Indian J. Anim. Nutr. 2021. 38 (1): 86-91
doi: 10.5958/2231-6744.2021.00013.X

Indian Journal of Animal Nutrition

In vitro Nutritional Assessment of Soy Nuggets based Dog Food

Mohneet Kaur, Udeybir Singh*, A.P.S. Sethi, J.S. Hundal, Haneet Kaur and O.P. Malav

Department of Animal Nutrition, College of Veterinary Science,
Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141 004, Punjab, India

ABSTRACT

The present study was conducted to evaluate the effect of different processing techniques on nutritive value and to determine appropriate inclusion level of soy nuggets (SN) in dog food. Standardized dog foods were subjected to different processing techniques *viz.* raw, boiling and extrusion. The *in vitro* study was performed with two incubation stages, with gastric phase of 2 hours in the presence of pepsin, gastric lipase in HCl solution, and intestinal phase of 4 h in presence of pancreatin and bile salts in buffer solution. Data analysis revealed that extrusion improved (P<0.01) *in vitro* nutrient digestibility and the best (P<0.01) response was observed in diets having 5% SN containing extruded dog food. Maximum (P<0.01) dry matter digestibility and organic matter digestibility was observed in extruded diet; No significant difference (P<0.01) was observed in crude protein digestibility at 5% SN supplementation. However, with 10% and 15% SN supplementation, no significant difference (P<0.01) was observed in raw and boiled diets, but crude protein digestibility increased (P<0.01) with extrusion as compared to raw and boiled diets. Ether extract digestibility decreased (P<0.01) in boiled diet at all the levels of SN supplementation. Irrespective of processing techniques, level of SN supplementation had shown non-significant difference in nutrient digestibility but the extent was lesser than no supplementation. It was concluded that SN can safely be included in dog diet at 5% level.

Key words: Boiling, Dog food, Extrusion, *In vitro* digestibility, Raw

INTRODUCTION

The ever increasing demand for pet food in India has abetted the prompt growth of pet industry. Advancement in research for improved health and disease prevention in dogs has led to the production of multitude of commercial dog foods with novel sources of protein (Zentek and Mischke, 1997; Dust *et al.,* 2005), carbohydrate (Fortes *et al.,* 2010; Kore *et al.,* 2008; Kore *et al.,* 2009) and fiber (Bednar *et al.,* 2001; Swanson *et al.,* 2001; Kore *et al.,* 2012). Consequently, the quality and quantity of commercial dog foods have also improved. Most of the pet food manufacturing industries use animal co-products as preferred protein sources; however, their bioavailability and component composition can be very capricious due to undesirable components and processing techniques employed. Most of pet owners are vegetarians (Sethi *et al.,* 2019), so the nutritional value of vegetable protein sources need to be ascertained. Soy by-products possess 23% carbohydrate, 20% fat and reasonable amount of minerals, vitamins and dietary fibre besides containing 40% protein and essential amino acids such as glycine, tryptophan and lysine *etc.* However, soyabean also contains some anti-nutritional factors. Quality of soya protein is sensitive to processing techniques employed. Various soy products like soy milk, soy nuggets, soy nuts *etc.* are available in the market but no work has been done to use them in canine diets. Hence, the present experiment was concocted to study the effect of processing and level of incorporation of soya nuggets on diet digestibility under *in vitro* condition.

MATERIALS AND METHODS

Samples of soy by-products (soy nuggets, soy chunks and soy flour) were procured from the local market and were analysed for proximate principle as per AOAC (2005). Soy nuggets were added at 0, 5, 10 and 15% level to formulate diets to meet specifications for puppy stage of dogs (AAFCO, 2014). The energy density was kept 3500 kcal ME/kg (ICAR, 2013). The formulated diets were subjected to different processing techniques *viz.* raw, boiling and extrusion, and were analysed for proximate principles and major minerals.

*Corresponding author: E-mail: udeybirchahal@gmail.com; Principal Scientist and Head, Department of Animal Nutrition, GADVASU, Ludhiana.

