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Evaluation of graded levels of corn fermented protein on extrusion processing and diet utilization in healthy adult dogs

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There has been increased interest among pet owners to feed vegetarian diets to their pets. However, the primary protein sources used in pet food today are animal based, warranting a need to evaluate novel plant-based ingredients to meet the protein demand. Corn fermented protein (CFP), a coproduct from ethanol production, may provide a plant-based alternative protein source for pet food. Therefore, the objectives of this study were to determine the effects of increasing levels of CFP on extrusion processing, stool quality, apparent total tract digestibility, and palatability in dog diets. Four extruded diets were fed to 12 adult beagle dogs in a replicated 4 × 4 Latin square design. The control diet contained 15% soybean meal (0C) and CFP was exchanged at either 5%, 10%, or 15% of soybean meal (5C, 10C, and 15C, respectively). Dogs were fed each dietary treatment for 9 days of adaption followed by 5 days of total fecal collection. Feces were scored on a 1–5 scale, with 1 representing liquid diarrhea and 5 representing hard pellet-like. Titanium dioxide (0.4%) was added to all diets as an external marker to estimate digestibility. Data were analyzed using orthogonal contrasts in SAS (version 9.4; SAS Institute, Inc., Cary, NC, USA). Dry bulk density of kibble decreased ($P < 0.05$), whereas kibble toughness increased ($P < 0.05$) with CFP inclusion. Fecal dry matter, dry fecal output, and defecations per day increased ($P < 0.05$) when dogs were fed increased levels of CFP. Dry matter and crude protein digestibility of CFP treatments were comparable ($P > 0.05$) to 0C. There was a decrease ($P < 0.05$) in organic matter, crude fat, gross energy, and total dietary fiber digestibility in the CFP treatments compared with 0C. A cubic relationship ($P < 0.05$) was observed in the digestibility of all nutrients except crude fat, indicating that 10C resulted in the lowest digestibility. For the palatability assessment, dogs had no preference when comparing the 5C treatment with the 0C treatment. Even with the differences in dietary treatments, inclusion of CFP at 5%, 10%, and 15% still resulted in acceptable processing parameters, kibble characteristics, and utilization when fed to dogs.

KEYWORDS

canine, corn fermented protein, extrusion, nutrient digestibility, stool quality, palatability

1 Introduction

As the human population has become more concerned about health, animal welfare, and the environment, there has been an increase in the vegetarian lifestyle (Pribis et al., 2010; Stahler and Mangels, 2022). The increase of affluent nations has shifted diets from plant-based diets to diets high in animal products, which has been identified as a contributor to the rise in chronic disease (Popkin and Du, 2003; Walker et al., 2005). In addition, over 66 billion terrestrial animals are slaughtered for consumption (Schlatzer, 2010), which has given rise to animal welfare concerns. Climate change is also becoming one of the biggest environmental issues, which is thought to be impacted by animal agriculture (Koneswaran and Nierenberg, 2008). It is estimated that 75 million people are vegetarian by choice, which is expected to rise as education and affluence spreads (Leahy et al., 2010).

Not surprisingly, the increase in the vegetarian population has resulted in the demand for vegetarian diets for pets. However, shifting dogs and cats to a vegetarian lifestyle is more challenging as they belong to the order Carnivora whose ancestors survived by consuming primarily or entirely captured prey animals. The domestic dog has evolved to become omnivorous, as they have increased gene expression for pancreatic amylase, the ability to convert maltose to glucose, and increased intestinal glucose uptake compared with wolves (Axelsson et al., 2013; Semp, 2014). Therefore, dogs can metabolize carbohydrates and endure on a lower-protein diet (Buff et al., 2014). However, often due to consumer perception, pet food today consists primarily of animal-based ingredients to mimic ancestral diets. For vegetarian pet food to be safe and nutritious, the development and evaluation of novel plant-based ingredients that are high in protein is warranted. Traditional ingredients, such as corn gluten meal, soybean meal (SBM), and pea protein concentrate, are currently available but new options may be valuable.

Ingredients like corn fermented protein (CFP) may be able to meet this demand. CFP, a coproduct from ethanol production, is produced using post-fermentation separation technology, which results in a high protein ingredient. The combination of zein and yeast protein results in an ingredient containing 50% protein, which is nearly double that of traditional distillers' dried grains. Graded levels of CFP have already been evaluated in cats, resulting in acceptable palatability, stool quality, and nutrient digestibility (Kilburn-Kappeler et al., 2022). Therefore, the objective of this study was to evaluate increasing levels of CFP on extrusion processing, stool quality, apparent total tract digestibility (ATTD), and palatability in adult dogs.

2 Materials and methods

The digestibility trial was conducted at the Kansas State University Large Animal Research Center (LARC) under the Institutional Animal Care and Use Committee (IACUC) #4097 protocol. The palatability trial was conducted at Summit Ridge Farms (Susquehanna, PA) under protocols KSUPALC00120, KSUPALC00220, and KSUPALC00320.

