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Comparative *in-vitro* Evaluation of Dog Food Using Either Rice Gluten or Maize Fibre with Commercially Available Dog Food

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ABSTRACT

The study was carried out with an objective of studying *in-vitro* nutrient digestibility and quality evaluation of dog foods incorporated with 15% rice gluten and 2.5% maize fibre and its comparison with commercially available dog food for its nutritional worth and physical quality parameters. After standardization of diets, raw diets were subjected to different processing techniques viz. boiling and extrusion. Boiling of feed reduced the ether extract content of diets. The *in-vitro* analysis of dog feed involved two incubation phases: first, gastric digestion simulation at 39ºC for 2h in HCl solution in presence of pepsin and gastric lipase enzyme; second, small intestine digestion simulation at 39ºC for 4h using bile salts and pancreatin in phosphate buffer solution. Statistical analysis revealed that boiling of diet reduced the *in-vitro* digestibility of ether extract. Among different processing techniques, *in-vitro* digestibility was best in-case of extruded diets. *In-vitro* digestibility of CP had non-significant difference among different processing techniques; however CPD of boiled dog feed with 15% RG used dog feed was comparable with extruded and raw diet. Comparative evaluation of best preformed extruded feeds with commercial diets revealed that all diets had equal nutritional digestibility of various nutrients. pH, FFA, PV and aflatoxin content of feeds were within permissible limits. It was concluded that RG and MF can be included in dog diet at 15 and 2.5% level respectively showing equal digestibility of feed.

HIGHLIGHTS

- Processing such as extrusion significantly improved the *in-vitro* digestibility of various nutrients.
- *In-vitro* studies confirmed the non-significant differences among commercial and GADVASU prepared feeds.

Keywords: Boiling, Digestibility, Dog food, Extruded, *In-vitro* analysis, Raw diet

Increase in no. of nuclear families ensued by upsurge in pet adoption rate and increasing cognizance among pet owners about health of their pet and has boosted the growth of Indian pet food industry. Pet food manufacturers now emphasize more on introducing different specified pet food products that are rich in vitamins, mineral and other specific nutrients, thereby ensuring a high-quality food for pets. Home cooked pet food is nutritionally unbalanced and lack proper microbial evaluation which make pet prone to no. of pathological and metabolic diseases (Sethi *et al.,* 2019). Tiwari *et al.* (2020) also observed that the consumption of milk is significantly (P≤0.05) higher in rural areas than urban areas. There are certain constraints in feeding commercially available dog food beside their high prices. Commercially available dog foods are formulated using wide variety of plant and animal based ingredients. The processing of commercial pet food can influence digestibility, nutrient bioavailability and safety. Digestibility of dog food is one of the imperative aspects for quality evaluation. Although much attention is paid to nutritional quality of dog food in the marketing of commercial food for dogs; there is limited literature on digestibility, keeping quality and aflatoxin content of

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commercially available dog foods. *In-vitro* digestibility methods are comprehensively used as screening tools to apprise large number of ingredients, thus reducing the number of animals involved in *in-vivo* trials (de Godoy *et al.,* 2016). For assessing the digestibility of commercially available diets for dogs, relatively simple, quick *in-vitro* protocol involving two incubation phases imitating gastric and small intestinal digestion was authenticated through *in-vivo* trials by Biagi *et al.* (2016). Keeping above points in mind, the present study was aimed (i) to study the *in-vitro* digestibility of dog feed containing 15% RG and 2.5 MF (ii) its comparative evaluation with commercially available dog food.

MATERIALS AND METHODS

Diets were formulated for puppy stage of dogs according to the requirements prescribed by AAFCO (2014) containing 22.5 % CP, 8.5 % EE, 1.2% Calcium and 1% Phosphorus. Energy density of diet was kept 3500 kcal ME/kg in accordance with Indian climatic conditions as described by ICAR (2013). The feeds were analyzed for proximate composition viz. dry matter (DM), crude protein (CP), ether extract (EE), total ash (TA), acid insoluble ash (AIA), crude fiber (CF) and phosphorus as per AOAC (2005) and calcium (Talapatra *et al.,* 1940).

