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THE EFFECT OF ALFALFA MEAL USED IN BROILER CHICKEN DIETS ON PRODUCTION PERFORMANCES, EFFICIENCY FACTORS, CARCASS PARTS, ORGANS DEVELOPMENT AND INTESTINAL MICROFLORA

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Abstract

In this study alfalfa meal effects on production performances, efficiency factors, carcass part, organs development and intestinal microflora were tested. For that, 60 Cobb 500 broiler chickens were divided into two groups of 30 broilers/ group and fed a control or experimental diet in the grower and finisher phases. The birds were raised in an experimental room, with controlled microclimate conditions and fed a control diet (C) based on corn, soybean meal and wheat or an experimental diet containing 5% alfalfa meal (A). The production performances showed that the experimental group, containing 5% alfalfa had a significant effect (P<0.05) on feed conversion ratio. Viability rate was higher in the A group, which influenced the European Production Efficiency Factor and the European Broiler Index but without significant effect. At the end of the trial, six broilers from each group were slaughtered, the carcass part and the organs were measured and samples of intestinal and caecal content were collected for microbiological analyses. The thigh muscle, liver and gizzard were significantly (P<0.05) higher in the A group compared with the C group. The effect of alfalfa meal was very efficient in increasing the beneficial bacteria in the intestinal and caecal segment such as Lactobacilli spp., and significantly decreased the total count of harmful bacteria like Staphylococcus spp., Escherichia coli, Clostridium spp., Enterococcus spp., and Coliforms.

Key words*: alfalfa meal; broiler chickens; feed additive; meat quality; performances*

INTRODUCTION

Alfalfa (*Medicago sativa*) is an important feedstuff for animal feeding being widely used due to its many functional components such as proteins, polysaccharides, saponins, minerals (phosphorus, calcium, potassium, sodium, chlorine, sulphur, magnesium, copper, manganese, iron, cobalt, boron, and molybdenum) and vitamins (A, D, E, K, C, B1, B2, B6, B12, pantothenic and folic acid, inositol, biotin, and niacin). Alfalfa is also rich in xanthophylls and carotenoids and is well-balanced in amino acids (Vlaicu et al., 2021). Alfalfa meal is presenting a broad range of advantages and it is an excellent dietary feed for poultry with different purposes. It has been used in previous research as a feed supplement in laying hens and broiler diets, as a commercially available leaf meal, rich in protein (up to 19% on a dry matter basis) and crude fiber (Jiang et al., 2012; Tufarelli et al., 2018). Although in a recent study, it was reported that the addition of more than 5% alfalfa meal negatively influenced broiler chicken performances (Pliedo et al., 2020), other study reported that 3%, 6%, or 9% alfalfa meal used in broiler diets improved carcass parts (Jiang et al., 2012). All these nutrients can be absorbed by the animals only if the gastrointestinal tract presents a balanced microbial community which further plays an important role in the overall health and function of the host (Shaufi et al., 2015). During the processes of nutrition, metabolism, physiology, and immunity, the gastrointestinal microbiota can promote digestion and absorption of nutrients, stimulate the immune response of the

host, and enhance resistance to infection (Zheng et al., 2019). Literature data reported that alfalfa meal can be a good candidate to enhance some carcass edible parts and intestinal health of the host, without negative effect on production performances if added in adequate amounts (Varzaru et al., 2020; Zheng et al., 2019).

The purpose of this experimental study was to test the effect of 5% alfalfa meal on broiler chickens production performances, efficiency factors, carcass parts, organ development and intestinal microflora.

MATERIALS AND METHODS

Ethical Considerations

This study was conducted in the experimental poultry facility located at the National Research and Development Institute for Biology and Animal Nutrition, Romania. All procedures concerning animals' care, handling, and sampling were conducted under the approval of the Ethical Committee of the institute, according to the Romanian legislation (Law 206/2004, ordinance 28/31.08.2011, Law 43/ 11.04.2014, Directive 2010/63/EU) before the initiation of the study and followed the Romanian guidelines.

