EFFECT OF A NATURAL OIL BLEND ON INTESTINAL MICROFLORA POPULATION IN LAYERS DIET

I.M. Stanel[1], Tatiana Dumitra Panaite[1], P.Al. Vlaicu[1*], Cristina Tabuc[1], Cristina Soica[1]

[1]National Research-Development Institute for Animal Biology and Nutrition (IBNA), Balotesti, Ilfov, Romania

Abstract

The feeding trial was conducted for 4 weeks on 48 Lohmann Brown layers (55 weeks) in order to evaluate the effect of a natural oil blend (OB) on intestinal microflora population. The trial was conducted in experimental halls with controlled microclimate (average temperature/total period 22.41±0.98°C; humidity 66.35±5.68%; ventilation/chick 0.50±0.24%; CO_2 level 686.39±104.38 ppm) and 16h/day light regimen. The layers assigned to 2 groups (24 hens/group, 4 hens/cage) received a conventional diet with the same basal formulation (16.80% crude protein; 2760 kcal metabolizable energy). The new formulations diet for the experimental (OB) group differed from the conventional diet C, by replacing the vegetal oil with a OB (0.50%). The compose of the OB was 20% buckthorn oil, 20% sesame oil, 20% rosehip oil, 20% grape oil and 20% walnut oil. At the end of the trial, 6 layers/group were slaughtered and samples of caecal and intestinal contents were collected for bacteriological examination. The colony forming units (CFU) of Escherichia Coli and Staphylococcus spp. in the caecum content in OB group was significantly (P≤0.05) decreased (10.02 and 8.33 CFU) compared with C group (10.09 and 9.12 CFU). Same trend was observed in the intestinal content, Escherichia Coli and Staphylococcus spp. decreased significantly (P≤0.05) in OB group (5.39 CFU Escherichia Coli and 5.24 CFU Staphylococcus spp.) compared with C group (5.41 and 5.25CFU). Lactobacillus spp. the favourable bacteria in the caecum and intestine content, was increased significantly (P≤0.05) in OB group (10.96 and 6.17 CFU) compared with that in C group (10.53 and 6.09 CFU). Salmonella was absent in all cases.

Key words: oil blend, laying hens, intestinal microflora population, animal health

INTRODUCTION

Feed additives derived from plants, including aromatic plants and oils, have gained popularity since when European Union legislations in 2006 phased out the use of antibiotics as feed additives in poultry feed legislation [1]. Natural oils and essential oils have been defined as plant essences obtained from plant material by steam, water distillation, or both [2]. Extracted oils may present a range of potentially beneficial properties including antimicrobial [3], antioxidant [4] antiviral, antitoxigenic and antiparasitic [5]. However, although there is a considerable literature providing *in vitro* evidence of the antibacterial, antifungal, and antiviral activity of plant extracts [6,7], but there are fewer *in vivo* studies that confirm growth-promoting effects. It is considered that plants and plant extracts have a number of beneficial effects on poultry. They increase feed consumption and improve the immune system since they have antibacterial, anticoxidical, antihelminthic, antiviral and antioxidant properties [7,8]. The essential oils are already used as feed supplements to improve growth performance under intensive management systems in poultry diets [9]. They help in the colonization and maintenance of balanced levels of beneficial microbial populations within the gastrointestinal system [10,11]. In addition to their antimicrobial properties [12], essential oils also exhibit antifungal [13, 14] properties. 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. Supplementation with some

*Corresponding author: alexandru.vlaicu@outlook.com
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dietary oils have also a beneficial effect on intestinal microflora [15]. Particulary, the antimicrobial activity of plant oils and extracts has formed the basis of many applications, including raw and processed food preservation. 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 [16]. Rosehips oils is rich in antioxidants [17], sesame oil has high phytic acid content [18], while sea buckthorn oils is in vitamins E, K and carotenoids [19], nut oils have extremely variable nutrient levels [20], and grape oil contains a wide range of bioactive compounds [21]. In the present study, was tested the effects of a natural oil blend (OB) on intestinal health and microflora population in layers diets.

