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ABSTRACT
Twenty-four adult Beagles were utilised to evaluate the partial replacement of wheat bran with corn gluten feed without steep water on digestibility and characteristics of faeces. The treatments were 0 (no substitution), 30, 60 or 90 g/kg of corn gluten without steep water. There was no effect (p > 0.05) on the digestibility coefficients (g/kg) of dry matter (0.771), organic matter (0.806), crude protein (0.813), ether extract (0.798), crude fibres (0.393), neutral detergent fibre (0.425), acid detergent fibre (0.286) and crude energy (0.812), whilst there was effect (p < 0.05) on the digestible and metabolisable energy. There were effects (p < 0.05) for dry matter and pH of faeces but no effect (p > 0.05) was found on the remaining faecal characteristics: excretion for 100 g of food (56.77 g), excretion (129.6 g/day and 49.0 g dry matter/day), score (3.90), dry matter excretion for 100 g of food (22.86 g), buffer capacity (BC) at pH 5 (57.81), ammonia nitrogen (1.46 g/kg of faecal dry matter) and water balance (333.25 mL/day), in vivo and in situ gas production (p > 0.05). Corn gluten feed without steep water can be utilised to replace up to 90 g/kg of wheat bran without causing negative effect on the digestibility and characteristics of faeces.

Introduction
The importance of fibre in non-ruminant nutrition has been previously questioned because its direct role as a nutrient was unknown, and certain functions, such as the maintenance of gastrointestinal tract transit, energy dilution and decrease in nutrient digestibility, were attributed to it (Roque et al. 2006). However, promoting intestinal health in dogs using fibre has aroused interest in using fibrous ingredients that do not decrease the digestibility of complete food (Kawauchi et al. 2011).

Different fibre sources have been tested, as corn gluten with 21% of crude protein (Kawauchi et al. 2011), beet pulp, tomato pomace, peanut hulls, wheat bran, alkaline hydrogen peroxide-treated wheat straw (Fahey et al. 1990a), alkaline hydrogen peroxide-treated oat hulls (Fahey et al. 1992), SolkaFloc® (a product based on carefully processed, highly purified cellulose; International Fiber Corporation, North Tonawanda, NY), citrus pulp and three blends, including SolkaFloc® and gum arabic, citrus pectin, gum talha, carob bean gum, locust bean gum, beet pulp, citrus pectin and guar gum (Sunvold et al. 1995).

The differences in the solubility and fermentability of these fibres can provide different physiological benefits and affect faecal characteristics (de-Oliveira et al. 2008; Calabrò et al. 2012). Several outcomes were evaluated to determine the physicochemical characteristics of faeces, such as faecal production (Kawauchi et al. 2011), faecal score (Laflamme et al. 2008), pH (de-Oliveira et al. 2008), buffering capacity (BC) (Zeyner et al. 2004) and ammonia nitrogen (Brito et al. 2010).

The study of the use of corn gluten feed without steep water as a fibre alternative source in dog nutrition is a pioneer study. The aim of this study was to evaluate the effect of partial replacement of wheat bran with increasing levels of corn gluten without steep water on the nutrient digestibility and physicochemical characteristics of dog faeces.
Materials and methods

The trial was conducted at the experimental kennel of the Federal University of Lavras (UFLA) Minas Gerais, Brazil. Twenty-four 5.17 ± 1.37 years old Beagles with a live weight of 15.37 ± 2.0 kg were utilised in this study. During the five adaptation days, the animals were maintained in individual boxes with a solarium area. During the five sample collection days, the dogs were housed in metabolic cages equipped with grouted flooring and a nipple drinker, receiving water *ad libitum*.

The daily supply of complete food was calculated using the equation 95 × (BW)0.75 recommended by the NRC (2006) for inactive adult dogs. The treatments consisted of partial replacement of wheat bran with corn gluten feed without steep water in increasing levels, i.e. 0 (no substitution), 30, 60 or 90 g/kg of corn gluten without steep water (Table 1). The complete foods were extruded, subsequently ground and then provided to the dogs with an added water ratio of 2:1 (water:food) in two daily meals at 8:00 am and 5:00 pm.

