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Effect of Tomato Pomace Supplementation on the Nutritional Value of Dog Diet as Assessed by *In-Vitro* Digestibility

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ABSTRACT

The present study was aimed to evaluate the effect on different processing techniques on nutritive value of dog food and to determine appropriate supplementation level of tomato pomace (TP) in dog food. Standardized diets were subjected to different processing techniques viz. raw, boiling and extrusion. Proximate analysis of feeds revealed that boiling of feed decreased the ether extract content of diets. *The in-vitro* analysis was done with two incubation phases, first gastric phase of 2 hours duration in the presence of pepsin, gastric lipase and HCl and a second intestinal phase with 4h duration in presence of pancreatin and bile salts. TP was included in diet at 0, 2.5 and 5% level. Results revealed that among different processing techniques, irrespective of level of TP in diet, best results were observed in case of extruded diets. DMD and OMD of boiled diets was lower (<0.001) than extruded diets and higher than raw diets. As far as level of TP is concerned, best results were obtained in diet without TP. However DMD and OMD was (<0.001) higher at 2.5 % inclusion level than at 5% inclusion level while CPD and EED were non-significant at 2.5 and 5% inclusion level. Parameters viz.pH, FFA, PV and aflatoxin content of prepared dog diets was with in permissible limits.

HIGHLIGHTS

- *In-vitro* study of tomato pomace in dog diet.
- Best inclusion level of tomato pomace in dog diet was 2.5%.

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

Utilization of agro-industry by products make a generous contribution towards better and more economical feeding of livestock and pets. The nutritive worth of a number of available agro industry by products is, nevertheless, inadequately known, this dejects their efficient utilization in pet foods. Due to the shortage and the high prices of pet food, some of these by-products could be used as an ingredient in pet food for production of economical and balanced pet food. In this deference, it is evident that the country's resources in such products need to be explored and thoroughly evaluated nutritionally. Fruit and vegetable fibers contain a good balance of soluble and insoluble fiber, which promotes gastrointestinal health (Larrauri *et al.,* 1996).Fibre sources from dehydrated vegetables and fruits are being used by the pet food industry to reduce the incidence of constipation and plasma glucose concentrations in diabetic animals. The processing of whole tomatoes into sauce, juice or paste generates the by-product, tomato pomace (TP) which contains seeds, pulp and skins. It is fed to animals after sun drying. The residue may be ground or pulverized into a powder. It contains a significant amount of soluble fiber. There is an increased demand of dry dog foods for which tomato pomace seems to be a suitable ingredient which could be incorporated. It is commonly amalgamated with supplementation levels of 3-7% (Aldrich, 2009). Typically, dried tomato pomace

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contains about 20% crude protein (CP), 12% ether extract (EE), 30% crude fiber (CF) and 4% total ash (Yuangklang *et al.,* 2015). Fiber content should be given almost importance while formulating dry pet food. Dry dog foods normally contain about 3-4% of CF. Substitution of 8% tomato pomace in diet in place of extruded corn reduced apparent digestibility of whole-diet; crude protein from 82.3 to 78.2% (Allen *et al.,* 1981). Substitution of dietary corn starch by 8.7% TP decreased the digestibility of whole-diet protein from 85.1 to 80.7% (Fahey *et al.,* 1990). Tomato pomace up to 5 % supplementation level in the diet had no adverse effect on the feed intake, gain in weight or feed conversion efficiency of broiler chicks (Sethi, 2012). Lot of research work has been done on incorporation of vegetable and fruit industry waste in livestock, but limited studies have been done on their use in dog feeding. Thus, the present study was aimed to study the effect of different processing method on dog food and to determine the appropriate level of inclusion of tomato pomace in 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). Physiochemical parameters of feed viz. pH (Trout *et al.,* 1992), free fatty acid and peroxide value (Koniecko, 1979) were determined. Extruded feeds and feed ingredient were analyzed for aflatoxin by column chromatography with VICAM series 4 and 4EX instrument.

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°C until constant weight was obtained. After drying, sample was finely ground (<1 mm 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; catalog no. 48305-F) 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 39C 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:

Dry matter digestibility = (100 – ([residue weight × 100] / 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:

Nutrient digestibility = 100 – {[nutrient% in residue× (100-diet digestibility)] / nutrient % in diet}

Statistical analysis

Data were analysed by simple ANOVA, as described by Snedecor and Cochran (1994), by using SPSS (2016) version 21. The differences in means were tested by Tukey's b.

