Digestibility of raw soybeans in extruded diets for dogs determined by different methods

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ABSTRACT

The objective was to evaluate the coefficients of total tract apparent digestibility (CTTAD) and metabolisable energy (ME) of raw soybeans (RSB) in extruded diets for dogs by three methods (difference, regression and substitution). We also evaluated the urease and trypsin inhibitor (TI) activity, CTTAD, ME and faecal characteristics of adult dogs fed diets containing increasing RSB levels. Six dogs, distributed in a 6 × 6 Latin square design, were fed extruded diets containing 0, 60, 120, 180, 240 or 300 g of RSB/kg. Urease and TI were reduced in all diets after extrusion (p < .01), but TI was not completely inactivated. The inclusion of RSB linearly reduced (p < .01) the digestibility of dry matter (DM), organic matter and crude protein (CP) and linearly increased (p < .05) the digestibility of fat and gross energy and the ME of diets. The CTTAD of DM, CP and fat, and ME of RSB, predicted by the substitution method were: 0.767, 0.758, 0.960 and 20.7 MJ/kg, respectively. The dietary inclusion of RSB reduced faecal DM content and promoted a quadratic increase (p < .01) of pH and ammonia. Increasing RSB in diet reduced CTTAD estimate errors (p < .05). When 300 g of RSB were added per kg of diet, CTTAD of RSB estimated by the three methods did not differ (p > .05). The three methods can be used to determine RSB digestibility. Diet extrusion may not be able to completely deactivate the protease inhibitors present in the RSB.

HIGHLIGHTS

- Extrusion may not be able to deactivate protease inhibitors of raw soybeans.
- Raw soybeans reduce nutrient digestibility of extruded diets in dogs.
- The substitution method is recommended to evaluate digestibility of raw soybeans.

Introduction

Knowledge of digestible nutrients and metabolisable energy (ME) of feedstuffs is essential for the formulation of balanced dog diets. The methods available for the determination of the coefficients of total tract apparent digestibility (CTTAD) and ME of feedstuffs are still inaccurate, imprecise, and impractical. In addition, results obtained by those methods are unreliable because of the lack of standardisation among studies. Considering that dogs are carnivorous animals, the evaluation of the digestibility of protein sources for this species is essential. This is particularly true for plant protein sources (Pires et al. 2018) such as soybeans. Despite being widely used in commercial dog foods, specific information on the digestible nutrients content and ME of soybeans are lacking for dogs.

However, to be used in non-ruminant diets, soybeans must be heat processed to deactivate the anti-nutritional factors present in raw soybeans (RSB). The trypsin inhibitor (TI) is the most important of these factors, as it impairs protein digestion (Rackis 1972). However, soybean processing needs to be properly controlled, because both under- and overheating may reduce the utilisation of its nutrients by the animals (Félix, Rivera et al. 2013).

Considering that extrusion is a process that involves heat treatment, the extrusion of toasted soybean products, which commonly occur in commercial dog foods, may result in over-heating these ingredients, thus compromising the availability of amino acids to animals (Félix, Rivera et al. 2013). To date, the results of the few studies on the inactivation of protease inhibitors in extruded soybean products have not been evaluated.
inhibitors of RSB included in extruded dog foods are controversial (Purushotham et al. 2007; Félix et al. 2010; Félix, Rivera et al. 2013), probably due to the low or high inclusion level of RSB in the diets.

Therefore, this study aimed at evaluating TI activity, digestibility and ME of diets containing increasing levels of RSB in dogs. In addition, three methods to determine CTTAD and ME content of RSB fed to dogs were evaluated.

Material and methods

Animals and facilities

Six healthy Beagle dogs (2.20 ± 0.18 years of age, 8.90 ± 1.10 kg body weight, three females and three males) were used for the experiment. Dogs were clinically healthy and were previously vaccinated and dewormed (Drontal plus, Bayer, Belford Roxo, RJ, Brazil). The dogs were individually housed in concrete kennels (5 m long × 2 m wide).

Diets

Six dry extruded diets, containing 0, 60, 120, 180, 240 or 300 g of RSB/kg were tested. Raw soybean levels were added in substitution of the same amount of the control diet (Cd). The diets containing RSB are referred as test diets (Td). The evaluated RSB (Table 1) belonged to a genetically modified soybean variety, which presented a semi-early maturing cycle (130 days, group VI), undetermined habitat and a height of 60 to 80 cm.

