Effect of dietary restriction and hay inclusion in the diet of slow-growing broilers

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Abstract

The objective of this study was to evaluate the effect of dietary restriction and inclusion of alfalfa (Medicago sativa L.) and Bermudagrass (Cynodon dactylon cv Coastal) hays in the diets of ISA Label JA57 slow-growing male broilers on performance, gastrointestinal tract characteristics, and economic viability. A total of 272 broilers at 21 days old were distributed in a randomized experimental design with four treatments, four replicates, and 17 birds per experimental unit. The treatments consisted of ad libitum fed control intake, feed restriction (80% of the control intake), and feed restrictions with supplementation of alfalfa hay (80% of the control intake+20% alfalfa) or Bermudagrass hay (80% control intake+20% Bermuda). Dietary restriction, with and without hay inclusion, negatively affected (P<0.05) the weight gain of the birds; however, feed conversion was improved (P<0.05) for animals that underwent only restricted feeding, which also had the best economic indices. Birds subjected to dietary restriction and inclusion of hays showed changes (P<0.05) in the gastrointestinal organs and intestinal morphology.

Introduction

In recent years, it has been observed that the poultry industry, grounded in unconventional designs, grows in a productive scenario, meeting the demands of the consumer market, which seeks foods with specific organoleptic characteristics (Fanatico et al., 2007), a presumed quality, and guaranteed nutritional security and health (Le Bihan-Duval, 2004). Rearing broilers in alternative production systems can ascribe distinguished physicochemical and sensory attributes to the meat, such as stronger flavours (taste and smell), colours, and a firm consistency, depending on the feeding and slaughter age (Eleru et al., 2013). However, late slaughter of birds would increase feed intake, burdening the cost of the animal feed. Thus, the reduction of grain consumption, such as corn and soybeans, which constitute approximately 80% of the composition of commercial diets, would become attractive due to the fluctuation in the availability and cost of these raw materials.

The use of alternative foods, such as dehydrated forage or in natura, associated with dietary-restriction programmes would enable the production of slow-growth broilers at relatively low cost, owing to the decrease in feed intake (Sacranie et al., 2012). However, depending on the duration and severity of the dietary restriction, problems may occur in terms of broiler performance (Tumová et al., 2002) as well as morphology and physiological changes in the organs of the gastrointestinal tract and intestinal mucosa (Susbilla et al., 2003). Although the mechanisms of action for the fibres present in forages are dependent on a number of factors, high levels of fibre in the diet of birds are still considered negative for voluntary intake and food utilisation. However, studies highlight the beneficial effects of fibre on animal metabolism, with the potential to improve the digestibility of nutrients, depending on the physicochemical properties of the fibrous fractions, the level of inclusion of fibre in the diet, and the age of the birds (Jiménez-Moreno et al., 2009; González-Alvarado et al., 2010). Therefore, this study was carried out with the objective of evaluating the effect of dietary restriction and hay inclusion of alfalfa (Medicago sativa L.) and Bermudagrass (Cynodon dactylon cv Coastal) in the diets of slow-growing broilers on performance, litter moisture, morphophysiology of the gastrointestinal tract organs, and economic viability.

Materials and methods

The experiments were carried out at the poultry sector of an experimental farm at Universidade Estadual de Maringá using alfalfa hay (Medicago sativa L.) and Bermudagrass hay (Cynodon dactylon cv Coastal) in a feed-restriction programme in slow-growing broilers. The chemical composition and energy of the alfalfa and Bermudagrass hays were 17.44 and 7.50% crude protein, 3.64 and 3.14% ether extract, 30.13 and 32.37% crude fibre, and 4.343 and 4.278 kcal/kg gross energy on dry matter, respectively, according to the methodology of Silva and Queiroz (2004).

Two hundred and seventy two male broilers of the ISA Label JA57 (naked neck) strain were used at 21 days of age, with an average initial body weight of 597±50 g, distributed in a completely randomised design with four treatments and four replications of 17 birds per experimental unit. The protocol for this experiment was approved by the Ethics Committee on Animal Use in Experimentation (CEAE) of the Universidade Estadual de Maringá (UEM, Maringá, Paraná, Brazil) (CEAE/UEM).