Aflatoxin estimation

After preparation of sample, extract was diluted and passed through column. Aflatoxin was estimated by coloumn chromatography using VICAM fluorometer.

RESULTS AND DISCUSSION

Chemical composition of formulated dog food containing tomato pomace (TP) at 0, 2.5 and 5% supplementation level subjected to different processing techniques viz raw, boiling and extrusion is presented in table 1. Non-significant ($P<0.05$) differences were observed between crude protein, total ash and organic matter content of all formulated diets confirming that formulated diets were iso-nitrogenous. Ether extract content decreased ($P<0.05$) after diets were subjected to boiling at 0, 2.5 and 5% TP level. Hefnawy (2011) also observed decrease in fat contents in the pulses after cooking. This may be due to their diffusion into cooking water. Ether extract content

Table 1: Chemical composition of formulated dog food

Parameters	Tomato pomace 0%				Tomato pomace 2.5%				Tomato pomace 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	23.18	22.75	23.20	0.649	22.70	22.75	23.18	0.431
EE	7.42^{c}	3.38^{a}	6.10^{b}	0.002	8.60^{b}	4.89^{a}	8.44^{b}	0.001	9.78^{c}	4.15^{a}	8.23^{b}	0.000
CF	3.80	3.50	3.70	0.529	4.13^{b}	3.60^{a}	3.77^{a}	0.029	4.10^{b}	3.60^{a}	3.95^{ab}	0.056
TA	7.60	7.56	7.32	0.270	7.32^{b}	7.15^{ab}	6.70^{a}	0.070	7.07	7.40	7.10	0.278
OM	92.40	92.44	92.68	0.262	92.68^{a}	92.85^{ab}	93.30^{b}	0.070	92.93	92.60	92.90	0.278

[1]Irrespective of level of tomato pomace in the diet;Means with different superscripts^{a,b,c} for different processing techniques and superscripts^{abc} for different levels of tomato pomace with in a row differ significantly; PSE–Pooled standard error.

remained (P<0.05) unaffected in dog food after extrusion. Calcium and phosphorus level in all the extruded diets were similar.

Aflatoxin content of apple pomace was 6.8 ppb. While Aflatoxin content of 0 and 2.5 % TP supplemented diets was 5.7 and 13 and ppb respectively Aflatoxin content of prepared diets was with in permissible limits.

Analyzed pH value of all ingredients was found to be acidic. Among the formulated feeds analyzed it was observed that control feed (pH 3.90) was highly acidic, followed by feed with 5% and 2.5% TP. Peroxide value and free fatty acid content was determined to check the rancidity of fat content in feed ingredients and feeds. Free fatty acid content of tomato pomace was within the critical limits which indicate that ingredient was of good quality. Free fatty acid content of formulated feeds was lowest where 5% TP was added (0.33%) and highest in control feed i.e. 1.13%. Peroxide value of tomato pomace and formulated feeds were 4 mEq/kg (Table 2). 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. 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% F.

In-vitro nutrient digestibility of TP based diets at different levels using different processing techniques is depicted in table 3. Dry matter digestibility (DMD) and organic matter digestibility (OMD) of boiled diet was significantly (P<0.01) higher than raw diet but lower (P<0.01) than extruded dog food at 0% TP level, however, non-significant (P<0.01) difference in DMD and OMD of boiled and extruded feeds was observed when 2.5 and 5% TP was included in diets. Inal *et al.* (2017) reported highest dry matter digestibility in extruded food. As far as crude protein digestibility (CPD) is concerned, Non-significant difference was observed in crude protein digestibility (CPD) of diets among different processing techniques with 0 and 5% TP inclusion level. At 2.5% inclusion level CPD of extruded diet was comparable with raw and boiled diet. Lankhorsta *et al.* (2007) observed that *in-vitro* protein digestibility was not affected by different extrusion conditions. While El-Adawy (2002) stated that *in-vitro* protein digestibility of 83.61% for unprocessed

Table 2: Physicochemical Properties of Dog Diet of ingredients and feed FA in view of their progressive increase during storage

Ingredients	pH	FFA(% oleic acid)	PV (mEq/kg)	P Value
Tomato pomace	5.80^{b}	0.6800^{b}	4	0.000
Control	3.90^{a}	1.1300^{c}	4	0.000
TP 2.5%	5.85^{b}	0.6700^{b}	4	0.000
TP 5%	5.45^{b}	0.3384^{a}	4	0.000

Means with different superscripts ^{a,b,c} for different processing techniques and superscripts ^{a,b,c} for different physiochemical properties of dog diet of ingredients with in a row differ significantly; PSE–Pooled standard error.

