DIGESTION AND MICROFLORA IN THE INTESTINAL CHYMUS OF CHICKENS AFTER FEED INTAKE OF ESSENTIAL OILS FROM SAGE AND OREGANO

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ABSTRACT

The objective of the study was the observation of the feed intake of essential oils from sage and oregano on enzymatic activities in the chymus of the jejunum and bacterial microflora in the caecum. The broiler chickens ROSS 308 (n=105, age one day) were divided into 3 groups (control, sage, oregano). The diets of experimental groups were supplemented with essential oils isolated from sage (*Salvia officinalis* L.) 2.306 g/kg and from oregano (*Origanum vulgare* L.) 1.179 g/kg. The increased enzymatic activities were observed as follows: a) amylolytic (glucose; μmol/l/min) by 0.09 ($p<0.01$, sage) on days 16 and by 0.05 and 0.04 ($p<0.001$, sage and oregano) on 29, b) cellulolytic (glucose; μmol/l/min) by 0.02 ($p<0.05$, sage) on days 16, by 0.06 ($p<0.001$, sage and oregano) on 29 and by 0.07 and 0.05

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(p<0.01, sage and oregano) on 42. The proteolytic activity (azocasein; µg/ml/min) decreased by 0.29 and 0.41 on day 16 (p<0.01, sage; p<0.05, oregano). The bacterial counts (log CFU/g wet digesta) in the chymus of caecum were increased in experimental groups compared to control on day 42: a) *Lactobacillus* spp. by 0.67 (oregano), b) *Enterococcus* spp. by 0.18 and 0.28 (sage and oregano). Counts (log CFU/g wet digesta) of *Escherichia coli* decreased by 0.66 (oregano) on day 29 and by 0.6 or 0.94 on day 42 (sage and oregano). The faecal digestibility of fibre was increased in all sampling periods on days 16 (p<0.01, exp1; p<0.001, exp2), 29 (p<0.01, sage; p<0.001, oregano) and 42 (p<0.01, sage and oregano). On the contrary, the digestibility of crude protein (CP) decreased on days 16 (p<0.01, oregano), 29 (p<0.05, exp2) and 42 (p<0.05, exp1 and exp2). The application of essential oils from sage and oregano has significantly positive effects on the enzymatic activities (amylolytic, cellulolytic), counts of *Lactobacillus* spp. and *Enterococcus* spp. and the faecal digestibility of fibre of the broiler chickens.

Keywords: poultry nutrition; plant extracts; zootechnical parameters; digestive enzymatic activities; faecal digestibility

INTRODUCTION

The gastrointestinal tract (GIT) of chickens is a complex and dynamic environment where digestion and microbial activities are closely intertwined. The composition of the gut microbiota significantly influences the health, nutrient absorption, and overall productivity of poultry. Recently, there has been a growing interest in utilizing essential oils (EOs) as natural feed additives to enhance poultry health

and performance (Windisch et al., 2008). Use of phytogenic products as feed additives for swine and poultry.

Essential oils derived from plants such as sage (*Salvia officinalis*) and oregano (*Origanum vulgare*) are particularly promising due to their high content of bioactive compounds like thymol and carvacrol, which possess potent antimicrobial, antioxidant, and anti-inflammatory properties (Zhang, 2021).

Research has shown that dietary supplementation with EOs can positively affect gut health by modulating the gut microbiota and improving digestive efficiency. Essential oils can stimulate the secretion of digestive enzymes and alter the microbial populations in the intestinal chyme, leading to enhanced nutrient absorption and growth performance in chickens. Specifically, EOs from sage and oregano have been highlighted for their ability to reduce pathogenic bacteria while promoting beneficial microbial communities in the gut (Hashemipour et al., 2013).

The objective of this study was to compare the influence of dietary intake of essential oils isolated from sage and oregano on some digestive enzymatic activities in the content of the jejunum and on bacterial microflora in the content of the caecum in the feeding trial with broiler chickens.

MATERIAL AND METHODS

The broiler chickens ROSS 308 (n=105, age one day) were from a commercial hatchery. They were randomly divided into 3 equal groups (control, exp1, exp2) and used in the feeding trial on a commercial farm for 42 days. The starting, growing and finishing diets were based on wheat, maize, extracted soybean meal (460 g/kg crude protein - CP),

extracted rapeseed meal (355 g/kg CP), sunflower meal, rapeseed oil and enzymes in commercial preparation Kenzyme W dry (Kemin Industries, Inc.).

