 2) were determined for dry matter, crude protein, crude fat, crude fiber, and ash according to the EC Commission Regulation (COMMISSION REGULATION (EC) No 152/2009, 2009). Diets were formulated as isoenergic and isonitrogenous. Wheat, maize and caraway were ground on a hammer mill with 3 mm sieve. Diets were fed in non-pelleted form. Bioactive substances in caraway were analyzed by FT-NIR Nicolet Antaris II DR instrument and given parameters were evaluated by means of the Omnic 8 programme (Horackova et al., 2019). Chemical analysis of caraway used in trials is shown in Table 3.

Table 3. Chemical analysis of used caraway (in dry matter basis).

Organic matter (%)	94.01
Crude protein (%)	25.87
Ether extract (%)	20.48
Crude fiber (%)	21.61
ADF (%)	22.88
aNDF (%)	47.33
ADL (%)	3.06
Nitrogen free extract (%)	26.05
Starch (%)	2.23
Celulose (%)	19.81
Crude ash (%)	5.99
P (%)	0.61
Ca (%)	0.04
K (%)	1.08
Mg (%)	0.33
Se (μg/kg)	31.03
Essential oils content (ml/100 g)	2.63
Limonene (%)	45.50
Carvone (%)	54.50

Abbreviations: ADF, acid detergent fiber; aNDF, neutral detergent fiber; ADL, acid detergent lignin.

End of the Experiments and Measurements of Digestive Tracts

At the end of the experiments, 6 average chickens from each group (one of each replicate/pen) were selected, weighed, and slaughtered by decapitation. At the same time, blood was collected for further biochemical analysis.

The entire digestive tracts were removed and divided into the following sections: proventriculus, gizzard, duodenum, jejunum, ileum, ceca, and colon. These sections were emptied and the remaining fat and mesenteries were removed. The segments removed from the small intestine were the region from the gizzard junction to the pancreatic and bile ducts (duodenum), the area between the end of the duodenum and Meckel's diverticulum (jejunum), and the segment between Meckel's diverticulum and the ileo-ceco-colic junction (ileum). The lengths (or widths) and empty weights of each segment were recorded. Gizzard height was measured as maximum distance between the proximal (distal limit of the proventriculus) and distal (proximal limit of the duodenum) part of the gizzard. Gizzard width was measured as maximal distance at right angles of the gizzard height. Gizzard depth was measured as maximum distance tendineal centers on the 2 flat sides of gizzard and gizzard muscle height was measured as maximum height at main muscle was measured along the maximal extension of the muscle. All gizzard measurements were measured using a slide calliper. The obtained values were recalculated and expressed in live weight of the chickens.

Blood Biochemical Profile Measurements

Blood was collected into heparinized tubes and centrifuged for 10 min at 3,000 rpm. The samples were centrifuged only after all samples were completed, but no later than 2 h after the collection. The separated blood plasma was frozen (−20°C) until the biochemical examination. The following parameters were determined from blood plasma samples (n = 6) using standardized biochemical methods using Erba Lachema (Czech Republic) commercial sets on the Ellipse automatic biochemical analyzer (AMS Spa, Italy): enzymes activity AST − aspartate aminotransferase (AST / GOT 500); GGT − gamma-glutamyltransferase (GGT 250); ALT − alanine aminotransferase (ALT / GPT 500); ALP − alkaline phosphatase (ALP AMP 500) and LD − lactate dehydrogenase (LDH-L 100). As other markers of hepatic metabolism, fat and nitrogen metabolism, as well as kidney activity, the following markers were determined: concentrations of the total bilirubin − Tbili (BIL T JG 350), cholesterol (Chol; CHOL 250); TG − triglycerides (TG 250), uric acid (UA − UA 500, no. 10010225 Erba Lachema, Czech Republic), CK − creatinkinase (CK − 100, no. 10004494 Erba Lachema, Czech Republic), glucose − GLU (GOD/POD, GLU 500, Erba Lachema, Czech Republic), Urea (Urea, no. UR 107; Randox, United Kingdom), and creatinine − Creat (CREA 500, no. 1010227 Erba Lachema, Czech Republic), TP − total protein (TP 500) and albumin (Alb 500). The globulin content (TP minus albumin) and albumin to globulin ratio were calculated.

