Effect of the dietary grape seeds and rosehip oils given to broilers (14-42 days) reared at 32°C on broiler performance, relative weight of carcass cuts and internal organs and balance of gut microflora

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SUMMARY

A feeding trial was conducted on 90, Cobb 500 broiler chicks (14-42 days) assigned to 3 groups (30 chicks/group), housed in an experimental hall with 32˚C air temperature, 36% humidity, and 23h light regimen. The conventional diet (group C), with corn and soybean meal as basic ingredients, had 3082.48 kcal/kg metabolisable energy and 19.99% crude protein. Unlike the conventional diet (C), the diets for the experimental groups included 2.5% grape seeds oil (E1) or 2.5% rosehip oil (E2). The broilers had free access to the feed and water. Throughout the experimental period we monitored broiler body weight and intake. At the age of 42 days, 6 broilers per group were slaughtered and the relative weight of carcass cuts and internal organs of broilers were measured. Samples of caecal content were collected upon slaughtering, for bacteriological examination (*Enterobacteriaceae, E. coli, Lactobacillus, staphylococci, Salmonella*). The use of grape seeds and rosehip oils in broiler diets (14-42 days) reared under heat stress (32^{0}C) didn't influence broiler performance compared to group C. The count of *Enterobacteriaceae, E. Coli, staphylococci* colony forming units was significantly (P≤0.05) lower in the groups treated with grape seeds oil (E1) or rosehip oil (E2) compared to group C. The number of *Lactobacillus* colony forming units was significantly (P≤0.05) higher in group E2 compared to groups C and E1. The two types of oils tested in broiler chicks reared under heat stress (32˚C), particularly the rosehip oil, inhibited the replication of the pathogen bacteria within the gut, which maintained the balance of gut microflora.

Keywords: broilers, heat stress, grape seed oil, rosehip oil, gut health

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INTRODUCTION

The heat, as stressing factor for poultry, has been intensely studied for decades. It bears effects on poultry, worldwide, and has a significant impact on poultry welfare and meat production (Lin et al., 2005; Akbarian et al., 2016). The high mortality rate, the decreasing feed intake and the poor feed conversion rate are frequent adverse effects of the heat stress on broilers (Yegani, 2008; Niu et al., 2009).

Broiler production under these circumstances, after the ban of antibiotic growth promoters in 2006, requires the use of alternative solutions in animal nutrition to replace them. Many studies showed the potential to use various phytogenic additives in feed formulations for poultry as alternative to antibiotics (Puvač et al., 2013; Jakubcova et al., 2015). The basic characteristics of phytoadditives is their wide antimicrobial activity (Jakubcova et al., 2014a), their use becoming more so important under conditions of heat stress when the gut microbial population can be affected. Suzuki et al. (1983) demonstrated that heat stress resulted in a marked change of bacterial composition in chicken intestine, which was subsequently associated with depression of body-weight gain.

The phytoadditives and their extracts generally have antioxidant properties, so that their use in diet formulations can improve the performance of chicken reared under heat stress (Lohakare et al., 2005; Akbarian et al., 2016). Several studies reported that the grape seeds powder and oil has strong antioxidant properties (Wren et al., 2002; Bloom, 2009; Poiana et al., 2009; Xia et al., 2010; Saracila et al., 2017); bactericide properties (Gordon and Wareham, 2010) and bacteriostatic properties too (Etxeberria et al., 2013); they decrease the adherence of the pathogen bacteria (E. coli, Clostridium) and inhibit the incidence of infections of the digestive tract (Dueñas et al., 2015; Brenes et al., 2016). Viveros et al., (2011) have also shown that the grape by-products, rich in polyphenols, given to broiler chicken alter intestine morphology and microflora and enhance the biodiversity of the gut bacteria in broiler chicken.

Rosehip has long been used in many European countries as it contains large amounts of vitamin C. Except to ascorbic acid, rosehips also contains carotenoids and phenols, which are also the important antioxidants (Yesilbag et al., 2011; Loetscher et al., 2013). The phenolic compounds from rosehip (powder or oil) may affect the growth and metabolism of bacteria. Criste et al., (2017) concluded that rosehip powder, given the broiler chicks reared under heat stress (32˚ C), had a favourable action in maintaining the health of the intestinal tract. Only few studies have yet been conducted on this property of the rosehip fruits.