2.1 Diet formulation

Four different diets with increasing levels of CFP (POET Bioproducts, Sioux Falls, SD) as a replacer of equal levels of SBM (Fairview Mills, Seneca, KS, USA) were formulated. The nutrient composition of the test ingredients (SBM and CFP) is reported in Table 1. The control diet contained 15% SBM (0C) and CFP was exchanged for either 5% (5C), 10% (10C), or 15% (15C) of SBM (Table 2). The formulated diets met the Association of American Feed Control Officials nutritional requirements of adult dogs. Titanium dioxide (0.40%) was added to serve as an indigestible marker to estimate apparent total tract nutrient digestibility. The dry raw materials, except for the CFP, SBM, and titanium dioxide, comprised the base ration and were purchased from a commercial mill (Fairview Mills, Seneca, KS, USA).

2.2 Diet production

Each diet was produced using a single screw extruder (model E525; ExtruTech, Inc., Sabetha, KS, USA). The preconditioner (model ADP 145; ExtruTech, Inc.) was configured with 12 45° back and 57 neutral beaters on each of the two shafts. The extruder profile and barrel temperatures were based on a typical commercial pet food configuration. At the end of the extruder barrel there were two round die inserts with an interior diameter of 3 mm. The dry matrix feed rate (318 kg/h), preconditioner (PC) cylinder speed (185 rpm), extruder (EX) water (0 kg/h), EX steam (0 kg/h), and EX knife speed (1,600 rpm) were kept constant during the processing of all treatments.

During processing, PC and EX parameters were collected from sensor readouts every 2 min to evaluate potential effects of CFP inclusion on the process. Output variables included PC discharge temperature, EX motor load, EX die temperature, total mass flow (TMF), specific mechanical energy (SME), and in-barrel moisture content (MC).

The TMF was calculated by adding the dry feed rate with water and steam injected in PC and EX, assuming that 80% of the water

TABLE 1 Analyzed chemical composition of experimental ingredients, soybean meal (SBM) and corn fermented protein (CFP), reported on a dry matter basis.

Nutrient (%)	SBM	CFP
Dry matter	88.03	94.87
Moisture	11.97	5.13
Organic matter	91.86	97.16
Ash	8.14	2.84
Crude protein	53.44	52.62
Crude fat	2.71	5.60
Insoluble dietary fiber	16.36	31.41
Soluble dietary fiber	3.52	3.58
Total dietary fiber	19.88	34.89

TABLE 2 Ingredient composition of canine diets with increasing levels of corn fermented protein (CFP).

Ingredient (%)	Treatment[1]			
	0C	5C	10C	15C
Corn	37.97	38.11	38.26	38.41
Chicken meal	20.86	20.23	19.59	18.96
Chicken meal, low ash	11.11	11.72	12.33	12.95
Soybean meal	15.00	10.00	5.00	–
CFP	–	5.00	10.00	15.00
Chicken fat	5.65	5.52	5.40	5.27
Other[2]	9.42	9.42	9.42	9.42

[1] 0C, 0% CFP; 5C, 5% CFP; 10C, 10% CFP; 15C, 15% CFP.

[2] Other ingredients: beet pulp, fish meal, flavor, titanium dioxide, salt, potassium chloride, vitamin and mineral premix, choline chloride, and natural antioxidant.

coming from the PC and EX steam is lost during flash-off as kibbles exit the die:

$$\text{TMF} = \text{dry feed rate} + \text{PC water} + (0.2{*}\text{PC steam}) + \text{EX water} + (0.2{*}\text{EX steam}) \quad (1)$$

SME was calculated using the following formula:

$$SME\left(\frac{kJ}{kg}\right) = \frac{\frac{\tau - \tau_o}{100} * \left(\frac{N}{N_r}\right) * P_r}{m} \quad (2)$$

where, τ is the EX torque percentage or EX motor load, τ_0 is the EX no-load torque percentage (25% at EX screw speed 425 rpm), N is the EX screw speed (rpm), N_r is the rated EX screw speed (425 rpm), P_r is the rated EX motor power (114 kW), and m is TMF (kg/s).

The MC was also calculated using the following formula:

$$MC = \frac{m_f \times X_f + m_{ps} + m_{pw} + m_{es} + m_{ew}}{m_f + m_{ps} + m_{pw} + m_{es} + m_{ew}} \quad (3)$$

where m_f is the feed rate, X_f is the moisture content of the raw material, m_{ps} is the percentage of added steam in the preconditioner, m_{pw} is the percentage of added water in the preconditioner, m_{es} is the percentage of steam added into the extruder, and m_{ew} is the percentage of water added into the extruder. A moisture content of 10% was assumed for X_f.

After extrusion, kibble was pneumatically conveyed through an 8-inch clean air hood system and deposited onto an oscillating belt spreader. The kibble was dried on a 1.5-m-wide single-pass two-zone dryer (model AFI; ExtruTech) to achieve a less than 10% moisture content. The kibble was dried at approximately 110°C for 22 min. The dried kibble was coated with chicken fat protected with natural antioxidants and a dry powdered flavor designed for dogs. The coated diets were stored in polylined Kraft paper bags until fed.

