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Comparative evaluation of *in vitro* and *in vivo* digestibility and gas production kinetics in commercial canine diets

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Abstract

The study evaluated the *in vitro* and *in vivo* digestibility and hindgut fermentative characteristics of four commercial maintenance diets for adult dogs: chicken (T₁), mutton (T₂), chicken and egg (T₃), and vegetarian (T₄). Diets were chemically analyzed and subjected to *in vitro* enzymatic digestion (pepsin, pancreatin, amylase), followed by 24-hour faecal inoculum fermentation using the gas production test. *in vitro* results showed significantly higher digestibility of dry matter, organic matter, and crude protein in T₃ and T₂, whereas T₄ exhibited greater fiber and fat digestibility. Total gas production was highest in T₄ (26.87 mL/g DM), indicating greater fermentable carbohydrate content. An *in vivo* trial was conducted with twelve adult Shih Tzu dogs randomly assigned to four groups, following a four-week adaptation and a five-day total collection digestion trial. Diets were fed according to NRC recommendations, and feed and faecal samples were analyzed for proximate composition and nutrient digestibility. Body weight gain was highest in T₃ (122 g) and lowest in T₄ (90 g). Dogs fed T₄ produced higher faecal output (59.73 g/day, as-is) with lower fecal dry matter (37.84%) and softer consistency. *in vivo* digestibility was highest for T₃ (DM 86.22%) and lowest for T₄ (79.65%). Overall, all diets achieved acceptable digestibility (>80% DM), with animal protein-based diets, particularly T₃, showing superior nutrient utilization and lower hindgut gas production. The findings support calibrated *in vitro* assays as reliable predictors of canine diet digestibility.

Keywords: Commercial diet, digestibility, *in vitro* and *in vivo*

Introduction

Dogs are increasingly valued as companion animals, providing emotional and social support, and require nutritionally balanced diets to maintain health and well-being. Commercial dog foods have evolved since the first formulated biscuit in England during the 1860s (Schaffer, 2009) [1]. Rising pet ownership, nuclear families, and awareness of pet health have driven growth of the Indian pet food industry. However, nutrient content listed on packaging does not always reflect actual composition, making independent evaluation essential (Rolinec *et al.*, 2016) [2]. Extruded dry dog food (kibble) is widely preferred due to convenience, palatability, and shelf life (Laflamme *et al.*, 2008; Daumas *et al.*, 2014) [3, 4]. Although extrusion improves stability, thermal and mechanical processing may reduce nutrient digestibility. Digestibility has traditionally been assessed *in vivo* using total collection, marker-based methods, or fistulated dogs (Yamka *et al.*, 2003; Brambillasca *et al.*, 2010) [5, 6]. These methods are laborious, costly, and raise ethical concerns, limiting feasibility for household pets. Consequently, *in vitro* digestion techniques, including two-phase gastric and intestinal models, are widely used to screen diets efficiently and reduce reliance on animals (Biagi *et al.*, 2016; De Godoy *et al.*, 2016) [7, 8]. The *in vitro* gas production method further evaluates substrate degradation and fermentation kinetics, originally developed for ruminants but applicable to monogastric diets (Coles *et al.*, 2005) [9]. This study aimed to determine the proximate composition of commercial adult dog foods and compare their *in vitro* and *in vivo* digestibility.

Materials and Methods

Four commercially available adult maintenance canine diets were selected in the form of kibbles: a chicken-based diet (T₁), a mutton-based diet (T₂), a chicken-and egg-based diet (T₃), and a vegetarian diet (T₄). All diets met the energy, protein, and nutrient specifications recommended by NRC (2006) [12]. The composition of all diets was obtained from the product labels. The chicken-based diet (T₁), included chicken and chicken by-products, cereals and cereal by-products, vegetables, vegetable by-products, and fish oil. The mutton-based diet (T₂) was composed of mutton and dehydrated lamb protein, cereals, dried fruits, vegetables, and fish oil. The chicken-and egg-based diet (T₃) contained chicken and chicken by-products, eggs, fish oil, and cereals. The vegetarian diet (T₄) contained cereals and cereal by-products, milk, vegetables, vegetable by-products, and vegetable oils.