Therefore, we conducted a feeding trial to determine the effects of the grape seed oil and rosehip oil given to broiler chicken (14-42 days) reared under heat stress (32° C) on broiler performance, relative weight of carcass cuts and internal organs and the digestive gut health.

MATERIAL AND METHODS

The study was conducted in the experimental halls of the Laboratory of Chemistry and Nutrition Physiology within the National Research-Development Institute for Animal Biology and Nutrition (IBNA-Balotesti, Romania) according to an experimental protocol (no. 1113/15.02.2017), approved by the Ethics Commission of the institute. The feeding trial was conducted on 90, Cobb 500 broiler chicks (14-42 days) assigned to 3 groups (30 chicks/group), housed in an experimental hall with 32°C air temperature, 36% humidity, and 23h light regimen, with 0.38% ventilation/broiler and 899 ppm CO_2 emission.

Table 1. Diet formulations

Ingredients/groups	Group C	Group E1	Group E2
Corn, %	62	62	62
Soybean meal, %	26.58	26.58	26.58
Gluten, %	4	4	4
Grape seeds oil, %	-	2.5	-
Rosehip oil, %	-	-	2.5
Plant oil, %	2.5	-	-
Monocalcium phosphate, %	1.36	1.36	1.36
Calcium carbonate, %	1.4	1.4	1.4
Salt, %	0.37	0.37	0.37
Methionine, %	0.26	0.26	0.26
Lysine, %	0.48	0.48	0.48
Choline, %	0.05	0.05	0.05
Premix A1 (IBNA)	1	1	1
Total	100	100	100
Metabolisable energy, (kcal/kg) - calculated	3082.48	3082.48	3082.48
Analysed			
Dry matter, %	88.749	89.629	88.550
Crude protein, %	19.99	19.52	19.58
Ether extractives, %	4.56	4.67	4.50
Fibre, %	3.75	3.96	3.79
Calcium, %	0.88	0.90	0.89
Phosphorus, %	0.94	0.91	0.93

1kg premix for group C 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.

Unlike the conventional diet (C), the diets for the experimental groups also included 2.5% grape seeds oil (E1) or 2.5% rosehip oil (E2) (Table 1). The broilers had free access to the feed and water. Diets formulations were calculated using the results of the chemical analysis of the feed ingredients in agreement with the feeding requirements (NRC, 1994) and the feeding requirements of Cobb 500 hybrid. The two types of oil, produced by cold pressing, came from a local SME (southern Romania), licenced for the production of food supplements.

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).

Feed samples were collected from each batch of compound feeds, analysed for their chemical composition and used for bacteriological determinations.

According to the experimental protocol, 6 broilers from each group were slaughtered at 42 days and measurements were performed regarding the relative weight of carcass cuts and internal organs of broilers. Caecal content samples were collected and submitted to bacteriological examination (*Enterobacteriaceae, E. coli, lactobacili, staphylococci, Salmonella*).

The samples of the manufactured compound feeds were assayed for their basic chemical composition and for the bacterial load (TGC, total coliforms, *E. coli, Salmonella*, TFC). 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 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; calcium by titrimetric method and phosphorus by spectrophotometry. TGC was determined according to standard SR 13178-1; the total number of coliforms/g was determined according to SR 13178-2; *E. Coli*/g was determined according to SR 13178-2; *Salmonella* Col/g was determined according to SR EN 12824; TFC Col/g was determined according to STAS 6953-81.

A classical medium of isolation, G.E.A.M. or Levine, was used to determine the *Enterobacteriaceae* and the *E. coli* in the samples of caecal content. The samples were first immersed into a medium with lauryl sulphate (enrichment medium), properly homogenized, and left for 20-30 minutes at room temperature (23-240C). 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 48h at 370 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 *Lactobacili* 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 Yi = Ti + ei, where Yi was the dependent variable, Ti is the treatment and ei 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 DISCUSSION

Table 1 shows the basic chemical composition of the three compound feeds, proving that they were balanced as energy and protein content.

