Dietary fibre type influences protein and fat digestibility in dogs

Fábio Ritter Marx, Geruza Silveira Machado, Alexandre de Mello Kessler and Luciano Trevizan

Abstract
Fibre can interfere with digestibility of fat by accelerating intestinal passage or by increasing chyme viscosity. Lecithin is an important emulsifier which can improve fat digestion. The study aims to determine if fibre solubility may interfere with apparent or true total tract digestibility (ATTD; TTTD) of macronutrients and energy and if adding lecithin could improve fat digestibility. Stool quality was evaluated regarding fibre and lecithin inclusion. Eighteen adult dogs fed 130 kcal metabolizable energy/BW kg0.75/day were assigned in a 3×2 factorial Design, two blocks of 10 days to complete six replications per treatment. Two factors were tested, the source of fibre replacing starch and the inclusion of lecithin. Six diets were formulated with 10% corn starch or cellulose powder, or beet pulp and then all of the diets were dressed with 10% poultry fat or 1% soy lecithin + 9% poultry fat. The fibre inclusion reduced ATTD of dry matter, organic matter, carbohydrates, and energy. The ATTD of fat, crude protein, acid detergent fibre (ADF), and energy, and the TTTD of fat were decreased by adding soluble fibre in the diet, while the ATTD of ADF was greater for the cellulose diet. Soluble fibre impairs absorption of fat and other nutrients, then energy, while insoluble fibre is more related to effects on energy dilution. Lecithin is not able to restore fat digestibility, but lecithin improves fat digestibility with insoluble fibre inclusion. Both sources can be used to produce low energy diets and modulate faecal score: soluble fibre holds water in the faecal content while insoluble fibre tends to produce dried faeces.

Highlights
- Soluble fibre inclusion reduces digestibility of protein, fat, and energy.
- The content of water in the faeces was increased by adding soluble fibre, and faecal score was damaged but remained under acceptable conditions.
- Insoluble fibre increases the faecal bulk but reduces faecal water content.
- Lecithin did not compensate the effects of soluble fibre on reducing fat digestibility, but improved fat digestibility with insoluble fibre inclusion.

Introduction
Fibre replacing energetic or protein sources has been used to reduce energy density in diets (Hervik and Svihus 2019). Different chemical composition and physicochemical properties reflect different patterns of fibre solubility and physiological outcomes (De Godoy et al. 2013). Soluble fibre, common in beet pulp, was found to decrease gastric emptying time, increases viscosity, binds bile acids, reduces digestibility of nutrients and energy, and improves fermentation increasing faecal water content (Fahey et al. 1990a, 1990b; Garcia-Diez et al. 1996; Donadelli and Aldrich 2019). On the other hand, insoluble fibre is minimally fermented (Sunvold et al. 2021). Cellulose, as an example, has been demonstrated to reduce diet energy content with minimal effect on fat and protein digestibility in dogs and cats, although faecal output increases firmer stools have been observed (Prola et al. 2010; Donadelli and Aldrich 2019). The distinct fibres modulate intestinal passage rate, then absorption of nutrients could be impacted due to interaction with fibre and nutrients in the lumen. Insoluble fibre by accelerating the passage ratio, potentially decreases dry and organic matter digestibility in dogs (Muir et al. 1996). Soluble fibre from beet pulp is found to decline digestibility of protein and fat (Fahey et al. 1990a, 1990b; Muir et al. 1996; Sabchuk et al. 2017) which is not desired when a diet dilution is proposed. Protein and fat carry essential amino and fatty acids, respectively. Based on previous effects reported we hypothesised that insoluble fibre could damage digestibility by increasing passage rate which impaired complete digestion and absorption of nutrients that would be compensated by adding an
emulsifier. In the same way, soluble fibre increases the chyme viscosity and reduces bile salts that could affect the gastrointestinal enzymes action on substrate, reducing digestibility. Lecithin as an emulsifying agent has hydrophilic and a lipophilic segment in its molecular structure and is concentrated at the interface between oil and water subsequently reducing the interfacial tension (van Nieuwenhuyzen and Tomás 2008). Adding lecithin would compensate the negative effects of fibres over nutrients digestibility. Therefore, the present study aimed to evaluate the effects of different fibre sources, combined or not with soy lecithin, on ATTD of macronutrients and energy and the true total tract digestibility (TTTD) of fat; complementary assessments were energy intake and stool quality.

