

EFFECT OF OREGANO AND ROSEHIP SUPPLEMENTSON BROILER (14-35 DAYS) PERFORMANCE, CARCASS AND INTERNAL ORGANS DEVELOPMENT AND GUT HEALTH

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Abstract

The study used 96 day-old Cobb 500 chicks, weighed individually and housed in an experimental hall with 32⁰C constant temperature and 23 h light regimen. During the starter stage (1-14 days), all chicks received a conventional diet formulation with sodium monensin (50 g/kg premix). During the growth stage (14-35 days), when the actual feeding trial started, the chicks were weighed, assigned to three groups (32 chicks/group) and housed in the same experimental hall. Throughout the experimental period, the temperature was maintained at 32⁰C, humidity 36%, 23 h light regimen. Like in the starter stage, the chicks had free access to the water and feed. Compared to the diet formulation for the control group (C), conventional formulation, with monensin in the premix, the formulations for the experimental groups included 2% dry oregano (E1), or 2% rosehip powder (E2), but no monensin in the premix. Throughout the experimental period were monitored the bodyweight and the intake. At the end of the feeding trial, 6 chicks from each group were slaughtered in order to make measurements of the relative weight of carcass cuts and internal organs of broilers. Samples of intestinal content were collected for bacteriological assessment (determination of the Enterobacteriaceae, E. coli and Lactic acid bacteria). Throughout the entire experimental period (14-35 days), the average daily feed intake was significantly higher in group C than in groups E1 and E2, but the average daily weight gain and the feed conversion ratio were not different among the three groups. The dietary phytoadditives given the broiler chicks reared under heat stress (32⁰C) had a favourable action in maintaining the health of the intestinal tract, by preserving the balance of the populations of microorganisms colonizing the intestine. Throughout the experimental weeks under heat stress no mortalities were recorded in any of the three groups.

Key words: bacteriological examination, broilers, digestive tract, fruits, heat stress, oregano, rosehip.

INTRODUCTION

Presently, the demand for animal foods is a challenge for animal nutrition and production, which must consider not only the amount of production, but also the societal demands. These requirements focus on quality, safe product, effect on consumer health and environmental protection (Magnin and Picot, 2015). Within this context, research and innovation in animal nutrition are basic for the support of EU animal industry, being the most profitable investment for this industry (Caprarulo et al., 2015). Such approach also became necessary with the European Union ban of antibiotics as growth promoters in animal feed, on January 1, 2006 (EC regulation No. 1831/20031) alternative methods are being

evaluated to improve the performance of agricultural livestock, especially in swine and poultry production (Windisch et al., 2008). The feeds with no chemical additives are increasingly used in poultry nutrition, because the use of antibiotics has been strongly condemned by the consumer associations, as well as by scientists (Demir, 2005). Several studies considered the possible alternatives to antibiotics: use of probiotics, organic acid, oligosaccharides, symbiotic materials, short or medium-chain fatty acids, botanic material or plant extracts, functional fibres, Cu, Zn (Magninand Picot, 2015). The vegetable feed additives are plants or plant derivatives, which have beneficial effects on animal performance

and health state (Peric et al., 2010). Compared with synthetic antibiotics or inorganic chemicals, plant-derived products are natural, less toxic than antibiotics, and typically residue free (Diaz-Sanchez et al., 2015). The manner of action of these feed additives is not entirely clear. Lee et al. (2003) consider that these additives work as digestibility promoters, stimulating the secretion of endogenous enzymes. Other authors (Williams and Losa, 2001; Ertas et al., 2005) show that there is a large variety of plants whose properties might improve feed intake, digestion and feed conversion and weight gain. Several studies (Ertas et al., 2005; Cross et al., 2007) show that the phytoadditives have antimicrobial, antiviral and antioxidant activity. Health and nutrition are interdependent and the interaction between the two occurs largely in the gut (Choct, 2009). Oregano (*Origanum vulgare* L. ssp.) is among the phytoadditives studied as natural alternatives to antibiotics. Several authors showed that oregano has the potential to enhance broiler performance and to reduce the bacterial populations from the gastrointestinal tract, such as *Clostridium perfringens* and *Escherichia coli* (Halle et al., 2001; Giannenas et al., 2003; Modeva and Profirov, 2003; Li Hua et al., 2007). Giannenas et al. (2004) showed that the body weight, average daily weight gain and feed conversion ratio improved in the broilers treated with dehydrated oregano (5 g/kg). Marcinčák et al. (2008) noticed that the dietary oregano in amount of 0.05% per kg feed, slightly increased the slaughter weight of the broilers.