MATERIAL AND METHOD

Experimental procedures were approved by the Ethical Committee of the National Research Development Institute for Biology and Animal Nutrition, in accordance with Romanian Law no. 305/2006 regarding handling and protection of animals used for experimental purposes. The trial was conducted 48 Lohman Brown laying hens (55 week old) which were housed in identical conditions, at temperature of 23.08 ± 0.98°C, humidity of 66.35±5.68% and ventilation/hen of 1.70 ± 0.14%. Lighting was provided for 16h of the 24h. Laying hens individually weighed were randomly divided into two groups (C and OB) with 24 hens/group. The groups of hens were reared in cages (4 hens/cage) structured by three levels, with *ad libitum* access to feed and water. For the elaboration of the combined feed formulations used in this trial, the age and feeding requirements of the birds (Lohman Brown Guide) were taken into consideration. The control group (C) received a conventional diet based mainly of corn, wheat, soybean meal and sunflower meal, characterized by (2760 kcal/kg metabolizable energy and 16.80% crude protein (Table 1), with a standard vitamin premix. The diet formulation for the experimental group (OB) included, unlike the C diet 0.50% OB composed of 20% rosehip oil, 20% sesame oil, 20% buckthorns oil, 20% nut oil and 20% grapeseed oil.

Table 1 Structure of diets used in the trial

Specification	C	OB
Corn %	28.87	30.00
Wheat %	35.80	35.80
Gluten %	4.00	4.00
Soybean %	18.17	18.17
Sunflower meal %	1.10	1.10
Vegetable oils %	0.50	0.00
Oil mixture %	0.00	0.50
Lysine %	0.03	0.03
Methionine %	0.04	0.04
Calcium Carbonate %	8.97	8.97
Monocalcium phosphate %	1.13	1.13
Salt %	0.34	0.34
Choline %	0.05	0.05
Premix* %	1.00	1.00
Total raw materials	100	100
Chemical analysis (Theoretical calculation)		
Metabolizable energy (kcal/kg)	2.760	2.760
Crude protein %	16.80	16.80
Lysine %	0.770	0.770
Methionine %	0.340	0.340

*Content/kg diet: vitamin A, 13500 IU; vitamin D3, 3000 IU; vitamin E, 27mg, vitamin K3, 2mg; vitamin B1, 2mg; vitamin B2 4.8mg; pantothenic acid, 14.85mg; nicotinic acid 27mg; vitamin B6, 3mg; vitamin B7, 0.04mg; vitamin B9, 1mg; vitamin B12, 0.018mg; vitamin C, 25mg; Mn, 71.9mg; Fe, 60mg; Cu, 6mg; Zn, 60mg; Co, 0.5 mg; I, 1.14 mg; Se, 0.18 mg.

At the end of the trial, according to the experimental protocol, six chicks/ group were slaughtered and 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 compound feeds samples and raw materials were analysed for the dry matter (DM) 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 (EE) 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.

Escherichia Coli was determined using a classical medium, G.E.A.M. or Levine, described by [22]. The samples were first soaked in enrichment medium (Lauryl-sulphate broth), homogenized and left for 20-30 minutes at room temperature (23-24ºC). 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. *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 analysed 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

The basic chemical analysis of the main raw materials (Table 2) showed a high CP level in the gluten and soybean meal compared with corn. The low CF level from all used raw materials showed that they can be included without restrictions in poultry diets. The values from Table 2 are similar regarding the DM, OM and EE, but comparable regarding the CP, CF and SEN.