Animals, Experimental Design and Diets

A total of 60, Cobb 500 broiler chickens were purchased from a local hatchery and randomly distributed into 2 homogeneous groups of 30 chickens each, with 6 repetitions of 5 replicates each. They were housed in an experimental hall equipped with three-tiered Big Dutchman digestibility cages. The temperature inside the experimental hall and the light regimen were set according to the Cobb 500 broiler management guide. For the starter phase (10 days) broilers were fed with the same basal diet. After these 10 days, the chickens were weighed individually and assigned to two groups (C and A) with homogenous weights (483 g/group), and the actual experimental trial started during the grower (11-28 days) and two finisher phases (29-42 days). Corn and soybean meal were used as the main ingredients in the control diet (C) for broilers and the experimental diet was supplemented with 5% alfalfa (A).

The experimental diet was individually prepared by mixing the control diet thoroughly with the 5% of alfalfa meal at the required incorporation levels, as presented in Table 1. Alfalfa was purchased in pelleted form and was milled to the powder and used in compound feeds, which was given to the broiler in mash form. Both diets were composed to meet the requirements suggested by the Cobb 500 Management Breeding Guide being isonitrogenous, iso-energetic, and iso-fibrous. The ingredients and nutritional composition of the diets are shown in Table 1.

Table 1. Ingredients of the control and experimental diets give to the broilers during grower and finishing phases

Ingredients, % as fed-basis	**C**	**A**	**C**	**A**	**C**	**A**
	Grower (11-28 days)		Finisher I (29-35 days)		Finisher II (36-42 days)	
Corn meal	44.94	45.00	45.00	44.10	44.95	44.86
Soybean meal	23.31	24.75	20.30	20.77	16.94	17.33
Corn gluten	5.00	5.00	5.00	5.00	5.00	5.00
Wheat	19.23	11.87	21.79	15.55	24.70	18.51
Sunflower vegetal oil	2.72	4.32	3.52	5.00	3.99	5.46
Alfalfa meal	-	5.00	-	5.00	-	5.00
Acidifying	-	0.10	-	0.10	-	0.10
L-Lysine HCl	0.38	0.10	0.33	0.08	0.36	0.11
DL-Methionine	0.29	0.18	0.26	0.16	0.25	0.14
L-Threonine	0.10	0.10	0.10	0.08	0.08	0.09
Choline	0.04	0.04	0.04	0.04	0.04	0.04
CaCO3	1.32	0.96	1.20	0.85	1.22	0.87
Monocalcium Phosphate	1.31	1.33	1.14	1.15	1.14	1.16
Chlorine	0.36	0.35	0.36	0.35	0.33	0.33
Premix*	1.00	1.00	1.00	1.00	1.00	1.00
Total ingredients, %	100	100	100	100	100	100

Calculated nutrients						
Metabolizable Energy, kcal/kg	3025	3025	3100	3100	3150	3150
Crude Protein, %	19.46	19.71	18.50	18.50	17.50	17.50
Crude fiber, %	3.45	4.43	3.31	4.25	3.15	4.08
Calcium, %	0.84	0.84	0.76	0.76	0.76	0.76
Available Phosphorus, %	0.42	0.42	0.38	0.38	1.05	0.38
Lysine, %	1.21	1.22	1.10	1.11	0.53	1.05
Methionine +Cysteine, %	0.93	0.94	0.88	0.88	0.84	0.84
Threonine, %	0.81	0.81	0.77	0.73	0.70	0.70
Tryptophan, %	0.19	0.19	0.18	0.18	0.17	0.16

The premix 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/kg Mn; 8000 mg/kg Fe; 500 mg/kg Cu; 6000 mg/kg Zn; 37 mg/kg Co; 152 mg/kg iodine; 18 mg/kg Se. C- control diet; A - a diet containing 5% alfalfa meal.