In vitro enzymatic digestion

Commercial diet samples were subjected to simulated gastrointestinal digestion following a modified Cone *et al.* (2005) [10] procedure. For each treatment, 10 g of dry matter (DM) was used to obtain sufficient residue for subsequent fermentation. Samples were incubated for 1.5 h in 250 mL of 0.1 M HCl containing 5 g/L pepsin (1:1000), then neutralized with 50 mL of 0.5 M sodium bicarbonate. A second 1.5 h digestion was conducted in 250 mL of 0.165 M phosphate buffer (pH 6.8) containing 2 g/L pancreatin (8NF/USP) and 2 mL/L amylase. Undigested residues were recovered by filtration through 40 µm nylon gauze, dried at 70 °C, and analyzed for proximate composition prior to fermentation.

Gas production test (GPT)

The dried residues were subsequently subjected to *in vitro* fermentation using modified protocols described by Cone *et al.* (2005) [10] and Becker *et al.* (2003) [11]. Fecal inoculum was prepared from fresh feces collected from three dogs fed a standard diet after a one-month adaptation period. Feces were pooled within treatment groups to prepare a 2% (w/v) fecal inoculum, resulting in four inocula (BS1-BS4) corresponding to treatments T₁-T₄. Approximately 0.5 g DM of each residue sample, in triplicate, was incubated anaerobically with 60 mL of buffered fecal medium under continuous CO₂ flushing. Gas production was continuously recorded over a 24-h incubation period, with blank incubations used to correct for background gas production.

In vivo feeding studies

Twelve adult Shih Tzu dogs (28-41 months of age; 4-8 kg body weight) were used in the experiment. Dogs were randomly assigned to four experimental groups to ensure comparable mean body weight and sex distribution among treatments. Animals were fed twice daily at 10:00 and 16:00 h. Prior to the morning feeding, dogs were provided with at least 30 min of physical activity and sunlight exposure. Fresh drinking water was available ad libitum throughout the experimental period.

The four dietary treatments consisted of a chicken-based diet (T₁), a mutton-based diet (T₂), a chicken-and egg-based diet (T₃), and a vegetarian diet (T₄). All diets were formulated to meet the nutrient requirements for adult dogs as recommended by the NRC (2006) [12].

- Maintenance energy requirement = 130 Kcal/Kg MBW

- Crude protein requirement = 3.28 g/Kg MBW
- Crude fat requirement = 1.8 g/Kg MBW

The metabolizable energy (ME) content of the commercial diet was calculated using predictive equations recommended by the NRC (2006) [12] for estimating ME in dog food.

The ME of commercial diet was calculated using following equations:

1. $GE \text{ (kcal)} = (5.7 \times \text{g protein}) + (9.4 \times \text{g fat}) + [4.1 \times (\text{g NFE} + \text{g fibre})]$
2. $\text{Per cent energy digestibility} = 91.2 - (1.43 \times \% \text{ crude fibre in dry matter})$
3. $DE \text{ (kcal)} = (GE \times \% \text{ energy digestibility}) / 100$
4. $ME \text{ (kcal)} = DE - (1.04 \times \text{g protein})$

Following a four-week adaptation period, a five-day digestion trial was conducted using the total collection method. Body weight was recorded weekly, and daily feed intake was monitored throughout the experimental period. During the trial, feed offered, feed refusals, and faecal samples were collected and analyzed for proximate composition according to AOAC (2016) [13]. Prior to the start of the experiment, all dogs were routinely vaccinated and dewormed to prevent infectious diseases and parasitic infestations.

Analysis

Descriptive statistics for all parameters were calculated following the procedures of Snedecor and Cochran (1989) [14]. Differences between groups were analyzed using one-way ANOVA, followed by Tukey's post hoc test, and unpaired Student's t-test, with all analyses performed using SPSS Version 16.0.

Results and Discussion

The Chemical composition of the diets used in the feeding trial was presented in the table 1. Four commercially available adult maintenance type dog foods, namely chicken based (T₁), mutton based (T₂), chicken and egg-based (T₃) and vegetable-based (T₄) groups were selected for evaluation. All diets had similar dry matter around 93 per cent and organic matter around (92-93%). Crude protein ranged from 20.54 to 25.67 per cent, while crude fiber varied between 2.86 to 4.27 per cent. Ether extract was highest in T₂ (11.87%) and lowest in T₃ (7.22%). Nitrogen-free extract ranged from 52.18 to 59.59 per cent and total ash content was about 6.7 to 7.4 per cent. The metabolizable energy content of the diets ranged from 3.65 to 3.93 kcal/g, with an average of 3.75 kcal/g, closely matching the typical energy density of commercial dry dog foods (3.5-4.0 kcal/g). The proximate analysis indicated that all diets were adequate to meet the nutritional requirements for adult maintenance type.