Processing of feeds

Raw, boiled and extruded diets were formulated in lab using dried ingredients. The formulated diets were analyzed for proximate composition.

Raw dried diets

Raw diets were formulated using dried ingredients which were kept overnight in hot air oven at 90°C.

Boiled Diets

100 g dried diets were boiled with 300 mL of water in pan for 15 minutes. Subsequently the boiled feed was transferred into trays to cool down and further kept overnight at 90°C in hot air oven for drying. After drying, the boiled feed was grinded and analyzed for proximate composition.

Extruded Diets

All ingredients were grinded in the mill and converted into the flour and were weighed as per the required level for formulation of complete dog feed and properly mixed in the mixer. The mixture was kept in vats. The die of desired product (bone shape) was fitted at the front portion of the extruder. The twin screw extruder and the main motor was switched on and the speed of the barrel was set to 35 Hz. Heating temperature at Heater 1: 80°C, Heater 2: 120°C, Heater 3: 160°C respectively was set. After reaching at set barrel temperature, the barrel of the extruder was cleaned with hot water. The mixture of the formulated complete feed (powder form) was filled in the feeding chamber. The feeding machine was switched on and the speed was fixed at 15 Hz. The ingredients mixture comes in the barrel and the finished product comes out of the machine. Now the cutter motor is switched on at the speed of 10 Hz. Extruded dog feed was collected in large vessels. The product was cooled for about 30 minutes.

In-vitro digestion

In vitro method validated by Baigi *et al.* (2016) was used with some modifications.

Sample preparation

One day before weighed amount of samples were kept in hot air oven at 95^{o}C until constant weight was obtained. After drying, sample was finely ground (<1mm particle size). Nylon bags were properly washed and kept in hot air oven at 65° C for collection of digested samples.

Gastric digestion simulation

10g of food samples was weighed and put in to the labeled 1 L bottles. Sample and 400 ml of a pepsin-HCl solution (HCl 0.075N; pepsin 2 g/L) containing gastric lipase (1 g/L) were incubated in a 1 L bottle in a bench top orbital shaking water bath at 39^{o}C for 2 h.

Small intestine digestion simulation

First, the pH of the above mixture was adjusted to 7.5 using 1N NaOH. Bile salts (Cholic acid-Deoxycholic acid sodium salt mixture) were added to each bottle at a final concentration of 25 g/L. Then, 400 ml of a pancreatin solution was added to each bottle. Finally, the bottles were placed in a bench top orbital shaking water bath at 39°C for 4 h.

Collection of the undigested fraction

Nylon bags were first labeled and weighed empty after cooling. The undigested residue was filtered through nylon bags and washings were given with cold water and were tied securely. These tied nylon bags were kept in hot air oven at 65°C until constant weight. The dried weight of nylon bags was recorded and residue was analyzed for the determination of crude protein, ether extract and total ash as per AOAC (2005).

Calculation and data analysis

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:

$$\text{Dry matter digestibility} = (100 - ([\text{residue weight} \times 100] / \text{sample weight})$$

The undigested fraction was analyzed for crude protein, ether extract, and ash as per standard methods (AOAC, 2005). Nutrient digestibility was calculated with the following equation:

$$\text{Nutrient digestibility} = 100 - \{[\text{nutrient\% in residue} \times (100 - \text{diet digestibility})] / \text{nutrient \% in diet}\}$$