Growth Performance, Production Efficiency and Sample Collection

The broiler chickens were individually weighed at 10 days and after the experimental feeding trial started. The weight was recorded (BWi, g), and then on the day of slaughter at 42 days (BWf, g). Body weight was recorded, and based on the differences; the average daily weight gain (ADWG, g/broiler/day) was calculated for the entire experimental period. Average daily feed intake (ADFI, g feed/broiler/day) was registered daily and the feed conversion ratio (FCR, kg feed/kg weight) was calculated. The viability (survival rate, %) of the chickens in each group was monitored during the experimental period.

The European Production Efficiency Factor (EPEF) and the European Broiler Index (EBI) were calculated with appropriate formulas (Vlaicu et al., 2023), based on the results obtained from the production performances.

After the rearing period, at the end of the feeding trial (42 days of age), 6 chickens/group with homogenous weights were selected for slaughter and sampling, following the procedures presented elsewhere (Vlaicu et al., 2020). After that, the defeathered carcass was dissected and eviscerated and the organs were measured. From the intestinal content, the samples from the caecum and small intestine were collected into plastic sterile tubes, placed on ice stored at -20°C until the analyses were performed.

Carcass Edible Parts and Organ Development Measurements

The organs' development was measured during the evisceration process. The weight of the carcass, breast muscle, thigh muscle, gizzard, liver, spleen, bile and full intestine were weighted with a Kern balance with 0.0001 precision. The results are expressed as % of total final body weight.

Intestinal Microflora Analyses

After samples defrost, decimal dilutions in phosphate-buffered saline pH 7.0 (PBS, Dulbecco A; Oxoid Livingstone Ltd., London, England) were performed for enumeration of microbial populations and assessed for analyses of Lactic Acid Bacteria as reported previously (Lefter et al., 2023) after the methods presented by Dumitru et al. (2018).

Statistical Analyses

One-way analysis of variance (ANOVA), using GraphPad Prism, version 9 was carried out to determine the effect of alfalfa meal versus control diet on production performances, efficiency factors and meat intestinal microflora. The significance of individual mean differences was considered at $p<0.05$.

RESULTS AND DISCUSSIONS

The production performances obtained at the end of the experiment are presented in Table 2. Although the average daily feed intake (ADFI) in the group receiving the experimental diet (A) was slightly lower than that of the control group (C), the difference was not statistically significant. This implies that the dietary inclusion of alfalfa did not have a substantial influence on the overall appetite and feed consumption. However, it is noteworthy that the feed conversion ratio (FCR), which measures the efficiency of converting feed into body weight, was significantly higher in experimental group (A) compared to control group C. This indicates that the broilers in

experimental group fed with alfalfa required more feed to produce the same amount of weight gain as the hens in control group, suggesting a reduced feed efficiency in the supplemented group. Additionally, the viability rate in group A was higher that in group C, indicating a potential positive impact of the experimental diet on the broilers survival rate.
Interestingly, despite these observed differences, there were no significant effects on the European production efficiency factor (EPEF) and European broiler index (EBI) between group A and group C. The EPEF and EBI are indices used to assess the overall performance and profitability of poultry production systems. The lack of significant effects it attributed to lower viability rate in the group C in comparison to the experimental group.

Table 2. Production performances and efficiency factors of broiler chickens fed control diet versus alfalfa diet

Item	C	A	SEM	P
BWi, g	483.1	483.5	0.020	0.995
BWf, g	3029	3056	0.055	0.321
ADWG, g	77.66	74.23	0.102	0.102
ADFI, g	150.2	144.1	1.035	1.070
FCR, kg feed/kg BW	1.63[b]	1.75[a]	0.022	0.003
Viability, %	96.20	100	-	-
EPEF, %	468.7	483.9	10.09	0.053
EBI, %	441.8	480.9	22.44	0.235

[a, b] - mean marked with a different superscript letter within each column are significantly different. C - control diet; A - experimental diet with the addition of 5% alfalfa meal; SEM - standard error of the mean; P - significance; BWi - initial body weight; BWf - final body weight; ADWG - average daily weight gain; ADFI - average daily feed intake; EPEF - European production efficiency factor; EBI - European broiler index.

