Effect of the Dietary Oil Mixture for Laying Hens on the Apparent Absorption Coefficients of Some Trace Elements

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Abstract

A 4-week feeding trial was conducted on 48 Lohmann Brown layers (55 weeks) to evaluate the effect on zootechnical parameters and coefficients of apparent absorption of micronutrients (Cu, Fe, Mn, Zn). The layers, assigned to 2 groups, were housed in three-tier digestibility cages (4 layers/cage) which allowed the daily recording of the feed intake and excreta, located in an experimental hall under controlled environmental conditions (temperature: 22.41±0.980C; humidity: 66.35±5.68%; ventilation/chick 0.50±0.24%; CO_2 level 686.39±104.38 ppm) and 16h/24h light regimen. The control group (C) received a conventional diet (2760 kcal/kg metabolizable energy and 16.8% crude protein). Compared to C formulation, the experimental diet (E) included 0.50% oils mixture (20% buckthorn oil, 20% sesame oil, 20% rosehip oil, 20% grape oil and 20% walnut oil). The daily feed intake (at weeks 3 and 4) recorded significant decreases ($P \leq 0.05$) in group E (113.29 respectively 114.38 g/chick/day) compared to C group (115 respectively 116.13 g/chick/day). Copper absorption in E group was significantly ($P \leq 0.05$) higher (with 32.21%) than in C group, which means that the concentrations of Cu in the excreta significantly ($P \leq 0.05$) decreased in E group (0.36 mg/chick/day) compared to C group (0.52 mg/chick/day). This is a positive aspect regarding the soil pollution with Cu. For Fe and Mn, the mineral concentrations in the excretion did not differ significantly ($P \geq 0.05$), showing that the supplement administered in the E diet did not affect the possible mineral load at the soil level.

Keywords: nutrient digestibility, oils, performances, poultry.

1. Introduction

Various medical plants have been used for years in daily life to treat disease all over the world [1]. Also, they have served humans for a wide variety of purposes for many years [2]. According to a study performed by the World Human Organization (WHO) based on publications on pharmacopoeias and medical plants in 91 countries, the plants that inhibit microorganisms and are important for human health have been researched in laboratories since 1926 [3]. Essential oils are already used as feed supplements to improve growth performance under intensive management systems [4]. 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. While some of oils used based on their reputed antimicrobial properties have well documented in vitro activity, there are few published data for many others [5]. Some studies have concentrated exclusively on one oil, while specific oils mixes (commercials or essentials) appear also to control coccidian infection [6] and consequently may help to reduce necrotic enteritis [7]. Also, a series of scientific reports and trials have demonstrated that the addition of essential oils, either individual oils or in combination, resulted in beneficial effects in terms of the bird's zootechnical performance, including body weight gain, feed efficiency, and viability [8]. The responses of laying pullets and hens to dietary essential oil supplementation are seemingly less pronounced than that of broiler chickens. Several reports in the scientific literature discuss administration the laying hen diet with extract and essential oils of aromatic herbs. According to

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those few reports available, supplementation of layer diets with herbal essential oils may affect performance parameters such as egg production rate, egg weight, feed intake and feed conversion ratio [9,10,11]. Layers diets are often supplemented with oils to meet the energy demands of modern genotypes [12]. Although there are many studies treating the use of by-products of these plants in animal feeding, but those regarding dietary nutrients digestibility are rather few. Rosehips oils contains carotenoids and phenols, which are important antioxidants [12; 13], sesame oil has high phytic acid content is deficient in lysine but high in other essential amino acids [14]. The buckthorn oils are rich in vitamins E, K [15; 16], carotenoids (lycopene, β-carotene), tocopherols (α-tocopherol is the most abundant), tocotrienols and sterols [17;18]. Nut oils have extremely variable nutrient levels (protein, lipids and fibre), depending on the extraction process [19]. Grape oil contains a wide range of bioactive compounds (polyphenols and flavonoids) which can offer many beneficial properties [20; 21]. However, the effects of oils mixture (OM) on performances and nutrient digestibility of laying hens have not been studied extensively. The purpose of this study, was to test the effect of an OM consisting of rosehip, sesame, grape, buckthorn and walnut oils on layers performances and coefficients of apparent absorption of micronutrients.