2.3 Physical characteristics of kibble

The wet and dry bulk density were measured off the extruder and off the dryer every 15 min during the processing of each treatment. The bulk density was measured using a 1-L cup in which kibble was leveled and weighed on a digital scale with a 0.1-g sensitivity. In addition, five kibbles were randomly selected every 15 min of each diet production off the extruder and off the dryer and measured for diameter and length using a digital caliper. Ten randomly selected kibbles off the dryer were also weighed using a digital scale with a 0.0001-g sensitivity (EX324 N; Ohaus Corporation, Parsippany, NJ, USA). The diameter, length, and mass measurements were used to determine sectional expansion index (SEI) and specific length.

The SEI was determined by comparing the squared diameter of the dried extruded kibbles by the squared die diameter of the extruder:

$$SEI = \frac{D^2}{d^2} \quad (4)$$

where D is the extrudate diameter and d is the extruder die diameter.

The specific length in mm/g was determined by the following equation:

$$Specific\ length = \frac{l}{m} \quad (5)$$

where l is the extrudate length and m is the extrudate mass.

A texture analyzer (model TA-XT2; Texture Technology Corp., Scarsdale, NJ, USA) with a 30-kg load cell was used to measure kibble texture. A cylindrical probe (with a 25-mm diameter) was used to compress 30 kibbles within each treatment. The procedure was adapted from Dogan and Kokini (2007), with a test speed of 2 mm/s and strain level set at 80%. The kibble hardness was considered to be the peak force in kilogram of the first major kibble breakage, and the energy to compress the kibbles to 80% was computed as the area under the curve in kg mm for each compressed kibble, not accounting for the negative values. The compression energy was considered as kibble toughness.

2.4 Feeding trial

For this study, 12 healthy adult (6.3 ± 0.45 years) beagle dogs (eight castrated males and four spayed females) were enrolled. The dogs had an average body weight of 11.4 ± 1.2 kg. The daily metabolizable energy requirement was calculated for laboratory kennel dogs [130 * $BW_{kg}^{0.75}$; NRC (2006)] to determine the amount of food offered to each dog per day. However, it was adjusted to 105 * $BW_{kg}^{0.75}$ to maintain the body weight of dogs. The body weight was measured at the beginning, middle, and end of each period. The experiment consisted of four periods, and each one was composed of 9 days of adaptation followed by 5 days of collection. Dogs were randomly assigned to each of the four treatments over the four periods. In this model, each animal served as its own control, and each treatment had 12 total observations.

The dogs were individually housed in pens (1.83 m × 1.20 m) equipped with an acrylic-coated mesh floor to allow for separation of urine and feces. Six animals were maintained per room in a temperature-controlled (23°C) modular building with a 12-h light cycle. The dogs received two feedings per day at 08:00 and 17:00, with water provided *ad libitum*. During the collection period, all feces were collected periodically throughout each day to prevent contamination and disturbance. The fecal samples were weighed, scored on a scale of 1–5 with 0.5 increments [with 1 representing liquid diarrhea and 5 representing dry hard pellets; Carciofi et al. (2008)]. A score of 3.5–4.0 was considered ideal. In addition, the pH of a fresh sample (within 15 min of defecation) was recorded in triplicate with a calibrated glass electrode pH probe (FC240B; Hanna Instruments, Smithfield, RI, USA). Fecal samples were stored in a labeled Whirl-Pak® bag in a freezer until further processing.

2.5 Digestibility calculations and nutrient analysis

After each collection period, feces from each dog were composited and dried at 55°C in a forced-air oven until at a constant weight (24–48 h). The dried samples were ground to pass through a 1-mm screen in a laboratory fixed-blade impact mill (ZM 200; Retsch, Verder Scientific, Haan, Germany). Titanium dioxide (TiO_2) concentration was measured in food and feces using a spectrophotometric plate reader (Gen5TM; Biotek® Instruments, Inc., Winooski, VT, USA) at 410 nm (Myers et al., 2004). ATTD was estimated by TiO_2 using the following equation:

$$ATTD = \left[1 - \frac{\%\ TiO_2\ in\ food * \%\ nutrient\ in\ feces}{\%\ TiO_2\ in\ feces * \%\ nutrient\ in\ food}\right] * 100 \qquad (6)$$

Food and partially dried fecal samples were analyzed in duplicate for moisture (AOAC 930.15), ash (AOAC 942.05), crude fat by acid hydrolysis and hexane extraction (AOAC 960.39), gross energy (Parr 6200 Calorimeter; Parr Instrument Company, Moline, IL, USA), and total dietary fiber (AOAC 991.43). Crude protein was determined by Dumas combustion (AOAC 990.03) using a nitrogen analyzer (FP928; LECO Corporation, Saint Joseph, MI, USA).