Table 3: *In-vitro* nutrient digestibility of Tomato based diets at different levels using different processing techniques

Nutrient Digestibility %	Diets											
	Tomato pomace 0%				Tomato pomace 2.5%				Tomato pomace 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	78.13^{a}	81.59^{b}	82.16^{b}	0.004	73.5^{a}	81.50^{b}	81.53^{b}	0.002
CP	90.16	91.59	92.80	0.145	87.9^{a}	89.67^{b}	89.09^{ab}	0.064	87.6	88.85	89.02	0.815
EE	94.18^{b}	83.34^{a}	94.92^{b}	0.032	94.38^{b}	76.1^{a}	93.56^{b}	0.000	95.84^{b}	75.65^{a}	93.08^{b}	0.016
OM	84.38^{a}	86.68^{b}	91.27^{c}	0.002	78.94^{a}	83.04^{b}	84.01^{b}	0.002	73.88^{a}	82.82^{b}	83.46^{b}	0.004

[1]Irrespective of level of tomato pomace 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 tomato pomace with in a row differ significantly; PSE–Pooled standard error.

Table 4: Effect of processing technique used and level of tomato pomace in the feed on *in vitro* digestibility (%) of nutrients

Parameters	Processing technique[1] (P)			Level of tomato pomace [2], % (L)			PSE	P-value		
	Raw	Boiled	Extruded	0	2.5	5.0		P	L	P×L
DM	77.36[a]	82.37[b]	85.30[c]	85.56[C]	80.63[B]	78.85[A]	0.31	<0.001	<0.001	<0.001
CP	88.55[a]	90.04[b]	90.24[b]	91.45[B]	88.89[A]	88.49[A]	0.32	0.009	<0.001	0.666
EE	94.80[b]	78.36[a]	93.86[b]	90.81[B]	88.02[A]	88.19[A]	0.74	<0.001	0.044	0.039
OM	79.06[a]	84.18[b]	86.76[c]	87.96[C]	82.00[B]	80.05[A]	0.38	<0.001	<0.001	0.002

[1]Irrespective of level of tomato pomace 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 tomato pomace with in a row differ significantly; PSE–Pooled standard error.

chickpea (*Cicer arietinum* L.) increased to 88.52% after cooking for 90 min. Non-significant difference was observed with respect to ether extract digestibility (EED) of raw and extruded diets. However, EED reduced significantly after boiling of diet. OMD was lowest in case of raw diets at 2.5 and 5% inclusion level of TP, however at non-significant difference was observed w.r.t. OMD of boiled and extruded diets at these levels.

Irrespective of inclusion of level, DMD and OMD was lowest in-case of raw diets, it improved after boiling, It further improved after extrusion of diet (Table 4). CPD was lowest in case of raw diets, however no significant difference was observed in CPD in case of boiled and extruded diets. Hullar *et al.* (1998) and Overland *et al.*(2007) reported that the crude protein digestibility in cat and dog foods was not affected by the extrusion. Non-significant difference was observed between raw and extruded diets w.r.t. EED while lowest ($P<0.01$) EED was found in-case of boiling. This may be due to the fact that liquification of fat in boiling water, resulting in decrease in fat content in dog food subsequent low EE digestibility. Cipollini (2008) reported that EE digestibility of dry dog food was more than wet dog food (94.9 vs. 87.7%).

Irrespective of processing techniques, Highest DMD and OMD was recorded in-case of diets without TP. OMD and DMD was better at 2.5% inclusion level however, it further it decreased on further increasing level of TP. CPD and EED was highest in-case of diet without TP. However, non-significant difference was recorded w.r.t. CPD and EED at 2.5 and 5%.

CONCLUSION

It was concluded that best results were observed in case of diet without TP. Results were comparatively better at 2.5% level than at 5% level inclusion level of TP. Among different processing techniques best results were observed in case of extruded diets.

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