The essential oils (100% v/v) isolated from sage leaves (*Salvia officinalis* L., family *Labiateae*) 2.306 g/kg (exp1) and oregano tops (*Origanum vulgare* L., family *Lamiaceae*) 1.179 g/kg (exp2) by steam distillation of plant materials in Calendula Inc. (Nová Ľubovňa, Slovak Republic) were added into the diets of experimental groups. The qualitative and quantitative parameters of both essential oils were determined by gas chromatography (GC) using a Hewlett-Packard 5890 Series II system (injection input split splitless, an HP-5 capillary column, detector FIF, an HP 7673 automatic injector) with nitrogen as carrier gas (Pavlišinová and Danielovič, 2007).

The control group was fed an identical ration without essential oils. Quantitative analyses of dry matter (DM), CP, crude fat (CF), crude fibre, ash, starch, Ca, P and Na were performed in diets (Faithfull, 2002).

The portion of insoluble ash in HCl was determined in feed mixtures as the ash residue after dissolving ash in diluted HCl by weighing (Daněk et al., 2005). The parameters of the diets that were determined are described in Table 1.

The body weights of chickens and the feed consumption were measured and the feed consumption and feed conversion ratio were calculated at 7-day intervals. The samples of chyme from the jejunum were prepared for the quantification of the activities of digestive enzymes as follows.

Table 1. Nutrients in the experimental diets

Ingredients [g/kg]	Experimental diets		
	Starting	Growing	Finishing
Dry matter	772.4	862.3	890.2
Crude protein	268.2	223.1	214.4
Crude fat	12.5	21.6	46.3
Crude ash	66	57.9	55.3
Starch	340.34	385.92	398.02
Ca	9.8	9.1	7.9
P	10.05	8.66	8.26
Na	2.8	2.5	2.3
Methionine	3.92	4.56	4.71
Lysine	10.05	13.39	13.42
Cystine	2.98	2.86	2.9
Insoluble ash	0.143	0.159	0.26
Metabolizable energy (MJ)	11.5	11.8	12

The fresh sample 1.0 g was mixed with 49 ml of sterile TBS buffer (10 mmol/l TRIS-hydroxymethyl aminomethane, 0.5 mol/l HCl, pH 7.0).

The samples were used for the measurement of the nonspecific proteolytic activity using azocasein as the substrate after homogenization. The analysis was performed using the method described by Broderick (1987). The cellulolytic and amylolytic activities were analyzed using methylhydroxyethylcellulose or starch as substrates, following the method by Lever (1977). The total protein concentration was quantified using the method by Bradford (1976).

The samples of chyme for the microbiological studies were prepared according to the method described by Muhl and Liebert (2007).

The identified bacterial groups and species comprised *Lactobacillus* spp., *Enterococcus* spp., and *Escherichia coli*. Total counts of *E. coli* were enumerated on McConkey agar, Enterococcus spp. on Slanetz-Bartley agar, and Lactobacillus spp. on Rogosa agar (Merck Ltd., Germany).

The digestibility checks were conducted on days 16, 29, and 42. The samples of faeces were analyzed for dry matter, crude protein, crude fibre, ash content, and insoluble ash fraction in HCl.

The data presented in this paper are expressed as means ± standard deviation (SD) of a single value (SAS, Version 8.2; SAS Institute Inc., 1999, Cary, NC, USA). The means of the results from treatments were compared using one-way analysis of variance.

RESULTS AND DISCUSSION

We expected that dietary supplementation with essential oils from sage and oregano could improve the growth parameters, digestion of nutrients, enzymatic activities in the jejunum and bacterial flora in the caecum. The results of the presented experimental study were in agreement with the expectations. The percentage ranges of the main components of essential oils used in the trial were as follows: from sage (eucalyptol 8.5%, alpha-thujone 14.8%, beta-thujone 7.2%, camphor 14.9%, borneol 3.7%) and oregano (carvacrol 60%).

The growth data of broiler chickens are summarized in Table 2. The essential oils from oregano significantly increased the average daily gain (ADG) ($p < 0.01$) during the periods of 1–16 days (14.55 ± 3.13 vs. 17.23 ± 4.04), 17–29 days (36.87 ± 13.14 vs. 46.60 ± 10.46), and ($p < 0.05$) 30–42 days of age (69.49 ± 17.99 vs. 80.77 ± 8.38). An increase in the same parameter was observed in the experimental

groups following the intake of essential oils from sage ($p < 0.05$), notably within the periods of 17–29 days (36.87 ± 13.14 vs. 44.89 ± 14.71) or 30–42 days (69.49 ± 17.99 vs. 98.19 ± 12.98) of age.