Subsequently, α-1 globulins, α-2 globulins, ß-globulins, and γ-globulins were determined using Interlab G26 electrophoretic analyzer (Interlab S.r.l., Italy) with the SRE607K set. Proteins were separated at alkaline pH by agarose gel electrophoresis. After separation, the gel was denatured, stained with acid violet, decolorized, and dried. Quantification of the divided zones was performed densitometrically (densitometer is a part of the instrument).

Histomorphological Measurements

Sample Collection and Histological Examination

After the slaughter and evisceration of 6 broilers, ileum tissue was removed from each one. Tissue samples of approx. 1 cm for histopathology were taken from defined areas (the mid-gut) of ileum (1 cm proximal to the ileocecal junction). Samples were taken immediately after the slaughter of the experimental animals and fixed in 10% neutral buffered formalin (pH 6.9−7.1; Merck, Czech Republic). Fixed samples of gut were dehydrated through alcohol, acetone and xylene series (all from Kulich Pharma Ltd., Czech Republic), embedded into paraffin (Paramix, Czech Republic) and 2 sections (5 μm) were cut using Microtome SM2000 R (Leica Biosystems, Lake Cook Road, USA) from each sample. Subsequently, the samples were de-waxed through xylene, rehydrated in alcohol series (all from Kulich Pharma Ltd.) and stained in Tissue-Tek Prisma automat with Meyer's Hematoxylin and Eosin according to the standard histological protocol (Aescht et al., 2010; Alshamy et al., 2018). The histopathological changes were evaluated using a BX-53 microscope (Olympus, Czech Republic).

Histomorphic Measurement Methodology Microphotography of all section submitted for morphometry was done using camera Canon EOS 2000D at 100 × magnification and histomorphometric measurements of intestinal villi lengths and crypts depths were performed via program Quick PHOTO CAMERA 3.2. Subsequently, the amount of *Eimeria* sp. was determined quantitatively, per 10 high-power fields (**HPF**) at 40 × magnification.

The villi height (**VH**) and depth of crypts (**CD**) with apparently complete, full-sized intestinal villi with no signs of autolysis or mechanical damage were measured for each sample (5 lengths of villi/depths of crypts per animal; n = 30). Measurements of VH and CD were based on the methods used by (Abdelqader and Al-Fataftah, 2016; Okpe et al., 2016; Shokryazdan et al., 2017). The villus height to crypt depth (VH:CD) ratio is a comparison of VH to CD (Santos et al., 2015). All measurements were made by the same person. The ratio between villi height and crypts depth was assessed.

Statistical Analysis

The data were processed by Microsoft Excel (Redmond, USA) and TIBCO Statistica version 12.0 (Palo Alto, USA). The experimental unit was the pen for body weight, weight gain, feed intake, feed conversion ratio, and for the other monitored parameters. Analyses were performed separately for each genotype (FG or SG) because the experiments were carried out separately. The Shapiro-Wilk W test was used to test the normality of the data distribution. The data set was well-modeled by a normal distribution. The diet effect on all monitored parameters was estimated by Student t test. $P < 0.05$ was regarded as a statistically significant difference. A statistical trend was considered for $P > 0.05$ to $P \leq 0.07$.

RESULTS

The nutrients and bioactive substances content of caraway is presented in Table 3. The results of the performance parameters of FG and SG broiler chickens are shown in Tables 4−6. Table 7 brings results of blood biochemical parameters. Table 8 shows relative sizes of individual sections of the chickens' digestive tract. Finally, Table 9 shows ileum villus height and crypt depth of SG broilers.

The mean body weights of broilers from both experiments (Table 4) were equal at the start of trials and there was not found significant differences in body weight through experiments. This also corresponds to the weight gain of the broiler chickens in which no significant differences were found during both experiments.

During the FG experiment, 3 deaths in the Control group and 6 deaths in the Caraway group of broilers occurred. In case of the SG trial, 3 deaths in the Control group and no death in the experimental group occurred.