2.6 Palatability trial

The experimental treatments (5C, 10C, and 15C) were evaluated for palatability compared with the control diet (0C) by dog panels at a commercial kennel (Summit Ridge Farms, Susquehanna, PA). Each experiment was conducted as a split-plate test, in which two stainless steel bowls containing 400 g of food were presented to dogs for a total of 30 min. Each comparison trial was repeated for 2 days, with bowl position switched daily. Twenty dogs were fed daily, providing 40 observations for each paired comparison test. Preference was determined based on the dogs' first choice and total food consumption. Data from consumption were represented as the following ratio:

$$Intake\ ratio = \frac{Consumption\ of\ diet\ A}{Total\ consumption\ of\ diet\ A + diet\ B} \qquad (7)$$

2.7 Statistics

The least squares means of the data were estimated by ANOVA using the GLIMMIX procedure in SAS (version 9.4; SAS Institute INC, Cary, NC, USA) with Tukey correction. Contrasts comparing control (0C) with treatments (5C, 10C, and 15C), and linear, quadratic, and cubic relationships among all diets were considered significant at a P-value < 0.05. For each diet production, sampling was conducted at evenly spaced intervals, which were considered replicates. The digestibility experiment was conducted as a replicated 4 × 4 Latin square design, with three dogs randomly assigned to each of the four diets in each period. Therefore, dog and period were considered random effects in the model for analysis of data from the digestibility trial.

In the palatability experiments, the intake ratio was analyzed using a *t*-test in a two-way ANOVA and the first-choice preference was analyzed using a chi-squared test. The 20 dogs were considered the experimental units for analysis.

3 Results

3.1 Extrusion processing and kibble characteristics

The PC cylinder speed was kept constant across all treatments at 185 rpm (Table 3). However, PC steam, PC water, and EX screw speed fluctuated among dietary treatments. There was slightly more steam and water added to the PC during the production of 0C than during the CFP treatments ($P < 0.05$). The 0C treatment resulted in the fastest ($P < 0.05$) screw speed at 425 rpm, compared with the CFP treatments at an average of 395 rpm.

The PC discharge temperature was lower ($P < 0.05$) in the 0C treatment than in the CFP treatments, and resulted in a significant quadratic relationship among dietary treatments (Table 3). The motor load was also lower ($P < 0.05$) in the 0C treatment than in the CFP treatments, and resulted in a significant cubic relationship among dietary treatments. The 0C treatment resulted in the lowest die temperature (109°C), compared with the CFP treatments (average 110°C). There was also a significant quadratic relationship in die temperature among dietary treatments. The 0C treatment had a greater ($P < 0.05$) TMF compared with the other treatments, and there was also a quadratic relationship ($P < 0.05$), indicating that 5C and 10C resulted in a lower TMF than 0C and 15C. SME was lower ($P < 0.05$) for 0C at 135 kJ/kg than for the other treatments at an average of 141 kJ/kg. There was also a cubic relationship ($P < 0.05$) among dietary treatments for SME, indicating that 10C had the greatest SME at 149 kJ/kg. The MC was higher ($P < 0.05$) during the production of 0C than during the production of the other treatments. There was also a quadratic relationship ($P < 0.05$) for MC, showing that 0C and 15C resulted in a higher MC than 5C and 10C.

There was a significant quadratic relationship in wet bulk density, wet kibble diameter, and wet kibble length among dietary treatments (Table 3). Dry bulk density was greater ($P < 0.05$) for the 0C treatment at 337 g/L compared with the CFP treatments at an average of 320 g/L. Dry bulk density also decreased linearly ($P < 0.05$) as CFP increased. Dry kibble diameter was not affected ($P > 0.05$) by CFP inclusion. The dry kibble length was smaller for 0C than for CFP treatments, and increased linearly ($P < 0.05$) with CFP inclusion. The specific length or SEI of kibble were not affected ($P > 0.05$) by CFP inclusion.

Inclusion of CFP linearly increased ($P < 0.05$) toughness of kibble, ranging from 5.1 kg mm to 6.4 kg mm. However, kibble hardness did not result in a significant linear relationship. Instead, there was a cubic relationship ($P < 0.05$), indicating that the 0C and 10C treatments resulted in the hardest kibble (Table 3).

3.2 Diet chemical analyses

The diets were drier than target at an average of 5% moisture. Overall nutrient composition for dry matter, organic matter, crude fat, and gross energy were maintained among dietary treatments at 94.7%, 91.2%, 12.3%, and 4970.4 kcal/kg, respectively (Table 4). The average crude protein content of CFP treatments was 36.7%, whereas the crude protein content of 0C was 38.4%. The total dietary fiber content was greatest for the 15C treatment at 16.1% and lowest for the 0C treatment at 13.8%.