The treatments were i) feeding ad libitum, consumption control; ii) feed restriction, 80% of the control intake; iii) feed restriction with alfalfa hay inclusion, 80% of the control intake+20% alfalfa hay; and iv) feed restriction with Bermudagrass hay inclusion, 80% of the control intake+20% Bermudagrass hay.

The calculation of feed restriction was based on the previous day’s feed intake of the control group (feed ad libitum), which was recorded and adjusted daily, early in the morning. Drinking water was not restricted. All experimental units received a diet that was formulated to meet the nutritional requirements of reg.
ular performance broilers, as proposed by Rostagno et al. (2005) for 21-49 and 50-70 days of age (Table 1), varying the amount of the feed concentrate supplied and the type of hay included in the diet.

Water was provided ad libitum in a feeding programme that consisted of a grower phase (21-49 days of age) and a final phase (50-70 days of age). The diets (Table 1) were based on corn and soybean meal and were formulated using the nutritional requirements for male broilers, according to Rostagno et al. (2005), varying the amount of the feed concentrate supplied and the type of hay included in the diet. Broiler mortality was recorded daily to correct the feed/gain ratio per pen. The birds and the feed were weighed weekly to evaluate the performance, which was measured as the feed intake, weight gain, and feed/gain ratio at 70 days of age, eight birds per treatment, with representative weights (mean±5%), were weighed, desensitized by electric shock, and sacrificed by cervical dislocation to determine the relative weights (g/100 g body weight) of the gastrointestinal tract organs (proventriculus, gizzard, small and large intestines, pancreas, and liver) and the length of the small and large intestines (in cm). For the morphometric analysis, 2 cm sections of each segment were opened longitudinally and transversely, washed with saline solution, and placed in containers containing formaldehyde solution, buffered at 10%, for tissue fixation. The sections were dehydrated in a series of alcohols with increasing concentrations, diaphanized in xylene, and embedded in a series of alcohols with increasing concentration, diaphanized in xylene, and embedded in alcohols with increasing concentration. The diets (Table 1) were based on corn and soybean meal and were formulated using the nutritional requirements for male broilers, according to Rostagno et al. (2005), varying the amount of the feed concentrate supplied and the type of hay included in the diet. Broiler mortality was recorded daily to correct the feed/gain ratio per pen. The birds and the feed were weighed weekly to evaluate the performance, which was measured as the feed intake, weight gain, and feed/gain ratio at 70 days of age, eight birds per treatment, with representative weights (mean±5%), were weighed, desensitized by electric shock, and sacrificed by cervical dislocation to determine the relative weights (g/100 g body weight) of the gastrointestinal tract organs (proventriculus, gizzard, small and large intestines, pancreas, and liver) and the length of the small and large intestines (in cm). For the morphometric analysis, 2 cm sections of each segment were opened longitudinally and transversely, washed with saline solution, and placed in containers containing formaldehyde solution, buffered at 10%, for tissue fixation. The sections were dehydrated in a series of alcohols with increasing concentrations, diaphanized in xylene, and embedded in paraffin. Slides containing semi-serial and transversal sections (7 µm), stained using the periodic acid Schiff (PAS) method, were prepared and images were obtained with a light microscope using the image capture Motic Image Plus 2.0 software (British Columbia, Canada). From each intestinal segment (duodenum, jejunum, ileum, and cecum) 30 measurements for height (apex to base), width (middle third), and crypt depth were taken to determine the villus/ crypt ratio. To count the goblet cells in the duodenum, jejunum, and ileum segments, ten measurements per sample were obtained in the middle-third of the villi in different cuts, totaling an area of approximately 1 cm² with a 40 objective.