The faeces samples were collected immediately after defaecation to determine their physicochemical characteristics using the following measurements: a consistency score according to de-Oliveira et al. (2008), weight, pH (determined directly in the faeces using a 0.01 accuracy digital pH metre (model Q400A, QUIMIS, Diadema, SP, Brazil), buffer capacity (BC) (Zeyner et al. 2004) and ammonia nitrogen (Preston 1995).

Following the methodology of Carciofi et al. (2005), the water balance was determined by the daily difference between ingested water (drinking water and water from the food) and water eliminated through urine and faeces without considering the metabolic water produced by protein and carbohydrate intake or eliminated by other methods. The water intake was measured by the difference between the amount delivered per day and the amount of leftover.

To determine the chemical composition and digestibility of nutrients, the stools, after dried and grounded, were routed to the Animal Research Laboratory of the Department of Animal Science at Federal University of Lavras (Lavras, MG, Brazil) where the analyses were carried out. The methodologies of the Association of Official Analytical Chemistry (AOAC 1995) were utilised to determine the dry matter (DM), mineral matter (MM), ether extract (EE), crude protein (CP), gross energy (GE) and crude fibre (CF). The acid detergent fibre (ADF) and neutral detergent fibre (NDF) analyses were conducted based on Van Soest et al. (1991) method. The coefficient of total apparent digestibility (CTTAD) of the nutrients was performed following the procedure of Andriguotto et al. (2002).

*For in vitro and in situ* gas determination, the faeces were incubated at 39°C for 6, 12 and 24 hours to determine *in vitro* production of gases (Citrignelli et al. 1999; Mauricio et al. 1999) and *in situ* gas generation was accomplished by radiographic examination in the lateral–lateral and ventral–dorsal position for intestinal gas identification through radiolucent images (Feliciano et al. 2010).

On the last day of the experiment, blood samples were collected in heparinised tubes, from jugular vein, pre-feeding and four and eight hours post-feeding. Samples were centrifuged at 15,000 rpm for 10 minutes, and then the plasma was placed in polypropylene tubes and stored at −20°C. For the analysis of short-chain fatty acid (SCFA) concentrations, 1 mL of plasma was added to 5 mL of ethyl alcohol and then centrifuged at 3000 rpm for 10 minutes. The supernatant was added to 40 μL of 1 mM NaOH and placed in a forced ventilation oven at 60°C until completely dry. Afterwards, the residues were dissolved with 99% formic acid and 1 mL of distilled water and stored in a freezer at −15°C until analysis of SCFA by gas chromatography using a gas chromatograph GC-2014 Shimadzu (Shimadzu, Kyoto, Japan), equipped with a capillary column Stabilwax® (30 m length, 0.53 mm internal diameter, 0.50 μm thickness; Restek

### Table 1. Ingredients and chemical composition of the complete foods formulated by replacing wheat bran with corn gluten without steep water, (g/kg DM), except otherwise stated.

| Item                          | Treatments, g/kg |
|-------------------------------|------------------|
| Ingredient composition        |                  |
| Wheat bran                    | 0 (control) 30 60 90 |
| Corn gluten without steep water | 0 30 60 90 |
| Corn grain                    | 290 280 280 280 |
| Rice grits                    | 210 210 210 210 |
| Soybean meal                  | 110 110 110 110 |
| Meat and bone meal            | 60 60 60 60     |
| Whole viscera meal            | 80 90 90 100    |
| Minor ingredients*            | 50 50 50 50     |
| Total                         | 1000 1000 1000 1000 |
| Chemical composition          |                  |
| Dry matter, g/kg              | 967.1 967.1 965.5 955.2 |
| Ashes                         | 76.2 71.4 70.3 59.3 |
| Crude protein                 | 239.9 250.2 235.1 224.0 |
| Ether extract                 | 54.8 52.8 51.4 59.8 |
| Crude fibre                   | 35.7 38.2 39.8 37.8 |
| Neutral detergent fibre       | 150.6 167.9 170.1 169.5 |
| Acid detergent fibre          | 56.7 55.1 57.4 61.0 |
| Nitrogen-free extract         | 593.4 587.4 603.4 616.1 |
| Crude energy, MJ/kg           | 18.20 18.27 18.17 18.59 |