Experiment with FG broiler chickens showed no differences between groups in feed intake and feed conversion ratio (**FCR**) in each phase of life. Approximately 2.7 kg feed intake and 1.3 FCR in both experimental groups were found. The broilers of both groups in the SG experiment had balanced feed intake and FCR per bird and trial (approx. 4 kg and 1.7, respectively). No significant differences were found in these parameters.

Table 7 brings blood biochemical parameters from both experiments with FG and SG broilers. Blood was taken at the end of each trial (35th day and 50th day of age for FG and SG, respectively). The experiment with FG broilers showed some differences in alkaline phosphatase activity (**ALP**) and total bilirubin concentration (Tbili). Statistically lowered ALP activity (58.28 vs. 97.66 μkat/l) and Tbili (4.28 vs. 5.62 μmol/l) were found in the group of broilers fed with 1% caraway in diet compared to the Control group. In the biochemical blood parameters of SG chickens, no statistical differences were found. However, some trends in the parameters of uric acid ($P = 0.07$) and triglycerides ($P = 0.06$) can be seen.

Table 8 shows the size, weight, and length of individual sections of the digestive tracts of FG and SG broilers. In the trial with FG broilers, there were found lower

Table 4. The mean fast-growing and slow-growing broiler chickens body weights during the trial.

		Control	Caraway	Control	Caraway		
	Days of age	n		mean (g)		SEM	P
Fast-growing	1	138	138	45	45	0.20	0.14
	8	137	135	165	161	1.44	0.13
	12	137	134	298	291	2.99	0.28
	15	137	134	429	418	4.51	0.21
	22	137	133	853	847	8.17	0.74
	29	136	133	1,456	1,441	13.03	0.56
	35	135	132	2,087	2,106	17.36	0.58
Slow-growing	1	108	108	38	38	0.26	0.67
	8	106	108	104	105	1.03	0.63
	15	106	108	264	266	2.37	0.65
	22	106	108	546	547	4.05	0.90
	28	106	108	881	893	5.95	0.31
	36	106	108	1,429	1,441	9.50	0.49
	43	105	108	1,967	1,963	11.97	0.87
	50	105	108	2,537	2,541	14.76	0.89

Differences between control and caraway groups were not statistically significant.

Table 5. The average weight gains during the experiment for fast-growing and slow-growing broiler chickens.

		Control	Caraway	Control	Caraway		
	Days of age	n		mean (g)		SEM	P
Fast-growing	1 to 8	138	138	119	112	1.85	0.05
	8 to 12	137	135	132	128	3.32	0.52
	12 to 15	137	134	132	127	5.09	0.62
	15 to 22	137	134	423	423	9.38	0.99
	22 to 29	137	133	593	594	15.24	0.98
	29 to 35	136	133	615	649	23.74	0.47
Slow-growing	1 to 8	106	108	66	67	1.06	0.56
	8 to 15	106	108	160	161	2.42	0.82
	15 to 22	106	108	282	281	4.38	0.90
	22 to 28	106	108	336	347	7.01	0.44
	28 to 36	105	108	548	548	9.93	1.00
	36 to 43	105	108	538	522	14.35	0.58
	43 to 50	105	108	570	579	18.89	0.83

Differences between control and caraway groups were not statistically significant.

Table 6. The mean feed consumption and feed conversion ratio for each phase and whole trial for fast-growing and slow-growing broiler chickens.