The pH of the gizzard contents was measured in the organ lumen with an electrode. The caeca collected were sectioned, and the collected contents were homogenized in distilled water (1:10 wt/wt) and agitated for 1 min; the pH value was then determined. At the end of the experimental period, a 500 g litter sample was taken from each experimental unit from three areas of the box, at least 10 cm away from drinking and feeding troughs, in order to analyse the litter moisture. The collected material was homogenized and dried in a forced-air oven at 55°C for 72 h. To verify the economic feasibility of hay inclusion in a feed restriction programme, the prices in the state of Paraná from January 2013 were used (converted to dollar price on 31/01/2013) to calculate the price paid to the producer per kilogram of naked neck chicken, live weight (US$ 1.89/kg), and the prices of the feed ingredients: corn US$ 0.30/kg, soybean meal US$ 0.60/kg, soybean oil US$ 0.84/kg, limestone US$ 0.09/kg, dicalcium phosphate US$ 0.66/kg, salt US$ 0.06/kg, premix US$ 3.27/kg, DL-methionine US$ 5.16/kg, L-lysine US$ 3.32/kg, L-threonine US$ 6.26/kg, butylated hydroxytoluene (BHT) US$ 8.55/kg, alfalfa hay US$ 0.33/kg, and Bermudagrass hay US$ 0.25/kg.

The average cost of feed (ACF) was calculated, according to the centesimal composition of the experimental diets for chickens for from 21 to 70 days of age, to obtain the average feed intake of the birds for each treatment. The average gross revenue (AGR) was calculated from the total weight gain of the birds and the price paid to the producer. The gross margin (GM) is the difference between the AGR and ACF. The profitability index (PI) is obtained from the quotient GM and ACF. The PI indicates the rate of return on the capital invested. The balance point (BP) shows the exact volume of production from which there is zero return (GR=ACF). The economic approach, presented in this study, only took into account the costs of feeding and, owing to the purpose of this research, does not include other components for the cost of production. Therefore, it is about the necessary weight gain to cover the costs of feeding. In this case, it only infers the point of partial equilibrium. Pen means were used as the experimental unit for all analyses. Data were analyzed by ANOVA followed by the Tukey test, using the statistical software System for Statistical and Genetic Analysis, Universidade Federal de Viçosa (SAEG, 1997), and a probability of P<0.05 was considered significant.

Table 1. Ingredients and nutrient composition of experimental diets.

| Ingredients, % | 21 to 49 d | 50 to 70 d |
|---------------|------------|------------|
| Corn          | 66.26      | 69.94      |
| Soybean meal  | 28.43      | 24.85      |
| Soybean oil   | 1.99       | 2.40       |
| Limestone     | 0.89       | 0.79       |
| Dicalcium phosphate | 1.14 | 0.88   |
| NaCl          | 0.400      | 0.400      |
| DL-Methionine | 0.226      | 0.170      |
| L-Lysine HCI  | 0.194      | 0.154      |
| L-Threonine   | 0.030      | 0.010      |
| Premix vitamins and minerals a,b | 0.400 | 0.400 |
| BHT           | 0.010      | 0.010      |
| Total         | 100.00     | 100.00     |

Calculated composition:

| Crude protein, % | 18.40 | 17.00 |
| Metabolisable energy kcal/kg | 3075 | 3150 |
| Calcium, % | 0.70 | 0.58 |
| Available phosphorus, % | 0.31 | 0.26 |
| Digestible Met+Cys, % | 0.74 | 0.65 |
| Digestible Lys, % | 1.01 | 0.89 |
| Digestible Thr, % | 0.66 | 0.58 |
| Digestible Trp, % | 0.20 | 0.18 |

BHT, butylated hydroxytoluene. "Vitamin mixture (content per kg of diet): vitamin A (retinyl acetate), 2250 U; vitamin D₃ (cholecalciferol), 450 U; vitamin E (DL-α-tocopheryl acetate), 7000 U; vitamin K₃ (menadione dimethylpyrimidinol), 438 U; vitamin B₁ (thiamine mononitrate), 300 mg; vitamin B₂ (cyanocobalamin), 2300 µg; niacin (niacinamide), 7000 mg; calcium pantothenate, 2500 mg; folic acid, 110 mg; D-biotin, 14 µg. aMineral mix (content per kg of diet): Fe (iron sulfate monohydrate), 12.5 g; Cu (copper sulfate pentahydrate), 3000 mg; Mg (magnesium oxide), 256 g; Mn (manganese oxide), 15 g; Zn (zinc oxide), 12.5 g; Co (cobalt sulfate), 50 mg.
Results and discussion

