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EFFECT OF A BLEND OF COMMERCIAL OILS ON GROWTH PERFORMANCE AND INTESTINAL MICROFLORA POPULATION IN BROILER CHICKENS

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Abstract

The present study was designed to evaluate if the use of a blend of commercial oils (BCO) obtained from different plants could affect growth performance and intestinal microflora of the gut in growing-finishing broiler chickens. A total of 80 Cobb 500, 14-day-old broiler chickens, with initial body weight 360 ±0.38g, were assigned to two groups, basal diet (C) and the basal diet supplemented (E) with 0.50% BCO. Throughout the entire feeding period (14-42 days), there were significant differences (P<0.05) in final body weight, daily weight gain, and for daily feed consumption (35-42days), for group C compared with BCO. At 35 and 42 days, respectively, 6 chicks/group were slaughtered and samples of caecal and intestinal contents were collected for bacteriological examination. Weights of digestive organs including the liver, gizzard, intestine and pH from cecum and intestine were not affected by the dietary treatment. The colony forming units (CFU) of Escherichia Coli and Staphylococcus spp. in the digesta of caecum in the BCO group showed a significantly (P≤0.05) lower number compared with that in the C group. The CFU of Staphylococcus spp. in intestine was significantly (P≤0.05) lower in BCO group compared with C. Salmonella was absent in all cases. The inclusion of 0.5% BCO in the chicken's diet (14-42 days) it has reduced the proliferation of pathogen bacteria and has stimulated the increase of favourable bacteria like Lactobacillus spp. in the intestine and cecum of BCO group.

Key words*: broilers; intestinal microflora; essential oil blend; antibiotics; Lactobacillus spp.*

INTRODUCTION

The increased use of antibiotics has given rise to a fear of the development of resistant pathogenic bacterial strains (Vondruskova et al., 2010) and the contamination with antibiotics of the food chain (Chen et al., 2005). Antibiotics, by being banned from the use as feed additives, has accelerated and led to investigations of alternative feed additives in animal production (Laxminarayan et al., 2013). The concerns about possible antibiotic residues and disease resistance have aroused great caution in the usage of antibiotics in the animal industry (Dibner and Richards, 2005) within the European Union since 2006. The need of new alternatives to replace them has gained increasing interest in animal nutrition. Plants and oil extracts have played a significant role in maintaining human health and improving the quality of human life. Also, they have served humans well for a wide variety of purposes for many thousands of years (Jones, 1996) like flavouring drinks (Lawless, 1995), application for the preservation of stored food (Mishra and Dubey, 1994). Also, as valuable components of seasonings, beverages, cosmetics, dyes, and medicines. Bedford (2000) pointed out that the growth-promoting effects of antibiotics in animal diets are clearly related to the gut microflora because they exert no benefits on the performance of germ-free animals. The 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 (Lee et al., 2001). As one of the alternatives, essential oils are already used as feed supplements to improve growth performance under intensive management systems (William and Losa, 2001). Generally, these essential oils are admitted as safe by the Food and Drug Administration (FDA). They inhibit microbial growth in the gut and enhance nutrient digestibility. Dietary supplementation of some oils has also a beneficial effect on intestinal microflora (Helander et al., 1998). In particular, the antimicrobial activity of plant