		Fast-growing				Slow-growing			
	Phase	Control	Caraway	SEM	P	Control	Caraway	SEM	P
Final body weight (g)	Starter	298	291	3.59	0.40	546	547	4.53	0.96
	Grower	1,757	1,784	22.26	0.58	876	895	7.13	0.20
	Finisher	-	-	-	-	1,108	1,100	9.95	0.71
	Overall	2,086	2,106	18.61	0.62	2,537	2,541	15.09	0.90
Feed intake (g/bird/period)	Starter	279.90	276.99	3.04	0.65	708.52	714.86	8.28	0.72
	Grower	2,478.31	2,446.61	22.46	0.51	1,254.91	1,260.56	6.76	0.70
	Finisher	-	-	-	-	2,234.73	2,222.22	26.27	0.82
	Overall	2,758.21	2,723.60	24.68	0.51	4,198.16	4,197.64	35.17	0.99
Feed intake (g/bird/day)	Starter	25.45	25.18	0.28	0.65	33.74	34.04	0.39	0.72
	Grower	107.75	106.37	0.98	0.51	96.53	96.97	0.52	0.70
	Finisher	-	-	-	-	148.98	148.15	1.75	0.82
	Overall	81.12	80.11	18.61	0.51	85.68	85.67	0.72	0.99
Feed conversion ratio	Starter	0.94	0.95	0.01	0.36	1.30	1.31	0.01	0.45
	Grower	1.41	1.37	0.01	0.16	1.43	1.41	0.01	0.12
	Finisher	-	-	-	-	2.02	2.02	0.01	0.92
	Overall	1.32	1.29	0.01	0.06	1.65	1.65	0.01	0.84

Differences were not statistically significant. n = 6.

First period – 1st to 11th day of life, 1st to 21st day of life (Ross 308 and Hubbard, respectively); second period – 12th to 35th day of life, 22nd to 35th day of life (Ross 308 and Hubbard, respectively); third period – 36th to 50th day of life (Hubbard only).

weight of proventriculus (3.27 vs. 3.95 g) and longer colon (4.14 vs. 3.40 cm) in chickens fed with 1% caraway in diet compared to the Control group. In the experiment with SG broiler chickens, there were found no statistical differences between the groups supplemented or not supplemented with caraway in the diet. In the Caraway group, a trend for longer gizzard ($P = 0.07$) was found compared to the Control group.

Significant differences were found in villus height and crypt depth of SG broilers ileum as shown in Table 9. The group of slow-growing broiler chickens supplemented with 1% of caraway in the diet showed longer villi and deeper crypt in the ileum after 50 days of life (and feeding a diet with a proportion of caraway). The ratio between the height of the villi and the depth of the crypts was without significant difference between the experimental groups.

From the histomorphological point of view, no surface epithelial injury was found in ileum of chickens from the control group. Intraepithelial lymphocytes were up to 10 high-power fields (**HPF**). Lacteals were of normal diameter – less than 25% of the villous width. Within the villous lamina propria, the lymphocytes and plasma cells occupy up to 25% of the area of one HPF. Granulocytes

Table 7. The mean values for blood biochemical parameters for fast-growing and slow-growing broiler chickens.

	Fast-growing				Slow-growing			
	Control	Caraway	SEM	P	Control	Caraway	SEM	P
ALT (ukat/l)	0.09	0.10	0.01	0.54	0.11	0.12	0.01	0.71
AST (ukat/l)	3.53	3.70	0.14	0.56	2.60	2.68	0.11	0.75
GGT (ukat/l)	0.25	0.25	0.01	0.80	0.26	0.30	0.02	0.24
ALP (ukat/l)	97.66[b]	58.28[a]	10.24	0.05	21.39	29.23	3.04	0.21
LD (ukat/l)	69.95	87.11	10.59	0.44	47.07	48.22	1.76	0.76
CK (ukat/l)	270.08	425.17	89.52	0.41	208.47	296.41	37.44	0.26
Tbili (umol/l)	5.62[b]	4.28[a]	0.29	0.01	6.40	8.18	0.78	0.27
Urea (mmol/l)	1.24	1.23	0.09	0.94	1.43	1.48	0.05	0.62
Creat (umol/l)	25.53	22.90	1.50	0.41	31.27	29.88	0.88	0.46
UA (umol/l)	243.67	245.37	18.28	0.97	264.40	216.32	13.38	0.07
Glu (mmol/l)	8.48	9.15	0.35	0.37	12.93	13.45	0.34	0.46
Chol (mmol/l)	2.79	2.92	0.06	0.34	3.45	3.49	0.08	0.80
TG (mmol/l)	0.75	0.81	0.04	0.53	0.77	0.95	0.05	0.06
TP (g/l)	29.75	28.92	0.58	0.50	34.30	34.25	0.49	0.96
Alb (g/l)	17.95	17.23	0.33	0.29	18.85	18.38	0.46	0.64
Glob (g/l)	11.80	11.69	0.35	0.88	15.45	15.87	0.56	0.73
α-1 glob (g/l)	7.08	6.88	0.19	0.63	8.59	8.41	0.20	0.68
α-2 glob (g/l)	1.37	1.53	0.08	0.33	2.02	1.98	0.09	0.84
ß-glob (g/l)	2.15	2.12	0.11	0.87	2.75	2.91	0.14	0.60
γ-glob (g/l)	1.20	1.16	0.06	0.81	2.10	2.57	0.26	0.38
A/G	1.54	1.48	0.04	0.49	1.24	1.18	0.06	0.66