Table 2 shows the results of the bacterial examination of the compound feeds for the three groups (C, E1. E2). The values range within the maximal regulated allowed limits, as published in the Official Gazette of Romania no. 362/ 2003. *Salmonella* was absent in all diet formulations (Table 2).

Table 2. Bacteriological examination of the compound feeds

Specification	TGC Col/g SR 13178-1	Total coliforms/g SR 13178-2	*E. coli*/g SR 13178-2	*Salmonella* Col/g SR EN 12824	TFG Col/g STAS 6953-81
C group formulation (14-42 d)	141×10^4	20	5	Absent	15500
E1 group formulation (14-42 d)	112×10^4	130	10	Absent	21250
E2 group formulation (14-42 d)	117.5×10^4	700	50	Absent	42500

Allowed maximal limits: (MO 362 bis/2003): TGC: maximum 15×10^6 col/g; total coliforms: maximum 3000 col/g; *E.coli*: maximum 100 col/g; *Salmonella sp.*: 0 col/g; TFC: maximum 5×10^4 col/g.
Where: SR= Romanian standard; STAS = State standards; SR EN= European standards.

The average daily feed intake, average daily weight gain and the feed conversion ratio, cumulated for the entire experimental period (14-42 days), were not different between the three groups (table 3).

Compared to the values from the Cobb 500 management guide, for the broilers reared under conditions of thermal comfort (23˚C), the body weight at 42 days was lower by: 33.17% (C), 35.01% (E1) and 35.59 %

(E2). Geraert et al., (1996) noticed that the exposure of broiler chicken to heat stress (32° C) decreased the feed intake by 14% (2-4 weeks) and by 24% (4-6 weeks of age).

Table 3. Effect of the grape seed and rosehip oil inclusion to diets on broiler performance (14-42 d)

Parameter/group	Period	Group C	Group E1	Group E2	SEM	p value
Body weight (g/broiler)	14 days	390.00	392.33	382.33	4.792	0.6769
	42 days	1909.17	1856.67	1840.00	28.199	0.5857
Average daily feed intake (g/broiler/day)	14-42 days	84.46	83.84	83.56	1.415	0.8717
Average daily weight gain (g/broiler/day)	14-42 days	54.36	52.14	52.08	1.023	0.5919
Feed conversion efficiency (g feed/g gain)	14-42 days	1.62	1.56	1.639	0.032	0.6078

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

Ozgan et al. (2009) showed that feeding diets supplemented with 2% grape seeds oil didn't have a significant influence on the average daily weight gain. However, the results from Table 3 are not in agreement with the report of Tekeli et al., (2014a; 2014b) who conducted a study on the effect of rosehip fruits on Ross 308 broiler performance. They noticed that the addition of 10 and 20 g dry rosehips/kg feed had beneficial effects on the body weight (Tekeli et al., 2014a). Also, Tekeli et al., (2014b) showed, in another study on Ross 308 broilers, that the supplement of grape seeds oil (15 g/kg) to broiler diets (1-42 d) improved the average daily weight gain and the feed conversion ratio.

Although the broiler chicks have been reared throughout the 4 experimental weeks under heat stress (32˚C) no mortalities were recorded in any of the three groups: C (conventional diet); E1 (with 2.5% grape seed oil in the diet); E2 (with 2.5% rosehip oil in the diet).

The physical measurements performed after slaughter (Table 4) show no significant (P> 0.05) differences in breast and thigh weight between the 3 groups. Except for the liver, the weight of all the other internal organs was not significantly different between groups (P> 0.05). Liver weight was significantly (P≤ 0.05) higher in the broiler chicken from group E1, treated with grape seeds oil (Table 4). Brenes et al., (2010) showed that the inclusion of graded concentrations of grape seed extract (0.6, 1.8 and 3.6 g kg−1) did not affect the relative organ weights (pancreas, spleen, liver). On the other hand, Criste et al., (2017), showed that the broiler chicken reared under heat stress (32^0 C) treated with rosehip powder had a significantly ($P \leq 0.05$) higher liver and spleen weight than the control group.