oils and extracts has formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies (Lis-Balchin and Deans 1997). While some of the oils used based on their reputed antimicrobial properties have well documented in vitro activity, there are few published data for many others (Kamazeri et al., 2012). Some studies have concentrated exclusively on one oil or one micro-organism, while specific blends of oils (commercial or essential) appear also to control coccidia infection (Saini et al., 2003a) and consequently may help to reduce necrotic enteritis (Saini et al., 2003b). The broiler diets are often supplemented with oils to meet the high energy demands of modern genotypes (Loetscher et al., 2013). Rosehips oils contains carotenoids and phenols, which are important antioxidants (Loetscher et al., 2013; Vlaicu et al., 2017), sesame oil has high phytic acid content is deficient in lysine but high in other essential amino acids (Ahammad et al., 2003). The sea buckthorn oils are rich in vitamins E, K (Vlaicu et al., 2017; Zeb, 2006), carotenoids (lycopene, β-carotene), tocopherols (α-tocopherol is the most abundant), tocotrienols and sterols (β-sitosterol, cholesterol, campesterol, stigmasterol) (Cenkowski et al., 2006; Kumar et al., 2011). Nut oils have extremely variable nutrient levels (protein, lipids and fibre), depending on the extraction process (Panaite et al., 2017). Grape oil contains a wide range of bioactive compounds (polyphenols and flavonoids) which can offer many beneficial properties (Turcu et al., 2018, Olteanu et al., 2017). In the present study, was tested the effects of a blend of commercial oils (BCO) on growth performance and intestinal microflora in growing-finishing broiler chickens.

MATERIALS AND METHODS

The feeding trial was conducted in the experimental halls of the National Research-Development Institute of Animal Biology and Nutrition (IBNA-Balotesti, Romania) according to a protocol approved by the Commission of Ethics of the institute. The feeding trial was conducted on 80 Cobb 500 broiler chicks (14-42 days), weighed individually, housed in an experimental hall with floor rearing, under 27.10±2.62˚C air temperature, 60.03±11.24% humidity, 32.71±23.38% ventilation and with 23 hours light regimen. The hall was split into 2 experimental compartments (3.5 sq. m/rearing area), each experimental compartment having a capacity of 16 chicks/ sq. m. The broiler chicks were reared on permanent litter of wood shaves (10-12 cm thick). Feed was provided *ad libitum* in some common feeders and water was supplied through automatic nipples with free access. The conventional diet formulation (group C) had corn and soybean meal as basic ingredients (Table 1).

The diet formulation for the experimental group (E) included, unlike the C diet 0.50% BCO in both phases growing and finisher. The BCO was composed of 20% rosehip oil, 20% sesame oil, 20% buckthorns oil, 20% nut oil and 20% grapeseed oil.

Diets formulations were calculated using the results of the chemical analysis of the feed ingredients and according to the nutritional requirements (NRC., 1994) of Cobb 500 hybrid management guide.

Table 1. Compound feeds formulation

Ingredients	C	E	C	E
%	**Growing**		**Finisher**	
Corn,	62.00	62	60.5	60.5
Soybean meal,	26.58	26.58	25.46	25.46
Gluten,	4.00	4.00	6.00	6.00
Oil,	2.50	2.00	3.75	3.25
Blend of commercial oils, (BCO)	0.00	0.50	0.00	0.50
Calcium carbonate,	1.40	1.40	1.33	1.33
Monocalcium phosphate,	1.36	1.36	1.13	1.13
Salt,	0.37	0.37	0.33	0.33
Methionine,	0.26	0.26	0.25	0.25
Lysine,	0.48	0.48	0.2	0.2
Choline,	0.05	0.05	0.05	0.05
Premix without coccidiosis,	1.00	1.00	1.00	1.00
Total,	100	100	100	100

Calculated:				
Metabolizable energy, kcal	3140.03	3140.03	3250.00	3250.00
Dry matter, %	86.48	86.48	86.49	86.49
Crude protein, %	22.00	22.00	20.00	20.00
Crude fat, %	4.46	4.46	5.66	5.66
Crude fiber, %	3.54	3.54	3.56	3.56
Calcium, %	0.84	0.84	0.78	0.78
Total Phosphorus, %	0.75	0.75	0.39	0.39

1kg premix contains: = 1100000 IU/kg vit. A; 200000 IU/kg vit. D3; 2700 IU/kg vit. E; 300 mg/kg vit. K; 200 mg/kg vit. B1; 400 mg/kg vit. B2; 1485 mg/kg pantothenic acid; 2700 mg/kg nicotinic acid; 300 mg/kg vit. B6; 4 mg/kg vit. B7; 100 mg/kg vit. B9; 1.8 mg/kg vit. B12; 2000 mg/kg vit. C; 8000 mg manganese /kg; 8000 mg iron / kg; 500 mg copper /kg; 6000 mg/ zinc kg; 37 mg/ cobalt kg; 152 mg/ iodine kg; 18 mg selenium /kg

Where: C= basal diet; E= basal diet +0.50% blendof commercial oils (BCO).