3.3 Feed intake and fecal characteristics

The food intake of dogs was lower for the 0C treatment than for the CFP treatments, and resulted in a cubic relationship ($P < 0.05$) among dietary treatments (Table 5). The wet fecal output of dogs was maintained ($P > 0.05$) among dietary treatments (Table 5). Fecal dry matter percent was lower for dogs fed the 0C treatment (32%) than for dogs fed the CFP treatments (average 33%), and increased linearly ($P < 0.05$) as CFP increased. The dry fecal output of dogs increased linearly ($P < 0.05$) with CFP inclusion, ranging from 35 to 40 g per day. Defecations per day also increased linearly ($P < 0.05$) with CFP inclusion, ranging from 2.2 to 2.4 times per day. The fecal score was lower ($P < 0.05$) for dogs fed the 0C treatment (3.7) than for dogs fed the CFP treatments (average 3.9). The fecal score of dogs also had a quadratic relationship ($P < 0.05$) among dietary treatments. The fecal pH of dogs was maintained similarly ($P > 0.05$) among dietary treatments.

3.4 Apparent total tract digestibility

There was a cubic relationship ($P < 0.05$) in dry matter, organic matter, crude protein, gross energy, and total dietary fiber digestibility among dietary treatments, indicating that 10C resulted in the lowest digestibility (Table 6). Dry matter and crude protein digestibility were not different ($P > 0.05$) when comparing the 0C treatment with CFP treatments. However, organic matter digestibility was higher ($P < 0.05$) for the 0C treatment (87.6%) than for the CFP treatments (average 86.7%). Crude fat digestibility was greater ($P < 0.05$) in the 0C treatment (97.8%) than in the CFP treatments (average 97.5%). Crude fat digestibility also resulted in a quadratic relationship ($P < 0.05$), indicating greater digestibility for the 0C and 15C treatments than for the 5C and 10C treatments. Gross energy digestibility was higher ($P < 0.05$) in the 0C treatment (88.1%) than for the CFP treatments (average 87.2%). The 0C treatment also resulted in greater ($P < 0.05$) total dietary fiber digestibility, compared with CFP treatments.

3.5 Palatability

There was no preference between the 5C and 0C treatments offered to dogs, indicated by the non-significant results in first choice and intake ratio (Table 7). However, dogs chose the 0C treatment first over the 10C treatment 27 out of 40 times, indicating a first-choice preference ($P < 0.05$). Conversely, based on the intake ratio, dogs did not consume significantly more of the 0C treatment compared with 10C treatment. When comparing the 15C and 0C treatments, dogs consumed more ($P < 0.05$) of the 0C treatment, with no preference based on first choice.

4 Discussion

4.1 Extrusion processing and kibble characteristics

The differences in PC steam, PC water, and PC discharge temperature were minimal and interpreted to be of no practical importance. On average, CFP treatments contained greater levels of soluble fiber than the 0C treatment, which may have increased viscosity within the extruder barrel (Donadelli et al., 2021), resulting in an increase in motor load. However, the difference in soluble fiber was minimal. Therefore, the decrease in screw speed with the CFP treatments was likely the major contributor to the increase in motor load, as a decreased screw speed would result in increased barrel fill, increasing motor load (Unlu and Faller, 2002). Therefore, the

TABLE 3 Least squares means and contrasts [0C *vs.* 5C–15C (T), linear (L); quadratic (Q); cubic (C) level of corn fermented protein (CFP)] for processing parameters and physical characteristics of diets with increasing levels of CFP.

	Treatment[1]								
Parameter	0C	5C	10C	15C	SEM	0C *vs.* T	L	Q	C
Preconditioner									
Cylinder speed (rpm)	185.00	185.00	185.00	185.00	0.000	1.000	1.0000	1.0000	1.000
Steam flow (kg/h)	47.94	47.67	47.58	46.62	0.194	0.0460	0.0862	0.2596	0.9268
Water flow (kg/h)	57.57	57.36	57.40	57.54	0.046	0.0005	0.7206	< 0.0001	0.3807
Discharge temperature (°C)	89.09	90.37	90.84	90.02	0.179	< 0.0001	< 0.0001	< 0.0001	0.3854
Extruder									
Screw speed (rpm)	425.00	415.74	375.00	394.44	9.220	0.0001	< 0.0001	0.0299	0.0022
Motor load (amps)	70.56	72.04	76.41	72.81	0.842	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Die temperature (°C)	108.70	110.68	111.23	108.42	0.354	< 0.0001	0.7834	< 0.0001	0.0839
TMF[2] (kg/h)	385.33	385.08	385.09	385.24	0.061	0.0001	0.1728	< 0.0001	0.4731
SME[3] (kJ/kg)	135.35	140.49	149.37	133.62	2.345	0.0031	0.6211	< 0.0001	0.0002
MC[4] (%)	32.41	32.34	32.33	32.36	0.032	0.0067	0.0825	0.0225	0.7755
Bulk density (g/L)	344.00	351.60	333.40	321.00	6.110	0.0967	0.0005	0.0353	0.1147
Kibble diameter (mm)	4.99	4.94	4.87	5.07	0.085	0.7299	0.5053	0.0383	0.2513
Kibble length (mm)	4.18	4.14	4.31	4.57	0.093	0.0555	< 0.0001	0.0286	0.6209
Dryer									
Bulk density (g/L)	336.65	337.10	316.03	308.03	6.670	0.0308	0.0019	0.4008	0.1301
Kibble diameter (mm)	4.85	4.83	4.77	4.99	0.093	0.8559	0.2305	0.0774	0.2823
Kibble length (mm)	3.66	3.74	3.99	4.12	0.113	0.0023	< 0.0001	0.7694	0.4357
Specific length (mm/g)	133.01	136.92	138.99	138.43	4.034	0.1253	0.1546	0.4356	0.9499
SEI[5] (mm^2/mm^2)	2.63	2.61	2.54	2.77	0.100	0.8691	0.2398	0.0742	0.2601
Hardness (kg)	2.45	2.22	2.49	2.24	0.158	0.3213	0.4708	0.8745	0.0435
Toughness (kg mm)	5.46	5.06	6.12	6.43	0.368	0.1749	0.0009	0.1795	0.0605