Materials and methods
All animal care and handling procedures were approved by the Animal Ethical Committee of the Universidade Federal de Lavras, protocol number 053/14.

Animals and experimental design
Eighteen intact adult different breed dogs (9 Beagle, 2 German Shepherd, 1 Labrador, 1 Weimaraner, 2 Border Collie, 1 Akita, 2 Pitbull) weighing 17.8 ± 8.5 kg, aged 4 ± 1.2 years were distributed in a 3 × 2 factorial Design, consisting of two 10-days periods, with three observations per treatment per period in a total of six observations per treatment. Dogs were randomised into the treatments in the first period and again in the second period. Each period included an adaptation phase (d 1–5) followed by a total faecal collection phase (d 6–10). All dogs were free of ectoparasites, regularly immunised and tested for complete blood count (CBC), biochemistry, and coproparasitological analyses before starting adaptation. During the adaptation to the diets and collection phase, dogs were housed in a research facility in individual kennels of 8 m² half area covered with free access to drinking water. Body weight (BW) was measured on d 1 and d 10. Temperature and daylight fluctuated with external conditions. Before start the trial and between periods, the dogs were housed in the same kennels for five days and washed in with a commercial dry premium diet.

Dietary treatments
Three similar basal diets were formulated and extruded with up to 10% of its content being from different carbohydrate sources (corn starch, cellulose powder, or beet pulp). The corn starch diet was formulated to have a lower fibre content, while the other two diets were formulated to have a higher and similar dietary fibre content. All diets were formulated to have the same fat content. Each basal diet was split in two and coated with 10% poultry fat or 9% poultry fat plus 1% soy lecithin to produce six experimental diets. All diets were added with 1.5% powdered palatability enhancer (hydrolyzed swine liver). The ingredient and chemical compositions of the six experimental diets are shown in Table 1.

The dogs were fed twice a day and the food offer was calculated to meet their maintenance energy requirement (MER) estimated at 130 kcal metabolisable energy (ME) × BW kg⁻⁰.⁷⁵/day (FEDIAF, 2021).

Sample collection
Diet samples were collected and stored during the trial. First two hours after morning feeding dogs were watched for spontaneous defaecation. Then each two hours during the day kennels were checked and total faeces were collected. Faecal samples were scored after each defaecation by a same person as follows; 1 = hard dry and crumbly, 'bullet like'; 2 = well formed, does not leave a mark when picked up, kickable; 3 = moist beginning to lose form, leaving a definite mark when picked up; 4 = the majority, if not all the form is lost, poor consistency, viscous; and 5 = watery diarrhoea (Moxham 2001). At the start and at the end of each faecal collection period, a gelatine capsule, containing 1.000 mg of iron oxide (III), Fe₂O₃ was given orally to the dogs as a marker for faecal outputs during the collection periods. The faeces were collected shortly after defaecation and stored at −20 °C. Total faecal output from each dog was weighted and mixed, then samples were taken and dried at 55 °C in a forced-air oven for 72 hours according AOAC (Association of Official Analytical Chemistry – AOAC 1995) followed by grinding 1-mm screen in a Willey hammer mill (DeLeo Equipamentos Laboratoriais, Porto Alegre, Brazil).