The *in vitro* nutrient digestibility of the diets is presented in Table 2. The *in vitro* digestibility results revealed significant differences among treatments. The dry matter digestibility (DMD) was highest in T₃ (85.37%) and T₂ (84.37%), followed by T₁ (83.01%) and lowest in T₄ (80.50%). Organic matter digestibility (OMD) followed a similar trend, with T₃ (87.73%) and T₂ (84.50%) showing higher values than T₁ (83.04%) and T₄ (80.85%). These results are consistent with findings by Madhusudhan (2010) [15] and higher than those reported by Biagi *et al.* (2016) [7]. Digestibility values above 80 per cent indicate high-quality

extruded dog foods, aligning with Tonglet *et al.* (2001) [16], who recommend a minimum DM digestibility of 80 per cent for good-quality dog food

Crude protein digestibility (CPD) was also higher in T₃ (84.60%) and T₂ (83.60%) compared to T₁ (82.87%) and T₄ (81.65%). These findings align with Madhusudhan (2010) [15] and support earlier observations by Meyer *et al.* (1991) [17] and Kendall and Holme (1982) [18], who reported that animal proteins typically exhibit greater digestibility than plant-based proteins. The lower CPD observed in the T₄ is likely due to its reliance on plant-based protein sources, which tend to be less bioavailable and more resistant to enzymatic degradation compared to animal-derived proteins. In contrast, crude fiber digestibility (CFD) was highest in T₄ (76.34%), while T₂ (67.92%), T₃ (65.33%), and T₁ (64.47%) showed lower values. Ether extract digestibility (EED) was greater in T₄ (70.53%) and T₂ (69.61%) compared to T₃ (64.12%) and T₁ (63.07%). Overall, T₃ and T₂ showed superior digestibility for most nutrients, while T₄ had higher fiber and fat digestibility.

Total gas production after 24 h *in vitro* incubation of diets were presented in the Table 3. The total gas production after 24 hours of *in vitro* incubation was found to be highest in the vegetable-based diet (26.87 ml/g DM), followed by chicken based (17.17 ml/g DM), mutton based (15.08 ml/g DM), and chicken and egg (13.82 ml/g DM) based diets. The higher gas production in the vegetable-based diet was attributed to the greater availability of fermentable carbohydrates and fibre. However, this excessive fermentation could lead to undesirable effects such as flatulence and frequent defecation in dogs, as they are non-ruminants and derive minimal energy benefits from hindgut fermentation. Faecal consistency and overall health status were also influenced by the type of diet. Dogs fed animal protein based diets exhibited firmer faeces, better coat and body condition whereas those on the vegetable based diet showed softer faeces and more frequent defecation.

The results of the *in vivo* digestion trial on nutrient digestibility are presented in Table 4. The mean *in vivo* digestibility (%) of various chemical components showed notable differences among the four diets (T₁-T₄). Dry matter digestibility (DMD) was highest in T₁ (85.19%), T₂ (85.09%) and T₃ (83.57%), while significantly lower in T₄ (84.41%). This may be attributed to the fact that non-vegetarian diets exhibit significantly ($p \leq 0.05$) higher digestibility efficiency compared to vegetarian diets, as noted by Debnath and Patil (2018) [19]. It also suggests that the T₄ may have included ingredients less digestible to dogs, such as higher fibre or plant-based components, which can reduce overall nutrient absorption (Fahey *et al.*, 1990; De Godoy *et al.*, 2016) [20, 8]. Organic matter digestibility (OMD) followed a similar pattern, being highest in T₃ (86.29%) and lowest in T₄ (79.42%).

Crude protein digestibility (CPD) was comparable among T₁ (85.55%), T₂ (86.94%) and T₃ (86.83%), but significantly reduced in T₄ (80.71%). This reduction may be attributed to the protein source used in the T₄ diet, as protein digestibility in dogs is strongly influenced by ingredient type, animal based proteins typically exhibit higher digestibility than plant-based sources (Case *et al.*, 2010; NRC, 2006) [21, 12]. Crude fibre digestibility (CFD) varied considerably, with the lowest value in T₂ (43.30%) and the highest in T₄ (57.05%).

