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Effects of dietary supplementation of alfalfa meal and rice bran on growth performance, carcass characteristics and intestinal microbiota in broilers

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ABSTRACT

The composition of gastrointestinal tract microbiota can be changed by dietary manipulation, to prevent gut health issues and to promote animal performance. This study was conducted to investigate the effects of rice bran and alfalfa meal on growth performance and intestinal microbiota in broilers. A total of 252 Cobb 500 broilers, aged 14 days, were randomly assigned into 3 groups: control (CON), 5 % rice bran (RB), 5 % alfalfa meal (AM), and housed in an environment-controlled hall 42 days. Throughout the experimental period, grow performance parameters were monitored and at the end relative weights of internal organs were measured. Samples of intestinal content were collected for bacteriological determinations.

Feed intake, daily weight gain and viability were not significantly affected by the dietary supplements. Alfalfa meal and rice bran decreased the populations of *Escherichia* coli and staphylococci in small intestine content, and Enterobacteriaceae, *Escherichia* coli and staphylococci in caecal content of broilers. The count of lactobacilli in both small intestine and caecal content registered a significantly increase in experimental groups, compared to CON group.

Supplementation of diets with rice bran and alfalfa meal tended to increase the population of beneficial bacteria and inhibit the potential pathogens.

Keywords: rice bran, alfalfa meal, gut microbiota, broiler

INTRODUCTION

Poultry industry is one of the most dynamic and growing sectors in the world (Alkhalf et al., 2010), especially in developing countries. The global poultry sector is expected to grow continuously since the demand for meat is continuously increasing by the growing populations (Mottet and Tempio, 2017). Broilers efficiently convert feed into body mass, as a consequence of intensive selection and low input required to produce high-quality meat protein (Stanley et al., 2014). For breeders it is crucial to maintain a high feed efficiency, being also a major challenge, since poultry production is frequently affected by infectious diseases (Diaz Carrasco et al., 2019). Thus, the challenge is to produce efficiently enough amount of poultry meat.

The microbiota within the gastrointestinal tract (GIT) plays an important role in overall health status and productivity in chickens (Wei et al., 2013), by promoting digestion and absorption of nutrients, modulating their immune system, and enhancing resistance to infection (Wang et al., 2016). Optimal gut health can be assured by the bacteria populations that are present in the small intestine (Rinttilä and Apajalahti, 2013). The digestion and absorption of nutrients can be influenced by the microbiota from the small intestine, being an important factor of growth rate (Gong et al., 2008). The composition of GIT microbiota can be changed by dietary manipulation, being a successful approach in preventing gut health disorders and promoting animal performance.

Rice bran is a by-product of rice grain processing, being considered a high value ingredient due to the content in proteins, vitamins, minerals, carbohydrates, phytonutrients, phospholipids and essential fatty acids (Ryan, 2011). The prebiotic properties of rice bran can influence significantly the intestine microbiota in broilers (Sheflin et al., 2015). Several studies have demonstrated that dietary rice bran supplementation reduced the *Salmonella* Typhimurium population in the poultry gastrointestinal tract (Kim et al., 2018; Rubinelli et al., 2017). Ricke et al. (2020) showed that the source of grain cultivar and maturity of microbiota in the cecum of poultry are key issues when assessing prebiotic potential of the rice bran.

Alfalfa meal is a conventional feedstuff for ruminants, containing 17–20% crude protein, with a balanced amino acids profile, being an important source of other bioactive compounds, such as minerals and vitamins (Jiang et al., 2012). Also, it contains many functional components such as polysaccharides, flavonoids, xanthophylls, β-carotene, tocopherol (Ouyang et al., 2016). Due to the high content of saponins (2–3% of dry matter), alfalfa meal has hypocholesterolaemic, anticarcinogenic, anti-inflammatory, and antioxidant activities (Englmaierova et al., 2019).

The aim of this study was to investigate the effect of alfalfa meal and rice bran on growth performance of broiler and intestinal and cecal microbiota.