Raw diets were formulated using dried ingredients which were kept overnight in hot air oven at 90°C. In case of boiled diets, 100 g of dried diets were weighed and boiled with 300 ml of water for 15 min. The boiled feed was kept for cooling and was further kept overnight in hot air oven at 90°C for drying. After drying, the boiled feed was grinded and analyzed for proximate composition. For formulating the extruded diets, all the ingredients were grinded in a mill, converted into flour, each ingredient was mixed in requisite proportion, properly blended and extrusion was done in the twin screw extruder. The product was cooled for about 30 minutes. Aflatoxin estimation of extruded feeds was done by column chromatography with VICAM series 4 and 4EX instrument.

In vitro method as validated by Biagi *et al.* (2016) was used with slight modifications such as nylon bags were used for collection of the undigested fraction. Nylon bags were properly washed and kept for drying in hot air oven at 65°C, labeled and weighed. Filtration of the undigested residue was done through nylon bags and washings were given with cold water and were tied firmly. Afterwards, nylon bags were kept in hot air oven at 65°C until constant weight was obtained. The dried weight of nylon bags was recorded and residue obtained was subsequently analyzed for the parameters such as crude protein (CP), ether extract (EE) and total ash as per AOAC (2005). In order to determine the dry matter digestibility of the food samples residue obtained from each bottle after the *in vitro* digestion was weighed and digestibility was calculated using following equation: Dry matter digestibility=(100-([residue weight×100]/sample weight). The undigested fraction was analyzed for different nutrients. Nutrient digestibility was calculated with the following equation: Nutrient digestibility=100-{[nutrient% in residue × (100-diet digestibility)] / nutrient % in diet}.

The data pertaining to digestibility of nutrients was subjected to statistical analysis using one-way ANOVA in Software Package for Social Sciences (SPSS, version 25.0). The treatment means were compared by Duncan's Multiple Ranged Test (Duncan, 1995).

RESULTS AND DISCUSSION

Data pertaining to nutrient composition of experimental diets are presented in Table 1. Proximate analysis of soy nuggets revealed that it contained 45.07%

Table 1. Nutrient composition of experimental diets

Treatments	Nutrient composition (% dry matter basis)			
	Crude protein	Ether extract	Crude fibre	Ash
Raw diets				
0 % soy nuggets	23.18	7.42	3.80	7.60
5% soy nuggets	22.75	8.67	3.68	6.80
10% soy nuggets	23.00	8.44	3.86	7.33
15% soy nuggets	22.75	8.20	3.75	6.25
Boiled diets				
0 % soy nuggets	22.75	3.40	3.50	7.56
5% soy nuggets	23.00	6.45	3.20	6.68
10% soy nuggets	22.75	6.20	3.34	7.26
15% soy nuggets	23.18	6.30	3.40	6.50
Extruded diets				
0 % soy nuggets	23.18	6.10	3.70	7.32
5% soy nuggets	22.97	8.55	3.50	6.95
10% soy nuggets	22.77	8.44	3.65	7.30
15% Soy nuggets	22.75	8.65	3.52	6.80

Table 2. *In vitro* nutrient digestibility of control feed using different processing techniques

Nutrient digestibility %	Control diet (0% soya nugget diet)			P-value
	Raw	Boiled	Extruded	
DM	80.46[a]	84.01[b]	92.2[c]	0.001
CP	90.16	91.59	92.80	0.145
EE	94.18[b]	83.34[a]	94.92[b]	0.032
OM	84.38[a]	86.68[b]	91.27[c]	0.002

[a,b,c] Means bearing different superscripts in a row differ significantly (P<0.05)

crude protein (CP), 5.70% ether extract(EE), 8.65% crude fiber (CF) and 7.68% total ash (TA), while soy chunks contained 48.12% CP, 0.53% EE, 14.40% CF and 7.33% TA. Soy flour had low nutritional profile as compared to soy nuggets and soy chunks. It contained 17.94 % CP, 0.38% EE and 2.02 % fibre. Though soy chunks had more CP than soy nuggets soy chunks were poorer in EE and higher in CF content.