While fibre is generally less digestible, certain fermentable fibres (e.g. beet pulp) can be partially broken down by colonic bacteria in dogs, contributing to improved stool quality and colonic health (Swanson *et al.*, 2002) [22]. The higher fibre digestibility in the T₄ group may reflect the inclusion of more fermentable fibre sources in the vegetarian-based formulation.

Ether extract digestibility (EED) ranged from 90.42 to 93.89 per cent with T₄ showing significantly lower digestibility than T₂ and T₃. Nitrogen free extract digestibility (NFED) was highest in T₃ (87.28) and lowest in T₄ (79.44). Overall, T₄ consistently exhibited significantly lower digestibility values across most nutrients compared to the other diets, while T₃ exhibited significantly higher digestibility. The reduced NFE digestibility in the T₄ may be attributed to the presence of resistant starches or less processed carbohydrate ingredients, which are more common in vegetarian formulations.

Comparison of *in vitro* and *in vivo* digestibility of nutrients of various diets were presented in the Table 5. The comparison of *in vitro* and *in vivo* digestibility of various nutrients revealed close agreement for most diets, with a few significant differences. For dry matter digestibility (DMD), values were statistically similar across T₂, T₃, and T₄, while T₁ showed a significantly higher *in vivo* value compared to *in vitro*. Organic matter digestibility (OMD) was generally comparable, though *in vivo* OMD was slightly lower than *in vitro*. For crude protein digestibility (CPD), *in vivo* values were significantly higher than *in vitro* for T₁ (85.55 vs. 82.87%), T₂ (86.94 vs. 83.60%) and T₃ (86.83 vs. 84.60%), while T₄ showed no significant difference. Ether extract digestibility (EED) was significantly higher *in vivo* than *in vitro* across all diets. The observed increase in CPD *in vivo* is likely due to the metabolic activity in the large intestine, where, microbial deamination of amino acids and breakdown of nitrogen-containing compounds such as urea lead to ammonia (NH₃) production. This ammonia can be absorbed through the intestinal wall and subsequently excreted in the urine, resulting in a net nitrogen loss not accounted for in *in vitro* models (Hendricks *et al.*, 2012) [23]. For crude fibre digestibility (CFD), the *in vitro* and *in vivo* values were 68.56 and 41.46 per cent respectively, indicating a significant difference. This discrepancy may be attributed to particle size and methodology differences. A study by Junejo *et al.* (2019) [24] on wheat bran with varying particle sizes reported a negative correlation between particle size and both macronutrient digestibility and dietary fiber solubility. In the present study, the *in vitro* samples were finely ground and sieved through a 1 mm mesh before analysis, whereas, in the *in vivo* trial, the animals were fed the diets without particle size reduction. The ether extract digestibility (EED) in T₁ increased from 63.07 per cent (*in vitro*) to 92.43 per cent (*in vivo*); in T₂ from 69.61 to 93.89 per cent; in T₃ from 64.12 to 93.07 per cent; and in T₄ from 70.53 to 90.42 per cent. This discrepancy can primarily be attributed to the absence of bile salts in the *in vitro* digestion method. Bile salts are essential for lipid emulsification and absorption in the gastrointestinal tract, and their absence in *in vitro* systems severely limits the breakdown and solubilization of dietary fats (Biagi *et al.*, 2016) [7]. Thus, *in vitro* EED tends to underestimate true fat digestibility in dogs.

Table 1: Chemical composition of the diets used in the feeding trial

Parameters	T ₁	T ₂	T ₃	T ₄
Dry matter %	92.96	93.27	93.06	93.10
Organic matter %	92.74	92.58	93.27	93.13
Crude protein %	20.54	25.67	22.70	23.92
Ether extract %	8.33	11.87	22.70	9.83
Crude fiber %	4.27	2.86	3.76	4.12
Nitrogen free extract %	59.59	52.18	59.59	55.26
Total ash %	7.25	7.41	6.72	6.86
Metabolizable energy (Kcal)	3.65	3.93	3.66	3.75