[1] 0C, 0% CFP; 5C, 5% CFP; 10C, 10% CFP; 15C, 15% CFP.

[2] TMF = total mass flow.

[3] SME = specific mechanical energy.

[4] MC = in-barrel moisture content.

[5] SEI = sectional expansion index.

variation in input variables (EX screw speed) likely caused the increase in motor load and not the CFP inclusion itself.

Surprisingly, the fluctuation in EX screw speed did not appear to affect the final product. It would be expected that a decrease in screw speed would result in less mechanical energy, decreasing material cook and expansion (Rokey, 2006). Therefore, the fastest screw speed should have produced the most expanded kibble; however, this was not the case, as the 0C treatment had the fastest screw speed, but the 15C treatment was the most expanded indicated by the lowest bulk density. This could be explained by the fact that a decreased screw speed would increase material retention time in the extruder barrel, allowing for an increased cook time (Yeh et al., 1992). This is supported by the increased TMF with the 0C treatment. Therefore, the fluctuations in screw speed could have counteracted, resulting in a similar process among dietary treatments. In other words, the degree of cook in the 10C and 15C treatments could have been comparable to the 0C and 5C treatments with the faster screw speeds. This is supported by the increased SME for CFP treatments, specifically 10C, compared with 0C. Regardless, the differences in bulk density were not of practical concern, as the average dry bulk density of the dietary treatments (324 g/L) resembled that of typical commercial kibble, which have densities between 280 and 400 g/L (Rokey, 2006). The increase in die temperature would also be expected to increase product expansion (Shukla et al., 2005). However, this was not observed in this study, as the 5C and 10C

TABLE 4 Analyzed chemical composition of canine diets with increasing levels of corn fermented protein (CFP) on a dry matter basis.

	Treatment[1]			
Nutrient	0C	5C	10C	15C
Dry matter (%)	93.50	95.47	95.20	94.61
Moisture (%)	6.50	4.53	4.80	5.39
Organic matter (%)	90.50	91.09	91.24	91.87
Ash (%)	9.50	8.91	8.76	8.13
Crude protein (%)	38.44	36.52	36.63	36.87
Crude fat (%)	12.88	12.15	11.39	12.79
Insoluble dietary fiber (%)	11.01	10.75	11.55	12.95
Soluble dietary fiber (%)	2.65	3.35	2.45	3.19
Total dietary fiber (%)	13.76	14.20	14.00	16.13
Gross energy (kcal/kg)	4992.05	4959.87	4933.84	4995.75

[1]0C, 0% CFP; 5C, 5% CFP; 10C, 10% CFP; 15C, 15% CFP.

TABLE 5 Least squares means and contrasts [0C *vs.* 5C–15C (T), linear (L); quadratic (Q); cubic (C) level of corn fermented protein (CFP)] for food intake and stool quality parameters of dogs fed diets with increasing levels of CFP.

	Treatment[1]								
Parameter	0C	5C	10C	15C	SEM	0C *vs.* T	L	Q	C
Food intake (g/d)	190.05	189.14	195.48	193.31	1.232	0.0151	0.0003	0.4757	0.0003
Wet fecal output (g/d)	113.16	109.20	111.69	116.88	3.326	0.8349	0.2041	0.0611	0.7232
Fecal dry matter (%)	31.59	32.43	33.59	34.00	0.296	< 0.0001	< 0.0001	0.3037	0.2640
Dry fecal output (g/d)	35.65	35.35	37.41	39.61	1.184	0.0706	0.0008	0.1477	0.5585
Defecations per day	2.25	2.18	2.30	2.42	0.080	0.4483	0.0200	0.1138	0.4726
Fecal score	3.67	3.82	3.87	3.87	0.034	< 0.0001	< 0.0001	0.0043	0.7839
Fecal pH	5.85	5.80	5.64	5.77	0.092	0.1271	0.1722	0.1579	0.1845

[1]0C, 0% CFP; 5C, 5% CFP; 10C, 10% CFP; 15C, 15% CFP.

