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Herbal extracts in diets for broilers

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ABSTRACT - The objective of this study was to evaluate the effect of feeding herbal extracts for broilers on performance and histology of the intestinal mucosa and its effects on the profiting from the metabolizable energy of experimental diets. For so, two experiments were conducted. In experiment I, the inclusion of different herbal extracts in diets on performance and intestinal histology of broilers was evaluated, and in experiment II, the values of apparent metabolizable energy and metabolizable energy corrected by the nitrogen balance of the experimental diets were studied. Treatments consisted of: positive control diet; positive control + avilamycin; negative control; negative control + 100 ppm of a complex containing three different herbal medicines (pepper, cinnamon and oregano); negative control + 75 ppm garlic extract; negative control + 150 ppm garlic extract. In the performance experiment, which comprised the period of 1 to 40 days of age, 960 male broilers were distributed in a randomized block design, with six treatments and eight replicates, with 20 birds per experimental unit. In experiment II, the method adopted was the traditional of total excreta collection with male broiler chicks in the age of 14 to 24 days, in a completely randomized design, with six treatments and eight replicates with five birds per experimental unit. The intestinal villus height was improved with addition of the composite containing the three herbal extracts; however, crypt depth and villus/crypt ratio were not affected. The use of herbal extract in diets for broilers promotes performance similar to that with the use of antibiotics. Herbal extracts can be incorporated into diets replacing antibiotics without compromising the metabolizable energy of diets, performance or intestinal mucosa for broilers in the period of 1 to 40 days of age.

Key Words: growth promoters, herbal medicines, intestinal histology, metabolizable energy

Introduction

The Brazilian aviculture has presented great advancements in production, conferring Brazil with an outstanding position among the main countries that produce and export chicken meat. As a result of the fast development, along with high productivity rates and relatively low production costs, chicken is, currently, the most consumed meat in the country.

Some antimicrobial agents have been incorporated in diets for broilers at sub-therapeutic doses, aiming at the improvement of birds performance (Kim et al., 2008). This practice derives from observations made since 1946, that birds raised intensively had greater feed efficiency when fed diets containing antimicrobial growth promoters.

The use of antibiotics, with prophylactic characters in aviculture, started to be seen as a risk factor to human health, for there is the chance that their residues be found in the tissues, and also by the probable induction of cross-resistance for pathogenic bacteria in humans (Costa et al., 2007), which could generate problems related to public health. Thus, restrictions and new regulations concerning the use of these products in animal feed have arisen. In the European Union, the use of any growth-promoting antimicrobial in animal production has been banned since January, 2006 (Brugalli, 2003); its use is only permitted for healing purposes. Therefore, the development of new alternatives to mend or minimize the impact of the removal of antimicrobial from diets as growth promoters becomes necessary.

Recent studies have been testing the inclusion of herbal extracts as replacers to antibiotics as growth promoters. These extracts present a mechanism of action based on the alteration of the intestinal microbiota, the increase of enzyme secretion, the improvement of the immune response, the morpho-histological maintenance of the gastrointestinal tract and the antioxidant activity (Brugalli, 2003). Several research studies have demonstrated their in vitro effect against many pathogens, with antimicrobial, antifungal and/or anthelmintic activity, in addition to antioxidant effects (Kamel, 2000).

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The objective of this study was to evaluate the effect of the utilization of herbal extracts in the feeding of broilers on the parameters performance and histology of the intestinal mucosa and their influence on the profiting from the metabolizable energy of experimental diets.

Material and Methods

A total of 960 male broiler chicks of the ROSS strain, in the period of 1 to 40 days of age were utilized. Chicks were distributed at the first day of age, in a completely randomized design with six treatments (Table 1), eight replicates and 20 birds per pen; each pen was considered an experimental unit.

Animals were housed in masonry shed divided in 1.0 × 1.5 m pens, with bedding of reused wood shavings, to increase the sanitation challenge. The shed utilized had concrete floor and as asbestos tiles covering and pens were provided with tubular feeders and nipple drinkers. Each pen was equipped with infra red bulb for heating, which was utilized until 16 days of age. A continuous lighting program (24 hours of light) was adopted, and maximum minimum thermometers were utilized inside the shed for daily temperature recording.