The RSB included in the diet was previously ground in a roller mill to 2.50 mm particle size. The experimental diets were ground to 0.8 mm particle size in a hammer mill and extruded in a single-screw extruder (Ferraz, E-130, Ribeirão Preto, SP, Brazil) at a feeding rate of approximately 1.950 kg/h, with average water addition and temperature in the pre-conditioner of 355.5 L/h and 89.7°C, respectively. Extrusion temperature ranged between 115 and 138°C. Extrusion parameters were controlled every 10 min by adjusting the density of each food being processed. After extrusion, the prepared diets were dried in a triple-deck horizontal drier for 20 min at 110°C and sprayed with poultry fat. All flavouring was added after cooling. The ingredients and the analysed and calculated chemical composition of the experimental diets are shown in Table 2.

Table 1. Analysed DM content, chemical composition, and qualitative variables of raw soybeans.

| Item                             | Raw soybeans |
|---------------------------------|--------------|
| DM, g/kg                        | 907.20       |
| Analysed chemical compositiona (on as-fed basis) |             |
| CP, g/kg                        | 361.30       |
| CF, g/kg                        | 63.20        |
| Acid-hydrolysed fat, g/kg       | 188.00       |
| Ash, g/kg                       | 41.30        |
| GE, MJ/kg                       | 21.83        |
| Qualitative variablesb (on as-fed basis) |             |
| Urease, APh                     | 1.86         |
| Trypsin inhibitor, mg/g         | 15.91        |
| Protein soluble in KOH, %       | 89.30        |

aIn triplicate.
bIn duplicate.
DM: dry matter; CP: crude protein; GE: gross energy.

Table 2. Ingredients and analysed DM and chemical composition of the experimental diets.

| Item                                      | Raw soybean/kg |
|-------------------------------------------|----------------|
| Diets, g raw soybean/kg                   | 0              |
|                                           | 60             |
|                                           | 120            |
|                                           | 180            |
|                                           | 240            |
|                                           | 300            |
| Ingredients, %                            |                |
| Maize (yellow dent)                       | 60.47          |
| Raw soybean                               | 56.84          |
| Poultry by-product meal                   | 32.16          |
| Raw soybean                               | 28.16          |
| Poultry by-product meal                   | 26.47          |
| Mineral-vitamin supplementa               | 0.50           |
| Sodium chloride                           | 0.50           |
| Ammonium propionate                       | 0.24           |
| Butyl-hydroxy-toluene                     | 0.10           |
| Poultry fat                               | 7.03           |
| Hydrolysed poultry liver                  | 3.00           |
| DM, %                                     | 91.84          |
| Chemical composition (DM basis)           | 91.96          |
|                                           | 92.61          |
|                                           | 92.81          |
|                                           | 93.36          |
|                                           | 92.68          |
| Ingredients, %                            |                |
| Ash, %                                    | 7.30           |
| CP, %                                     | 21.59          |
| Acid-hydrolysed fat, %                    | 11.01          |
| CF, %                                     | 2.40           |
| ME, MJ/kg                                 | 15.53          |
| Provided per kilogram of feed: vitamin A (retinol) = 20.000 IU; vitamin D3 = 2.000 IU; vitamin E (α-tocopherol) = 48 mg; vitamin K3 = 48 mg; vitamin B1 = 4 mg; vitamin B2 = 32 mg; pantothenic acid = 16 mg; niacin = 56 mg; choline = 800 mg; Zn as zinc oxide = 150 mg; Fe as ferrous sulphate = 100 mg; Cu as copper sulphate = 15 mg; I as potassium iodide = 1.50 mg; Mn as manganous oxide = 30 mg; Se as sodium selenite = 0.20 mg; antioxidant = 240 mg. |
| DM: dry matter; CP: crude protein; GE: gross energy; ME: metabolisable energy. |                |

Digestibility

The digestibility trial used the method of total faeces collection according to the recommendations of the Association of American Feed Control Officials (2003). The experimental diets were offered for a five-day adaptation period, followed by five days of total faecal collection pooled per individual animal. Faeces were collected and weighed twice daily and individually stored in the freezer (−15°C) for subsequent analyses.

Dogs were fed twice daily (at 07:30 and 15:00 hours) in sufficient amount to supply their ME requirements, as recommended by the National
Research Council (2006): MJ/day = 0.54 × BW0.75. Water was offered ad libitum.