Chemical analysis
Diets and faeces were analysed for DM (Dry Matter) (AOAC 934.01), ash (AOAC, 1995), crude protein (AOAC 954.01), total fat content by acid hydrolysis (AOAC 954.02); neutral detergent fibre (NDF) and acid detergent fibre (ADF) were analysed according to Silva & Queiroz (2002); Diets were analysed for total dietary fibre (TDF), soluble fibre (SF) and insoluble fibre (IF) (AOAC 991.43). Dietary and faecal gross energy (GE) were determined by bomb calorimeter (Parr Instrument Co., model 1261, Moline, IL, USA).
Calculations

The dietary ME was calculated according to the following equation:

\[
MEFEDIAF \text{ kcal/kg} = \frac{\left( GE \text{ intake} - \text{faecal GE} \right) - \left( CP \text{ intake, g} - \text{faecal CP, g} \right) \times 1.25}{DM \text{ intake, g} (FEDIAF, 2021)}
\]

The following equation was used to determine apparent total tract digestibility:

\[
ATTD: \frac{\text{nutrient intake, g/d} - \text{faecal output, g/d}}{\text{nutrient intake, g/d}} \times 100
\]

The organic matter (OM) content was calculated by:

\[
OM (\%) = \%DM - \%Ash
\]

Carbohydrate content was calculated by difference as follows:

\[
\text{CARBOHYDRATES} (\%) = \%DM - (\%Ash + \%Fat + \%CP)
\]

The endogenous fat loss (EFL) of dogs were considered as 155 mg/kg BW/d (Marx et al. 2017). The TTTD of fat was determined according to the equation:

\[
TTTD \text{ OF FAT} : \frac{\text{fat intake, g/d} - \text{(fat faecal output, g/d)}}{\text{fat intake, g/d} \times 100}
\]

Statistical analysis

Differences in the ATTD of macronutrients and energy, TTTD of fat, energy intake, and faecal outputs between the dietary treatments were normal distributed according Shapiro–Wilk test \(p > 0.05\). Means were tested by ANOVA using GLM procedure (Statgraphics Plus for Windows 4.1). Dog was used as a random
effect, diet and time as a fixed effect. Preplanned contrasts were used to evaluate the effects of dietary fibre sources, soy lecithin, and its interaction on intake, digestibility coefficients, and faecal characteristics. The means were compared by Student-Newman-Keuls test at 5% probability.

Results and discussion

All dogs remained healthy throughout the study.

Feed intake

The dogs had a similar intake (kcal ME x BW kg\(^{0.75}\)/day) for all experimental diets (p > 0.05; Table 2). The fibre inclusion did not decrease diets acceptance, despite decreasing energy density (kcal ME/kg of diet). The ME (kcal/kg) of diet was decreased by adding cellulose and beet pulp (Table 1). This effect was predicted, and food offers compensations were made before starting the trial once dietary fibre inclusion could depress feed intake. However, no limitation on energy feed intake was observed (kcal ME x BW kg\(^{0.75}\)/day). The inclusion of dietary fibres in diets with reasonable amounts of fat (19% DM basis) did not reduce food acceptance by dogs.

Apparent total tract digestibility

The diets with cellulose and beet pulp had lower ATTD of DM, OM, carbohydrates, and energy (p < 0.05) compared to the corn starch diet, as we expected. In addition, cellulose did not interfere with ATTD of CP, fat, and TTTD of fat, compared to the corn starch diet. Also, cellulose diet showed the highest ATTD of ADF (p < 0.05). Finally, the beet pulp diets increased faecal moisture (p < 0.0001) and impaired faecal score (p < 0.0099), but it remained within the standard range (Table 2).