2. 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 chicks from the two experimental groups (C and E) were housed in digestibility cages (4 chicks/cage) stacked in 3 tiers, which allowed the daily recording of the feed ingestion and of the excretion.
The trial was conducted in an experimental hall with controlled environmental conditions (average temperature/total growth period 22.41 ± 0.98^{0}C; humidity 66.35±5.68%; ventilation/chick 0.50±0.24%; CO_2 level 686.39±104.38 ppm). The light regimen was adequate to layers age (16h light). The chicks had free access to the feed and water. The objective of the feeding trial, the age and feeding requirements of the birds (Lohman Brown Guide) were taken into consideration when the diet formulations were developed.
All diets were composed mainly of maize, soybean meal and sunflower meal (Table 1), with a standard vitamin premix. Compared to the formulation for group C, the E group formulations included 0.50% OM (rosehip, sesame, grape, buckthorn and walnut oils) in equal proportions (0.20%) (Table 1). Samples of the finished compound feeds (about 500 g/group) were collected for chemical analyses. The coefficients of apparent absorption of the nutrients were determined using the balance technique in week 4. The amounts of ingested feed and of excreted droppings were recorded for 5 consecutive days. The droppings were collected daily, for 5 days, at the same hour, weighed and stored in refrigerator (4^{O}C); average weekly samples (6 samples/group) were formed, homogenized and dried in the drying oven, for 48 h, at 65^{O}C.

Table 1. Compound feeds formulations

Ingredients	C	E
	%	
Corn	30.00	30.00
Wheat	35.80	35.80
Soy meal	18.14	18.14
Gluten	4.00	4.00
Vegetal oil	0.50	0.00
Oils mixture	0.00	0.50
Lysine	0.03	0.03
Methionine	0.04	0.04
Choline	0.05	0.05
Calcium carbonate	8.97	8.97
Phosphate	1.13	1.13
Salt	0.34	0.34
Vit-min premix*	1.00	1.00
Total	100.00	100.00
➢ Calculated		
Metabolizable Energy, kcal/kg	2760.00	2760.00
Crude protein, %	16.80	16.80
Ether extractives, %	1.96	1.96
Crude fiber, %	2.99	2.99

*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.

The compound feeds samples and the average weekly samples of droppings/group, were

analysed for the matter, at 65-103^{0}C (DM); protein (CP); fat (EE); fibre (CF); Ash (ash).
The chemical analyses were performed according to Regulation (CE) no. 152/ 2009 (Sampling and analytical methods for the official inspection of feeds): gravimetric method for the dry matter (DM); the Kjeldahl method for the crude protein (CP); extraction in organic solvents for the ether extractives (EE); acid hydrolysis followed by alkaline hydrolysis for the crude fibre (CF); gravimetric method for the ash (Ash). The recorded data on the amounts of ingested feeds and excreted droppings, corroborated with the analytical data regarding the concentrations of nutrients in the feeds and droppings, allowed making the balance calculations. The absorbed amount was considered to be the difference between the ingested amount of nutrients and the amount of excreted droppings. The coefficient of apparent absorption was calculated as the ratio of the absorbed amount of nutrient to the amount of ingested nutrient, multiplied by 100.
Statistical analysis: The analytical data were compared by variance analysis (Anova), using StatView for Windows (SAS, version 6.0). The differences between the average values within the groups were considered significant for P <0.05.

3. Results and discussion

The trance minerals (Cu, Fe, Mn, Zn) from compound feeds were analysed from one batch. The concentrations of trace minerals from the two experimental diets did not exceed the requirements specific to the species and category and are detailed in table 2. Because of the regulatory restrictions, the dietary Cu level is limited; solutions are sought for indirect stimulation of Cu absorption in the organism [22], its absorption being limited by homeostatic mechanisms after the requirement was met.

Table 2. Content of trace elements in the compound feeds

Item	C	E
	ppm	
Copper (Cu)	7.06	6.45
Iron (Fe)	499.55	490.17
Manganese (Mn)	124.67	121.33
Zinc (Zn)	103.58	107.23

Throughout the experimental period, the following zootechnical 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 3 shows layer performance.