*Supplement per kg of complete food: vitamin A, 10,000 U; vitamin D₃, 1500 U; vitamin E, 50 U; vitamin K, 0.55 mg; vitamin B₁₂, 30 mg; vitamin B₂, 5.5 mg; vitamin B₆, 4 mg; vitamin B₉, 5.5 mg; niacin, 50 mg; biotin, 0.012 mg; pantothenic acid, 12 mg; folic acid, 0.8 mg; choline, 1200 mg; cobalt, 0.2 mg; manganese, 40 mg; zinc, 120 mg; copper, 18 mg; iodine, 1.8 mg; Iron, 80 mg; selenium, 0.12 mg; antioxidant, 150 mg (BHT, China); antifungal, 2 g (calcium propionate, China); zeolite, 10 g (Celpec, Celta Brasil); Yucca extract, 0.25 g (Alitech, Brazil); flavour agent, 10 g (SPF, Brazil); poultry viscera oil, 20 g (Big Frango, Brazil).
Corporation, Bellefonte, PA) at 145°C (isothermic condition), an injector split/splittless and a detector dual FID at 250°C, according to the method described by Getachew et al. (2005). The trial was performed using completely randomised design, with six replicates per treatment, and each dog was the experimental unit.

A Kruskal–Wallis test was utilised to evaluate the faecal score results. For data analysis, a linear mixed model by the Statistical Analysis System, version 9.1.3 (SAS 2004) was utilised, considering as fixed effect of the corn gluten without steep water inclusion levels (0, 30, 60, 90 g/kg) as well as the effect of animals and residue. In case of significant effects for inclusion level, regression analyses were conducted.

Results and discussion

During this study, the dogs showed no change in their body weight. There was no effect (p > .05) of the treatment on the apparent digestibility coefficients of DM, organic matter (OM), CP, EE, CF, NDF, ADF and GE (Table 2). However, an effect was observed for apparent digestible energy (DE, expressed as MJ/kg DM; \(Y = 15.27 - 0.0228 \times X + 0.0002 \times X^2; p = .097\)) and for apparent metabolisable energy (ME, expressed as MJ/kg DM; \(Y = 14.27 - 0.0252 \times X + 0.0002 \times X^2; p = .0049\)), results that comply with those obtained by Sá (2011). The major digestible energy (DE, ME) coefficients were observed with 0 replacement level and can be attributed to the lesser amount of crude fibre observed in these diets, followed by 90 g/kg diets, which presented higher amounts of EE and NFE.

On the other hand, Burkhalter et al. (2001), using soybean hulls, observed a decrease (p < .05) in the digestibility of the nutrients regardless of the proportion of insoluble fibre, as well as Fahey et al. (1992) that observed that the DM, OM and TDF digestibility coefficients decreased as the hulls were added to the food. The disparity between these data may be attributed to the difference in the quality of the substituted ingredients in the treatments and the type and amount of fibre in these ingredients. For this study, the formulated diets maintained similar NDF and ADF values (Table 1), unlike in Fahey et al. (1992). The digestibility of GE was higher than that observed by Fahey et al. (1990b) and Cole et al. (1999), which may be because their diets had higher energy concentration and lesser EE concentration as observed in the diets from this study.