Data indicates that ATTD of macronutrients and energy could be affected, at least in part, by increasing the solubility of dietary fibre (Sunvold et al. 2021). Beet pulp reduced the ATTD of CP and fat, and the TTTD of fat. These effects were not observed when complete insoluble fibre was added. Some researchers have already reported a decrease in ATTD of nitrogen for beet pulp diets compared to cellulose diet (Fahey et al. 1990a, 1990b; Bosch et al. 2009; Donadelli and Aldrich 2019; Sunvold et al. 2021). Unlike cellulose, beet pulp contains protein (9–10% CP) which is in a high extent linked to cell wall (around 50%) and consequently shows low apparent digestibility, as measured in other non-ruminant animals (De Blas et al. 2010). Thus, as beet pulp contributes with a low digestible protein as a small part of the dietary CP, it has an effect in lowering overall CP digestibility. Also, the dietary inclusion of beet pulp or other fermentable fibre, and the subsequent fermentation in the colon, may enhance the fermentation of protein or enhance microbial nitrogen due to increased energy availability. By providing energy, fermentable fibre encourages

Table 2. Food intake, faecal output, apparent total tract digestibility (ATTD) of macronutrient and energy, true total tract digestibility (TTTD) of fat and stool quality of adult dogs fed diets with different fibre sources and soy lecithin.

| Fibre sources | Soy lecithin | p-Value |
|---------------|-------------|---------|
| Corn Starch   | FS          | SL      |
| Intake        | FS vs. SL   |         |
| Food intake, kcal ME/BW (kg\(^{0.75}\)/day) | 131 | 131 | 128 | 132 | 129 | 1.60 | 0.6050 | 0.3581 | 0.4310 |
| ATTD, %       |             |         |
| DM            | 83.3\(^a\)  | 78.7\(^b\) | 78.0\(^b\) | 79.7 | 80.3 | 0.32 | <0.0001 | 0.3139 | 0.2940 |
| OM            | 87.9\(^a\)  | 82.7\(^b\) | 82.1\(^b\) | 84.0 | 84.4 | 0.27 | <0.0001 | 0.4626 | 0.2655 |
| CP            | 86.3\(^a\)  | 85.6\(^b\) | 82.9\(^b\) | 84.8 | 85.0 | 0.34 | 0.0011 | 0.7350 | 0.0890 |
| NDF           | 26.0\(^a\)  | 35.6\(^b\) | 35.5\(^b\) | 29.7 | 35.1 | 1.45 | 0.0172 | 0.0718 | 0.5700 |
| ADF           | 15.2\(^a\)  | 35.4\(^b\) | 19.1\(^b\) | 21.3 | 25.2 | 1.65 | <0.0001 | 0.2824 | 0.4453 |
| Carbohydrates\(^d\) | 87.3\(^a\) | 76.7\(^b\) | 78.1\(^b\) | 80.5 | 80.9 | 0.36 | <0.0001 | 0.5313 | 0.8111 |
| Fat           | 91.8\(^a\)  | 91.8\(^b\) | 89.8\(^b\) | 91.1 | 91.1 | 0.27 | 0.0049 | 0.9419 | 0.3482 |
| Energy        | 87.7\(^a\)  | 83.7\(^b\) | 82.5\(^b\) | 84.5 | 84.8 | 0.28 | <0.0001 | 0.6322 | 0.0274 |
| TTTD of Fat, %|             |         |
| EFL corrected by mg/kg BW/day | 97.0\(^a\) | 96.9\(^a\) | 94.8\(^b\) | 96.2 | 96.3 | 0.29 | 0.0075 | 0.9608 | 0.4465 |
| Faecal output |             |         |
| Faecal DM, %  | 38.1\(^a\)  | 40.1\(^a\) | 27.4\(^b\) | 34.9 | 35.6 | 0.68 | <0.0001 | 0.6061 | 0.7551 |
| Faecal score\(^e\) | 2.50\(^a\) | 2.40\(^a\) | 2.70\(^b\) | 2.50 | 2.50 | 0.04 | 0.0099 | 0.8533 | 0.6895 |

\(^a\)Within a row, means lacking a common superscript differ (p < 0.05).
\(^b\)Standard error of the mean.
\(^c\)Carbohydrates = %DM – (%Ash + %Fat + %CP).
\(^d\)Faecal score based on the following scale: 1 = hard dry and crumbly, ‘bullet like’; 2 = well formed, does not leave a mark when picked up, kickable; 3 = moist beginning to lose form, leaving a definite mark when picked up; 4 = the majority, if not all the form is lost, poor consistency, viscous and 5 = watery diarrhoea.