Experimental diets (positive control) were formulated to meet the requirements of birds at the starter and growth phases (Tables 2 and 3), according to Rostagno et al. (2005), for all the nutrients. In the diets of negative control, 75 kcal were reduced from the metabolizable energy (ME) value and 2% of the amino acids of diets. Feed and water were supplied *ad libitum* during the entire experimental period.

All the treatments underwent sanitation challenge, as proposed by Menten (2002), where the author claims the existence of a field sanitation challenge enough for growth promoters to produce significant effect on animal performance is essential.

Animals were weighted at the beginning, at 21 days of age and at the end of the experiment, at 40 days of age, for assessment of weight gain. Feed and leftovers were also weighed for determination of feed intake, weight gain, feed conversion and productive efficiency index at 40 days of age. Bird vilability was also monitored.

Table 1 - Treatments utilized

	Treatments
T 1	Positive control (Brazilian charts)
T 2	Positive control (Brazilian charts) + 10 ppm avilamycin
T 3	Negative control (Brazilian charts) – Reduction of ME (75 kcal) and AA (2%) levels
T 4	Negative control + 100 ppm herbal complex (5 ppm carvacrol + 3 ppm cinnamaldehyde + 2 ppm capsaicin)
T 5	Negative control + 75 ppm garlic extract
T 6	Negative control + 150 ppm garlic extract

T - treatment; ME - metabolizable energy; AA - amino acid.

Table 2 - Feed and nutritional composition of the starter diet (1 to 21 days)

Ingredient	Positive control	Negative control
Corn, g/kg	548.95	581.91
Soybean meal (46%), g/kg	380.00	364.70
Soybean oil, g/kg	30.00	12.80
Dicalcium phosphate, g/kg	18.30	18.30
Limestone, g/kg	9.00	8.60
Salt, g/kg	4.90	4.90
DL-methionine (99%), g/kg	2.40	2.26
L-lysine HCl, g/kg	1.00	1.08
L-threonine, g/kg	0.10	0.10
Choline chloride (60%), g/kg	1.00	1.00
Vitamin supplement, g/kg[1]	1.20	1.20
Mineral supplement, g/kg[2]	0.50	0.50
Anticoccidial, g/kg[3]	0.55	0.55
Antioxidant, g/kg[4]	0.10	0.10
Starch, g/kg[5]	2.00	2.00
Calculated values		
Metabolizable energy, kcal/kg	3000	2925
Crude protein, g/kg	219.8	21.56
Digestible lysine, g/kg	11.63	11.37
Digestible methionine, g/k	5.44	5.26
Digestible met. + cys., g/kg	8.39	8.19
Digestible threonine, g/kg	7.55	7.39
Digestible tryptophan, g/kg	2.46	2.39
Digestible arginine., g/kg	14.14	13.77
Digestible valine, g/kg	9.25	9.07
Calcium, g/kg	9.02	8.84
Available phosphorus, g/kg	4.51	4.51
Sodium, g/kg	2.13	2.13
Potassium, g/kg	8.49	8.30
Chlorine, g/kg	3.77	3.79

met.+cys. - methionine + cysteine.
[1] Vitamin supplement containing, per kg of product: vit. A - 10,000,000 U.I.; vit. D3 - 2,000,000 U.I.; vit. E - 30,000 U.I.; vit. B1 - 2.0 g; vit. B2 - 6.0 g; vit. B6 - 4.0 g; vit. B12 - 0.015 g; pantotenic acid - 12.0 g; biotin - 0.1 g; vit. K3 - 3.0 g; folic acid - 1.0 g; niacin - 50.0 g; selenium - 250.0 mg; q.s. - 1,000 g.
[2] Mineral supplement containing, per kg of product: iron - 100.0 g; cobalt - 2.0 g; copper - 20.0 g; manganese - 160.0 g; zinc - 100.0 g; iodine - 2.0 g; q.s. - 1000 g;
[3] Salinomycin (10%).
[4] 99% Butylated hydroxytoluene.
[5] Herbal extracts replaced the same quantity of starch in the diet.