MATERIALS AND METHODS

Experimental design

The birds were kept in accordance with the Romanian Law 43/2014, EU Council Directive 98/58/EC and Directive 2010/63/EU. The experiment was approved by the Ethical Commission of the Institute.

The experiment was conducted for 42 days on 252 one-day-old unsexed broilers (COBB 500). During the first 14 days, all broilers received a conventional diet characterized by 3000kcal / kg metabolizable energy and 23% crude protein. After 14 days, the chicks were individually weighed and assigned to 3 groups (CON, RM, AM). During the grower (14-35 days) and finisher (35-42 days) stages, the administered dietary treatments were: (1) Control diet (CON) representing a commercial diet; (2) Diet RB included 5% rice bran; (3) Diet AM included 5% alfalfa meal (Table 1). Diets were formulated in agreement with the requirements stipulated in NRC (1994) and with The Management guide of Cobb 500 hybrid (2015). Water and feed were provided ad libitum.

Microclimate parameters were monitored throughout the experimental period. The average temperature / period was 25.97±4.15º C; the humidity was 66.24±89.72%; the light regimen was adequate to broiler age (23h light/1h darkness).

Body weight (g), average daily feed intake (g feed/broiler/day), average daily weight gain (g/broiler/day), feed conversion ratio (g feed/g gain) and broilers' viability (%) were monitored throughout the experimental period. At the end of the feeding trial (42 days of age), 6 chicks from each group were slaughtered in order to make measurements of the relative weight of carcass and of the liver, spleen, bile, gizzard of broilers. The small intestinal and caecal contents were collected for bacteriological analyses (*Enterobacteriaceae, E. coli, lactobacilli, staphylococci, Salmonella spp*).

Chemical analysis

Proximate composition

In order to determine the crude nutrients composition, standard methods were used: crude protein by Kjeldahl method using a Kjeltec 2300 analyser unit (Tecator Instruments, Hoganas, Sweden); ether extractives by ether extraction using a Soxtec 2055 extraction unit (Foss Tecator, Sweden); crude fibre by intermediary filtration method using an automatic analyser (Fibertec 2010 System, Foss Tecator, Sweden); ash by gravimetric method using a Caloris CL 1206 oven (Romania).

Table 1. The structure of diets

Item	Grower (14-28 days)			Finisher (28-42 days)		
	CON	**RB**	**AM**	**CON**	**RB**	**AM**
Corn	36.42	31.51	49.83	50.28	55.98	56.41
Wheat	20.00	20.00	0	10.00	0	0
Soybean meal	34.47	33.78	35.62	30.51	29.48	28.97
Rice bran	0	5.00	0	0	5.00	0
Alfalfa meal	0	0	5.00	0	0	5.00
Vegetable oil	4.82	5.28	5.34	5.25	5.12	5.63
Monocalcium phosphate	1.51	1.52	1.62	1.41	1.48	1.48
Calcium carbonate	0.79	0.78	0.51	0.66	0.87	0.41
Salt	0.39	0.39	0.40	0.37	0.41	0.38
DL-methionine	0.30	0.31	0.31	0.27	0.29	0.29
Lysine HCl	0.18	0.20	0.14	0.17	0.21	0.20
L-threonine	0.07	0.08	0.08	0.03	0.05	0.08
Choline	0.05	0.05	0.05	0.05	0.05	0.05
Biotronic top 3	0	0.10	0.10	0	0.10	0.10
Premix	1	1	1	1	1	1
Total	100	100	100	100	100	100
Calculated metabolisable energy, kcal/kg	3107.21	3100.00	3100.00	3200.00	3206.27	3200.00
Chemical composition (calculated)						
DM, %	87.23	87.48	82.92	86.97	86.96	82.80
CP, %	21.50	21.50	21.50	19.50	19.00	19.00
EE, %	6.50	6.92	7.07	7.06	7.00	7.42
CF, %	3.66	3.62	4.49	3.54	3.47	4.32
Ca, %	0.87	0.87	0.87	0.79	0.87	0.79
P, %	0.72	0.72	0.74	0.68	0.69	0.69
Available phosphorus, %	0.44	0.44	0.44	0.40	0.40	0.40
Lys, %	1.29	1.29	1.29	1.16	1.16	1.16
Meth, %	0.63	0.63	0.65	0.58	0.59	0.60
Meth + Cys, %	0.99	0.99	0.99	0.91	0.91	0.91
Thre, %	0.88	0.88	0.88	0.78	0.78	0.78
Triptophan, %	0.25	0.25	0.25	0.22	0.21	0.21

Note: CON= control group; RB= group with 5 % rice bran inclusion; AM= group with 5 % alfalfa meal inclusion.