Table 3. Zootechnical parameters (average values/group)

Specification		C	E	SEM	P Value
Daily feed consumption, (g feed/chick/day)	1st week of trial	112.86	113.29	1.652	0.9028
	2nd week of trial	115.00	116.29	0.551	0.2589
	3rd week of trial	115.00^{b}	113.29^{a}	0.361	0.0104
	4th week of trial	116.13^{b}	114.38^{a}	0.413	0.0285
	Average 1st to 4th week	114.79	114.31	0.452	0.5975
Feed conversion ratio, (kg feed/kg egg)	1st week of trial	1.87	1.93	0.050	0.6045
	2nd week of trial	1.88	1.88	0.024	0.9123
	3rd week of trial	1.85	1.84	0.033	0.9022
	4th week of trial	1.90	1.87	0.035	0.7233
	Average 1st to 4th week	1.87	1.88	0.018	0.8873
Egg weight, (g)	1st week of trial	64.14	63.76	0.302	0.5513
	2nd week of trial	64.09^{b}	65.11^{a}	0.183	0.0013
	3rd week of trial	64.61	64.74	0.197	0.7690
	4th week of trial	63.98	64.57	0.213	0.1733
	Average 1st to 4th week	64.20	64.55	0.117	0.1406
Laying intensity, (%)	1st week of trial	95.24	96.87	1.716	0.6538
	2nd week of trial	95.92	94.16	1.151	0.4662
	3rd week of trial	96.60	95.46	1.663	0.7457
	4th week of trial	95.83	95.46	1.642	0.9128
	Average 1st to 4th week	95.90	95.48	0.766	0.7900

*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.

Table 3 data show that the average daily feed intake (g feed/chick/day) was significantly ($P \leq 0.05$) higher in group C than in group E, in the experimental weeks 3 and 4. However, overall experimental period, there were no significant differences between the two groups. Feed conversion ratio (kg feed/kg egg) and laying percentage (%) were not affected by the experimental diet. The average egg weight (g) was significantly ($P \leq 0.05$) higher in experimental week 2 in E group than in C group, but there were no statistically significant differences for the overall experimental period. The average egg weight for E group was 0.54% than for C group, but the difference was not statistically significant ($P \geq 0.05\%$). According to [23], dietary oil mixture supplementation has no effect on body weight gain, egg production, egg weight, egg mass, feed intake, feed conversion ratio, and mortality during the experiment ($P>0.05$). These results are in agreement with the findings of [8;24], and [25] who showed that essential oil mixture had no effect on performance in laying hens. On the other hand, some researches have showed the positive effect of essential oils on performance in laying hens [9;8]. Bolukbasi [9], reported that egg weight and feed conversion ratio were improved by supplementation of thyme, sage, and rosemary oil in laying hens. Bozkurt [26] reported similar results. Çabuk [27] also confirmed that supplementation with oil mixture to the basal diet significantly increased egg production and improved ($P<0.01$) feed conversion ratio.

The same author, stated that the addition of OM, to the layer diet did not affect the daily feed intake of the laying hens significantly, but regarding the feed conversion ratio, there were significant differences between the treatment groups. Bozkurt [26] also stated that the egg production rate of hens fed a diet supplemented with OM was 2.19% higher, than that of hens receiving the standard control diet ($P<0.01$) and the egg production did not differ for hens on the OM supplemented diet.

Table 4 shows the manure content of trace elements (Cu, Fe, Mn and Zn). Copper is an essential trace element for animals, present in many enzyme systems in the body, [28] where it serves as a co-factor.

Table 4. Manure content of trace elements

Items	C	E	SEM	P Value
		ppm		
Copper (Cu)	20.800^{b}	14.110^{a}	1.337	0.0045
Iron (Fe)	1715.21	1713.59	8.752	0.9313
Manganese (Mn)	405.49	434.14	7.809	0.0627
Zinc (Zn)	318.02^{b}	332.62^{a}	3.114	0.0102

*Where: a-b mean values within a row having different superscripts are significantly different by least significant difference test ($P<0.05$);