At 21 days of age, one animal from each experiment unit, chosen randomly, was slaughter by cervical dislocation, then bleeding and evisceration were performed, with subsequent collection of approximately 1.0 cm of the intestine, corresponding to the jejunum. Afterwards, this portion of the intestine was open by the mesenteric and extended by the serosal border, and, next, fixed in Bouin solution for 24 hours. After this period, the tissue sample was transferred to absolute and 90% alcohol and processed by standard paraffin method, according to the suggestion of Labiocel (2002). After this stage, 4 to 6- μm sections were cut and colored by hematoxylin technique.

Histology slides were prepared at the Histology Laboratory of the Veterinary Department of Universidade

Table 3 - Feed and nutritional composition of the growth/finishing diet

Ingredient	Positive control	Negative control
Corn, g/kg	603.45	633.40
Soybean meal (46%), g/kg	315.70	301.72
Soybean oil, g/kg	42.50	25.97
Dicalcium phosphate, g/kg	16.50	16.54
Limestone, g/kg	8.50	8.52
Salt, g/kg	4.70	4.69
DL-methionine, g/kg	2.40	2.15
L-lysine HCl, g/kg	1.00	1.62
L-threonine, g/kg	0.10	0.24
Choline chloride (60%), g/kg	1.00	1.00
Vitamin supplement, g/kg[1]	1.00	1.00
Mineral supplement, g/kg[2]	0.50	0.50
Anticoccidial, g/kg[3]	0.55	0.55
Antioxidant, g/kg[4]	0.10	0.10
Starch, g/kg[5]	2.00	2.00
Calculated values		
Metabolizable energy, kcal/kg	3150	3075
Crude protein, g/kg	194.10	190.30
Digestible lysine, g/kg	10.50	10.29
Digestible methionine, g/k	5.05	4.87
Digestible met. + cys., g/kg	7.75	7.55
Digestible threonine, g/kg	6.84	6.69
Digestible tryptophan, g/kg	2.13	2.07
Digestible arginine., g/kg	12.27	11.93
Digestible valine, g/kg	8.20	8.04
Calcium, g/kg	8.24	8.24
Available phosphorus, g/kg	4.10	4.10
Sodium, g/kg	2.05	2.05
Potassium, g/kg	7.46	7.29
Chlorine, g/kg	3.56	3.59

met.+cys. - methionine + cysteine.
[1] Vitamin supplement containing, per kg of product: Vit. A - 10,000,000 U.I.; Vit. D3 - 2,000,000 U.I.; Vit. E - 30,000 U.I.; Vit. B1 - 2.0 g; Vit. B2 - 6.0 g; Vit. B6 - 4.0 g; Vit. B12 - 0.015 g; Pantotenic acid - 12.0 g; Biotin - 0.1 g; Vit. K3 - 3.0 g; Folic acid - 1.0 g; Naicin - 50.0 g; Selenium - 250.0 mg; q.s. - 1,000 g.
[2] Mineral supplement containing, per kg of product: Iron - 100.0 g; Cobalt - 2.0 g; Copper - 20.0 g; Manganese - 160.0 g; Zinc - 100.0 g; Iodine - 2.0 g; q.s. - 1000 g;
[3] Salinomycin (10%)
[4] 99% Butylated hydroxytoluene.
[5] Herbal extracts replaced the same quantity of starch in the diet.

Federal de Viçosa, and villus height and crypt depth measurements were conducted at the Animal Nutrition Laboratory of the Animal Science Department of Universidade Federal de Viçosa, by means of image analyzer Imagepro Plus 1.3.2 (1994) (40X increase) and optical microscope. For each slide, 33 villi and 33 crypts were chosen and measured. Three extreme values were discarded for statistical analysis, so that the average of each slide was constituted of 30 measures.

In the second experiment, 240 male broiler chicks from the ROSS strain, in the period of 14 to 24 days of age, with average weight of 530 g were utilized for determination of apparent metabolizable energy and apparent metabolizable energy corrected for nitrogen (AME and AMEn, respectively), through the standard total excretion collection method. Chicks were raised in masonry shed from the first to the 14th days of age under the same raising and management conditions adopted for birds of experiment I, then transferred to metallic batteries constituted of cages, which were distributed in two floors and equipped with feeder and nipple drinker.

Treatments and diets were the same described for experiment I (Table 1); in this experiment, only the starter diet was utilized (Table 2). The experimental period was of 10 days: five for birds to adapt to cages, diets and management, and five for total excreta collection. The experimental design was completely randomized, with six treatments and eight replicates of five birds.