The following parameters were monitored throughout the experimental period: body weight (g), average daily feed intake (g feed/chick/day), average daily weight gain (g/chick/day) and feed conversion ratio (g feed/g gain). At 35 and 42 days of age, according to the experimental protocol, six broilers per group were slaughtered by sectioning the jugular vein and carotid artery, after which they were let to bled for 2 minutes, scalded in hot water and manually defeathered. Immediately after were performed measurements to determine the relative weight of some internal organs of broilers (liver, heart, bile, gizzard), by using Kern scales (0.001% precision, Germany) and the pH from cecum and intestine. The pH value of the intestinal and caecal contents was determined with a Mettler Toledo pH-meter. Samples of caecal and intestinal content were collected, in sterile tubes, from the slaughtered chicks, for bacteriological examination (determination of the *Escherichia Coli*, *Salmonella* spp., *Staphylococcus* spp. and *Lactobacillus* spp.).

Samples were collected from each batch of compound feed, for each group, and assayed for the basic chemical composition.

- the dry matter (DM) was determined with the gravimetric method, according to SR ISO 6496:2001;
- crude protein (CP) was determined with the Kjeldahl method, according to SR EN ISO 5983-2:2009;
- the fat was determined by extraction in organic solvents according to ISO 6492/2001;
- the crude fiber (CF) was determined by successive hydrolysis in alkaline and acid environment, according to SR EN ISO 6865:2002;
- the ash (Ash) was determined with the gravimetric method, according to SR EN ISO 2171:2010;
- calcium (Ca) by titrimetric method and phosphorus (P) by spectrophotometry.

Escherichia Coli was determined using a classical medium, G.E.A.M. or Levine. The samples were first soaked in enrichment medium (Lauryl-sulphate broth), homogenized and left for 20-30 minutes at room temperature (23-24^{o}C). Decimal dilutions were made up to 10-5 in the Lauryl-sulphate medium. The dilutions of 10^{-2} – 10^{-5} were used to seed 3 Petri dishes each per dilution, on selective medium used.

The Petri dishes were incubated for 48 h at 37^{o}C and the colonies were count. Escherichia Coli was interpreted by appearance of dark violet with metallic shine colonies.

The other Staphylococcus spp. formed either dark red opaque colonies (lactic-positive species) or pale pink semi-transparent or colourless colonies (lactic-negative species). *Lactobacillus* spp. were determined on selective medium (MRS broth and MRS agar Merck). The colonies counter was determined by Scan 300, INTERSCIENCE (France).

The effects of treatments were analyzed using one-way variance (ANOVA) with STATVIEW for Windows (SAS, version 6.0). The experimental results were expressed as mean values and the differences being considered statistically significant for $P < 0.001$.

RESULTS AND DISCUSSIONS

Table 2 data show that the body weight and the daily weight gain recorded for the entire experimental period (14-42 days) were signifycantly ($P \leq 0.05$) higher in C group compared with E group.

Amerah et al., (2011), stated that some essential oils supplementation significantly improved weight gain.

Table 2. Broiler performance (average values/group)

Item	Days	C	E	SEM	P Value
Body weight (g)	14	360.21	360.55	5.634	0.9761
	35	2183.09	2109.4	27.04	0.1749
	42	2822.2^{b}	2669.2^{a}	38.03	0.0435
Daily weight gain (g/day/bird)	14-35	86.96	82.87	1.249	0.1020
	35-42	91.30^{b}	79.98^{a}	6.429	0.3825
	14-42	88.05^{b}	82.15^{a}	1.387	0.0324
Daily feed consumption (g CF/bird/day)	14-35	125.22	123.21	4.451	0.8250
	35-42	156.52^{b}	145.88^{a}	2.717	0.0458
	14-42	133.87	129.47	3.662	0.5539

*Where: a-b Mean values within a row having different superscripts are significantly different by least significant difference test (P<0.05); SEM-standard error of the mean; *CF: consumption feed.