Abbreviations: Alb, albumins; ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; A/G, albumins/globulins; Chol, cholesterol; CK, creatinkinase; Creat, creatinine; GGT, gamma-glutamyltransferase; Glu, glucose; Glob, globulins; LD, lactate dehydrogenase; Tbili, total bilirubin, TG, triglycerides; UA, uric acid; TP, total protein.

[a,b]Different letters in a row are statistically different; n = 6.

Table 8. Size, weight and length of individual sections of the digestive tract for FG and SG broiler chickens.

	Fast-growing				Slow-growing			
	Control	Caraway	SEM	*P*	Control	Caraway	SEM	*P*
Final live weight (g)	2,171	2,329	59.36	0.19	2,505	2,596	44.88	0.33
Proventriculus weight (g)	3.95[b]	3.27[a]	0.15	0.02	3.87	3.45	0.16	0.22
Gizzard weight (g)	12.17	10.80	0.82	0.43	12.21	13.12	0.43	0.31
Gizzard height (mm)	25.23	26.26	1.00	0.63	18.75	20.42	0.47	0.07
Gizzard width (mm)	15.73	17.57	1.20	0.47	14.85	15.73	0.55	0.45
Gizzard depth (mm)	12.18	11.37	1.09	0.73	10.66	11.17	0.74	0.75
Gizzard muscle height (mm)	6.32	5.25	0.47	0.28	8.74	8.59	0.34	0.83
Duodenum weight (g)	5.51	5.20	0.19	0.44	4.51	4.07	0.16	0.18
Duodenum length (mm)	156.12	142.84	5.92	0.28	123.92	113.40	3.56	0.15
Jejunum weight (g)	9.23	8.49	0.38	0.35	7.92	8.47	0.25	0.28
Jejunum length (mm)	393.74	365.91	11.24	0.23	244.76	258.64	4.65	0.14
Ileum weight (g)	8.47	8.42	0.48	0.96	6.39	6.64	0.39	0.77
Ileum length (mm)	392.42	386.47	9.85	0.78	215.80	218.39	4.92	0.81
Ceca weight (g)	3.42	3.26	0.14	0.59	3.04	3.30	0.20	0.54
Ceca length (mm)	86.62	84.48	1.38	0.46	66.83	72.52	2.24	0.22
Colon weight (g)	1.41	1.50	0.05	0.36	1.08	1.16	0.05	0.52
Colon length (mm)	34.00[a]	41.35[b]	1.54	0.01	39.84	38.65	2.31	0.81
Liver weight (g)	20.68	20.81	0.81	0.94	15.58	16.14	0.41	0.53
Heart weight (g)	4.81	4.44	0.22	0.42	5.29	5.77	0.19	0.21

[a,b]Different letters in a row are statistically different; n = 6.

Table 9. The average ileum villus height and crypt depth in slow-growing broiler chickens.

n	Control 30	Caraway 30	SEM	*P*
Villus height (μm)	890.97[a]	987.30[b]	18.66	0.01
Crypt depth (μm)	93.63[a]	108.03[b]	3.12	0.02
Villus/crypt ratio	9.75	9.58	0.27	0.76

[a,b]Different letters in a row are statistically different.

were found up to 30/HPF. Crypt dilation was up to 2% crypts. Normal amount of mucosal fibrose tissue – it was up to 2 fibrocyte separating the crypts. Secondarily, the occurrence of *Eimeria* sp. was monitored during the morphological examination. There is a possible relationship between bioactive substances and the reduced incidence of coccidia, which could have potential to reduce antimicrobial use. *Eimeria* sp. 0/10 HPFs were found.