The excreta of all experimental units were collected daily (8:00 a.m. and 3:00 p.m.) on trays covered with plastic and conditioned in freezer until the end of the experiment. At the end of the experimental period, excreta were defrosted, weighed, homogenized for sampling and oven-dried (55 ºC). After, they were sent to analysis at the Animal Nutrition Laboratory of the Animal Science Department of UFV, according to techniques described by Silva & Queiroz (2002), for determination of metabolizable energy and nitrogen balance values.

At the end of the experiment, the amount of feed consumed per experimental unit during the five collection days was determined. Once the results of the laboratory analysis of the feedstuffs, reference diet, test diets and excreta were obtained, the AME and AMEn values were calculated, by means of equations suggested by Matterson et al. (1965).

$$\text{AME of the diet (kcal/kg)} = \frac{\text{GE ingested} - \text{GE excreted}}{\text{DM ingested}}$$

$$\text{AMEn of the diet (kcal/kg)} = \frac{\text{GE ingested} - (\text{GE excreted} - 8.22 \times \text{NB})}{\text{DM ingested}}$$

GE = gross energy; NB = nitrogen (N) balance = N ingested - N excreted; DM = dry matter.

The experimental results were submitted to variance analysis, and when there was significant effect, means were compared by the Student-Newmann-Keuls test, at 0.05% significance level, through the software SAEG (Sistema para Análises Estatísticas, version 9.0)

Results and Discussion

Maximum and minimum temperatures, with their standard deviations, were: 31.55±2.99 ºC and 22.84±2.24 ºC for the phase of 1-21 days and 29.47±2.89 ºC and 21.76±1.52 ºC for the phase of 22-42 days, respectively. In the period of 1 to 21 days (Table 4), no effects (P>0.05) of treatments on feed intake were observed, which agrees with the results obtained

Table 4 - Performance of 1 to 21-day of age broilers

Treatments	Body weight (g)	Weight gain (g)	Feed intake (g)	Feed conversion (g/g)
PC	867.8a	822.8a	1198.5a	1.457a
PC + 10 ppm avilamycin	875.2a	829.5a	1199.8a	1.446a
NC	802.6b	758.0b	1163.4a	1.534b
NC + 100 ppm HC[1]	842.9a	798.1a	1178.4a	1.476a
NC + 75 ppm GE	851.4a	807.4a	1193.2a	1.479a
NC + 150 ppm GE	870.0a	825.0a	1213.6a	1.471a
P	0.003	0.003	0.107	0.001
CV (%)	3.19	3.36	3.09	2.30

[1] Herbal complex - 5 ppm carvacrol + 3 ppm cinnamaldehyde + 2 ppm capsaicin.
Means followed by different letters on the same column indicate significant difference (P<0.05) by SNK test.
PC - positive control; NC - negative control; GE - garlic extract; CV - coefficient of variation.

by Barreto et al. (2008), who did not verify any difference in broilers intake when assessing diets containing cinnamon, clove, oregano and pepper extracts, in addition to a control treatment and another with avilamycin addition.

In the starter phase, differences (P<0.05) were found for weight gain and live weight of birds when comparing the negative control treatment with the others. This was because of the lower energy and amino acid concentration of the diet (75 kcal and 2% fewer amino acids compared with the treatments of the positive control), and absence of extracts. Such findings evinced that the extracts were capable of stimulating enzymatic action, improving the use of the nutrients from the diet, as described by Wang & Bourne (1998) and Manzanilla et al. (2004), for pigs and Lee et al. (2004), for birds. According to these authors, the presence of extracts containing capsaicin (red pepper) and cinnamaldehyde (cinnamon) stimulated production and secretion of salivary amylase and pancreatic and intestinal enzymes.

The presence of carvacrol (oregano) in the herbal complex also contributed to the improvement in the parameters evaluated by its anti microbial potential, as affirmed by Fukayama et al. (2005). The combination of different compounds boosted its effect, which were superior to its use singly. Kamel (2000) and Langhout (2000) consider that, for the obtainment of better results with the utilization of herbal medicines, combinations of essential oils from different plants should be administered and strengthened by the addition of more relevant active ingredients.