The analyzed value of crude protein of control group and feed containing different levels of raw, boiled and extruded feeds were around 23%. This confirms that all formulated diets were iso-nitrogenous. Different processing method had not affected the nutrient composition of diet except the EE and CF content which was reduced in boiled diets. The reduction in these parameters could be due to the diffusion of soluble nutrients into water and softening of fiber fraction during boiling. Hefnawy (2011) also observed decreased fat content in pulses after cooking. Nsa *et al.* (2011) reported that crude fiber content of un-decorticated castor oil seeds reduced after boiling.

In vitro nutrient digestibility of control feed i.e without soy nuggets supplementation using different processing techniques (raw, boiling and extrusion) revealed that DM digestibility and OM digestibility of boiled feed was higher (P<0.05) than raw feed, but lower (P<0.05) than extruded feed. No significant (P<0.05) difference was observed in CP digestibility among

Table 3. *In vitro* nutrient digestibility of soy nuggets based dog feed at different levels using different processing techniques

Diets	Dry matter	Crude protein	Ether extract	Organic matter
Soy nuggets 5%				
Raw	66.74[a]	87.51	91.06[c]	66.84[a]
Boiled	84.27[b]	87.59	83.70[a]	84.26[b]
Extruded	88.20[c]	88.13	88.85[b]	87.94[c]
P-value	0.000	0.896	0.002	0.000
Soy nuggets 10%				
Raw	64.98[a]	85.22[a]	90.35[b]	64.48[a]
Boiled	83.80[b]	85.92[a]	83.36[a]	83.74[b]
Extruded	89.25[c]	89.72[b]	91.75[b]	89.90[c]
P-value	0.000	0.016	0.000	0.000
Soy nuggets 15%				
Raw	67.96[a]	85.49[a]	90.05[b]	67.22[a]
Boiled	83.42[b]	86.02[a]	84.09[a]	83.40[b]
Extruded	88.36[c]	89.00[b]	90.20[b]	88.30[c]
P-value	0.000	0.002	0.000	0.000

[a,b,c] Means bearing different superscripts in a row differ significantly (P<0.05)

different processing techniques. Boiled feeds had lower (P<0.05) EE digestibility than raw and extruded diets, while no significant difference was observed in EE digestibility between raw and extruded diets. Extrusion of feed improved the *in vitro* digestibility of nutrients (Table 2).

Nutrient digestibility of feed containing soy nuggets is depicted in Table 3. Digestibility of DM of boiled feed containing 5% SN was significantly (P<0.05) higher (84.27 %) than raw diet (66.74 %) and lower (P<0.05) than extruded (88.20 %) diet. No significant (P<0.05) difference was seen in CP digestibility of raw (87.51%), boiled (87.59 %) and extruded diets (88.13%). EE digestibility was lower (P<0.05) in case of boiled diets and was higher (P<0.05) in raw diets. The OM digestibility of boiled feed (84.26 %) was lower (P<0.05) than extruded (87.94 %) and higher (P<0.05) than raw diet (66.84 %). Lankhorst *et al.* (2007) also observed that *in vitro* protein digestibility was not affected by different extrusion conditions.

The *in vitro* digestibility of nutrient of boiled dog feed containing 10% soy nuggets showed better (P<0.05) DM digestibility than raw diet (64.98%). However, it was lower (P<0.05) than extruded diet (89.25%). Non-significant (P<0.05) difference was recorded between raw and boiled diets with respect to CP digestibility. However, CP digestibility was higher (P<0.05) in case of extruded diet (89.72%). Non-significant (P<0.05) difference was observed in EE digestibility of raw (90.35%) and extruded (91.75 %) diet, however, EE digestibility was lowest (P<0.05) *i.e.*, 83.36 % in boiled diet. OM digestibility of boiled diet (83.74 %) was significantly higher than raw diet (64.48 %) but was significantly lower than extruded diet (89.90 %).

DM and OM digestibility of boiled feed containing 15% SN was higher (P<0.05) than raw diet and lower (P<0.05) than extruded diet. There was no significant (P<0.05) difference in CP digestibility of boiled (86.02%) and raw diets (85.49%). CP digestibility of extruded diet (89.00%) was highest (P<0.05). Park *et al.* (2010) reported that apparent ileal digestibility's for extruded pea seeds with respect to CP digestibility were higher than unprocessed pea seeds, hence extrusion improved protein digestibility.