Table 2 Chemical composition of raw materials

Specification	Corn	Wheat	Gluten	Soybean
Dry matter, (%)	88.31	89.13	93.97	88.73
Organic matter, (%)	86.64	87.18	92.46	82.06
Crude protein, (%)	7.87	12.01	60.39	44.43
Ether extractives, (%)	2.14	1.09	1.15	1.55
Crude fiber, (%)	3.64	7.26	0.35	4.40
Extractives substances, (%)	73.00	66.83	30.57	31.68
Ash, (%)	1.68	1.95	1.51	6.67

The data shown in Table 3 reveal that the level of omega 3 polyunsaturated fatty acids (ω 3 PUFA) was higher in the OB diet formulation containing 20% buckthorn oil, 20% sesame oil, 20% rosehip oil, 20% grape oil and 20% walnut oil than in groups C, with vegetal oil. In terms of the oxidative status of the compound feeds (Table 3), the highest concentration of polyphenols (2.42 mg galic acid equivalents/g) was determined in group OB. Also, it can be noticed that group OB had the highest level of xantophiles (luthein and zeaxantine) with 0,711% compared with group C (Table 3).

Table 3 Chemical composition of compound feeds

Specification	C	OB
• NC chemical composition		
Dry matter, (%)	90.10	90.21
Organic matter, (%)	77.39	78.58
Crude protein, (%)	17.36	18.09
Ether extractives, (%)	1.88	1.69
Crude fiber, (%)	3.68	3.13
Extractives substances, (%)	54.47	55.66
Ash, (%)	12.71	11.63
• Lutein and zeaxanthin content		
Lutein + zeaxanthin content, ppm	8.232	8.291
• Total polyphenols content		
Total polyphenols	1.97	2.42
• PUFA Ω3 fatty acid content		
Ω3	2.38	2.49
Ω6/ Ω3	15.45	14.68

From table 4 data, it can be noticed that at both 14 and 28 days after manufacture, the Kreiss reaction was negative, resulting in the rancid process was not installed in the combined feeds. Fat degradation rates were within the maximum allowable limits for combined feeds, for both storage periods, 14 and 28 days respectively: 1.2 ml of 0.01N / g fat for the peroxide index and 50 mg KOH / g fat for fatty acidity (table 4).

Table 4 Indices of fat degradation in the compound feeds

Specification		C	OB	Reference values
Indice peroxide (mlTiosulfate 0,01 Ng/gr)	initial	0.46	0.42	1.2
	14 days	0.58	0.57	
	28 days	0.85	0.84	
Fat acidity (mg KOH)	initial	13.86	12.4	50
	14 days	16.51	16.25	
	28 days	19.1	19.26	
Kreiss reaction	initial	negative	negative	negative
	14 days	negative	negative	
	28 days	negative	negative	

Throughout the experimental period, the following parameters were monitored: daily feed consumption (g feed/chick/day); feed conversion ratio (kg feed/ kg egg); the egg weight (g) and the intensity of laying (%) (table 5). The data from table 5 regarding the zootechnical parameters showed that the values are comparable between the groups. Daily feed consumption (FC), had almost similar average values, while feed conversion ratio (FCR) and egg weight, had some higher values in the favour of OB group. Otherwise, these parameters have not been influenced by the new OB diet.

Table 5 Zootechnical parameters (average values/group)

Specification	C	OB	SEM	P Value
FC,(g feed/chick/day)	114.79	114.27	0.342	0.0132
FCR, (kg feed/kg egg)	1.87	1.88	0.012	0.2429
Egg weight, (g)	64.20	64.55	0.082	0.0024
Laying intensity, (%)	96.25	95.31	0.482	0.0206

Just like us, other authors reported no effects on using different oils in layers diets, [23] reported that the use of an OB in laying hens diet tended to decrease all the productive performances. Contrary to him, [24] concluded that supplementation of OB did not affect (P>0.05) the body weight and mortality rate of hens and was beneficial in terms of an increased egg production rate and eggshell weight. [25] reported that an OB (oregano, cinnamon, and pepper) fed to chichens gave good performance levels like those of the antibiotic growth promoter. These differences among the researchers results may be due to different active ingredient from used plants.