There was no diet effect (p > .05) on the remaining faecal characteristics: excretion for 100 g of food, daily excretion, score, dry matter excretion for 100 g of food, buffering capacity, ammonia nitrogen and water balance, faecal production in relation to the amount of food intake regarding OrM (original matter) and DM (Table 2). These results differed from those found by Fahey et al. (1990b), Sá (2011), Kawauchi et al. (2011) and Burkhalter et al. (2001) who observed an increase (p < .05) in faecal weight with fibre inclusion, and may be attributed to the similar digestibility of

### Table 2. Mean apparent digestibility coefficients of nutrients, digestible and metabolisable energy, and the physicochemical characteristics of dog faeces as a result of consuming complete food with the partial replacement of wheat bran by corn gluten without steep water.

| Coefficient of total tract apparent digestibility | Treatments, g/kg | SEM* | p linear | p quadratic |
|--------------------------------------------------|------------------|------|----------|------------|
| Dry matter                                       | 0.792            |      |          |            |
| Organic matter                                   | 0.829            |      |          |            |
| Crude protein                                    | 0.824            |      |          |            |
| Ether extract                                    | 0.825            |      |          |            |
| Crude fibre                                      | 0.393            |      |          |            |
| Neutral detergent fibre                          | 0.449            |      |          |            |
| Acid detergent fibre                             | 0.290            |      |          |            |
| Nitrogen-free extract                            | 0.872            |      |          |            |
| Crude energy                                     | 0.835            |      |          |            |
| Daily faecal excretion                           | 0.230            |      |          |            |
| Faecal DM, g/kg                                  | 384.90           |      |          |            |
| Daily DM faecal excretion                        | 46.06            |      |          |            |
| Faecal score                                     | 3.93             |      |          |            |
| Excreted OrM for every 100 g of ingested food, g | 52.48            |      |          |            |
| Faecal pH                                        | 6.06             |      |          |            |
| Ammonia nitrogen, g/kg faeces DM                 | 1.38             |      |          |            |
| Water balance, ml/day                            | 396.39           |      |          |            |
| Gas production – 6 hs, ml/g DM                   | 33.88            |      |          |            |
| Gas production – 12 hs, ml/g DM                  | 76.56            |      |          |            |
| Gas production – 24 hs, ml/g DM                  | 126.17           |      |          |            |

*Standard error of the means.
the fibre fraction (NDF, ADF and CF) among the treatments considered in the present study. The average faecal score remained in a desirable classification between three and four (Maia et al. 2010; Laflamme et al. 2011). From a commercial perspective, the volume and consistency of the faeces produced by the dogs are relevant characteristics; therefore, it is important that the foods result in firmer stools (Maia et al. 2010; Sá 2011).

In this experiment, there were diet effects ($p < .05$) on faeces DM ($Y = 38.87 - 1.5380X + 0.1889X^2$) and faeces pH ($Y = 6.36 - 0.1952X + 0.0234X^2$), which differed from the studies by Sá (2011) and Kawauchi et al. (2011). Lower pH values can inhibit the proliferation of pathogenic bacteria (Hussein 1999), thus favouring animal health. Therefore, it can be suggested that the treatments may have had a prebiotic effect, which is desired in complete foods for dogs, and can be supported by the lower in vitro and in situ gas production (Table 2).

The amount of faecal DM had an effect ($p < .05$) with drier faeces at 90 g/kg corn gluten feed and wetter faeces at 60 g/kg, which differed from Fahey et al. (1990b) who observed a linear decrease ($p < .05$) in faecal DM with the inclusion of beet pulp. Little variation was observed in the faecal DM (g/kg) by Guevara et al. (2008), who considered the corn fibre to be a potential source of dietary fibre in dog food. The results of the present study confirm previous studies that have shown significant effects of dietary fibre on DM and moisture in dog faeces (Fahey et al. 1990a; Fahey et al. 1990b). The ammonia nitrogen was quantified to analyse the effect of the diets with different level of corn gluten inclusion on faeces odour, and there was no effect ($p > .05$) of the diets. The lowest level of ammonia nitrogen observed can be characterised as a positive factor because lower ammonia concentrations are associated with reduced faecal odour (Swanson et al. 2002).