Table 2: *In vitro* digestibility of nutrients of various diets

Nutrients	T ₁	T ₂	T ₃	T ₄	SEM	P VALUE
DMD	83.01 ^b	84.37 ^c	85.37 ^c	80.50 ^a	0.289	0.001
OMD	83.04 ^b	84.50 ^c	85.73 ^c	80.52 ^a	0.286	0.001
CPD	82.87 ^a	83.60 ^b	84.60 ^b	81.32 ^a	0.289	0.001
EED	63.07 ^a	69.61 ^b	64.12 ^a	70.53 ^b	0.560	0.001
CFD	64.47 ^a	67.92 ^b	65.53 ^a	76.34 ^c	0.523	0.001
NFED	87.42 ^b	89.21 ^c	90.05 ^c	82.68 ^a	0.231	0.001

Values in a row with different superscript are statistically significant ($p < 0.05$)

Table 3: Total gas production after 24 h *in vitro* incubation of diets

Parameters	T ₁	T ₂	T ₃	T ₄	SEM	P VALUE
A (ml/g residue)	101.13 ^b	96.53 ^a	94.46 ^a	140.47 ^c	0.586	0.001
B (ml/DM food)	17.17 ^b	15.08 ^a	13.82 ^a	26.87 ^c	0.363	0.001
C (ml/Mean DMI)	2098 ^a	1646 ^a	1623 ^a	2990 ^b	158.31	0.001

A, Amount of gas produced per g of residue of enzymatic digestion of diet (ml g⁻¹ DM);

B, Amount of gas produced per g of DM of food);

C, Amount of gas produced per mean dry matter intake by animal (ml/mean DMI)

Values in a row with different superscript are statistically significant ($p < 0.05$)

Table 4: *In vivo* digestibility of nutrients of various diets

Nutrient	T ₁	T ₂	T ₃	T ₄	SEM	P VALUE
DMD (%)	85.19 ^b	85.09 ^b	86.22 ^b	79.65 ^a	0.479	0.001
OMD (%)	84.48 ^b	85.10 ^b	86.29 ^b	79.42 ^a	0.730	0.001
CPD (%)	85.55 ^b	86.94 ^b	86.83 ^b	80.71 ^a	0.324	0.001
EED (%)	92.43 ^{ab}	93.89 ^b	93.07 ^b	90.42 ^a	0.478	0.005
CFD (%)	52.32 ^b	43.30 ^a	51.52 ^b	57.05 ^b	1.506	0.001
NFED (%)	86.66 ^b	84.57 ^b	87.28 ^b	79.44 ^a	0.631	0.001

Values in a row with different superscript are statistically significant ($p < 0.05$)

Table 5: Comparison of *in vitro* and *in vivo* digestibility of nutrients of various diets

Parameters	Diets	<i>In vitro</i>	<i>In vivo</i>	SEM	P VALUE
DMD	T ₁	83.01	85.19	0.37	0.009
	T ₂	84.37	85.09	0.25	0.053
	T ₃	85.37	86.22	0.20	0.254
	T ₄	80.50	79.65	0.37	0.188
OMD	T ₁	83.04	84.48	0.67	0.38
	T ₂	84.50	85.10	0.26	0.12
	T ₃	85.73	86.29	0.29	0.39
	T ₄	80.52	79.42	0.43	0.11
CPD	T ₁	82.87	85.55	0.22	0.01
	T ₂	83.60	86.94	0.22	0.01
	T ₃	84.60	86.83	0.30	0.009
	T ₄	81.32	80.71	0.37	0.05
EED	T ₁	63.07	92.43	0.10	0.001
	T ₂	69.61	93.89	0.50	0.001
	T ₃	64.12	93.07	0.51	0.001
	T ₄	70.53	90.42	0.68	0.004
CFD	T ₁	64.47	52.32	0.71	0.011
	T ₂	67.92	43.30	1.32	0.005
	T ₃	65.53	51.52	0.64	0.018
	T ₄	76.34	57.05	0.56	0.008
NFED	T ₁	87.42	86.66	0.49	0.504
	T ₂	89.21	84.57	0.27	0.006
	T ₃	90.05	87.28	0.25	0.03
	T ₄	82.68	79.44	0.50	0.008

Values in a row with different superscript are statistically significant ($p < 0.05$)