Calcium and phosphorus content

Sample preparation was performed according to the method previously described by Untea et al. (2008). Calcium was determined by flame atomic

absorption spectrometry, after the microwave digestion. Phosphorus was determined by spectrophotometry (wavelength 420 nm) with a molecular absorption spectrophotometer (UV-Vis spectrophotometer Jasco V530 Tokio, Japan).

Lutein and zeaxanthine determination

The preparation of samples was carried out using the method previously described by Varzaru et al. (2015). Samples were saponified with an ethanolic solution of potassium hydroxide and extracted with petroleum ether. Lutein and zeaxanthin were eluted with 13% water and 87% acetone on a C18 column (250 × 4.60 mm i.d., 5 μm) (Nucleodur, Macherey-Nagel, Germany), using a HPLC (Perkin Elmer 200 series, Shelton, CT, USA).

Microbial population determination

Microbial populations from the small intestinal and caecal contents were determined by counting the colonies on selected media for each microorganism. Briefly, the classical isolation medium were used to determine the *Enterobacteriaceae* and *E. Coli*, De Man-RogosaSharpe (MRS) agar for *Lactobacillus* and Rambach-Salmonella agar was used to determine the presence or absence of Salmonella spp.

Statistical analysis

Results were statistically analysed by one-way analysis of variance (ANOVA) followed by the Fisher test to calculate the interrelation between the groups, using the StatView software 6.0 (SAS Institute, Inc., Cary, NC).

RESULTS AND DISCUSSION

The chemical analysis of the 2 supplemented raw materials is presented in Table 2. When analyzing rice bran samples with NIRS, close results were reported by Bagchi et al. (2016): 13.29 % crude protein, 14.12 % ether extract and 9.36 % ash. However, lowered values for calcium and phosphorus were observed by Kumari et al. (2018), which reported a content of 0.069 % calcium and 1.331 % phosphorus. The major carotenoid in rice bran is lutein, as confirmed by Belefant-Miller and Grace (2010), but the types and the content of carotenoids may vary among cultivars.

In line with the results of this study, Yıldız et al. (2020) reported that alfalfa meal has 1650 kcal/kg metabolizable energy, showing 17–20% crude protein, 20–25% crude cellulose, 1.50% Ca, 0.25% P.

Table 2. Chemical composition of the rice bran and alfalfa meal

Item	Rice bran	Alfalfa meal
Crude protein, %	15.29	19.89
Ether extract, %	15.51	0.77
Ash, %	8.00	8.39
Calcium, %	0.25	1.24
Phosphorus, %	1.79	0.28
Lutein and zeaxanthine, mg/kg	1.53	29.24

Alfalfa is most often added to poultry diets as a source of carotenoids for improving yolk colour and skin pigmentation (Olgun and Yıldız, 2015). Analyzing the content of carotenoids in dried alfalfa, Englmaierova et al. (2019) found 86.30 mg/kg lutein and 90.30 mg/kg zeaxanthin, values higher than that observed in this study, when it was analyzed alfalfa meal.

The results of growth performance are shown in Table 3.