The fruit of rosehip (*Rosa canina*) might be another interesting phytoadditive. *In vitro* assays have shown a high antioxidant capacity of rosehip, especially in the lipophilic extract fraction, which is likely due to phenolic compounds (Gao et al., 2000). Currently, rosehip is widely used as aromatic and medicinal plant with high antioxidant activity (Yesilbag et al., 2011; Jakubcova et al., 2015). Loetscher et al. (2013) reported that the dietary rosehip influenced positively carcass weight. Tekeli (2014) showed that rosehip supplements decreased TBARS numbers in the meat and increased carcass weight, compared to the broilers treated with rosemary.

However, there are few studies on the effects of the dietary phytoadditives given to broilers reared under heat stress. Therefore, we conducted a feeding trial to determine the effects of the oregano and dry rosehip fruits given to broilers (14-35 days) reared under heat stress (32°C) on broiler performance, development of the carcass and internal organs and the digestive gut health.

MATERIALS AND METHODS

The trial was conducted within the experimental halls of the National Research-Development Institute for Animal Biology and Nutrition (IBNA-Balotesti, Romania), according to the provisions of the protocol approved by the Ethics commission of the institute. The study used 96 day-old Cobb 500 chicks (1 day), weighed individually and housed in an experimental hall with 32°C constant temperature and 23h light regimen. During the *starter stage* (1-14 days), all chicks received a conventional diet formulation (Table 1), with sodium monensin (50 g/kg premix), as COXIDIN (monensin concentration, 20%) supplied by HUVEPHARMA (Sofia, Bulgaria). The chicks had free access to the water and feed. During the *growth stage* (14-35 days), when the actual feeding trial started, the chicks were weighed again, individually, and assigned to three groups (32 chicks/group). They were housed in the same experimental hall. Throughout the experimental period (3 weeks), the temperature was maintained at 32°C, humidity 36%, 23 h light regimen; ventilation/broiler 0.38% and CO₂ 899 (ppm). Like in the starter stage, the chicks had free access to the water and feed. Compared to the diet formulation for the control group (C), conventional formulation, with monensin in the premix, the formulations for the experimental groups included 2% dry oregano (E1), or 2% rosehip powder (E2), but no monensin in the premix (Table 1). The whole dry oregano plant and the rosehip powder were supplied by two local SMEs producing natural food supplements. Diet formulations were calculated using the results of the chemical analysis of the feed ingredients in agreement with the feeding requirements (NRC, 1994) and with the feeding requirements of Cobb 500 hybrid.

Table 1. Diet formulations

Ingredient	Starter (1 – 14 days)	Growth (14 - 35 days)		
		Group C	Group E ₁	Group E ₂
Corn, %	58	59.8	56	56.87
Soybean meal, %	30.82	28.2	28.83	28.18
Gluten, %	5	5	5	5
Plant oil, %	2.2	3.3	4.47	4.22
Lysine, %	0.31	0.24	0.23	0.25
Methionine, %	0.27	0.22	0.23	0.24
Choline, %	0.05	0.05	0.05	0.05
Calcium carbonate, %	0.53	0.54	0.53	0.54
Monocalcium phosphate, %	1.45	1.33	1.33	1.33
Salt, %	0.37	0.32	0.33	0.32
Premix, %	1	1	1*	1*
Oregano, %	-	-	2	-
Rosehip powder, %	-	-	-	2
Total	100	100	100	100
<p>1 kg premix for group C (in both stages) contains: = 1100000 UI/kg vit. A; 200000 UI/kg vit. D₃; 2700 UI/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/kg manganese; 8000 mg/kg iron; 500 mg/kg copper; 6000 mg/kg zinc; 37 mg/kg cobalt; 152 mg/kg iodine; 18 mg/kg selenium; 50 g sodium monensin /kg.</p> <p>*1 kg premix for groups E₁ and E₂ (growth stage) has the same structure as for group C, but with no monensin.</p>				

Throughout the experimental period we monitored the following parameters: bodyweight (g); average daily feed intake (g feed/broiler/day); average daily weight gain (g/broiler/day); feed conversion ratio (g feed/g gain).

According to the experimental protocol, at the end of the feeding trial (35 days of age), 6 chicks from each group were slaughtered in order to make measurements of the relative weight of carcass cuts and internal organs of broilers. Samples of intestinal content were also collected and assayed bacteriologically to determine the Enterobacteriaceae, *E. coli* and Lactobacilli.