Even if in our trial the OB (20% buckthorn oil, 20% sesame oil, 20% rosehip oil, 20% grape oil and 20% walnut oil), did not influenced the zootechnical parameters, the blend contributes at mantaining the health and the balance of intestinal microflora in the caecum and intestinal microflora population (table 6 and 7). Tables 6 and 7 show the results of the bacteriological determinations on ileal and caecal contents collected after slaughter at the end of the trial. The concentration of the analyzed microorganisms Escherichia Coli, *Staphylococcus spp., Lactobacillus spp. and Salmonella spp.* are within normal limits [26]. Regarding the effects of OB on the intestinal microbial population of laying hens (table 6), the number of *Staphylococcus* spp. CFU was significantly lower (P≤0.05) in OB group compared to C group.

Escherichia coli count was also significantly lower in OB group compared with C group. *Lactobacillus* spp. count, the beneficial bacteria, as it can be noticed had significantly higher (P≤0.05) values in the samples collected from OB group compared with C group.

Other authors [27], stated that the intestinal bacterial community of chickens changes with age as indicated by both culture based and culture-independent studies [28]. Also, the intestinal bacterial composition and activity in birds has been found to be influenced by the composition and physical structure of the feed [29].

Table 6 Microbiological values of intestinal content

Specification	C	E	SEM	P
E. coli	5.41^{b}	5.39^{a}	0.004	0.0007
Staphylococci	5.25^{b}	5.24^{a}	0.014	0.0237
Lactobacilli	6.09^{b}	6.17^{a}	0.013	<0.0001
Salmonella	abs	abs		

Where: a-b mean values within a row having different superscripts are significantly different by least significant difference test (P<0.001);

The colony forming units of *Escherichia Coli* (10.02 CFU) and *Staphylococcus* spp. (8.33 CFU) in the caecum content (table 7) in the OB group were significantly (P≤0.05) lower compared with that in C group (10.09 CFU and 9.12 CFU). These negative intestinal bacteria, mantained the same trend in the caecum as in the intestine. On the other hande, *Lactobacillus* spp. the favourable bacteria in the caecum and intestine content, has increased significantly (P≤0.05) in OB group (10.96 CFU) compared with that in C group (10.53 CFU). *Salmonella*, same as in intestine, was absent in all cases.

Table 7 Microbiological values in caecum

Specification	C	E	SEM	P
E. coli	10.09^{b}	10.02^{a}	0.011	<0.0001
Staphylococci	9.12^{b}	8.33^{a}	0.130	<0.0001
Lactobacilli	10.53^{b}	10.96^{a}	0.097	0.0165
Salmonella	abs	abs		

Where: a-b mean values within a row having different superscripts are significantly different by least significant difference test (P<0.001);

It is suggested that the establishment of *Lactobacillus* spp. prevents the colonization of pathogenic bacteria by competitive exclusion [30]. *Lactobacilli* and bifidobacteria compete against potential pathogens for nutrients and binding sites, thereby reducing the intestinal population of pathogens [31]. Furthermore, *Lactobacilli* and bifidobacteria produce organic acids and other bactericidal substances all of which can suppress the colonization of the intestine by pathogenic bacteria.

CONCLUSIONS

The use of this oil mixture has had beneficial effects on the health of the intestinal microflora. Significant differences in the concentrations of E.coli, Staphylococcus and lactic acid bacteria has reduce the number of colonies of E.coli and Staphylococci in the intestinal tract of the hens' from experimental group compared to the C group. The colony forming units of Escherichia Coli and Staphylococcus spp. in the caecum content in the E group showed a significantly lower number compared with that in C group. Lactobacillus spp. the favorable bacteria in the caecum and intestine content, has increased significantly in E compared with that in C group. Salmonella was absent in all cases.

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