TABLE 6 Least squares means and contrasts [0C *vs.* 5C-15C (T), linear (L); quadratic (Q); cubic (C) level of corn fermented protein (CFP)] for apparent total tract digestibility, estimated by titanium dioxide as a dietary marker, of diets with increasing levels of CFP.

	Treatment[1]								
Nutrient (%)	0C	5C	10C	15C	SEM	0C *vs.* T	L	Q	C
Dry matter	82.87	83.16	81.68	82.83	0.420	0.3623	0.2344	0.1557	0.0024
Organic matter	87.59	87.46	86.01	86.56	0.332	0.0021	0.0002	0.1594	0.0037
Crude protein	88.32	88.81	87.92	88.89	0.285	0.3534	0.3674	0.2436	0.0012
Crude fat	97.78	97.50	97.32	97.64	0.150	0.0198	0.1979	0.0073	0.3972
Gross energy	88.06	87.95	86.49	87.17	0.300	0.0015	0.0001	0.0699	0.0009
Total dietary fiber	59.91	58.12	45.29	48.04	1.409	< 0.0001	< 0.0001	0.4465	< 0.0001

[1]0C, 0% CFP; 5C, 5% CFP; 10C, 10% CFP; 15C, 15% CFP.

treatments resulted in the highest die temperature, but not the lowest bulk density. The differences in MC among dietary treatments were minimal (< 0.1%) and unlikely to affect processing or final kibble characteristics.

It would have been expected that the CFP treatments, specifically 15C, would result in denser kibble due to the increase in dietary fiber. Previous studies have reported decreased kibble expansion with dietary fiber (Monti et al., 2016; Alvarenga et al.,

TABLE 7 First choice (FC) and intake ratio (IR) of dogs fed diets with increasing levels of corn fermented protein (CFP).

Diet comparison (A *vs.* B)[1]	FC[2]	IR[3]
5C *vs.* 0C	22	0.471
10C *vs.* 0C	13*	0.399
15C *vs.* 0C	20	0.325*

[1] 0C, 0% CFP; 5C, 5% CFP; 10C, 10% CFP; 15C, 15% CFP.
[2] Number of first visits to bowl A out of 40 observations.
[3] IR = intake (g) of diet A/total intake (g) of diets A + B.
*Comparison differs ($P < 0.05$).

2018). According to the Guy Classification System, fibers are dispersed phase fillers and known to have very poor functionality in extrusion, meaning that they lead to less-expanded final products (Guy, 2001). Therefore, it was surprising that the 15C treatment resulted in the most expanded kibble. However, protein is also considered a dispersed phase filler (Guy, 2001), which was greatest in the 0C treatment. Therefore, the higher protein content could help to explain the decreased expansion observed in the 0C treatment. In contrast to the current study, Shukla et al. (2005) reported an increase in bulk density with increased inclusion of traditional distillers' dried grains with solubles (DDGS). This could indicate that CFP has less of an effect on expansion, regarding bulk density, compared with DDGS.

On average, the CFP treatments resulted in greater levels of insoluble fiber compared with the 0C treatment, which may affect longitudinal expansion and radial expansion of kibble. Donadelli et al. (2021) reported a greater longitudinal expansion, compared with radial expansion, in kibble containing ingredients with a higher concentration of insoluble fiber. Monti et al. (2016) also reported an increase in kibble length with the addition of an insoluble fiber compared with a soluble fiber. In addition, Alvarenga et al. (2018) observed an increase in kibble length with a decrease in kibble diameter as insoluble fiber increased. In the current study, CFP inclusion did increase kibble length, but kibble diameter was not affected. However, even with the differences in kibble length, specific length and SEI of kibble were maintained among dietary treatments, indicating that overall expansion was not impacted by CFP inclusion. Previous studies have reported a decrease in radial expansion with inclusion of distillers' dried grains (Satterlee et al., 1976; Breen et al., 1977; Walker, 1980; Anderson et al., 1981; Shukla et al., 2005). These results could indicate that traditional distillers' dried grains have a greater effect on radial expansion than CFP.

Previous research has reported that kibble expansion has an impact on hardness and compression energy (Moraru and Kokini, 2003; Yanniotis et al., 2007). Therefore, it would be expected that the densest treatment (5C) would have resulted in the greatest hardness and toughness. However, this was not the case, as the 10C treatment resulted in the greatest hardness, while the 15C treatment resulted in the greatest toughness. The increased toughness in the 15C treatment could be explained by the increase in dietary fiber. This corresponds to a previous study that reported a higher cutting force in kibble containing sugarcane fiber compared with kibble containing wheat bran (Monti et al., 2016). In addition, Kantrong et al. (2018) reported a correlation of increased hardness in rice-based snacks, with a decrease in screw speed, which supports the results in the current study.

Due to the varying results, there does not appear to be a direct correlation between increased levels of CFP on processing conditions or kibble characteristics. Instead, there seems to be a greater effect from the variation in input processing conditions, specifically EX screw speed.