Several studies have reported beneficial effects of many combinations of essential oils (mix, blends or just oils) on weight gain (Bento et al., 2013; Péron et al., 2009; Yang et al., 2009), while others reported no effect (Jang et al., 2007; Vlaicu et al., 2017). These differences have been attributed to the type of essential oils used and inclusion level (Cross et al., 2007). Hernandez et al., (2004) reported that an BCO, containing oregano, cinnamon, and pepper and *Labiatae* extract from sage, thyme, and rosemary extracts, fed to broilers gave good performance levels like those of the antibiotic growth promoter, Avilamycin.

The effect of BCO on feed conversion ratio (FCR) is presented in Figure 1. For the entire trial period, FCR was higher in the E group compared with C.

Osman et al., (2005) improved FCR by approximately 12%, by using essential oils in broiler diets. These differences among the researcher's results may be due to different active ingredient from used plants.

The weight of the internal organs (Table 3) didn't show significant differences between the two groups, for any growing period. The measurements performed after slaughter (35 days) show that the relative weight of the liver, heart, bile and the gizzard was lower in the E group compared with C, but not statistically significant.

The same differences were found also after slaughter at 42 days. Amerah et al. (2010), said that essential oils supplementation increased the relative gizzard weight and reduced the caecal weight in birds.

Hernandez et al. (2004), by using a blend of three extract of essential oils didn't find any differences for some internal organs like: proventriculus, gizzard, liver, pancreas, or large or small intestine weight, concluding that the use of BCO had no effects on organ weights.

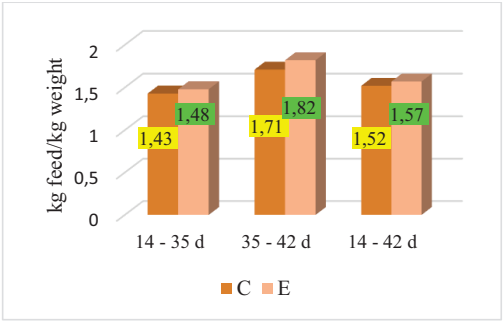

Figure 1. Feed conversion ratio

Table 3. Effect of the BCO on development of the internal organs

Group / Organs	C	E	SEM	P Value	C	E	SEM	P Value
	35 days				42 days			
	grams							
Liver,	42.20	38.45	1.404	0.2077	52.02	47.78	0.099	0.1864
Heart,	9.40	9.01	0.287	0.5302	12.42	11.28	0.554	0.3548
Bile,	3.17	2.66	0.299	0.4291	2.08	2.74	0.394	0.4956
Gizzard,	35.53	39.76	2.643	0.4496	53.83	46.15	2.981	0.2120

Where: a-b: Mean values within a row having different superscripts are significantly different by least significant difference test (P<0.05). SEM: standard error of the mean;

Tables 4 and 5 show the results of the bacteriological determinations on ileal and caecal contents collected after slaughter at 35 and 42 days. The concentration of the analyzed microorganisms *Escherichia Coli*, *Staphylococcus* spp., *Lactobacillus* spp. and*Salmonella* spp. (table 4and 5) are within normal limits (Gournier-Chateau et al., 1994). Regarding the effects of BCO on the intestinal microbial population of broilers (table 4), at 35 respectively 42 days, the number of *Staphylococcu*s spp. CFU was significantly lower (P≤0.05) in E group compared to C group. *Escherichia coli* count was also significantly lower in E group compared with C group. The *Lactobacillus* spp. count was significantly higher (P≤0.05) in the samples collected from E group at 35 and 42 days. Mead & Adams (1975), stated that the intestinal bacterial community of broilers changes with age as indicated by both culture-based and culture-independent studies (Gong et al., 2002a; Wise and Siragusa 2007). Also, the intestinal bacterial composition and activity in broilers has been found to be influenced by the composition and physical structure of the feed (Engberg et al. 2002, 2004).