**Faecal characteristics**

The faecal characteristics evaluated were: dry matter (DM) content, dry and wet faecal output/kg DM intake, faecal score, ammonia and faecal pH. The faecal score was always determined by the same researcher, according to a scale from 1 to 5 as follows: 1 = watery faeces; 2 = soft, unshaped stool; 3 = soft, shaped and moist stool, leaving spots on the floor; 4 = firm, shaped, dry stool; and 5 = hard, dry pellets (small, hard masses).

Faecal pH and ammonia were measured in faeces collected up to 15 min after excretion. Faecal pH was measured using a digital pHmeter (331; Politeste Instrumentos de Teste Ltda, São Paulo, SP, Brazil) in 3.0 g of fresh faeces diluted in 30 mL of distilled water. The ammonia content was determined according to Félix, Rivera et al. (2013). A 3 g sample of faeces collected within 15 min of defaecation was incubated for 1 h in a 500-mL volumetric flask containing 100 mL distilled water, to which three drops of octyl alcohol (1-octanol) and 2 g of magnesium oxide were added. The solution was distilled in a Macro-Kjeldahl apparatus (Vapodest 200, Gerhardt, Koenigswinter, Germany) and recovered in a beaker containing 50 mL boric acid. Ammonia was then titrated using 0.1 N sulphuric acid. Faecal ammonia content was calculated according to the equation: ammonia-N (g/kg) = N × correction factor × 17 × [(volume of acid in the tested sample – volume of acid in blank sample)/sample weight in grams]. Faecal ammonia content was corrected for faecal DM content (g).

**Laboratory analyses**

After the collection period, faeces from each replicate were thawed to room temperature and individually homogenised. Faeces were then dried in a forced-ventilation oven at 55 °C (320-SE, Fanem, São Paulo, Brazil) until a constant weight was achieved. The experimental diets, RSB and faeces were grounded (Arthur H. Thomas Co., Philadelphia, PA, USA) at 1 mm sieve diameter and submitted in duplicate to analyses to determine DM content at 105 °C, crude protein (CP, method 954.01), ether extract after acid hydrolysis (EEAH, method 954.02), and Ash (method 942.05) according to the Association of Official Analytical Chemists (1995). The gross energy (GE) was determined in a calorimetric bomb (Parr Instrument Co., model 1261, Moline, IL, USA). The neutral detergent fibre (NDF) was determined in RSB and diets according to Silva and Queiroz (2002). Raw soybeans and diets before and after extrusion were analysed in triplicate for urease activity (American Oil Chemists’ Society, 1980) and TI (Kakade et al. 1974 modified by Hamerstrand et al. 1981). Protein solubility in KOH was determined only for RSB in triplicate (Araba and Dale 1990).

**Calculations and statistical analysis**

The CTTAD of DM, organic matter (OM), CP, EE, and GE and ME content of the diets were calculated according to the Association of American Feed Control Officials (2003) using the following equations:

\[
\text{CTTAD} = \frac{[g \text{ of nutrient intake} - g \text{ of nutrient excretion}]}{g \text{ of nutrient intake}}
\]

\[
\text{ME (kJ/g)} = \left(\frac{kJ/g \text{ GE intake} - kJ/g \text{ faecal GE}}{g \text{ CP intake} - g \text{ faecal CP}} \right) \times 5.23\text{kJ/g} / g \text{ DM intake}
\]

The methods of substitution (1) (Matterson et al. 1965), regression (2), and difference (3) (Fan and Sauer 1995) were applied to calculate the CTTAD of DM, OM, CP, EE, and GE and ME of RSB, as shown below:

1. \[
\text{CTTAD}_{\text{RSB}} = \text{CTTAD}_{\text{Cd}} + \left[\frac{\text{CTTAD}_{\text{Td}} - \text{CTTAD}_{\text{Cd}}}{g/\text{kg RSB substitution/1000}}\right]
\]
2. \[
\text{CTTAD}_{\text{Td}} = \text{CTTAD}_{\text{RSB}} + \left(\frac{\text{CTTAD}_{\text{Cd}} - \text{CTTAD}_{\text{RSB}}}{\% \text{ contribution of the nutrient of the Cd in the Td}}\right)
\]
3. \[
\text{CTTAD}_{\text{RSB}} = \left(\frac{\text{CTTAD}_{\text{Td}} - \text{CTTAD}_{\text{Cd}}}{\% \text{ contribution of the nutrient of the Cd in the Td}}\right) / \% \text{ contribution of nutrient in RSB/100}
\]

where CTTAD_{RSB}: Coefficients of total tract apparent digestibility of raw soybeans; CTTAD_{Cd}: Coefficients of total tract apparent digestibility of test diet; CTTAD_{Td}: Coefficients of total tract apparent digestibility of control diet; Cd: Control diet; Td: Test diet.