The basic chemical composition of the phytoadditives, feed ingredients and compound feeds was assayed: 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 ether extractives (EE) were determined by extraction in organic solvents, according to SR ISO 6492:2001; the crude fibre (CF) was determined by successive hydrolysis in alkali

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.

A classical medium of isolation, G.E.A.M. or Levine, to determine the enterobacteriaceae and the *E. coli*. The samples were first immersed into a medium with lauryl sulphate (enrichment medium), properly homogenized, and left for 20-30 minutes at room temperature (23-24°C). Decimal solutions up to 10⁻⁵ in medium with lauryl sulphate were prepared. Dilutions 10⁻² – 10⁻⁵ were used to seed 2 Petri dishes/dilution, on Levine medium. The Petri dishes were incubated for 48 h at 37°C, and the colonies which developed in the dishes were thereafter counted. *E. coli* developed characteristic colonies (dark violet with metallic shining). The other Enterobacteriaceae formed either intense red, opaque colonies (lactose-positive species), or pale pink or colourless, semi-transparent colonies (lactose-negative species). The colony forming units from Enterobacteriaceae, *E. coli* and *lactobacilli* was determined by a colony counter (Scan 300, INTERSCIENCE France).

The effects of treatments were tested by analysis of variance using the GLM procedure of the Minitab software (version 17, Minitab® Statistical Software), with treatment as fixed effect, according to the model $Y_i = T_i + e_i$, where Y_i was the dependent variable, T_i is the treatment and e_i is the error. When overall F-test was significant, differences between means were declared significant at $P < 0.05$ using the test of Tukey.

RESULTS AND DISCUSSIONS

The results of the chemical analysis of the 2 phytoadditives (oregano and rosehip powder) revealed a high level of crude fibre in both products (Table 2). Therefore, the compound feeds used during the growth stages, which included 2% dry oregano (E_1), or 2% rosehip powder (E_2), had high levels of crude fibre (Table 2).

Table 2. Chemical composition of the phytoadditives and of the compound feeds*

Specification	Dry matter (%)	Crude protein (%)	Ether extractives (%)	Crude fibre (%)	Ash (%)
Dry oregano	91.29	5.26	1.17	37.58	6.66
Rosehip powder	92.37	10.53	4.84	49.35	2.47
Starter formulation (1-14 days)	88.15	22.77	3.60	3.45	6.80
C group formulation (14-35 days)	87.29	21.15	5.37	3.42	5.50
E_1 group formulation (14-35 days)	87.61	21.22	6.35	4.02	4.90
E_2 group formulation (14-35 days)	88.05	21.49	6.39	5.26	5.45

*Chemical composition on dry matter (DM) basis

The insoluble fibres in the diets for monogastric animals it is considered just an inert nutritional diluent with low feeding value. Nevertheless, there are researchers who consider that monogastric animals have "fibre requirement", which may contribute to the development and health of the digestive tract (Hetland et al., 2004; Walugembe et al., 2010). Table 3 shows broiler performance throughout the experimental period (14-35 days). The bodyweight of C broilers, both at 21 and at 35 days, was significantly ($P < 0.05$) higher than that of E_1 and E_2 broilers. Throughout the whole experimental period (14-35 days), the average daily feed intake was significantly ($P < 0.05$) higher in group C than in groups E_1 and E_2 . The average daily weight gain and the feed conversion ratio, cumulated for the entire experimental period (14-35 days), were not different between the three groups (Table 3). The literature data are not in agreement as to the effects of the dietary phytoadditives on broiler performance. Thus, Giannenas et al.

(2004) showed that the bodyweight, average daily weight gain and feed conversion ratio improved in the broilers treated with dry oregano (5 g/kg) as unique supplement, or in combination with α -tocopheryl acetate. However, oregano effects were higher at 5.0 and 7.5 g/kg, than at 2.5 and 10.0 g/kg. Botsoglou et al. (2002) studied the effect of dietary oregano essential oil (50 and 100 mg/kg of feed) on the performance of broilers and showed that the performance of the birds was unaffected by the experimental diets. They concluded that dietary oregano oil exerted no growth-promoting effect on broilers.

Table 3 data converge towards the same conclusion, with the difference that E_1 used dry oregano in the diet. Cross et al. (2007) reported the same effects when they studied the effect of herbs, among which oregano, and their associated essential oils on performance, dietary digestibility and gut microflora in chickens from 7 to 28 days of age.