ATTD: Apparent total tract digestibility; NDF: Neutral detergent fiber; ADT: Acid detergent fiber; TTTD: True total tract digestibility; EFL: Endogenous fat loss.
microbial growth and, thus, contributes to the production of nitrogenous constituents that are lost in the faeces (Silvio et al. 2000; De Godoy et al. 2013; Sunvold et al. 2021). Thus, the greater ATTD of CP in dogs consuming corn starch and cellulose diets may reflect the lower microbial protein present in their faeces.

The lower ATTD of fats could also be partially explained for similar reasons. But, although soluble fibre can promote an increase in the microbial population on large intestine it may not explain the complete reduction on the TTTD of fat, as bacteria is not rich in lipids in its composition. In general, bacteria composition in DM can vary between 3 and 9% (Brown et al. 1996). However, after the EFL corrections, the TTTD of fat still showed significant differences between cellulose and beet pulp.

The digestive passage rate through the intestine could explain the reduction on ATTD and TTTD of fat associated with beet pulp addition in the diet (Fahey et al. 1990a). The emulsification of fat may be compromised due to the higher viscosity of food on stomach and small intestine, causing a reduction on micelles formation and a decrease on effectiveness of the digestive enzymes. However, 1% soy lecithin inclusion did not improve dietary fat digestion.

Diets contain similar carbohydrate concentration, but the ATTD of carbohydrates were clearly greater in corn starch diets ($p < 0.0001$). Diets containing fibre as part of the starch were replaced by fibre. Starch is completely digestible for dogs (Fortes et al. 2010) then replacing starch with fibre account to reduce digestible of energy in the diets.

The ATTD NDF was greater for the fibre sources which improved the amount of NDF in the diet ($p = 0.0172$). The cellulose diet which presented the greatest concentration of ADF has the best digestibility coefficient, differing from basal and beet pulp diets. This higher ATTD of the ADF is contrary to the results of Silvio et al. (2000) where the substitution of cellulose by pectin led to an increase of ADF ATTD. One difficulty in comparing the results lies in the fact that when different fibre sources are experimentally compared, not only does the ratio of soluble to insoluble fibre change, but also the amount of insoluble fibre of the diets is quite different. In the present study it was attempted that the inclusion of cellulose or beet pulp would bring similar ADF levels, but some discrepancies were found in the diet analysis. Furthermore, it could be argued that cellulose’s contribution to ADF is primarily devoid of lignin and perhaps more digestible than ADF from beet pulp or grain sources. In horses, the ADF digestibility of hay was shown to be greater than the ADF digestibility of beet pulp or grain sources (Braga et al. 2008; Jensen et al. 2014).

Irrespective of the fibre source, the ATTD of energy was reduced ($p < 0.0001$), similarly to the ATTD of the OM ($p < 0.0001$). This is a direct result of lower carbohydrate digestibility of cellulose and beet pulp when compared to corn starch. The addition of soy lecithin had no effect on the energy ATTD, but as the significant interaction indicated, lecithin acted in an opposite way in the fibre diets, increasing the energy ATTD when the fibre source was the cellulose and decreasing the ATTD of the when the source of fibre was the beet pulp. The lack of a physiological explanation for this behaviour demands further studies to understand the results.

The inclusion of 1% soy lecithin in dog diets did not interfere with ATTD of macronutrients and energy ($p > 0.05$). Significant interactions between fibre sources and lecithin effects were not observed for most responses, except for ATTD of energy ($p = 0.0274$) that showed a crossed effect between lecithin and fibre sources. Soy lecithin increased ATTD of CP and energy in cellulose diet and decreased in beet pulp diet (Figure 1). Contrarily to the expectation, lecithin did not work with soluble fibre to improve digestibility. Lecithin improved energy digestibility when insoluble fibre was added. If cellulose promotes an increase on the intestinal rate of passage, lecithin may play a role in the emulsification stage improving absorption of energy. However, no effect on nutrient digestibility was observed for lecithin. Based on analytical methods energy has less error than other methods and must be more accurate to detect difference when it exists.