Dietary restriction and restriction with the inclusion of hay alfalfa and Bermudagrass hays resulted in a decreased weight gain (P<0.05) of the birds (Table 2). The animals submitted to food restriction showed the best feed conversion (P<0.05), demonstrating that, in some situations, birds subjected to feed restrictions can increase the efficiency of digestion of food ingested, improving feed conversion (Pietsch et al., 2009).

The lowest performance and worst feed conversion of the birds under feed restriction and with milled hay (80% control+alfalfa hay or 80% control+Bermudagrass hay) can be attributed to changes in the rate of passage and low nutrient availability, which is caused by the ingestion of fibrous food (Jiménez-Moreno et al., 2013).

Depending on the concentration of the insoluble fibres found in the feed, the residence time of the bolus in the gastrointestinal tract is smaller, reducing the available time for digestion and nutrient absorption (Savón, 2002), which interferes with the availability of energy and proteins that are needed for the growth and maintenance of birds (Ponte et al., 2008). The increase in endogenous losses and dilution of the diet may also hinder the penetration of the enzymes in the digesta, reducing the fraction of nutrients absorbed by the organisms and reducing the metabolizable energy content of the feed (Tabook et al., 2006).

There was no effect (P>0.05) of the treatments on the litter moisture (Table 2), although this relates to the presence of high concentrations of fibre with the addition of water retention in the gut and the hydration capacity of fibre in the production of more wetary excreta, with consequent increase in litter moisture (Rezaei et al., 2011).

The relative weight of the gizzard as well as the weight and length of small and large intestines were altered by dietary restriction and restrictions with the inclusion of hay, regardless of the type of hay used in the diet (P<0.05; Table 2).

Birds that were only subjected to food restriction had lower (P<0.05) relative weights of the small intestines and small intestine lengths compared to the other treatments. This decrease in weight and length of the organs may have been caused by lower mechanical and metabolic activity, although Govaerts et al. (2000) and Silva and Kalubowila (2012) suggested that, even when there is limitation in nutrient supply, the birds prioritize the growth of the organs of the digestive tract.

The feed-restriction group that was supplemented with alfalfa and Bermudagrass hays showed higher (P<0.05) relative weights of the gizzard, small intestine, and large intestine. This effect may be related to higher levels of fibre and the volume of diet, with consequent digesta expansion, causing changes in gastrointestinal tract development (Mourão et al., 2008).

The inclusion of alfalfa and Bermudagrass hays favoured the relative weight of the gizzard, possibly due to the increase in volume and particle size of the food bolus, requiring higher mechanical activity of the muscle, with consequent muscular hypertrophy (Mateos et al., 2012).

The increase in dietary fibre (in both the soluble and insoluble fractions of the hays) may have triggered an increase in the physical and chemical activities of the small intestine, causing adaptive hyperplasia to the intestinal mucosa, with consequent increase in intestinal volume in an attempt to improve digestive capacity (Hetland et al., 2004). The increased weight of the large intestine may have occurred by higher fermentation in the cecum and multiplication of replacement cells in the cecum mucosa (Olkowski et al., 2005), both caused by the intake of hay.

The influence of the treatments was observed in the villus height, crypt depth, villus/crypt ratio, and number of goblet cells, without changes in the villus width (Table 3). The lowest (P>0.05) villus height in the duodenum and crypt depth in jejunum, demonstrated by the chickens that had their food restricted, may be associated with regression of the intestinal mucosa, caused by a decrease in the rate of renewal and cell division in the crypts (Hansen et al., 1992). Birds subjected to restriction and inclusion of hay had higher (P>0.05) villous heights in the duodenum and cecum as well as increased crypt depths in the duodenum. A lower villus/crypt ratio was observed in the duodenum and jejunum segments, and an increased (P>0.05) number of goblet cells was observed in the ileum.