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References

- Schaffer M. One nation under dog. New York: Macmillan; 2009.
- Rolinec M, Bíro D, Gálík B, Šimko M, Juráček M, Tvarožková K, Ištáková A. The nutritive value of selected commercial dry dog foods. Acta Fytotech Zootech. 2016;19(1):25-28.
- Laflamme DP, Abood SK, Fascetti AJ, Fleeman LM, Freeman LM, Michel KE. Pet feeding practices of dog and cat owners in the United States and Australia. J Am Vet Med Assoc. 2008;232:687-694.
- Daumas C, Paragon BM, Thorin C, Martin L, Dumon H, Ninet S, Nguyen P. Evaluation of eight commercial dog diets. J Nutr. 2014;3:63.
- Yamka RM, Jamikorn U, True AD, Harmon DL. Evaluation of low-ash poultry meal as a protein source in canine foods. J Anim Sci. 2003;81(9):2279-2284.
- Brambillasca S, Purtscher F, Britos A, Repetto JL, Cajarville C. Digestibility, faecal characteristics, and plasma glucose and urea in dogs fed a commercial diet once or three times daily. Can Vet J. 2010;51(2):190.

7. Biagi G, Cipollini I, Grandi M, Pinna C, Vecchiato CG, Zaghini G. A new *in vitro* method to evaluate digestibility of commercial diets for dogs. *Ital J Anim Sci*. 2016;15(4):617-625.
8. De Godoy MRC, Hervera M, Swanson KS, Fahey GC Jr. Innovations in canine and feline nutrition: technologies for food and nutrition assessment. *Annu Rev Anim Biosci*. 2016;4(1):311-333.
9. Coles LT, Moughan PJ, Darragh AJ. *In vitro* digestion and fermentation methods for nutritive evaluation in the hindgut. *Anim Feed Sci Technol*. 2005;123-124:421-444.
10. Cone JW, Jongbloed AW, Van Gelder AH, De Lange L. Estimation of protein fermentation in the large intestine of pigs using gas production technique. *Anim Feed Sci Technol*. 2005;123:463-472.
11. Becker PM, Van Gelder AH, Wikselaar V, Jongbloed AW, Cone JW. Carbon balance for *in vitro* digestion and fermentation of roughages for pregnant sows. *Anim Feed Sci Technol*. 2003;110:159-174.
12. National Research Council. Nutrient requirements of dogs and cats. Washington, DC: National Academy Press; 2006.
13. AOAC. Official methods of analysis. 20th ed. Washington, DC: Association of Official Analytical Chemists; 2016.
14. Snedecor GW, Cochran WG. Statistical methods. 8th ed. Ames (IA): Iowa State University Press; 1989.
15. Madhusudhan HS. Evaluation of canine diets based on *in vitro* hindgut fermentation and faecal microbiota [PhD thesis]. Bidar: Karnataka Veterinary, Animal and Fisheries Sciences University; 2010.
16. Tonglet C, Jeusette I, Istasse L, Diez M. Prediction of protein digestibility in dog food by a multi-enzymatic method. *J Anim Physiol Anim Nutr*. 2001;85(8):189-194.
17. Meyer H, Kienzle E. Digestive enzymes in the dog and cat. *J Nutr*. 1991;121(11 Suppl):S99-S100.
18. Kendall PT, Holme DW. Digestibility of soybean products and cereals in dog diets. *J Sci Food Agric*. 1982;33(9):813-822.
19. Debnath BC, Patil MB. Comparative evaluation of vegetarian and non-vegetarian dry pet food in dogs. *Indian J Anim Res*. 2008;42(3):219-221.
20. Fahey GC Jr, Merchen NR, Corbin JE, Hamilton AK, Serbe KA, Lewis SM, Hirakawa DA. Dietary fiber for dogs: effects of graded levels of beet pulp. *J Anim Sci*. 1990;68(12):4221-4228.
21. Case LP, Daristotle L, Hayek MG, Raasch MF. Canine and feline nutrition: a resource for companion animal professionals. St Louis: Elsevier Health Sciences; 2010.
22. Swanson KS, Grieshop CM, Flickinger EA, Merchen NR, Fahey GC Jr. Effects of fructooligosaccharides and mannanoligosaccharides in dogs. *J Nutr*. 2002;132(6):1717S-1719S.
23. Hendriks WH, van Baal J, Bosch G. Ileal and faecal protein digestibility measurement in humans and non-ruminants. *Br J Nutr*. 2012;108(S2):S247-S257.
24. Junejo SA, Geng H, Wang N, Wang H, Ding Y, Zhou Y, Rashid A. Effects of particle size on physicochemical and *in vitro* digestion properties of durum wheat bran. *Int J Food Sci Technol*. 2019;54(1):221-230.