Table 2. Zootechnical parameters of broiler chickens (n = 105; mean ± SD)

	IBW [g]	FBW [g]	ADG [g/day]			ADFI [g/day]			FCR [g/day]		
day	1	42	1-16	17-29	30-42	1-16	17-29	30-42	1-16	17-29	30-42
Control	38.86±6.71	1746.67 ±340.52	14.55^{a} ±3.13	36.87^{a} ±13.14	69.49^{a} ±17.99	29.39 ±6.33	80.45 ±28.67	160.63±41.58	2.02	2.18	2.31
Sage	40.57±4.75	1947.06 ±274.14	14.49^{a}±3.24	44.89^{b} ±14.71	98.19^{b} ±12.98	28,69 ±6.42	89.52 ±29.33	203.30±26.87	1.98	1.99	2.07
Oregano	39.29±8.31	1840.0 ±231.0	17.23^{c} ±4.04	46.60^{c} ±10.46	80.77^{b} ±8.38	32.80 ±7.69	91.44 ±20.52	160.35±16.64	1.90	1.96	1.99

The enzymatic activities related to digestion (amylolytic, cellulolytic, proteolytic) were assessed in the chyme of the jejunum (Table 3).

The application of both essential oils into feed mixtures significantly increased amylolytic activity ($p < 0.001$) on day 29 of age. The essential oils from sage led to both enhancement ($p < 0.01$) and

reduction ($p < 0.05$) of the mentioned enzymatic activity on days 16 or 42 of age.

Table 3. Enzymatic activities in the chymus of the jejunum of broiler chickens (n=54; mean ± SD)

Age [day]	Group	Amylolytic (glucose) [µmol/l/min]	Cellulolytic (glucose) [µmol/l/min]	Proteolytic (azocasein) [µg/ml/min]
16	Control	$0.17^{a} \pm 0.011$	$0.11^{a} \pm 0.025$	$0.77^{a} \pm 0.063$
	Sage	$0.26^{c} \pm 0.038$	$0.13^{b} \pm 0.023$	$0.48^{c} \pm 0.062$
	Oregano	$0.17^{a} \pm 0.062$	$0.12^{a} \pm 0.017$	$0.36^{b} \pm 0.038$
29	Control	$0.1^{a} \pm 0.013$	$0.07^{a} \pm 0.015$	$0.41^{a} \pm 0.048$
	Sage	$0.15^{d} \pm 0.032$	$0.13^{d} \pm 0.038$	$0.41^{a} \pm 0.035$
	Oregano	$0.14^{d} \pm 0.013$	$0.13^{d} \pm 0.03$	$0.40^{a} \pm 0.055$
42	Control	$0.14^{a} \pm 0.025$	$0.16^{a} \pm 0.025$	$0.60^{a} \pm 0.066$
	Sage	$0.12^{b} \pm 0.015$	$0.23^{c} \pm 0.018$	$0.55^{a} \pm 0.07$
	Oregano	$0.13^{a} \pm 0.021$	$0.21^{c} \pm 0.025$	$0.56^{a} \pm 0.085$

Means with various letters differ significantly: (a,b) $p<0.05$, (a,c) $p<0.01$, (a,d) $p<0.001$

In spite of the fact that the mixture of enzymes was applied to all feed mixtures, the significant increase of amylolytic and cellulolytic activities in the chymus of jejunum was observed only in both experimental groups. The favourable effect of essential oils on amylolytic activities was in agreement with the conclusions of Jang et

al. (2007). Their observation was that activities of pancreatic alpha-amylase and intestinal maltase were elevated in the gastrointestinal apparatus of broiler chickens after the application of a blend of essential oils extracted from herbs. Recent research further supports these conclusions, demonstrating significant enhancements in enzymatic function upon treatment with essential oils (Marcin and Nad', 2017).

The cellulolytic activity significantly increased following the application of both essential oils ($p < 0.001$) on day 29 and ($p < 0.01$) on day 42. The sage essential oils significantly enhanced cellulolysis ($p < 0.05$) on day 16. Because the cellulolytic activity is not endogenous, the significant increase in the chyme of jejunum was caused by the stimulation of a limited population of bacteria with this enzymatic activity with the used essential oils.

A significant decrease in proteolytic activity was observed in both experimental groups ($p < 0.001$, $p < 0.01$) on day 16 of age.

The bacterial counts in the cecal chyme of broiler chickens are summarized in Table 4.

The dietary addition of a blend of essential oils extracted from herbs decreased the population of *E. coli* in ileocaecal digesta (Jang et al., 2007).

Several studies provided evidence for the antimicrobial activities of thymol and carvacrol *in vitro* (de Sousa et al., 2023).