The ME of RSB was predicted only by the substitution and regression methods, because the difference method requires the contribution of the nutrient (i.e. ME) in the ingredient to be calculated. Digestible nutrients were calculated as: g/kg of nutrient in RSB × CTTAD of the estimated nutrient of RSB.

Urease activity and TI results before and after diet extrusion were presented as descriptive statistics due to the insufficient number of replicates (n = 3). The CTTAD of the nutrients and energy, ME and faecal
characteristics of the diets were analysed according to a 6 × 6 Latin square design (treatments × periods), totalling six replicates per treatment. The experimental unit was the individual dog. The sums of squares of the ANOVA of the model were separated into animal, period, and treatment effects, totalling six replicates per treatment. Differences were considered significant when the F test revealed differences at a 5% probability level. Data were then submitted to regression analysis using the REG procedure of SAS statistical package (SAS Inst. Inc., Cary, NC), considering p < .05.

The CTTAD of nutrients and energy and ME values obtained for RSB by the different methods were submitted to the Q-Cochran test to evaluate the effect of calculation method and RSB inclusion level on the results variances. When the Q-Cochran test indicated that variances were heteroscedastic, data were analysed by the test of Kruskal–Wallis at 5% significance level. Data presenting homoscedastic variances were submitted to ANOVA, using the GLM procedures of SAS (SAS Inst. Inc., Cary, NC).

Results

The regression equations obtained from urease activity and TI content in diets according to increasing RSB levels before and after extrusion are presented in Table 3 and the averages in Table 4. There was a quadratic effect in urease and TI activity with the inclusion of RSB in the mash (p < .01) and extruded diets (p < .01), although extrusion reduced the values of these parameters.

No diarrhoea, vomiting, or BW changes were observed. There were no differences in food intake among treatments (p > .05, Table 5).

The regression equations of nutrient and GE CTTAD and ME content against the inclusion of increasing RSB dietary levels are presented in Table 3, and their means are shown in Table 5. There was a linear reduction of DM, OM and CP CTTAD and a linear increase in AHF and GE CTTAD and ME content as RSB inclusion levels increased (p < .05).

The results of faecal characteristics are presented in Tables 3 and 5. Increasing dietary RSB levels promoted a quadratic effect (p < .05) in faecal DM, score, faecal ammonia content, and pH and a linear increase in faecal output (p < .05).

Table 6 presents the values of the CTTAD of RSB nutrients and GE and RSB ME content estimated by

| Item             | Equation<sup>a</sup>,<sup>b</sup> | R²    | $s_y$  | p-value L | p-value Q |
|------------------|-----------------------------------|-------|--------|-----------|-----------|
| Mash diet        |                                   |       |        |           |           |
| Urease, ΔpH      | $0.0001x^2 + 0.0013x + 0.0118$    | 0.9900| 0.0060 | <.0010    | <.0010    |
| TI, mg/g         | $0.0002x^2 + 0.0016x + 0.1214$    | 0.9800| 0.0280 | <.0010    | <.0010    |
| Extruded diet    |                                   |       |        |           |           |
| Urease, ΔpH      | $0.0001x^2 - 3E-06x + 0.0039$     | 0.7200| 0.0070 | <.0010    | <.0010    |
| TI, mg/g         | $0.0005x^2 + 0.0004x + 0.0168$    | 0.9800| 0.0280 | <.0010    | <.0010    |
| CTTAD            |                                   |       |        |           |           |
| DM               | $-0.0005x + 0.8229$               | 0.9900| 0.0050 | <.0010    | .9340     |
| OM               | $-0.0004x + 0.8585$               | 0.8000| 0.0070 | <.0010    | .0640     |
| CP               | $-0.0008x + 0.8286$               | 0.9300| 0.0080 | .0020     | .2030     |
| AHF              | $0.0005x + 0.8949$                | 0.7900| 0.0100 | .0170     | .4210     |
| GE               | $0.0004x + 0.8601$                | 0.9200| 0.0050 | .0030     | .2710     |
| ME, MJ/kg DM     | $0.1607x + 18.1802$               | 0.8700| 0.0320 | .0070     | .7920     |
| Faeces           |                                   |       |        |           |           |
| DM, %            | $-0.0082x^2 + 0.0761x + 36.3611$  | 0.9900| 3.6710 | <.0010    | .0020     |
| Score<sup>c</sup> | $-0.0009x^2 - 0.0089x + 4.1786$  | 0.9400| 0.0310 | <.0010    | .0330     |
| pH               | $-0.0023x^2 + 0.0075x + 6.4043$   | 0.5900| 0.3980 | <.0010    | .0010     |
| Ammonia, g/kg    | $-0.0017x^2 + 0.0080x + 1.4211$   | 0.9400| 0.3090 | <.0010    | <.0010    |
| Output<sup>d</sup> | $0.0045x + 0.4505$                | 0.9200| 0.0850 | <.0010    | .1920     |