Table 3. Effect of dietary supplements on growth performance of broilers (mean values/group)

Item	CON	RB	AM	SEM	p-Value
Body weight, g					
14 days	574.69	574.75	574.97	3.141	0.9993
28 days	1776.20	1767.62	1733.42	10.145	0.1907
42 days	3196.44	3130.12	3119.63	20.436	0.2542
Grower (14–28 days)					
Feed intake, g/d	123.16	121.02	122.09	3.115	0.9633
Daily weight gain, g/d	85.65	85.18	82.72	0.769	0.2492
Feed conversion ratio, g/g	1.48	1.45	1.50	0.014	0.3842
Viability, %	99.76	99.92	99.84	0.072	0.6770
Finisher (28–42 days)					
Feed intake, g/d	175.16	175.82	180.63	1.447	0.2462
Daily weight gain, g/d	101.48	97.32	99.02	1.720	0.6147
Feed conversion ratio, g/g	2.29	1.95	1.96	0.141	0.5403
Viability, %	99.82	100.00	100.00	0.041	0.1282
Overall (14–42 days)					
Feed intake, g/d	150.46	149.60	152.69	3.431	0.9319
Daily weight gain, g/d	93.56	91.25	90.87	0.735	0.2741
Feed conversion ratio, g/g	1.91^{b}	1.95^{ab}	2.02^{a}	0.016	0.0325
Viability*, %	99.83	99.96	99.91	0.042	0.4387

Note: *Viability was calculated with the following formula: (100 – mortality in percent).
CON= control group; RB= group with 5 % rice bran inclusion; AM= group with 5 % alfalfa meal inclusion.

During the overall period, feed intake and daily weight gain were not significantly affected by the rice bran nor alfalfa meal supplementation. The viability range in normal limits and was not affected by dietary treatments.

The feed conversion ratio recorded for the entire experimental period (14-42 days) was significantly different (P≤ 0.05) for AM group, compared to the other groups. Different results were reported by Zheng et al. (2019), who observed that supplementation of different levels of alfalfa meal (5 %, 8 %, 10 %) decreased (P < 0.05) feed conversion ratio and mortality compared to the control in Beijing-you chickens. On the contrary, higher feed conversion than control (P < 0.05) was observed when 7.3 % alfalfa meal was included in the basal diet of broilers (Gulizia and Downs, 2020). This increased in the feed conversion ration of the AM group can be attributed to the antinutritional factors present in alfalfa meal, several detrimental effects been associated with the ingestion of high levels of saponins by poultry (Ponte et al., 2004). Moreover, the indigestible fiber contained in alfalfa meal can decrease the digestibility of feed and increase the impact on the gastrointestinal tract (Gulizia and Downs, 2020).

The carcass traits and the relative weight of internal organs are presented in Table 4. Between the RB and AM diets, there were significant treatment effects (P < 0.05) on % breast. Also, RB group registered a significant (P < 0.05) lower % gizzard compared to CON group, while a significant increased (P < 0.05) in % bile was observed for AM group compared to CON group. However, there were no significant treatment effects observed for thigh, liver, heart and spleen between control, RB, and AM groups (P > 0.05).

Table 4. Effect of dietary supplements on carcass traits and relative weight of selected internal organs (mean values/group)

Item	CON	RB	AM	SEM	p-Value
	Age at slaughter (42 d), g				
Live body weight	3141.67	3120.00	3160.00	13.707	0.5192
	% live weight				
Eviscerated carcass	82.72	81.22	81.32	0.547	0.4822
Breast	23.82^{ab}	21.44^{b}	24.51^{a}	0.566	0.0572
Thigh	16.99	18.34	16.75	0.405	0.2319
Gizzard	1.14^{a}	0.87^{b}	1.08^{ab}	0.052	0.0916
Liver	1.65	1.69	1.77	0.037	0.4582
Heart	0.42	0.46	0.46	0.013	0.3178
Spleen	0.08	0.09	0.10	0.05	0.1850
Bile	0.05^{b}	0.05^{b}	0.08^{a}	0.005	0.0082

Note: CON= control group; RB= group with 5 % rice bran inclusion; AM= group with 5 % alfalfa meal inclusion.

In a study conducted on Muscovy ducks, Jiang et al. (2012) reported an improvement of carcass characteristics without an adverse effect on performance, as a consequence of 3, 6, and 9% alfalfa meal supplementation of diets. Dietary inclusion of alfalfa meal (5 % and 10 %) in Hubbard broilers diets showed a significant increase ($P < 0.05$) in the relative weight of abdominal fat, relative weight of gizzard and relative weight of intestines, with no significant effect in terms of consumption, feed conversion and carcass yield (Paredes and Risso, 2020).