RESULTS AND DISCUSSION

Wet chemistry analysis of formulated control dog feed, feed with 15% rice gluten and 2.5 % maize fibre supplementation level subjected to different processing techniques viz raw, boiling and extrusion is presented in table 1. Crude protein (CP), total ash (TA) and organic matter(OM) content of all formulated diets were within the desired levels. Ether extract (EE) content reduced (P<0.05) after boiling of diets. Hefnawy (2011) observed that lipid content reduced in pulses after cooking. It may be due to the diffusion of liquids into water. Crude fiber (CF) content of diets remained unaltered (P<0.05) with processing except at 15% RG level where it reduced (P<0.05) after boiling and extrusion. Nsa *et al.* (2014) asserted that CF content of un-decorticated castor oil seeds decreased after boiling. This may be due to softening of fiber fraction during boiling. Calcium (Ca)and phosphorus (P) level in all the extruded diets were similar. Commercial diet contained 25% CP, 6.8% EE, 4.1 % CF, 7.25% TA, 1.59 Ca and 0.61% P.

In-vitro nutrient digestibility of control feed, feed containing RG 15% and 2.5% MF subjected to different processing techniques is depicted in table 2. Dry matter digestibility (DMD) of control boiled diet was significantly (P≤0.05), higher than raw diet but lower (P<0.05) than extruded dog feed. Non-significant (P<0.05) difference was observed in DMD of raw and boiled feeds when 15% RG and 2.5% MF was included in dog feed. However, DMD was highest in case of all extruded dog feeds (0, 15% RG and 2.5% MF). Inal *et al.* (2017) testified highest dry matter digestibility in case of extruded food. Non-significant difference was discerned in crude protein digestibility (CPD) of diets among different processing techniques with control and 2.5% MF containing feed; however, at 15% RG inclusion level, CPD of boiled dog food was comparable with raw and extruded dog food. Lankhorst *et al.* 2007 observed that *in-vitro* protein digestibility (IVPD) was not affected by different extrusion conditions on other hand El-Adawy (2002) stated that IVPD for unprocessed chickpea (*Cicer arietinum* L.) increased from 83.61% to 88.52% after cooking for 90 min. Singh *et al.* (2020) also reported that extrusion significantly (P<0.01) increased the dry matter (DMD) and organic matter digestibility (OMD) in diets. DMD and OMD was lowest in-case of raw diets which improved after boiling and further improved after extrusion of diets. However, Overland *et al.* (2007) reported that the crude protein digestibility in cat and dog foods was not affected by the extrusion. In another study conducted by Kaur *et al.* (2021) also concluded that extrusion has remarkable (P<0.05) effect on the nutrient digestibility of different diets prepared by using *dal churi* at various levels.

Ether digestibility decreased after boiling of dog feed but there were non-significant difference in EED of raw and extruded diets at all levels. Cipollini (2008) studied that EE digestibility of dry dog food was higher than wet dog food (94.9 vs. 87.7%).

Table 1: Chemical composition of formulated dog food

Parameters %	Control feed				Rice gluten 15%				Maize fibre 2.5%			
	Raw	Boiled	Extruded	P-value	Raw	Boiled	Extruded	P-value	Raw	Boiled	Extruded	P-value
CP	23.18	22.75	23.18	0.650	22.75	22.84	22.75	0.465	23.18	22.75	23	0.697
EE	7.42^{c}	3.38^{a}	6.1^{b}	0.002	9.6^{c}	4.3^{a}	8.24^{b}	0.000	8.4^{b}	4.78^{a}	7.65^{b}	0.001
CF	3.8	3.5	3.7	0.529	4.8^{b}	4.2^{a}	4.3^{a}	0.021	5	4.5	4.1	0.324
Total Ash	7.6	7.56	7.32	0.270	6.6	6.2	6.37	0.185	7.69	6.7	7.34	0.213
OM	92.4	92.44	92.68	0.262	93.4	93.8	93.63	0.185	92.31	93.3	92.66	0.213

Means with different superscript differ significantly.