DM: dry matter; OM: organic matter; CP: crude protein; AHF: acid hydrolysed fat; GE: gross energy; ME: metabolisable energy; $R^2$: linear (for linear equation) or quadratic (for quadratic equation) coefficient of determination.

<sup>a</sup>$x = $ estimated.

<sup>b</sup>$x =$ dietary raw soybean content (g/kg).

<sup>c</sup>Faecal score = 1 (watery stools) to 5 (dry stools).

<sup>d</sup>Faecal output = g wet faecal output/g DM intake.

Table 3. Linear (L) and quadratic (Q) effects of increasing raw soybean dietary inclusion levels on trypsin inhibitor (TI), urease activity, apparent total tract digestibility (CTTAD), ME, and faecal characteristics of dogs.
the substitution and the difference methods. No DM or OM CTTAD differences were detected when the diets contained 60 to 300 g RSB/kg as estimated by both methods (p > .05). However, the substitution and the difference methods underestimated CP digestibility and ME content and overestimated AHF and GE digestibility when the diet contained 60 g RSB/kg (p < .05). The variance of the CTTAD of nutrients and GE and ME content was reduced as dietary RSB levels increased (p < .05).

The CTTAD estimated for RSB by the substitution and the difference methods (both using diets with

300 g RSB/kg) were not different from those calculated by regression (p > .05, Table 7). The variances of the CTTAD of nutrients and GE and ME content estimated for RSB were not different among methods (p > .05). Based on the CTTAD values obtained by the substitution method (using 300 g RSB/kg) and on analysed nutrient and energy levels, RSB contained 0.273 g digestible protein, 0.180 g digestible fat and 19.70 MJ of DE/kg (not shown).

### Table 5. Dry matter intake, apparent total tract digestibility (CTTAD) of nutrients and energy, ME, and faecal characteristics of dogs fed diets containing increasing raw soybean levels (n = 6).

| Item | 0 | 60 | 120 | 180 | 240 | 300 | SEM |
|------|---|----|-----|-----|-----|-----|-----|
| DMI, g/d | 209 | 208 | 208 | 210 | 209 | 208 | 1.800 |
| CTTAD | 0.823 | 0.819 | 0.817 | 0.814 | 0.811 | 0.808 | 0.005 |
| DM | 0.856 | 0.856 | 0.855 | 0.852 | 0.850 | 0.841 | 0.005 |
| OM | 0.832 | 0.821 | 0.818 | 0.813 | 0.808 | 0.806 | 0.006 |
| CP | 0.891 | 0.902 | 0.903 | 0.903 | 0.905 | 0.910 | 0.007 |
| AHF | 0.858 | 0.864 | 0.866 | 0.867 | 0.870 | 0.871 | 0.005 |
| GE | 0.823 | 0.819 | 0.817 | 0.814 | 0.811 | 0.808 | 0.005 |
| ME, MJ/kg DM | 18.410 | 18.420 | 18.500 | 18.930 | 19.110 | 19.090 | 0.032 |
| Faeces | | | | | | | |
| DM, % | 36.410 | 36.320 | 36.530 | 34.810 | 33.560 | 31.340 | 0.573 |
| Score | 4.200 | 4.000 | 4.100 | 3.600 | 3.500 | 3.100 | 0.012 |
| pH | 6.430 | 6.380 | 6.540 | 6.520 | 6.570 | 6.600 | 0.064 |
| Ammonia, g/kg | 1.340 | 2.010 | 2.090 | 2.280 | 2.330 | 2.330 | 0.063 |
| Output | 0.470 | 0.470 | 0.480 | 0.530 | 0.570 | 0.590 | 0.018 |

DM: dry matter; OM: organic matter; CP: crude protein; AHF: acid hydrolysed fat; GE: gross energy; ME: metabolisable energy; SEM: standard error of the mean.