Irrespective of processing methods inclusion of

Table 4. Effect of processing technique and level of inclusion of soy nuggets (SN) on *in vitro* digestibility of nutrients

	Dry matter	Crude protein	Ether extract	Organic matter
Processing Technique[1] (P)				
Raw	70.04^{a}	87.09^{a}	91.41^{b}	70.73^{a}
Boiled	83.88^{b}	87.78^{a}	83.62^{a}	84.52^{b}
Extruded	89.50^{c}	89.86^{b}	91.43^{b}	89.35^{c}
Level of soy nuggets[2] % (L)				
0	85.56^{B}	91.45^{B}	90.81^{B}	87.44^{B}
5	79.74^{A}	87.75^{A}	87.87^{A}	79.68^{A}
10	79.35^{A}	86.95^{A}	88.49^{A}	79.37^{A}
15	79.91^{A}	86.84^{A}	88.11^{A}	79.64^{A}
PSE	0.22	0.35	0.49	0.22
P-value				
P	<0.001	<0.001	<0.001	<0.001
L	<0.001	<0.001	0.007	<0.001
P×L	<0.001	0.118	0.050	<0.001

[1]Irrespective of level of soy nuggets in the diet; [2]Irrespective of processing technique; Means with different superscripts^{a,b,c} for different processing techniques and superscripts^{A,B,C} for different levels of soy nuggets with in a row differ significantly; PSE Pooled standard error

soy nuggets had significant effect on the digestibility of nutrients. Digestibility of DM, OM, CP and EE was highest (P<0.01) in feed containing no soya nuggets. However, non-significant difference was observed between different levels of soy nuggets inclusion in dog feed.

FDA (2019) suggests 20 ppb of aflatoxin in pet foods is safe. Analysis revealed that aflatoxin content of soy nuggets was 21 ppb. Lowest aflatoxin content was found in control diet *i.e.*, 5.7 ppb whereas for 5% soy nuggets diet it was 16 ppb, which was within permissible limits. Rumbeiha (2001) observed that dogs are susceptible to the noxious effects of mycotoxins mainly aflatoxin B_1 in feed (> 60 ìg/kg of feed) with an LD50 of 0.5 to 1.5 mg/kg of body weight).

CONCLUSIONS

In vitro study revealed that soy nuggets can be incorporated into the extruded dog's diet up to 5% level and extrusion improved the digestibility of nutrients.

ACKNOWLEDGEMENTS

This study was conducted as part of DBT-GADVASU Canine Research Centre and Network vide order no.BT/ADV/Canine Health/GADVASU/2017-2018 under the sub-head entitled "Nutritional and processing interventions for developing pet foods" under the aegis of Department of Biotechnology, Ministry of Science & Technology, Govt. of India. Authors are thankful to Govt. of India, Department of Biotechnology for giving us such opportunity.

REFERENCES

AAFCO. 2014. *AAFCO Methods for Substantiating Nutritional Adequacy of Dog and Cat Foods.* pp 1-17. In: Official Publication. Champaign, IL: Association of American Feed Control Officials Inc.

AOAC. 2005. *Official Methods of Analysis*. Association of Official Analytical Chemist.18th Ed.Washington, DC, USA.

Bednar, G.E., Patil, A.R., Murray, S.M., Grieshop, C.M., Merchen, N.R. and Fahey Jr, G. C. 2001. Starch and fiber fractions in selected food and feed ingredients affect their small intestinal digestibility and fermentability and their large bowel fermentability *in vitro* in a canine model. *J. Nutr.* 131: 276-286.

Biagi, G., Cipollini, I., Grandi, M., Pinna, C., Vecchiato, C.G. and Zaghini, G. 2016. A new *in vitro* method to evaluate digestibility of commercial diets for dogs. *Ital. J. Anim.* 15: 617-625.

Duncan D B. 1995. Multiple range and multiple F-test. *Biometrica.* 11:1-42.

Dust, J. M., Grieshop, C.M., Parsons, C.M., Karr-Lilienthal, L.K., Schasteen, C.S., Quigley, J.D., Merchen, N.R. and Fahey, G.C. 2005. Chemical composition, protein quality, palatability and digestibility of alternative protein sources for dogs. *J. Anim. Sci.* 83: 2414-2422.