The development of the gastrointestinal tract, especially that of the intestinal mucosa, is related to the stimulation of the process of cell mitosis and the modulation of the enteric microbial flora, where the addition of carvacrol reduces the microbial population, diminishing the competition for nutrients between the bacteria and the host, along with the reduction of direct injuries to the intestinal mucosa.

Broiler chicks, at birth, have a little developed gastrointestinal tract, with low production of certain enzymes such as amylase, lipase and trypsin, and, because of this, chickens at the starter phase, specifically in the period of 1 to 10 days of age, use the nutrients of the diet with lower efficiency (Dunnington & Siegel, 1995; Noy & Sklan, 2000). As the age passes, enzyme production increases, mainly according to the stimulus originated from the substrates (diet) and the increase in the size of the organs of the gastrointestinal tract (Nitsan et al., 1991). Thus, the increase in the use of diet ingredients, influenced by the stimulation to the enzyme activity generated by the plant extract in the first days of age compensated the low production that birds would usually present at this phase, promoting, therefore, significant improvement to weight gain. Besides, these compounds promote improvement in the intestinal mucosa, by augmenting the height of the intestinal villi and the absorptive area, consequently increasing nutrient absorption. This situation occurs due to the decrease in the intestinal microbial load, which promoted reduction in the desquamation of the villi apex, associated to the effect of mitosis stimulation in the intestinal crypts, increasing the intestinal turnover speed.

Diets containing garlic extract had beneficial effect on bird performance when compared with negative control, probably due to their great antibacterial power, as confirmed by Huyghebaert (2003). The author states that allicin has inhibitory effect against certain pathogens, such as *E. coli*, *Salmonella* sp., *Campylobacter* sp., *Staphylococcus aureus* and *Clostridium perfringens*. The same basis is applied to carvacrol, which is present in the herbal complex, in which Butolo (2005) affirms that the active ingredient of oregano also presents inhibitory effect against the microorganisms cited above.

It was not possible to observe significant effects on weight gain in the starter phase between the different herbal extracts, the antibiotic and positive control. Such results corroborate those found by Toledo et al. (2007), when testing a composite with several herbal medicines in comparison with the antibiotic, where they also did not find difference as for weight gain in this phase. Moreover, the results of the present study are in agreement with those of Dieumou et al. (2009), who, when evaluating garlic and ginger extracts in diets for broilers, did not find significant differences in the parameters evaluated.

Feed conversion in the period of 1 to 21 days showed behavior similar to that of weight gain, in which the negative control treatment had the worst values (P<0.05). The other treatments were not different from each other (P>0.05). This finding is in agreement with those of Hernández et al. (2004), who, when evaluating two combinations of herbal extracts (cinnamon + pepper + oregano and thyme + sage + rosemary) in relation to a control treatment and another with avilamycin, also did not find differences in the feed conversion of birds. Fukayama et al. (2005), working with oregano extract in lieu of the antibiotic in the diets also did not find influence on feed conversion, although in the two studies there was no negative control treatment for comparisons.

Overall, the same trends were observed on performance for the total period (Table 5). No differences (P>0.05) of intake were observed between the treatments. Weight gain and body weight values were lower in treatments negative control, negative control + 100 ppm herbal extract and negative control + 75 ppm garlic extract, which did not differ from each other (P>0.05). This occurrence for the negative control treatment is probably due to the fact that this diet presents nutritional deficiency. As for treatments negative control + 100 ppm of the herbal extract and negative control + 75 ppm, the results indicate that such extracts, at the levels utilized, do not have any effect on the improvement of nutrient digestibility, and consequently, on birds weight gain. Also, birds, from 21 days of age, have their enzyme production ability elevated enough for a great use of the nutrients of the diet; small increases in the enzyme production, stimulated by the herbal extracts in this phase, become imperceptible. Yet, as for weight gain, final weight and feed conversion of birds, it was found that treatments positive control, positive control + 10 ppm avilamycin, negative control + 100 herbal composite, negative control + 75 and 100 ppm avilamycin and negative control + 100 ppm garlic extract did not show difference in the values (P>0.05).

However, the treatments showed influence (P<0.05) on the productive efficiency index (PEI), in which the negative control treatment presented lower value. Other treatments were not different from each other (P>0.05). This finding shows that herbal extracts promoted improvement to the diets, since the values were similar to positive control and the treatment with antibiotic.