Similarly, no surface epithelial injury was found in ileum of broilers from the group with 1% of caraway proportion. Intraepithelial lymphocytes were up to 10/HPF. Lacteals are of normal diameter – less than 25% of the villous width. Within the villous lamina propria, the lymphocytes and plasma cells occupy 20 to 50% of the area of one HPF. Granulocytes were more than 31/HPF in some sections. Crypt dilation was up to 2% crypts. Normal amount of mucosal fibrose tissue – up to 2 fibrocyte separating the crypts were found. *Eimeria* sp. was not found (0/10 HPFs).

DISCUSSION

Some studies (Alizadeh et al., 2011; Jafari, 2011; Khajeali et al., 2012) show that caraway in the diet can increase body weight (**BW**), body weight gain (**BWG**), and improve feed conversion ratio. These facts may be caused by 1) herbal plant natural compounds which may enhance digestion and absorption of some nutrients; 2) increased intestinal villi and deepening of crypts, which may cause increase of the area for nutrient absorption. Moreover, these studies also found out the possibility to decrease of hematological or biochemical values of some blood parameters (cholesterol and triglycerides).

Regarding this fact, Khajeali et al. (2012) data showed that the use of caraway in Ross 308 diets for 42 d of life caused decrease of FCR with increasing BW. Increasing amount of caraway in the broiler diets (0, 1, 1.5 or 2%) caused significant decrease of blood triglyceride (**TG**) content. The TG content in blood was lowest in the diet with 2% caraway addition. In accordance with this, Jafari (2011) found out that serum total cholesterol and TG concentration were significantly reduced in Japanese quails diets supplemented with 1.5 or 2% of caraway compared to the control group. In our experiment with FG broilers, no difference in TG parameter was found. But in the experiment with SG broilers, the opposite trend in blood TG content was found, for example, with the addition of 1% caraway there is a trend ($P = 0.06$) of increasing triglycerides in the blood. This may be explained by the fact that caraway contains many unsaturated fatty acids, such as myristic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid (n-6), linolenic acid (n-3), and arachidonic acid, that are effective on saturated fat metabolism in the organism (Ketels and De groote, 1989; Laribi et al., 2010). Differences in our results and those of other authors may be, for example, due to the different chemical composition of caraway. The content of bioactive substances in caraway may vary by agronomic cultivation technique, environmental conditions, habitat, etc. According to Crowell (1999) and Khajeali et al. (2012), decrease in blood TG and cholesterol may also be caused by bioactive substances found in caraway which act as inhibitors to the active enzyme hepatic 3-hydroxyl-

3methylglutaryl which synthetized the cholesterol (Crowell, 1999). Another reason could be a reduction in the activity of hormones secreted by the cortex of adrenal glands which can cause reduction in the secretion of fatty acids from adipose tissue or reduction of fat oxidation which can lead to the reduction of fatty acids level, including cholesterol and TG (Gaong, 2005).

Khajeali et al. (2012) also reported that small intestine mucosa and sub mucosa diameters were significantly increased with diet with 1.5% of caraway. Muscularis and serosa parts diameter were higher in 1.5% caraway group compared to others. These improvements may be due to the biological functions of caraway to improve growth or they may be caused by its role as a stimulant, carminative, enhanced digestibility, antimicrobial properties, and the prevention of gastric toxicity. Gut can respond to changes in the animal diet by varying its weight, length, absorptive area, and rate of enterocyte turnover (Bedford, 1996). This is one of the reasons for which intestinal morphological attributes were examined to determine the effect of caraway on intestinal development in our study. According to Sharma and Schumacher (2001), crypt depth and villus height are useful indicators of the size of the absorptive and proliferative compartments in the intestinal mucosa. A high villus:crypt is related to a well-differentiated intestinal mucosa with high digestive and absorptive capabilities (Jeurissen et al., 2002). A smaller intestinal tract is an indication of higher absorptive efficiency per unit of intestinal weight, thus allowing for greater feed efficiency (Mitchell and Smith, 1991). Furthermore, Bedford (1996) showed that maintaining the rate of digestion with a smaller gastrointestinal tract would enable a greater proportion of absorbed energy to be utilized for carcass accretion, as the nutrient requirement for maintenance of the intestine would be reduced. However, the addition of 1% caraway did not result in a considerable increase in the length of the individual intestinal segments, nor caused an increase in BW and BWG in our experiments. Moreover, the increase in villus height and crypts depth in SG broilers did not increase BW or BWG of broilers.