The intestinal microbiota of poultry is an important issue, due to the fact that any change in the GIT populations can alter the uptake and availability of nutrients, having functional consequences for the health of the bird. Moreover, a significant effect can be observed in bird performance, being important when regulations do not allow the use of in-feed antibiotics.

There are limited studies showing how alfalfa meal supplementation of broilers diets can influence the GIT microbiota. Escarcha et al. (2012) showed that alfalfa meal supplementation can reduce the pathogen populations in the poultry GIT, due to the fact that alfalfa bacterial fermentation can produce volatile fatty acids, being toxic to some pathogenic bacteria. Moreover, as far as we know, the effect of rice bran on broilers microbiota has been studied only *in vitro*.

The effect of rice bran and alfalfa meal supplementation on the microbiota from small intestine and caecal content is presented in Table 5. Between the CON, RB and AM groups, there were no significant effects ($P > 0.05$) on *Enterobacteriaceae* count from small intestine content.

Table 5. Effect of dietary supplements on the composition of broiler intestinal microbiota (log10 CFU* /g wet intestinal digesta)

Item	CON	RB	AM	SEM	p-Value
Small intestine microbiota					
Enterobacteriaceae, log10	7.289	7.392	7.385	0.053	0.7062
Escherichia coli, log10	6.278^{c}	6.233^{b}	6.223^{a}	0.006	<0.0001
Staphylococci, log10	5.940^{c}	5.888^{b}	5.847^{a}	0.010	<0.0001
Lactobacilli, log10	7.010^{a}	7.046^{b}	7.088^{c}	0.008	<0.0001
Salmonella spp.	nd	nd	nd	-	-
Caecal microbiota					
Enterobacteriaceae, log10	11.454^{c}	11.397^{b}	11.386^{a}	0.007	<0.0001
Escherichia coli, log10	10.207^{c}	10.180^{b}	10.153^{a}	0.006	<0.0001
Staphylococci, log10	8.882^{c}	8.828^{b}	8.748^{a}	0.014	<0.0001
Lactobacilli, log10	11.708^{a}	11.805^{b}	11.854^{c}	0.022	<0.0001
Salmonella spp.	nd	nd	nd	-	-

Note: *colony forming units; CON= control group; RB= group with 5 % rice bran inclusion; AM= group with 5 % alfalfa meal inclusion.

It can be noticed that the total counts of *Escherichia* coli and Staphylococci decreased significantly ($P < 0.0001$) in RB and AM groups compared to the control group, while the count of lactobacilli increased significantly ($P < 0.0001$) for the same groups compared to the control group.

This positive effect of the dietary supplements is in agreement with the results of Zheng et al. (2019), who stated that supplementation of alfalfa meal can positively influence the populations of beneficial bacteria and inhibit potential pathogens, including the Clostridium. Mutlu and Yıldız (2020) also reported that 2.5 % alfalfa meal in the diet of Japanese quails increase significantly ($P < 0.05$) intestine weights and crypt depth, promoting intestinal development without any adverse effect on performance.

The populations of Enterobacteriaceae, *Escherichia* coli and staphylococci in caecal content of broilers were significantly influenced ($P < 0.0001$) by dietary treatments, being decreased in the experimental groups compared to control group. The count of lactobacilli in the caecal content registered a significantly increase ($P < 0.0001$) in AM and RB groups, compared to CON group. The ceca has a low passage rate, being favorable to diverse groups of bacteria proliferation, which influence nutrient utilization and overall health of poultry (Yadav and Jha, 2019).

Conclusion

Utilization of feed additives supplements in diets can influence the gut microbiota for better growth and improved health of poultry. During the experimental period, feed intake, daily weight gain and viability were not significantly affected by the rice bran nor alfalfa meal supplementation.

Supplementation of broilers diets with rice bran and alfalfa meal can increase the population of beneficial bacteria and inhibit the potential pathogens.

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