In line with these results, no significant effect (P>0.05) on BW, ADWG or ADFI when alfalfa was used at 3%, 5%, 6%, or 7.5% was also reported in other studies (Jiang et al., 2012; Zheng et al., 2019; Gulizia and Downs 2020; Varzaru et al., 2020). Regarding the increased FCR, when 7.5% alfalfa meal was used in broilers the authors Gulizia and Downs (2020) reported significantly higher FCR from 0 to 21 days of growing and a 5.56% mortality rate. In the study of Varzaru et al. (2020), the FCR increased from 1.91 g/g in the C group, to 2.02 g/g in the experimental group supplemented with 5% alfalfa meal. Contrary, Zheng et al. (2019) declared that 5%, 8% or 10% alfalfa meal used in laying-type chickens decreased (P < 0.05) FCR and mortality rate. Moreover, the utilization of 3%, 6%, and 9% alfalfa meal in growing ducks also did not significantly differ in terms of ADG, ADFI, and feed efficiency from those fed the control diet. No effect on production performances when adding 3%, 6%, and 9% alfalfa meal to laying quails diets was also observed by Güçlu et al. (2004). Based on these findings, it can be concluded that alfalfa meal has a different effect on poultry species, due to a variety of biologically active compounds which act through different pathways and influence differently the production performances and other parameters in poultry. This theory was also recently confirmed by other authors (Cui et al., 2022). Although a lot of contradictory findings are published on the optimal inclusion level of alfalfa in poultry diets, the processing techniques such as pelleting, milling or micronizing are believed to increase the digestibility of protein, starch and apparent metabolizable energy (Tufarelli et al., 2018).
The effect of alfalfa diet compared with the control diet, on carcass, edible parts with commercial interest and organ development is presented in Table 3.

Table 3. Organ development of broiler chickens fed control diet versus alfalfa diet

Item, %	C	A	SEM	P
Carcass	2288	2343	32.55	0.424
Breast muscle	619.4	598.9	23.70	0.105
Thigh muscle	499.7[b]	554.1[a]	12.45	0.019
Liver	60.85[b]	67.82[a]	2.240	0.009
Gizzard	33.90[b]	41.24[a]	1.495	0.005
Heart	15.75	15.85	0.347	0.895
Spleen	2.70	2.71	0.147	0.971
Bile	1.50	1.49	0.135	0.976
Full intestine	164.1	187.7	6.328	0.056

[a, b] - mean marked with a different superscript letter within each column are significantly different. C - control diet; A - experimental diet with the addition of 5% alfalfa meal; SEM - standard error of the mean; P - significance.

The results showed that the thigh muscle, the liver, and the gizzard were significantly (p<0.05) higher in the A group compared with the C group. In line with our results, other authors (Jiang et al., 2012; Zheng et al., 2019; Varzaru et al., 2020) reported that alfalfa meal could improve the carcass traits of poultry. However, when used at 7.5% addition, it was

reported that had no effect on organ development or carcass parts of eviscerated broilers (Gulizia & Downs, 2020). Other authors reported that the early introduction of 5% alfalfa leaves or high alfalfa leaf content (15-20%) significantly decreased performance and carcass weights, due to antinutritional substances such as saponins that naturally occur in alfalfa (Pleger et al., 2020). In contrast to broilers, the ducks fed diets with 3, 6, and 9% alfalfa meal decreased abdominal fat percentage and improved carcase traits, without an adverse effect on performance (Jiang et al., 2012). The hardly controllable dose of phytogenic substances and antinutritional factors could also explain these inconsistencies.

A thorough investigation of the chicken gastrointestinal microbiota is essential to understand their roles in host function, as it is well known that the intestinal microbiota is of great importance to host health and production.