Alizadeh et al. (2011) also tested 0, 0.5, 1, and 2% *Carum carvi* addition in Ross 308 diets between 1st and 42nd day of broilers' life. Authors reported that 2% addition of *Carum carvi* in Ross 308 diet caused the best FCR and higher activity of serum ALP. Authors stated that 1% *Carum carvi* in diet in their study can be used for improved performance of the broiler chickens. In our study, the opposite phenomenon was found, that is, a decrease in serum ALP activity in FG broilers. Generally, the ALP enzyme was localized in all tissues of the organism in various activities. It has been localized, for example, in osteoblasts, intestinal mucosa, hepatocytes, renal tubules, or leukocytes (Kraft and Dürr, 2001). According to Fasina et al. (2004), increased ALP activity and expression in small intestinal segments may be caused by impaired intestinal mucosal integrity, that is, brush border membrane (**BBM**). Brush border enzymes (such as sucrase-isomaltase, aminopeptidases, and ALP) are synthesized by villi-attached enterocytes and transported during the process of enterocyte differentiation into the apical membranes (Uni, 1999). Enzymes mentioned above are responsible for the final stages of macromolecules digestion and they are important in regulating the amount of nutrients available for absorption, nutrient transport from intestine, reception of signals into cells, and regulation of cell growth and differentiation (Kenny, 1986; Iji et al., 2001; Sklan, 2001). Brush border enzymes can be used as markers of intestinal (i.e., enterocyte) maturity because they are specific for enterocytes and their levels increase as enterocytes mature (Ortega et al., 1995; Uni, 1999). Activities of the brush border enzymes are reduced with damaged BBM (Hong et al., 1991).

In addition, it seems that many bioactive substances (including those in caraway) can affect the health and function of the digestive tract. For example, it was found out that a pretreatment with oral dose of *Carum carvi* L. (500 mg/kg BW) to ethanol treated rats protects against ulcerogenic effects of necrotizing agents, ethanol-induced histopathological lesions, depletion of stomach wall mucus and non-protein sulphydryl groups (**NP-SH**) and pylorous ligated accumulation of gastric acid secretions. The protective effect of caraway against ethanol-induced damage of the gastric tissue appears to be related with the free-radical scavenging property of its constituents. The exact mechanism of action of the gastroprotective activity is not exactly known. However, it might be due to flavonoid-related suppression of cytochrome P450 1A1 (**CYP1A1**) which is known to convert xenobiotics and endogenous compounds to toxic metabolites (Alhaider et al., 2006). It is well known that the metabolism of Aves and Mammalia is different, but some biochemical processes may be similar to both classes.

CONCLUSIONS

Based on our results, it can be stated that the proportion of 1% caraway in fast-growing and slow-growing broiler chickens' diet did not influence performance parameters, blood biochemical profile and relative organ sizes. In the experiment with slow-growing broilers supplemented with caraway, a significant difference in the height of the villi and the depth of the crypts was found.

Thus, based on results of our study, it can be preliminary concluded that *Carum carvi* can be used as feed for fast-growing and slow-growing broiler chickens. For more general explanations and recommendations for the evaluation of caraway as poultry feed, further tests are needed. It would be appropriate to perform other studies to verify higher proportions of caraway and/or its byproducts (nonstandard grains or broken grains) in the diets of broiler chickens. Alternatively, to test the effect in animals with coccidiosis or in experimentally infected animals.

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DISCLOSURES

The authors declare that they have no conflict of interest.

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