Table 4. Bacterial microflora in the wet digesta in the caecum of broiler chickens (n=54)

Age [day]	Group	*Lactobacillus* spp. [log CFU/g wet digesta]	*Enterococcus* spp. [log CFU/g wet digesta]	*E. coli* [log CFU/g wet digesta]
16	Control	8	5.6	7.04
	Sage	8.18	6.36	6.85
	Oregano	8.2	6.32	6.78
29	Control	8.32	6.2	7.15
	Sage	8.64	6.6	7.11
	Oregano	8.85	6.88	6.49
42	Control	8.48	6.9	7.3
	Sage	8.94	7.08	6.7
	Oregano	9.15	7.18	6.36

On the contrary Cross et al. (2007) observed that dietary treatment with herbs, such as thyme, oregano, marjoram, rosemary and varrow had not any effect on the population of intestinal microflora, apparent metabolisable energy or the calculated coefficients of digestibility. Results obtained in the experiments *in vitro* (Marcin et al., 2006) with the agar spot test and agar diffusion paper disc test demonstrated higher antimicrobial activity of essential oils from oregano than from sage against tested bacterial strains: a/ *E. coli* – pig isolates, haemolytic,

K antigen positive, b/ *Salmonella enterica* var. *enteritidis* – pig isolate, c/ *Enterococcus faecium* M-74 – probiotic strain. The populations of *Lactobacillus* spp., *Enterococcus* spp. and *E. coli* were not significantly influenced by the dietary treatments.

Table 5. Faecal digestibility of broiler chickens (n=72; mean ± SD)

Age [day]	Group	CP (dc)	Fibre (dc)	Ash (dc)
16	Control	$64.12^{a} \pm 0.705$	$28.48^{a} \pm 0.712$	$39.04^{a} \pm 2.467$
	Sage	$61.94^{a} \pm 2.329$	$43.36^{c} \pm 0.997$	$44.64^{a} \pm 3.162$
	Oregano	$58.06^{c} \pm 0.749$	$46.32^{d} \pm 0.787$	$40.94^{a} \pm 1.556$
29	Control	$66.51^{a} \pm 2.501$	$33.99^{a} \pm 0.51$	$55.80^{a} \pm 3.471$
	Sage	$65.26^{a} \pm 2.088$	$43.51^{c} \pm 1.65$	$52.94^{a} \pm 1.853$
	Oregano	$57.61^{b} \pm 3.60$	$50.58^{d} \pm 1.518$	$48.82^{a} \pm 2.289$
42	Control	$64.18^{a} \pm 1.091$	$33.60^{a} \pm 0.874$	$46.18^{a} \pm 3.048$
	Sage	$57.41^{b} \pm 0.861$	$46.07^{c} \pm 0.848$	$49.84^{a} \pm 3.489$
	Oregano	$57.25^{b} \pm 1.202$	$47.18^{c} \pm 1.180$	$45.84^{a} \pm 3.897$

Means with various letters differ significantly: (a,b) $p<0.05$, (a,c) $p<0.01$, (a,d) $p<0.001$; dc – digestibility coefficient

The direct transfer of the results with the essential oils from the *in vitro* experiments to *in vivo* conditions of the digestive tract is very difficult.

The data on faecal digestibility are summarized in Table 5.

Significant differences were observed across all sampling periods for fibre. The digestibility coefficients of crude protein were notably lower with the feed mixture containing oregano in all samples, and with the feed mixture containing sage specifically on day 42 of age.

The fibre digestibility showed a significant increase in both experimental groups during the sampling intervals on days 16, 29, and 42.

The observed increase in fibre digestibility in both experimental groups on days 16, 29, and 42 suggests that oregano and sage positively influence the breakdown and absorption of fibre. This could be attributed to the bioactive compounds present in these herbs, which have been shown to enhance digestive processes. For instance, Castanon (2007) reported that essential oils and plant extracts could improve gut health by stimulating the growth of beneficial microbiota, which aids in fibre degradation. Similarly, Giannenas et al. (2003) found that the inclusion of oregano oil in animal diets enhanced fibre digestibility by increasing the activity of fiber-degrading enzymes.

In the case of the feed mixture containing sage, a notable decrease in crude protein digestibility was observed on day 42. This time-specific effect may indicate a delayed response or adaptation process in the gut microbiota or enzymatic activity in reaction to sage. Kamel (2001) suggested that the effects of herbal supplements on digestive processes might vary over time as the gut environment adapts to the new diet.

CONCLUSION

The addition of essential oils from sage and oregano to the diet of broiler chickens led to positive changes in amylolytic and cellulolytic activities in the chyme of jejunum, as well as in the digestibility

coefficient of fibre and the average daily weight gains of the broiler chickens.

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