The intestines are populated by a complex and dynamic microbial community, which contributes to the health status of the host animals and is the first barrier against pathogens. In this study, it was found that the alfalfa meal was very efficient in decreasing the harmful bacteria in the intestine and caecum of broiler chickens.

The CFU of *Staphylococcus* spp. (Figure 1) was significantly lower in both intestinal segments (intestine and caecum).

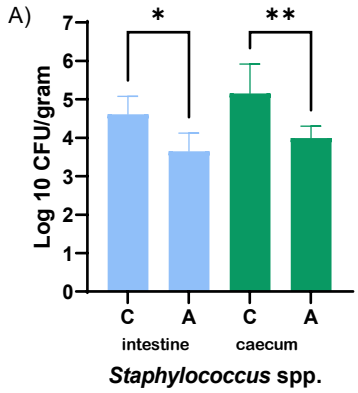

Figure 1. The effect of alfalfa meal on the colony forming units (CFU) of *Staphylococcus* spp. in broiler chickens intestine and caecum

The *Enterococcus* spp. (Figure 2) was significantly altered only in the intestine segment while the *Coliforms* (Figure 3) were significantly decreased only in the caecum segment.

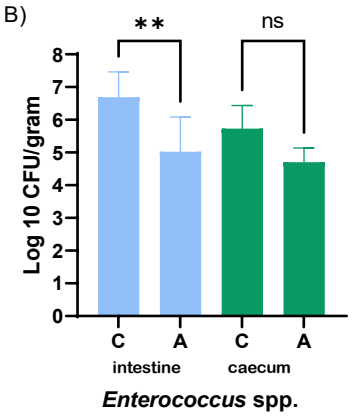

Figure 2. The effect of alfalfa meal on the colony forming units (CFU) of *Enterococcus* spp. in broiler chickens intestine and caecum

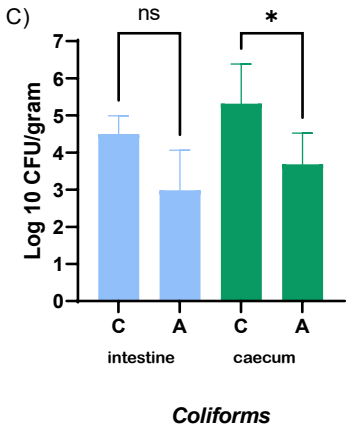

Figure 3. The effect of alfalfa meal on the colony forming units (CFU) of *Coliforms*, in broiler chickens intestine and caecum

No effect was observed for *Clostridium* spp. (Figure 4) while the CFU of *Escherichia coli* (Figure 5) were absent in both segments of the group A compared with the group C, where only the caecum was absent.

The total count of *Enterobacteriaceae* (Figure 6) was lower in both intestinal segments of the group A, but a significant effect was noted only in the caecum.

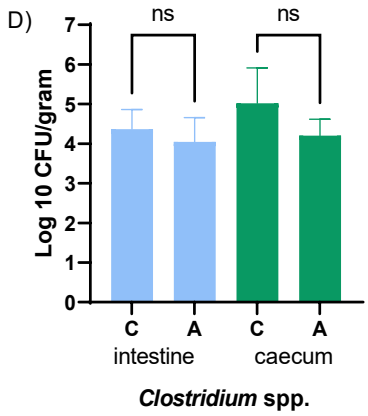

Figure 4. The effect of alfalfa meal on the colony forming units (CFU) of *Clostridium* spp. in broiler chickens intestine and caecum

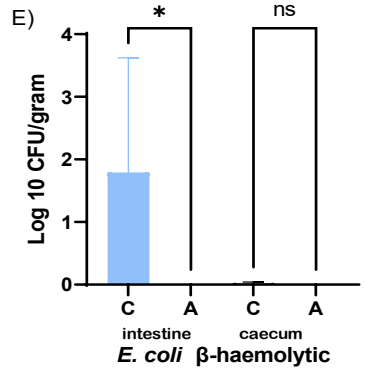

Figure 5. The effect of alfalfa meal on the colony forming units (CFU) of *Escherichia coli* in broiler chickens intestine and caecum