### Table 6. Average and variances (between parentheses) of the apparent total tract digestibility (CTTAD) of nutrients and energy and ME (MJ/kg) of raw soybean (RSB) estimated by the substitution and difference methods in dogs (n = 6).²

| Item | 60 | 120 | 180 | 240 | 300 | p-value Qc² |
|------|----|-----|-----|-----|-----|-------------|
| Substitution method | | | | | | |
| CTTAD | 0.7590 (0.0029) | 0.7710 (0.0023) | 0.7640 (0.0017) | 0.7650 (0.0002) | 0.7670 (0.0001) | .9890 0.4000 |
| DM | 0.8530 (0.0177) | 0.8440 (0.0017) | 0.8290 (0.0014) | 0.8290 (0.0004) | 0.8000 (0.0002) | .3970 0.8230 |
| OM | 0.6340d (0.0077) | 0.7020c (0.0020) | 0.7160c (0.0018) | 0.7250c (0.0005) | 0.7580c (0.0002) | .0350 0.9020 |
| CP | 0.7120c (0.0124) | 0.7540d (0.0019) | 0.7620d (0.0007) | 0.7670d (0.0006) | 0.7730d (0.0001) | .0410 0.7890 |
| AHF | 0.8590c (0.0164) | 1.0040d (0.0145) | 1.0600c (0.0069) | 0.9570d (0.0021) | 0.9600d (0.0021) | .0110 0.6170 |
| GE | 0.9700 (0.0035) | 0.9290 (0.0018) | 0.9130 (0.0003) | 0.9130 (0.0002) | 0.9040 (0.0001) | .0210 0.6180 |
| ME | 18.7000 (1.2781) | 19.5000 (0.9835) | 21.6000 (0.0972) | 21.6000 (0.0971) | 20.7000 (0.0842) | <.0010 0.6170 |
| Difference method | | | | | | |
| CTTAD | 0.7590 (0.0018) | 0.7710 (0.0023) | 0.7640 (0.0032) | 0.7650 (0.0002) | 0.7670 (0.0001) | .9890 0.4160 |
| DM | 0.8530 (0.0164) | 0.8450 (0.0016) | 0.8390 (0.0004) | 0.8390 (0.0004) | 0.8030 (0.0002) | .7040 0.8180 |
| OM | 0.7120 (0.0012) | 0.7540 (0.0019) | 0.7620 (0.0007) | 0.7670 (0.0006) | 0.7700 (0.0001) | .0410 0.7890 |
| CP | 0.9700 (0.0035) | 0.9290 (0.0018) | 0.9130 (0.0003) | 0.9130 (0.0002) | 0.9040 (0.0001) | .0210 0.5780 |
| AHF | 1.0430 (0.0091) | 0.9760 (0.0045) | 0.9510 (0.0018) | 0.9460 (0.0014) | 0.9500 (0.0002) | .0310 0.5780 |
| GE | 1.0420 (0.0002) | 0.9760 (0.0004) | 0.9440 (0.0005) | 0.9420 (0.0004) | 0.9260 (0.0001) | .0110 0.6140 |

DM: dry matter; OM: organic matter; CP: crude protein; AHF: acid hydrolysed fat; GE: gross energy; ME: metabolisable energy.

²It was not possible to estimate the ME of RSB by the difference method.

²Qc = A calculated Q value greater than the observed value in the table (0.506) indicates difference between the highest variance and the total sum of the other variances by the Q-Cochran test at 5% significance level.

Percentage followed by different letters are different by the Kruskal–Wallis test (p < .05).
residence time of the diet in the extruder (approximately 30 to 60 seconds) and high fat content of the mash diet (Félix, Rivera et al. 2013). In the present study, the AHF content of the diets containing 60 to 300 g of RSB/kg ranged between 85 to 128 g/kg. The detrimental effects of high-fat content in the mash diet during single-screw extrusion were described by Félix et al. (2010) in a study with different soybean products. Those authors reported a negative correlation (−0.97) between AHF content and starch gelatinisation in extruded dog foods, with 90 to 66% reduction in starch gelatinisation in extruded dog foods containing 52 g AHF/kg (diet with 300 g of defatted soybean meal/kg) to 109 g AHF/kg (diet with 300 g soybean/kg), respectively. The addition of fat to the diet before extrusion compromises starch gelatinisation due to its lubricating effect, which reduces shear strength and temperature inside the extruder, thereby reduces water absorption by the starch granules (Lin et al. 1997).