The daily water balance of dogs did not change with the diets and this result reinforcing the similarities of the chemical and physical characteristics of the treatments, given that these food characteristics influence the water balance of animals (Carciofi et al. 2005). The two different fibre sources utilised in this study showed identical behaviour since no difference in the amount of water retained by the animal or in the total amount of excreted water was observed in dogs differently fed.

When the in vitro and in situ gas production are considered (Table 2), there was no difference ($p > .05$) between treatments in the different periods of in vitro incubation, and there was no effect ($p > .05$) on the quantification of intestinal gases identified by radiographic examination, which can be benefited by the pH stability, BC, physicochemical characteristics of faeces and ammonia production, strengthening the possibility of the use of diets based in corn gluten feed without steep water.

It was observed an increasing gas production between 6 and 24 hours of incubation. The main SCFA observed in faeces was acetate that showed a quadratic evolution ($Y = 398.67 + 34.174X - 4.5239X^2; p = .0186$) with the highest values observed with levels of 30 and 60 g/kg of inclusion. For the concentration of propionate and butyrate, no effect ($p > .05$) of the diet was observed (Table 3), a result that disagrees with that previously obtained by Ferreira et al. (2017), but working with cats. The predominance of acetate can be probably attributed to the type of fibre (hemicelluloses) present in the diet, the higher contribution of NDF and ADF in 90 g/kg diet, which favoured the fermentation.

For the blood concentrations of short-chain fatty acids (SCFAs), we observed a significant effect of the treatment on blood acetate that showed a linear behaviour ($Y = 1.9957 - 0.036X; p = .0409$) whilst for propionate and total SCFA there was not verified a significant effect. By analysing the effect of collection time on the blood concentrations of SCFA, we observed that the acetate concentration ($Y = 4.9678 - 0.3085X$) and total SCFA concentration ($Y = 6.4924 - 0.3127X$) were

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Table 3. Short-chain fatty acid (SCFA) concentration in faeces and blood of dogs fed increasing levels of corn gluten without steep water in partial substitution of wheat bran after 24 hours of incubation.

| Treatment, g/kg | Faeces SCFA concentration, mmol/g DM | Blood SCFA concentration, mmol/l |
|----------------|-------------------------------------|---------------------------------|
|                | Propionic acid | Butyric acid | Total SCFA | Propionic acid | Total SCFA |
| 0              | 0.156          | 0.005        | 0.623      | 1.597          | 4.752      |
| 30             | 0.170          | 0.0007       | 0.638      | 1.567          | 5.304      |
| 60             | 0.165          | 0.0004       | 0.571      | 1.638          | 6.089      |
| 90             | 0.119          | 0.003        | 0.616      | 1.508          | 5.242      |

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$p$ Value (significant effects):
- Propionic acid: $p = .1074$ (faeces), $p = .1889$ (blood)
- Butyric acid: $p = .1481$ (faeces), $p = .1074$ (blood)
- Total SCFA: $p = .6684$ (faeces), $p = .0622$ (blood)

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affected by the time of collection \((p < .05)\), on the contrary to what was observed \((p > .05)\). In general, for fatty acids, the blood concentrations presented the same profile of the faeces, with a clear predominance of the acetate on the other SCFA (Table 3).

**Conclusions**

The partial replacement of wheat bran with increasing levels of corn gluten without steep water in diets for dog did not affect the physicochemical characteristics of faeces, nor the digestibility coefficients of dry matter, organic matter, gross energy and chemical components. A significant effect was observed for digestible and metabolizable energy and for the concentration of acetate in faeces and in blood. Based on the results obtained in the trial, corn gluten feed without steep water can be utilised to replace up to 90 g/kg of wheat bran without causing deleterious effects on nutrient digestibility and on the physicochemical characteristics of faeces.

**Ethical approval**

All procedures in this study were approved by the Ethics Committee on Animal Research of the College of Animal Science and Food Engineering (FZEA) of the University of São Paulo (USP), protocol number 2012.1.1327.74.0.

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**Disclosure statement**

No potential conflict of interest was reported by the authors.

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