The absence of significant difference between treatments with and without growth promoters allows us to conclude that the use of reused bedding was not enough to promote sanitary challenge and to point differences between treatments on the parameters of bird performance in the total period.

The values of intestinal villus heights of birds which received diet containing the composite with different herbal extracts were similar to those of positive control treatment with the addition of avilamycin and negative control with 75 ppm garlic extract (Table 6). It could also be observed that the treatments used, except for that with the herbal composite, presented similar values between each other (P>0.05).

The presence of the composites capsaicin (red pepper) and cinnamaldehyde (cinnamon) seems to have promoted increase in villi heights, as verified by Wang & Bourne (1998) and Platel & Srinivasan (2000), when they affirmed that capsaicin has showed to be efficient in stimulating salivation in pigs (amylase production) and by (Brugalli, 2003), who reported that there is increase in the secretion of pancreatic and intestinal enzymes, promoting, thus, reduction in the intestinal viscosity and improving the digestive process. Wang & Bourne (1998) and Manzanilla et al. (2004) cite that cinnamaldehyde presented stimulating

Table 5 - Performance of 1 to 40-day-old broilers

Treatments	Body weight (g)	Weight gain (g)	Feed intake (g)	Feed conversion (g/g)	Viab. (%)	PEI
PC	2500.5a	2456.7a	4113.7a	1.674a	98.75	362.3a
PC + 10 ppm avilamycin	2527.8a	2482.1a	4138.7a	1.667a	95.62	355.6a
NC	2395.6b	2351.0b	4026.8a	1.712b	96.87	332.6b
NC + 100 ppm HC[1]	2442.1ab	2397.3ab	4057.9a	1.693ab	99.37	351.8a
NC + 75 ppm GE	2442.9ab	2399.1ab	3995.7a	1.665a	99.73	357.9a
NC + 150 ppm GE	2521.9a	2469.6a	4163.1a	1.686ab	97.5	357.4a
P	0.002	0.004	0.087	0.001	—	0.035
CV (%)	2.56	2.70	2.47	1.51	—	4.53

[1] Herbal complex - 5 ppm carvacrol + 3 ppm cinnamaldehyde + 2 ppm capsaicin.
Means followed by different letters in the same column indicate significant difference. P<0.05 by SNK test.
PC - positive control; NC - negative control; GE - garlic extract; Viab. - viability; PEI - Productive Efficiency Index; CV - coefficient of variation.

Table 6 - Intestinal histology values of the jejunum of broilers slaughtered at 21 days of age (μm)

Treatments	Villus height	Crypt depth	Villus/crypt ratio
PC	725.9b	150.0a	5.1a
PC + 10 ppm avilamycin	789.2ab	138.7a	5.8a
NC	704.6b	154.5a	4.8a
NC + 100 ppm HC[1]	842.0a	145.5a	5.8a
NC + 75 ppm GE	767.7ab	148.6a	5.2a
NC + 150 ppm GE	734.1b	135.7a	5.5a
P	0.011	0.104	0.183
CV (%)	10.08	14.9	17.6

[1] Herbal complex - 5 ppm carvacrol + 3 ppm cinnamaldehyde + 2 ppm capsaicin.
Means followed by different letters in the same column indicate significant difference. P<0.05 by SNK test.
PC - positive control; NC - negative control; GE - garlic extract; CV - coefficient of variation.

effect on the pancreatic enzymes. Another effect of cinnamaldehyde concerns the digestion process, where the oxygen radicals (superoxide radicals) can be produced through oxidation reactions, attacking the surface of the intestinal mucosa, damaging nutrient absorption.

Cinnamaldehyde optimizes the antioxidant activity of enzymes superoxide dismutase, glutathione S-transferase and catalase (Brugalli, 2003), and this enzyme complex is responsible for converting the superoxide radicals into water and molecular oxygen, keeping, thus, intestinal health. Consequently, there will be greater intestinal absorption, since the oxidation process, resulting from the digestion of the feedstuffs results in the formation of free radicals harmful to the intestinal mucosa, which promotes oxidation at the apex of the villi, diminishing their height.

The intestinal villi height is one datum of extreme importance, for when they elevate, there is an increase in the intestinal absorption area, which improves the use of the nutrients. However, the difference found between some treatments did not reflect on bird performance. Fukayama et al. (2005), when assessing the villi height of the duodenum of broilers at 21 days of age fed oregano extract, did not find significant differences in villi height.