Egana, J.I., Lopez, A. and Quezada, Q. 1991. Effect of extrusion on acceptability and digestibility of growing dog diets. *Arch. Latinoam. Nutr.* 41: 111-20.

Food and Drug Administration. 2019. Sec. 683. 100. Action levels for aflatoxins in animal food (https://www.fda.gov/ICECI/ComplianceManuals/CompliancePolicyGuidanceManual/default.htm)

Fortes, C.M.L.S., Carciofi, A.C., Sakomura, N.K., Kawauchi, I.M. and Vasconcellos, R.S. 2010. Digestibility and metabolizable energy of some carbohydrate sources for dogs. *Anim. Feed. Sci. Technol.* 156: 121-125.

Hefnawy, T.H. 2011. Effect of processing methods on nutrient composition and anti-nutritional factors in lentils (*Lens culinaris*). *Ann. Agric. Sci.* 56: 57-61.

ICAR. 2013. *Nutrient Requirements of Animals - Companion, Laboratory and Captive Wild Animals*. Indian Council of Agricultural Research, New Delhi.

Kore, K. B., Pattanaik, A. K., Das, A. and Sharma, K. 2008. Nutritional and metabolic response of adult Spitz dogs fed pearl millet (Pennisetum typhoides) based diets to exogenous enzyme supplementation. *Anim. Nutr. Feed Technol.* 8: 193-202.

Kore, K.B., Pattanaik, A.K., Das, A. and Sharma, K. 2009. Evaluation of alternative cereal sources in dog diets: effect on nutrient utilisation and hindgut fermentation characteristics. *J. Sci. Food Agric.* 89: 2174-2180.

Kore, K.B., Pattanaik, A.K., Das, A. and Sharma, K.2012. Evaluation of mannanoligosaccharide as prebiotic functional food for dogs: effect on nutrient digestibility, hind gut health and plasma metabolic profile. *Indian J. Anim. Sci*. 82: 81-86.

Lankhorst, C., Tran, Q.D., Havenaar, R., Hendriks, W.H. and Van der Poel, A.F.B. 2007. The effect of extrusion on the nutritional value of canine diets as assessed by *in vitro* indicators. *Anim. Feed Sci. Technol*. 138: 285-297.

Lola, A. 2009. The effect of boiling on the nutrients and anti-nutrients in two non-conventional vegetables. *Pakistan J. Nutr.* 8: 1430-1433.

Nsa, E.E., Ukachukwu, S.N., Isika, M.A. and Ozung, P.O. 2011. Effect of boiling and soaking durations on the proximate composition, ricin and mineral contents of undecorticated castor oil seeds (*Ricinus Communis*). *Int. J. Plant Environ. Sci.* 91: 5344-5352.

Park, S.J., Kim, T.W. and Baik, B.K. 2010. Relationship between proportion and composition of albumins and *in vitro* protein digestibility of raw and cooked pea seeds (*Pisum sativum L.*). *J. Sci. Food Agric.* 90: 1719-1725.

Rumbeiha, W. 2001. *Mycotoxins in pets*. DCPAH-Newsletter.Vol.18, No 2.

Sethi A. P. S., Kaur A. and Singh U. 2019. Nutritional strategies for improving farm profitability and clean animal production. In: "Proc. XVIII Biennial Conference of Animal Nutrition Society of India on Prospects and research priorities in canine nutrition" (Das, A., Mohini, M., Samanta, I., Pakhira, M.C., Das, S., and Mandal, G.P., eds.) held at Kolkata, West Bengal from Dec. 17-19, 2019. pp 209-212.

Swanson, K.S., Grieshop, C.M., Clapper, G.M., Shields, R.G., Belay, T., Merchen, N. R. and Fahey, G.C. 2001. Fruit and vegetable fiber fermentation by gut microflora from canines. *J. Anim. Sci*. 79: 919-926.

Zentek, J. and Mischke, R. 1997. Soya and casein as dietary proteins for dogs. *J. Anim. Physiol. Anim. Nutr*. 77: 1-5.

Received on 10-05-2021 and accepted on 27-07-2021