Table 2: *In-vitro* nutrient digestibility of control feed, feed containing RG 15% and 2.5 MF subjected to different processing techniques

Nutrient digestibility %	Diets											
	Control feed				Rice gluten 15%				Maize fibre 2.5%			
	Raw	Boiled	Extruded	P-value	Raw	Boiled	Extruded	P-value	Raw	Boiled	Extruded	P-value
DM	80.46^{a}	84.01^{b}	92.2^{c}	0.001	84.76^{a}	86.82^{a}	92.7^{b}	0.004	81.90^{a}	83.98^{a}	91.42^{b}	0.003
CP	90.16	91.59	92.80	0.145	91.25^{a}	91.89^{ab}	92.6^{b}	0.064	90.01	91.02	91.87	0.150
EE	94.18^{b}	83.34^{a}	94.92^{b}	0.032	95.52^{b}	75.88^{a}	94.92^{b}	0.000	96.2^{b}	76.89^{a}	94.4^{b}	0.000
OM	84.38^{a}	86.68^{b}	91.27^{c}	0.002	86.51^{a}	88.76^{b}	93.2^{c}	0.002	83.22^{a}	84.14^{a}	92.3^{b}	0.004

Means with different superscript differ significantly.

Table 3: *In-vitro* digestibility of commercial feed and its Comparative evaluation with control, 15% RG and 2.5% MF containing feeds

Digestibility of Parameters %	Commercial feed	Control group	MF 2.5%	RG 15%	PSE	P- value
DM	91.80	92.20	91.42	92.70	0.274	0.477
CP	92.46	92.80	91.87	92.60	0.211	0.674
EE	93.69	94.92	94.40	94.92	0.315	0.568
OM	90.40	91.27	92.30	93.20	0.446	0.070

In dog foods, with 0 and 15% RG, organic matter digestibility (OMD) was highest ($P<0.01$) in case of extruded diets and lowest in case of raw diets. Diet with 2.5% MF, non-significant difference was recorded in OMD of raw and boiled dog food. Extrusion of dog food proved significant improvement in the digestibility of nutrients.

Formulated extruded feeds were compared with commercially available feed (table 3). Data analysis revealed that there was non-significant difference with respect to nutrient digestibility (DMD, CPD, EED and OMD) between commercially available feed and formulated feeds.

Physico-chemical properties and aflatoxin content of formulated dog feeds and commercial dog feed are depicted in table 4. Among the formulated and commercial feeds analyzed it was observed, commercial feed was highly acidic, followed by feed with 15% RG, 2.5% MF and without RG or MF inclusion. Free fatty acid (FFA) content of formulated feeds was lowest where 2.5% MF was added and highest in feed with 15% RG. FFA content in all feeds was within the permissible limits. Pearson (1968) reported that minced beef had FFA content in the range of 0.38 to 1.74% and had a maximum acceptability limit of 1.8% FFA in view of their progressive increase during storage.

Table 4: Physico-chemical properties and aflatoxin content of formulated feeds and commercial feed

Feeds	pH	FFA(% oleic acid)	PV (mEq/kg)	Aflatoxin (ppb)
Commercial feed	4.87	0.3948	8	7.5
Control group	5.34	0.536	10	5.7
Maize fibre 2.5% feed	5.14	0.3384	8	9.1
Rice gluten 15 % feed	4.98	1.1280	6	10

Maximum per oxide value (PV) was observed where 0% RG or MF was added other feeds. However, it was closely followed by 2.5% MF and 15% RG supplemented diets. Osawa *et al.* (2008) reported the value of FFA and PV of pet food in the range of 4.6 ±0.1 to 28.0±0.6% oleic acid and 1.4 ± 0.1 to 6.8 ± 0.3 meq O_2/kg respectively. Aflatoxin content in all the feeds formulated and commercial feed was with-in desired limits. According to FDA (2019), maximum permissible limit for Aflatoxin in complete pet food of all ages should not be more than 20 ppb.

CONCLUSION

The *in-vitro* studies conclusively indicated that extrusion improves the digestibility of nutrients and there was no-significant difference (P≤0.05) between overall nutrient digestibility of commercially available dog feed and GADVASU prepared extruded dog feeds. Thus RG and MF can be incorporated in dog feed at 15 and 2.5% level, respectively.

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