Table 4. Effects of grape seed oil and rosehip oil inclusion to diets on relative weight of carcass cuts and internal organs of broilers (42 d of age), (% BW)

Specification	Heat stress treatment			SEM	p value
	Group C	Group E1	Group E2		
Body weight, g	2091.667	2073.333	2063.333	13.483	0.7105
Breast (%)	21.997	20.854	20.408	0.424	0.3170
Thigh (%)	19.756	19.637	20.336	0.215	0.3879
Gizzard (%)	1.579	1.641	1.645	0.048	0.8357
Heart (%)	0.372	0.403	0.396	0.013	0.6117
Liver (%)	1.642^b	1.942^a	1.849^{ab}	0.057	0.0801
Spleen (%)	0.103	0.105	0.099	0.007	0.9482
Bile (%)	0.055	0.047	0.066	0.004	0.2156

* Where % are the weight expressed as g/100 g body weight (BW); SEM: standard error of the mean; means in the same row with no common superscript are significantly different ($p \leq 0.05$)

Table 5 shows the caecal microbial populations of broilers (42 d of age). The concentration of the analysed microorganisms *Enterobacteriaceae, E. coli,* staphylococci and lactobacili (table 5) is within normal limits (Gournier-Chateau et al., 1994).

The number of *Enterobacteriaceae* and *E. coli* colony forming units, was significantly ($P \leq 0.05$) lower in group E2 than in groups C (conventional diet formulation) and E1 (2.5% grape seeds oil). The number of *Enterobacteriaceae* and *E. coli* colony forming units was significantly ($P \leq 0.05$) lower in group E1 than in group C (Table 5). Jakubcova et al., (2014a) have also showed the positive effect of grape seeds, grape and rosehip pressings on *C. perfringens* and *E. coli* bacteria.

Table 5. Effects of the dietary grape seed oil and rosehip oil on the caecal microbial population of broilers (42 d of age)

Specification	Group C	Group E1	Group E2	SEM	P value
Enterobacteriaceae, log 10	12.113^a	12.081^b	12.036^c	0.008	<0.0001
E. coli, log 10	10.012^a	9.993^b	9.973^c	0.005	0.0005
Staphylococci, log 10	9.951^a	9.924^b	9.525^c	0.047	<0.0001
Lactobacilli, log 10	9.322^c	9.345^b	10.303^a	0.111	<0.0001
Salmonella	Absent	Absent	Absent	-	-

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

The caecal content of the broiler chicken from group E2 (2.5% rosehip oil) had the lowest count of staphylococci colony forming units, significantly (P≤0.05) lower than in groups C and E1. The count of staphylococci colony forming units was significantly (P≤0.05) lower in group E1 than in group C. Rotava et al. (2009) showed the high antibacterial activity of the extract of defatted grape seeds extract, which inhibits the replication of pathogen bacteria such as *Staphylococcus aureus* and *E. coli*.

The beneficial activity of the rosehip oil (E2) in maintaining the balance of the intestinal microflora has been also shown by significantly (P≤0.05) higher count of lactobacilli in the caecal content, compared to groups C and E1 (Table 5). Viveros et al. (2011) showed that the numbers of *Lactobacillus spp*. in the cecum were increased in birds fed the grape seed extract diet. Viveros et al., (2011) concluded that the dietary polyphenol-rich grape products modify the gut morphology and intestinal microflora and increase the biodiversity degree of intestinal bacteria in broiler chicks. Lichovnikova et al., (2015) reported that the use of red grape pomace in broiler diets had a positive effect on the lactobacilli count in the ileum, meaning that it can be an alternative to the dietary antimicrobial growth promoters.

CONCLUSIONS

The use of 2.5% grape seeds oil and of 2.5% rosehip oil in broiler diets (14-42 days) reared under heat stress (32^{0}C) didn't affect broiler performance compared to the broilers which received the conventional diet formulation. Except for the liver (higher weight in the group treated with grape seeds oil), the weight of the other internal organs, related to the live weight, was not different between groups. The two types of oils used in broiler diets inhibited the replication of the pathogen bacteria in the intestine, which promoted the balance of the intestinal microflora. The rosehip oil had a particularly positive effect on the lactobacilli count in the caecum, which was significantly (P≤0.05) higher than in the control group and than in the group treated with grape seeds oil.

ACKNOWLEDGEMENTS

This work was supported by a grant of the Romanian Ministry of Education and Research (Project PN 16. 41-0401).

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