Table 4. Effects of the BCO on the intestinal microbial population of broilers

Items	C	E	SEM	P value
35 days				
CFU/g intestinal content				
Escherichia Coli	6.102[b]	6.018[a]	0.013	<0.0001
Staphylococcus spp.	5.528[b]	5.374[a]	0.027	0.0003
Lactobacillus spp.	6.189[b]	6.802[a]	0.093	<0.0001
Salmonella spp.	absent	absent	NA	NA
42 days				
Escherichia Coli	6.360[b]	6.340[a]	0.004	<0.0001
Staphylococcus spp.	6.160[b]	6.130[a]	0.006	<0.0001
Lactobacillus spp.	7.420[b]	7.450[a]	0.004	<0.0001
Salmonella spp.	absent	absent	NA	NA

The results were expressed as logarithm of colony forming units/ml.Where: a-b: Mean values within a row having different superscripts are significantly different by least significant difference test (P<0.05). SEM: standard error of the mean; NA=not applicable

As it can be noticed, the number of *Escherichia coli* CFU in the caecal content at 35 and 42 days of broilers (Table 5) was significantly (P≤0.05) lower in E group compared with group C. Also, at 42 days, the number of Staphylococcus spp. has significantly (P≤0.05) decreased. *Lactobacillus* spp. count was significantly (P≤0.05) higher in the samples collected at 35 and 42 days in cecum from E group. *Salmonella* spp. was absent in all cases.

Table 5. Effects of the BCO on the caecal microbial population of broilers

Items	C	E	SEM	P value
35 days				
CFU/g caecal content				
Escherichia Coli	10.04[b]	9.98[a]	0.010	0.0009
Staphylococcus spp.	8.750	8.74	0.005	0.3871
Lactobacillus spp.	11.40[b]	11.44[a]	0.006	<0.0001
Salmonella spp.	absent	absent	NA	NA
42 days				
Escherichia Coli	10.30[b]	10.16[a]	0.057	0.2359
Staphylococcus spp.	8.830[b]	8.72[a]	0.017	<0.0001
*Lactobacillu*s spp.	11.56[b]	11.80[a]	0.037	<0.0001
Salmonella spp.	absent	absent	NA	NA

The results were expressed as logarithm of colony forming units/ml.Where: a-b: Mean values within a row having different superscripts are significantly different by least significant difference test (P<0.05). SEM: standard error of the mean; NA=not applicable

It is suggested that the establishment of *Lactobacillus* spp. prevents the colonization of pathogenic bacteria by competitive exclusion (van der Wielen et al., 2002). *Lactobacilli* and

bifidobacteria compete against potential pathogens for nutrients and binding sites, thereby reducing the intestinal population of pathogens (Rolfe, 2000). Furthermore, lactobacilli and bifidobacteria produce organic acids and other bactericidal substances (Jin et al., 1998) all of which can suppress the colonization of the intestine by pathogenic bacteria. According to Hammer et al., (1999), when comparing data obtained in different studies, most publications provide generalizations about whether or not a plant oil, extract or blend/mix possesses activity against bacteria. Some publications (Saracila et al., 2018; Turcu et al., 2018; Vlaicu et al., 2017) also show the relative activity of plant oils and extracts by comparing results from different oils tested against the same organism(s), but in different growing conditions. Comparing the data obtained in this study with other published results could be problematic. As stated by Hammer et al., (1999), the composition of plant oils and extracts is known to vary according to local climatic and environmental conditions (Sivropoulou et al., 1995). Furthermore, some oils with the same common name may be derived from different plant species (Reynolds 1996). Unfortunately, reports on the value of commercial oils used as blends in poultry are limited.

CONCLUSIONS

This study showed that the supplementation of 0.50% BCO (include seabuckthorn, sesame, rosehip, nut and grape oils) in broiler diets significantly improves the health of intestinal microbiota. BCO could be considered as a potential growth promoter for poultry due to digestive stimulating effect, and antimicrobial effect.

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