**True total tract digestibility of fat**

As hypothesised, ATTD of fat was affected negatively by soluble fibre ($p < 0.0049$). Discounting the EFL content of 155 mg/kg BW/day (Marx et al. 2017) remaining in dog faeces was possible to calculate the TTTD of fat. The digestibility of fat tends to be almost complete and could be reaffirmed in this trial reaching levels close to 97%. The ATTD was increased around 5 percentual points when TTTD of fat was estimated, but significant differences remained between fibre sources, with lower values for the beet pulp diet. As mentioned before, the solubility of the beet pulp could interfere with digestion. The viscosity of the diet
could play some detrimental effect during digestion what was not reversed by adding 1% of lecithin. It reveals that beet pulp reduces energy digestibility in part by reducing fat digestibility.

**Faecal characteristics**

The evaluation of stool quality is a useful tool for vets and nutritionists since diarrhoea and constipation are undesirable conditions. Also, stool quality is important for dog owners, often used as an indication of animal health. The optimal stool quality range varies from the faeces firm enough to prevent diarrhoea until faeces soft enough to prevent constipation.

In this study, diets with 10% corn starch or 6.5% cellulose, induced a greater DM faecal content and better faecal scores (2.4 and 2.5, respectively) than diets with 10% beet pulp (2.7). Still, all the diets produced a firmer stool, easier to collect. Sunvold et al. (2021) observed similar faecal characteristics in dogs fed diets with approximately 8% cellulose or 12.5% beet pulp. The authors reported faecal DM content of 46.6 and 24.7%, and faecal scores of 2.4 and 2.8, for cellulose and beet pulp diets, respectively. Also, Donadelli and Aldrich (2019) replaced the basal diet for dogs with 10% of cellulose sources and beet pulp and noticed a reduction of 28.5% on faecal DM by including beet pulp. There is a strong indication that beet pulp inclusion in the diet has impact on fermentation and then faeces hold more water, and faecal score tends to be softer. In both studies the faecal score was under acceptable conditions, but it must get worse with higher inclusion of fermentable fibre (Sunvold et al. 2021; Donadelli and Aldrich 2019).

Bosch et al. (2009) evaluated diets with 8.5% cellulose or beet pulp content and their results corroborate these findings. Dogs fed cellulose had higher DM faecal content, 37.9% and lower faecal score (2.44), while dogs fed beet pulp had lower DM faecal content 23.1% and slightly higher faecal score (2.5). Burkhalter et al. (2001), reported the DM faecal content of 30% and faecal score of 3.1, for dogs fed diets with 7.5% beet pulp. These results showed a minor discrepancy between the previously mentioned and ours, but still with similar values.

The higher fermentability of beet pulp induces the increase of water binding on faeces, while the low fermentability of cellulose induces the opposite effect. However, both fibre sources may be included, according to tested levels, in adult dog diets without causing any significant detrimental effect on stool quality. The
balance between non fermentable fibre and fermentable fibres must take place during feed formulation.

**Conclusion**

Fibre is an excellent choice for reducing energy content in diets for dogs. The simple replacement of starch impacts directly on disponible energy. While insoluble fibre works as a diluent, soluble fibre interferes negatively with fat and protein digestibility. Lecithin does not have a clear role but improves energy absorption when insoluble fibre is included. Soluble fibre holds water in the faecal content and insoluble fibre tends to produce dry faeces in greater amounts. The balance between soluble and insoluble fibre must be acquired to reach the faecal score desired.

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**Data availability statement**

Data are evaluable at doi: 10.17632/r357twhj68.1 or you can email corresponding author.

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