Differently from the present study, Purushotham et al. (2007) reported that TI was efficiently inactivated (<2.0 mg/g) when a complete dog containing 150 g of RSB/kg was extruded at 125–140 °C. Longer retention times and the use of greater mechanical and thermal energy levels in the extruder may allow the complete inactivation of RSB protease inhibitors. However, the consequent reduction of feed mill output should be considered.

The negative effects of high soybean inclusion levels in dog foods on faecal texture are well documented (Yamka et al. 2003; Swanson et al. 2004; Félix, Rivera et al. 2013; Félix, Zanatta et al. 2013). Although no diarrhoea was observed in the present experiment, the inclusion of increasing RSB levels in the diet resulted in looser stools. Soya oligosaccharides and soluble non-starch polysaccharides undergo fermentation in the large intestine, producing lactate and short-chain fatty acids. Excessive levels of these end-products of fibre fermentation in the large intestine increase osmotic pressure in the intestinal lumen, and, in association with the high water holding capacity of soluble fibres, increase faecal moisture and output (Roberfroid 1993; Silvio et al. 2000).

Soybean oligosaccharides may reduce faecal ammonia concentrations in dogs and may have a prebiotic effect in the intestine (Félix, Rivera et al. 2013). However, as previously reported by Félix, Rivera et al. (2013), the high faecal ammonia concentrations observed in the present study in dogs fed RSB diets may be due to the greater availability of non-digested protein in the large intestine of dogs caused by the incomplete inactivation of protease inhibitors. In addition, the increasing faecal pH in dogs fed RSB diets is consistent with the increase in faecal ammonia concentrations, which cause pH to become alkaline.

The microbial fermentation of non-digested nitrogen compounds produces several putrefactive compounds, including ammonia and branched-chain fatty acids (iso-butyrate and iso-butyrate) (Musco et al. 2016) by deamination, and account for the foul odour of faeces (Hussein et al. 1998). Many of these compounds are harmful to intestinal health. Ammonia and other nitrogen compounds may cause the development of tumours (Lin and Visek 1991) and aggravate colitis (Hussein et al. 1998).

Regarding the methods to evaluate ingredient digestibility, studies with canola and barley in pig diets (Fan and Sauer 1995) and corn gluten meal in dog diets (Kawauchi et al. 2011) also did not report any CTTAD differences when applying the methods of regression, difference and/or substitution. Besides, Fan
Ananda Portella Félix (1995) also observed a reduction in estimation error when higher test ingredient levels were included in the diet. According to these authors, the highest of the contribution of a nutrient of the test ingredient to the total dietary content, the more reliable the measurement of its digestibility with the difference method. Although no studies comparing different methods to determine soybean digestibility in dogs were found in literature, Félix, Rivera et al. (2013), using the substitution method with 300 g RSB/kg of the test diet, obtained a CP CTTAD value of 0.789 for RSB in dogs. This value is close to that obtained by the three methods evaluated in the present study (0.768, on average).

Therefore, considering that the regression method requires at least four diets and that is not possible to determine ME by the difference method, the substitution method is recommended to evaluate ingredient digestibility in dogs, because it requires only two diets. The limitation of the substitution and difference method is that they require greater test feedstuffs inclusion in the Cd to reduce estimation errors, which is not possible for all kinds of ingredients, as fat and fibre sources.

Conclusions

Soybeans must be heat-treated before single-screw extrusion to completely inactivate protease inhibitors, which may reduce soybean protein utilisation by dogs and increase ammonia production in the large intestine. The substitution, regression and difference methods can be used to estimate nutrient and GE digestibility of RSB. However, the substitution method is recommended because it requires a lower number of experimental diets and can predict ME.

Ethical approval

The experiment was approved by the Committee of Ethics on Animal Use of the sector of Agricultural Sciences of the Federal University of Paraná (UFPR) under protocol n. 012/2008.

Disclosure statement

No potential conflict of interest was reported by the authors.

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