Table 3. Effect of the dietary phytoadditives on broiler performance

Group/parameter	Period	Group C	Group E ₁	Group E ₂	SEM	P value
Bodyweight (g/chick)	1 day	38.41 ^a	38.3 ^a	37.872 ^b	0.055	<0.0001
	14 days	412	392.083	402.500	4.831	0.2568
	21 days	850 ^a	794.762 ^{ab}	781.905 ^b	12.025	0.0583
	28 days	1359.412	1289.048	1270.952	19.143	0.1588
	35 days	2016.923 ^a	1876.667 ^{ab}	1860.588 ^b	29.748	0.0820
Average daily feed intake (g/chick/day)	14-21 days	87.956 ^b	80.883 ^a	78.951 ^a	1.068	0.0013
	21-28 days	115.210 ^b	107.597 ^a	105.000 ^a	0.767	<0.0001
	28-35 days	119.845	119.600	116.039	1.189	0.3347
	14-35 days	106.523 ^a	102.433 ^{ab}	99.750 ^b	1.286	0.1141
Average daily weight gain (g/chick/day)	14-21 days	62.017 ^a	57.532 ^{ab}	53.247 ^b	1.699	0.1217
	21-28 days	72.773	70.714	69.026	2.201	0.8016
	28-35 days	93.247	85.079	83.193	4.485	0.6867
	14-35 days	73.714	70.207	67.283	1.813	0.3808
Feed conversion ratio (g feed/g gain)	14-21 days	1.397	1.408	1.557	0.042	0.2201
	21-28 days	1.568	1.484	1.522	0.041	0.7295
	28-35 days	1.413	1.430	1.489	0.082	0.9261
	14-35 days	1.538	1.569	1.618	0.042	0.7393

*Where: SEM: standard error of the mean; means in the same row with no common superscript are significantly different ($P \leq 0.05$)

Halle et al. (2004) reported that graded amounts of oregano and its essential oil reduced daily feed intake of broilers and significantly improved feed conversion compared with that of control birds. Roofchae et al. (2011) reported completely different results when they investigated the effects of dietary oregano (*Origanum vulgare* L.) essential oil on broiler performance. Birds of experimental groups were treated with basal diet supplemented with 300, 600 and 1200 mg/kg of oregano oil. They found that the inclusion of 600 mg/kg of

oregano oil in grower diet significantly increased body weight gain when compared with the control group ($P < 0.05$). Supplementation of 600 and 1200 mg/kg of oregano oil significantly improved feed conversion ratio compared with the control group in grower and overall experimental periods ($P < 0.05$)

A study by Tekeli (2014) on the effect of the rosehip fruits on broiler performance showed that the groups treated with 10 and 20 g rosehip fruits /kg feed had the highest live weight. A

higher level of rosehip fruits (30 g/kg) depressed significantly the live weight. In this feeding trial, we used 20 g/kg (E₂), but at 35 days, the weight of broilers reared under heat stress was lower (P<0.05) than that of group C broilers. Loetscher et al. (2013) considered that the potential for the application of rosehip as a growth promoter for broilers certainly deserves further attention.

Although the broiler chicks have been reared throughout the 3 experimental weeks under heat stress (32⁰C) no mortalities were recorded

in any of the three groups: C (with monensin in the premix); E₁ (with 2% dry oregano in the diet); E₂ (with 2% rosehip powder). On the other hand, at 14, 21, 28 and 35 days, the body weight of the broilers in all three groups was lower than that mentioned in the management guide of Cobb 500 hybrid. For instance, the bodyweight at 35 days of group C broilers was 7.94% lower than that mentioned in the management guide (2191 g); the bodyweight was 14.35% and 15.08% lower in groups E₁ and E₂, respectively (3).

Table 4. Effect of the dietary phytoadditives on development of the carcass and of the internal organs

Weight	Group C	Group E ₁	Group E ₂	SEM	P value
Broiler carcass (g)	1423.5 ^a	1413.4 ^a	1338.4 ^b	15.788	0.0211
Breast (g)	452.3	454.7	418.5	10.695	0.3446
Gizzard (g)	46.17	45.66	46.28	1.686	0.9905
Heart (g)	8.83	9.96	9.17	0.301	0.3299
Liver (g)	27.80 ^b	31.75 ^a	32.78 ^a	0.846	0.0074
Spleen (g)	1.57 ^a	2.12 ^b	2.09 ^a	0.119	0.0738
Bile (g)	1.79	1.65	1.21	0.193	0.5049
Bursa of Fabricius (g)	2.98	3.70	3.41	0.215	0.4508
Empty intestine (g)	47.18 ^a	51.96 ^b	47.52 ^a	0.874	0.0110

*Where: SEM: standard error of the mean; means in the same row with no common superscript are significantly different (P≤0.05)