The abrasive effect of the digesta on the mucosa, caused by the intake of a high fibre content, may have promoted hyperplasia of the intestinal cells, generating an increase in the size of the villus and increased crypt activity. This would ensure the constant replacement of cells lost in the apical region of the villi, as well as an increase in goblet cell production, in an

Table 2. Performance, litter moisture, organ weights, intestine length and pH of contents from gizzard and caecum (mean±standard error) of slow-growing broilers at 70 days of age, under feed restriction and hay inclusion diets.

| Treatments               | CV, % |
|--------------------------|-------|
| Control                  | 2440.25±7.60b | 6734.25±82.52a |
| 80% control              | 2180.00±20.68b | 5413.00±99.00a |
| 80% control+alfalfa      | 2025.00±30.37b | 6470.50±48.45a |
| 80% control+coast cross  | 2065.75±14.17b | 6365.00±34.24b |
| Weight gain, g           | 1.84   | 1.63   |
| Feed intake, g           | 3.081±0.06a  | 3.67   |
| Feed to gain ratio°, g/g  | 21.63±0.06b | 7.85   |
| Litter moisture, %       | 0.41±0.02c   | 12.96  |
| Proventriculus, %        | 2.78±0.11b   | 9.14   |
| Gizzard, %               | 2.08±0.03b   | 8.17   |
| Small intestine, %       | 3.14±0.09b   | 3.67   |
| Large intestine, %       | 2.08±0.03b   | 5.13   |
| Small intestine, cm      | 0.37±0.02c   | 11.99  |
| Large intestine, cm      | 0.37±0.02c   | 11.99  |
| pH gizzard               | 0.48±0.05b   | 5.13   |
| pH caecum                | 195.63±3.52b | 11.99  |
| CV, coefficient of variation. °Means followed by different letters in same row differ significantly by Tukey’s test (P<0.05). *Values corrected for mortality.
attempt to protect the mucosa enhancing the production of glycoproteins, which act as a protective mucus in order to maintain the integrity of the epithelial tissue (Forder et al., 2007).

The lowest values of the villus/crypt ratio were obtained in the duodenum and jejunum segments, demonstrated by the animals with restricted diets with the inclusion of alfalfa and Bermudagrass hays. This indicates that there was deterioration in the processes of digestion and absorption of nutrients and increased energy losses with cell renewal, providing less energy for the growth and maintenance of other tissues (Montagne et al., 2003).

The results obtained in the analysis of the economic viability, within the period of 21-70 days of age, show that the GM was positive in all treatments (Table 4). Yet, only birds subjected to food restriction (80% of the consumption control) had the highest GM, as well as the lowest cost of feed per kilogram of live weight of the produced chickens; this resulted in the best PI and BP. The highest weight gain of the birds occurred in the feed treatment ad libitum (consumer control), yielding the highest AGRs. Although the food-restriction treatments supplemented with alfalfa hay or Bermudagrass hay (80% of the consumption control+20% alfalfa hay or Bermudagrass hay) have shown inferior results, regarding AGR and BP, compared to the other treatments, it was found that the PI was better than that presented by feeding ad libitum. When assessing the economic feasibility of the feeding programmes used in the rearing of slow growing broilers, from 21-70 days of age, it was observed that the feed cost (R$kg) and the BP were worse for the feed treatment ad libitum; and the volume of feed consumed was 19.62, 10.14, and 11.10% higher than the other treatments (feed restriction and feed restriction with inclusion of alfalfa hay or Bermudagrass hay, respectively). Although the weight gain of feed ad libitum was superior to the others, the ratio of total feed cost to GM determined that the best PI could be attributed to animals subjected to food restriction.