Our results showed that the coefficient of apparent absorption of the Cu was significantly ($P \leq 0.05$) lower in E group compared to C group. Kim [29] evaluated the potential efficacy of high dietary doses of organic Cu and the results showed that diet supplementation with both organic Cu sources improved the growth performance of poultry. On the other hand, the amount of zinc eliminated in the excreta was significantly ($P \leq 0.05$) in group E than in group C, but did not affect the soil. The results of this experiment are in agreement with those reported by [28]. Zinc, essential trace element for live organisms, is present as a co-factor in over 300 metalo-enzymes, representing all six classes of enzymes, and plays an important role in many metabolic processes, including protein synthesis [30]. Deficiency of Zn exerts a negative influence on protein and carbohydrate metabolism in animals and leads to reduced feed intake, decreased growth, poor feed conversion ratio, abnormalities in immunological and reproductive processes and skeletal and skin issues [31]. The Fe and Mn concentrations in the excreta were not significantly different, although in E group, the excreted amount of Mn was 6.59% higher than in C group. This proves that the dietary OM given to E group did not affect the mineral load of the soil [32]. The absolute bioavailability of inorganic minerals is usually low, and a significant portion of these nutrients pass through the gastrointestinal tract and end up in the excreta [33]. Manganese, as a crucial component of enzymes which are involved in the antioxidative defence system, protein metabolism, and bone formation [34;35] is an important trace element for live organisms. In a study with laying hens, a dietary organic source of Mn gave better results, in terms of weight gain, egg weight, percentage of undamaged eggs [36].

Table 5. Data regarding the absorption of trace elements

Specification	C	E	SEM	P Value
Copper				
Ingested (mg/chick/day)	0.76[b]	0.67[a]	0.016	0.0003
Excreted (mg/chick/day)	0.52[b]	0.36[a]	0.031	0.0029
Absorbed (mg/chick/day)	0.24	0.31	0.022	0.1217
Absorption coefficient (%)	31.84[b]	46.97[a]	3.526	0.0230
Iron				
Ingested (mg/chick/day)	53.60[b]	50.68[a]	0.730	0.0381
Excreted (mg/chick/day)	42.47	39.59	0.923	0.1229
Absorbed (mg/chick/day)	11.13	11.09	0.781	0.9822
Absorption coefficient (%)	20.66	21.92	1.407	0.6762
Manganese				
Ingested (mg/chick/day)	13.38[b]	12.55[a]	0.191	0.0207
Excreted (mg/chick/day)	10.06	10.08	0.273	0.9690
Absorbed (mg/chick/day)	3.32	2.46	0.297	0.1588
Absorption coefficient (%)	24.73	19.62	2.118	0.2453
Zinc				
Ingested (mg/chick/day)	11.11	11.09	0.126	0.9223
Excreted (mg/chick/day)	7.87	7.69	0.171	0.6154
Absorbed (mg/chick/day)	3.24	3.40	0.157	0.6416
Absorption coefficient (%)	29.05	30.71	1.641	0.5617

*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.

Table 5 shows the results for trace element balance parameters. There were statistically significant differences between the experimental and control treatments regarding the ingested Cu ($P<0.05$), Fe ($P<0.05$) and Mn ($P<0.05$). The digestibility parameters of Cu showed a significant decrease for the excreted copper. The digestibility parameters of Cu showed a significant improvement for the E group. Copper is a trace element essential to animal health, but this element must be used in very low amounts. Exceeding the recommended dose leads to hazardous health effects [37]. It is an essential trace element that fulfils a very large variety of functions in animal organisms. Cu and Zn are synergistic elements, Zn functions being improved by copper-containing enzymes. For example, lysyl oxidase is an enzyme that contains copper, helping to synthesize collagen-derived proteins. With the occurrence of Cu deficiency, elastin and collagen cannot be synthesized, leading to problems with the elasticity of the cardiovascular system and the skeletal system [38]. From the data presented, it can be concluded that supplements used (0.50% OM) in the experimental diets of the laying hens improved the absorption of Cu and Zn, thus reducing the negative impact of manure on the environment. The Fe and Mn levels were also modified by the dietary supplementation with OM, but the effects were not observed clearly. The results of this feeding trial show a higher copper absorption in the organism under the influence of an OM.

4. Conclusions

The average daily feed intake (g feed/bird/day), was significantly ($P\leq0.05$) higher in group C than in group E in the experimental weeks 3 and 4. Feed conversion ratio (kg feed/kg egg) and laying percentage (%), were not affected by the experimental diet formulation. The average egg weight (g) was significantly ($P\leq0.05$) higher in the second experimental week, in group E compared to group C. The average egg weight in group E was 0.54% higher than in group C, but the difference was not statistically significant.

The excreted amounts of Cu decreased significantly ($P\leq0.05$) in group E compared to group C, which is a good thing in terms of environmental pollution with Cu.

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