The measurements performed after slaughter (35 days) show that the carcass of E₂ broilers was significantly (P<0.05) smaller than the carcass of the other groups (Table 4). Except for the liver and the spleen, the weight of all the other internal organs was not significantly different between groups (P>0.05). A study of Amad et al. (2011) on the effects of a phytogenic feed additive on growth performance and ileal nutrient digestibility in broiler chickens, showed that the relative weights of the pancreas, spleen, liver, and heart were not affected. Amad et al. (2011) showed that their results were consistent with those of Hernandez et al. (2004), Cabuk et al. (2005),

and Demir et al. (2008). They concluded that herbal powders and essential oils do not affect the relative weights of internal organs. In this study, however, the liver was significantly (P<0.05) smaller in group C broilers compared to the experimental groups (E₁ and E₂), while the spleen of E₁ broilers was significantly (P<0.05) larger than in groups C and E₂. Loetscher et al. (2013) showed that in the broilers supplemented with rosemary leaves, rosehip fruits, chokeberry pomace, and entire nettle, from the internal organs dissected, only the proportions of pancreas and liver were affected by treatment.

Table 5. Effect of the dietary phytoadditives on the ileal microbial population of broilers (35 d of age) (colony forming units per gram)

Specification	Group C	Group E ₁	Group E ₂	SEM	P value
Enterobacteriaceae lg10	7.252 ^a	7.205 ^b	7.227 ^{ab}	0.009	0.0941
Escherichia coli lg 10	5.600 ^a	5.613 ^a	5.427 ^a	0.089	0.6935
Lactic acid bacteria lg 10	6.312 ^a	6.806 ^b	6.947 ^b	0.109	0.0115

*Where: SEM: standard error of the mean; means in the same row with no common superscript are significantly different ($P \leq 0.05$)

The results on the ileal microbial population of broilers (35 d of age) (Table 5) show no significant differences between group C (with coccidiostat) and the experimental groups, and no significant differences between the experimental groups either. The concentration of the analysed microorganisms Enterobacteriaceae, *E. coli* and lactobacilli (Table 5) is within normal limits (Gournier-Chateau et al., 1994). The number of Enterobacteriaceae colony forming units, significantly ($P \leq 0.05$) lower in E₁ than in C and E₂ (Table 5), supports the bactericide action of the dietary oregano. The fact that the *Escherichia coli* colony forming units was not different between groups, shows that the two phytoadditives (oregano and rosehip fruits) inhibited the development of the colony forming units of this bacteria. As the number of Lactic acid bacteria was significantly ($P \leq 0.05$) lower in group C than in the two experimental groups (Table 4) shows that both the dietary oregano and rosehip fruits had a favourable influence on the colonization of the digestive tract with these beneficial bacteria. Unlike these results, Roofchae et al. (2011) showed that the supplementation of experimental broilers with 300 and 600 mg/kg, essential oregano oil, significantly lowered the viable counts of cecal *E. coli* compared with both the control and with the 1200 mg/kg, essential oregano oil supplemented group. Jakubcova et al. (2014a) studied the antimicrobial and antioxidative effect of phytogetic additives (grapevine seeds, grape and rosehip pressings). Their results highlighted the impact of grapevine seeds, grape and rosehip pressings on bacteria *Clostridium perfringens* and *E. coli*.

CONCLUSIONS

Even though the broiler chicks have been reared throughout the 3 experimental weeks under heat stress (32⁰C) no mortalities were recorded in any of the three groups: C (with monensin in the premix); E₁ (with dry oregano in the diet); E₂ (with rosehip powder). On the other hand, at 14, 21, 28 and 35 days, the body weight of the broilers in all three groups was lower than that mentioned in the management guide of Cobb 500 hybrid.

The bodyweight of group C broilers, both at 21 and at 35 days, was significantly ($P < 0.05$) higher than that of the experimental groups (E₁ and E₂). Throughout the entire experimental period (14-35 days), the average daily feed intake was significantly ($P < 0.05$) higher in group C than in groups E₁ and E₂, but the average daily weight gain and the feed conversion ratio were not different among the three groups.

The measurements performed after slaughter (35 days) show that except for the liver weight, which was higher ($P < 0.05$) in groups E₁ and E₂ than in group C, and the spleen weight, higher ($P < 0.05$) in group E₁ than in groups C and E₂, the weight of all the other internal organs was not significantly different between groups ($P > 0.05$).

The dietary phytoadditives given the broiler chicks reared under heat stress (32⁰C) had a favourable action in maintaining the health of the intestinal tract, by preserving the balance of the populations of microorganisms colonizing the intestine.

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