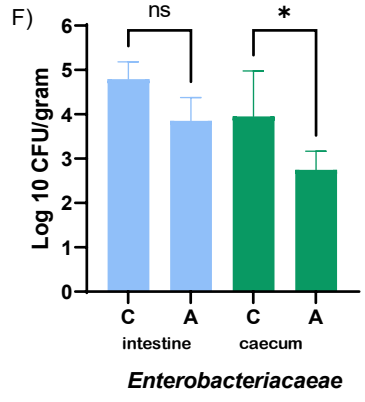

Figure 6. The effect of alfalfa meal on the colony forming units (CFU) of *Enterobacteriaceae* in broiler chickens intestine and caecum

Due to the numerous bioactive compounds present in the alfalfa meal, the CFU of beneficial bacteria determined in this study (*Lactobacillus* spp.) was significantly increased in the intestine and caecum (Figure 7). Although the alteration of the microflora population was made by the bioactive compounds present in the added feed ingredient, however, the composition of the intestinal microbiota can be also altered by good environmental conditions.

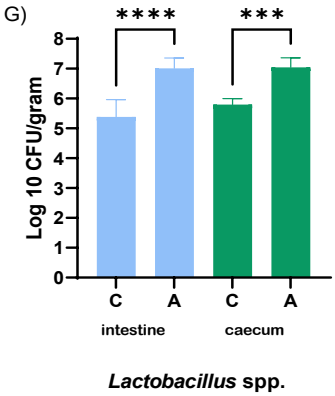

Figure 7. The effect of alfalfa meal on the colony forming units (CFU) of *Lactobacillus* spp. in broiler chickens intestine and caecum

It has been reported that plant bioactive compounds can be fermented to produce metabolites by the gut microbiota in the distal gastro intestine, and further change and reshape the intestinal microbial community via fermentation (Zhang et al., 2022). In line with our findings, it was reported by Varzaru et al. (2020), that the addition of 5% alfalfa meal had a significant effect on altering the microbial population in the intestine and caecum. Other authors (Cui et al., 2022) reported that dietary inclusion of 6% alfalfa was beneficial to improve the small intestinal morphology, caecal microbiota diversity and caecal metabolic function in Zhuanghe Dagu chickens These results were previously sustained by Zheng et al. (2019), when alfalfa meal was tested during the growing phase of the broiler chickens. The beneficial effects have been also supported in other studies when different plant feed additives have been used in broiler diets to promote the health of intestinal microbiota (Turcu et al., 2018; Saracila et al., 2018;

Panaite et al., 2022). In one of the above-mentioned papers, it was reported that obtaining different results among different parts of the microbiota (intestine or caecum) is normal because each part of the intestine develops its unique microbial profile. Inulin and citrus wastes supplementation were also reported to be efficient in decreasing the intestinal and caecal CFU of *Escherichia coli*, and *Salmonella*, promoted the proliferation of *Lactobacillus* spp. in broilers (Yusrizal et al., 2003; Vlaicu et al., 2020). To date the main explanation is given by the mechanism of pathogen inhibition in the microbiome which includes the competition for nutrients and binding locations on the intestinal epithelium, creating short-chain fatty acids and decreasing the pH (Yaqoob et al., 2021). Lower intestinal pH, improve the intestinal microbial balance by reducing the population of pathogenic species, and thus improves the health of the host, resulting in improved performances and health, without affecting these parameters. Although probiotics still have a top place as feed additives to replace antibiotics usage (Yaqoob et al., 2021; Lefter et al., 2023), the need for new dietary ingredients makes the plants a significant competitor to them.

CONCLUSIONS

Alfalfa meal can be used safely for up to 5% in broiler chickens' diets, during the grower and finisher phases, without altering the body weight and with a beneficial effect on some edible carcass parts and intestinal microbiota. However, alfalfa meal can significantly affect the feed conversion ratio, due to anti-nutritional factors.

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