### Table 3. Intestinal morphometry and goblet cells (mean±standard error) from segments of small intestine and cecum of slow-growing broilers at 70 days of age, under feed restriction and hay inclusion diets.

| Segments | Control | 80% control | 80% control+alfalfa | 80% control+coast cross | CV, % |
|----------|---------|-------------|---------------------|------------------------|-------|
| **Villus height, µm** |         |             |                     |                        |       |
| Duodenum  | 1524.58±28.37 | 1357.83±52.07 | 1662.45±25.08 | 1657.65±17.09 | 25.26 |
| Jejunum   | 1001.36±46.24 | 967.45±58.47 | 944.54±19.08 | 1058.66±48.05 | 7.91  |
| Ileum     | 677.59±59.22  | 539.21±30.36 | 650.17±37.23 | 691.78±49.10 | 22.08 |
| Cecum     | 201.67±3.26   | 193.82±3.64  | 217.77±3.04  | 214.49±2.29  | 14.94 |
| **Villus width, µm** |         |             |                     |                        |       |
| Duodenum  | 125.71±13.76 | 127.81±1.59  | 120.80±7.20 | 117.43±5.50 | 11.66 |
| Jejunum   | 124.14±4.82  | 145.95±24.72 | 105.38±7.49 | 112.89±7.49 | 18.97 |
| Ileum     | 151.28±9.91  | 168.75±13.60 | 151.98±5.49 | 129.40±21.18 | 18.72 |
| **Crypt dept, µm** |         |             |                     |                        |       |
| Duodenum  | 117.42±7.74  | 107.44±2.54  | 155.39±2.54 | 173.40±8.06 | 26.56 |
| Jejunum   | 128.18±5.55  | 114.42±7.26  | 143.26±5.54 | 167.50±8.00 | 28.11 |
| Ileum     | 117.83±12.69 | 87.87±5.48  | 115.24±5.05 | 110.63±11.16 | 17.19 |
| Cecum     | 104.14±5.53  | 93.65±5.34  | 113.14±3.69 | 113.62±2.15 | 8.47  |
| **Villus/crypt ratio** |         |             |                     |                        |       |
| Duodenum  | 14.32±0.93   | 13.86±0.52  | 11.20±0.22 | 10.27±0.29 | 7.88  |
| Jejunum   | 8.37±0.47    | 8.98±0.11   | 6.93±0.13 | 6.42±0.32 | 6.71  |
| Ileum     | 8.02±1.04    | 4.81±0.32   | 6.04±0.63 | 6.55±0.54 | 22.40 |
| Cecum     | 2.11±0.05    | 2.09±0.10   | 1.93±0.08 | 1.91±0.02 | 6.95  |
| **Goblet cells, cm² of villi** |         |             |                     |                        |       |
| Duodenum  | 359.75±23.57 | 298.25±67.81 | 371.50±28.36 | 364.25±34.73 | 24.29 |
| Jejunum   | 780.50±30.16 | 439.00±31.46 | 517.75±9.92 | 520.00±24.80 | 10.50 |
| Ileum     | 508.00±31.95 | 473.25±50.55 | 699.75±40.27 | 682.25±25.29 | 12.93 |

CV, coefficient of variation. *Means followed by different letters in same row differ significantly by Tukey’s test (P<0.05).

### Table 4. Economic analysis of feeding programmes using feed restriction and hay supplementation of slow-growing broilers from 21 to 70 days of age.

| Treatments | Control | 80% control | 80% control+alfalfa | 80% control+coast cross |
|------------|---------|-------------|---------------------|------------------------|
| Feed cost, US$ | 2.90 | 2.33 | 2.74 | 2.67 |
| ACF, US$/kg | 1.19 | 1.07 | 1.35 | 1.29 |
| AGR, US$ | 4.60 | 4.11 | 3.82 | 3.90 |
| GM, US$ | 1.71 | 1.78 | 1.08 | 1.23 |
| PI, % | 0.59 | 0.76 | 0.39 | 0.46 |
| BP, kg | 1.54 | 1.23 | 1.45 | 1.41 |

ACF, average cost of feed; AGR, average gross revenue; GM, gross margin; PI, profitability index; BP, balance point.
Conclusions

The feed restriction resulted in the weight gain of birds; dietary alfalfa and Bermudagrass hay supplementation impaired the feed conversion, promoting gastrointestinal tract organ modifications and deleterious effects on the intestinal morphology. The best economic indices were obtained for birds that were only subjected to feed restriction (without hay supplementation).

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