Variables crypt depth and the villi height/crypt depth ratio were not affected (P>0.05) by the different treatments. These results are in agreement with those achieved by Fukayama et al. (2005). The crypts of Lieberkuhn are responsible for the production of the enterocytes which will compose the intestinal villi, and their depth reflects the degree of exigency of the synthesis of these cells. The more the villi are exposed to injuries, be it by pathogens, anti-nutritional or toxic factors, the greater the synthesis of enterocytes by the crypts to try to promote adequate cell renewal, in order to try to keep the integrity of the intestinal mucosa (to augment the cell turnover). In this manner, the more the crypt is demanded in terms of cell renewal, the greater its depth. The accented renewal of the cells of the intestinal mucosa is the main factor of induction to morpho-functional changes, which can affect directly or indirectly the mechanisms of nutrient absorption, and consequently, bird performance.

The villous height/crypt depth ratio indicates the degree of maintenance of intestinal villi in relation to the degree of exigency of the synthesis of enterocites by the crypts; so it is a great indicator of intestinal health.

Maximum and minimum temperatures, with their standard deviations, of experiment II were 30.95±2.32 ºC and 23.5±2.84 ºC, respectively. No significant effects (P>0.05) were observed with the addition of the herbal extracts on apparent metabolizable energy (AME) and apparent metabolizable energy corrected by nitrogen balance (AMEn) (Table 7). These results corroborate those

Table 7 - Dry matter and energy values of experimental diets expressed on an as fed basis

Treatments	DM (%)	AME (kcal/kg)	AMEn (kcal/kg)
PC	88.01	3170	2990
PC + 10 ppm avilamycin	88.01	3189	3011
NC	87.91	3029	2857
NC + 100 ppm HC[1]	87.91	3034	2860
NC + 75 ppm GE	87.91	3027	2871
NC + 150 ppm GE	87.91	3024	2877
P	—	0.067	0.081
CV (%)	—	1.28	1.30

[1] Herbal extract - 5 ppm carvacrol + 3 ppm cinnamaldehyde + 2 ppm capsaicin.
Means followed by different letters in the same column indicate significant difference. P<0.05 by SNK test.
DM - dry matter; AME - apparent metabolizable energy; AMEn - apparent metabolizable energy corrected for nitrogen; PC - positive control; NC - negative control; GE - garlic extract; CV - coefficient of variation.

obtained by Lee et al. (2003) and Barreto et al. (2008), in which no significant differences were found with the addition of extracts containing carvacrol, capsaicin, cinnamaldehyde, thymol, or even avilamycin in the diet. This fact could be attributed to the supply of highly-digestible diets to the animals, in which the increase in digestibility would be almost imperceptible (Barreto et al., 2008). Besides, since the birds utilized in the experiment were in the phase of 14-24 days of age, all their digestive and enzymatic system was developed, hampering the improvement in digestibility through stimulation to increase of the endogenous enzymatic synthesis. The diets of the positive control treatments had apparent metabolizable energy values compatible with the formulation of experimental diets.

According to Leeson & Summers (2001), it is necessary to correct the estimated values of energy by nitrogen balance because, during a metabolism assay, it is impossible to ensure that all birds will present the same growth rate. The AME values were superior to those of AMEn, and according to Nery et al. (2007), this characteristic is normal when metabolizable energy values are determined in growing birds, for in this phase there is higher retention of nitrogen so that there is deposition of protein tissue. Although no effects of plant extracts were verified on the AME and AMEn values, there are studies in the literature that affirm that the presence of the active ingredients in these extracts in the diet promotes increase in the production of pepsin and hydrochloric acid by the organism, contributing to reduction in the stomach and small intestine pH and promoting reduction in the proliferation of pathogenic bacteria and lower competition for nutrients, associated to stimulation to the pancreatic secretion, which improves digestibility of nutrients (Mellor, 2000).

Conclusions

The use of herbal extracts in diets for broilers promotes performance similar to the use of antibiotics. Herbal extracts can be incorporated into diets, replacing the antibiotic, without compromising performance, the intestinal mucosa or the metabolizable energy of diets for broilers in the period of 1 to 40 days of age in environments of high sanitation challenge.

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