4.2 Diet chemical analyses

The lower than targeted moisture content was likely due to the small kibble size, which would have required less dry time than the standard 22 min. The small kibble size was intentional, as diets were produced for both dogs and cats. Of note, the ideal moisture content of kibble is ≤ 10% to prevent mold growth (Gautam et al., 2018). Therefore, the low moisture content in experimental treatments was not of concern. The decrease in crude protein content in the CFP treatments compared with the control was unexpected, as the test ingredients SBM and CFP are comparable in protein content on a dry matter basis, at 53.4% and 52.6%, respectively. Therefore, the slight increase in protein for the 0C treatment could be due to the normal variation among laboratory analysis. The increase in total dietary fiber in the 15C treatment was expected because CFP contained 34.9% total dietary fiber, whereas SBM contained 19.9% total dietary fiber.

4.3 Feed intake and fecal characteristics

The calculation used to determine food amounts was the same among all dogs for each period. In addition, all dogs readily consumed the entire portion offered each day. Therefore, there should not have been any differences in food intake among dietary treatments. Of note, differences in food intake were minimal (< 7 g/day) and unlikely to affect stool quality or nutrient digestibility.

The increase in fecal dry matter of dogs consuming the CFP treatments explains the consistent wet fecal output and the increase in dry fecal output with CFP inclusion. Kilburn-Kappeler et al. (2022) reported a similar relationship in fecal dry matter and fecal output of cats fed increased levels of CFP. The increase in fecal dry matter also resulted in firmer stool, which was observed with the increase in stool quality score of dogs fed CFP treatments. In addition, an increase in dry fecal mass resulted in an increase in the number of defecations per day for dogs fed CFP. The differences in stool quality with increased CFP inclusion is likely due to the increased fiber content in CFP treatments, specifically 15C, compared with the 0C treatment, as previous studies have attributed an increase in fecal bulk to increased dietary fiber in dogs (Fahey et al., 1992; Sunvold et al., 1995). In terms of its fiber profile, CFP would be considered more of an insoluble fiber type (Kilburn-Kappeler et al., 2022). In agreement with the current study, Wichert et al. (2002) reported that the addition of cellulose (an insoluble fiber) increased dry matter content of feces and frequency of well-formed feces when fed to dogs. Fecal pH was not affected by CFP, indicating that the increase in dietary fiber did not alter microbial fermentation. This is supported by results from the work of Wichert et al. (2002), which also reported that fecal pH of dogs was not impacted by an insoluble fiber source.

4.4 Apparent total tract digestibility

The significant cubic relationships among dry matter, organic matter, crude protein, gross energy, and total dietary fiber digestibility, with the 10C treatment having the lowest digestibility, were surprising. Rather, it was expected that the 15C treatment would result in the lowest digestibility due to the increased dietary fiber content and greatest fecal output. Previous studies have reported the effects of dietary fiber on gastric emptying, digesta transit time, and nutrient digestibility in dogs (Burrows et al., 1982; Russell and Bass, 1985; Fahey et al., 1990). Russell and Bass (1985) concluded that an increase in dietary fiber content and viscosity resulted in slowed gastric emptying in dogs. However, Burrows et al. (1982) reported a decrease in intestinal transit time with added dietary fiber in dogs. Therefore, decreased transit time could explain a decrease in nutrient digestibility (Burrows et al., 1982). Fahey et al. (1990) reported that increased dietary fiber did not impact digesta mean retention time of dogs, but still decreased dry matter and organic matter digestibility. The differing results in the current study and previous studies indicate that fiber type, inclusion level, and diet matrix can impact the effect of fiber on nutrient digestibility. Of note, the decrease in organic matter, crude fat, and gross energy digestibility with CFP treatments compared with the 0C treatment was minimal (< 1%), and unlikely to be of practical concern.

The digestibility of diets containing increasing levels of CFP when fed to dogs differed relative to that observed in cats. Kilburn-Kappeler et al. (2022) reported that digestibility of diets containing 5% and 10% CFP was comparable to the control when fed to cats. However, a significant decrease in digestibility was observed when cats were fed diets containing 15% CFP. The study in cats indicated a clear level of inclusion in which digestibility was affected, which was not observed in the current study with dogs. This could be explained by the fact that cats have a shorter digestive tract than dogs, decreasing their ability to utilize fiber (Verbrugghe and Hesta, 2017).

4.5 Palatability

The palatability of CFP in dogs differed from that in cats. Wherein, Kilburn-Kappeler et al. (2022) observed that cats preferred a 5% inclusion of CFP compared with a control (0% CFP), but had no preference with increased inclusion levels (10% and 15% CFP). However, in the current study, dogs had no preference between the control and the low inclusion level (5% CFP), but appeared to prefer the control over the higher CFP inclusion levels of 10% and 15%.

In addition to ingredients, palatability may be affected by processing and final kibble characteristics (Koppel et al., 2015). Therefore, the preference for the 0C treatment over the 10C and 15C treatments could be due to product texture, not the increased CFP inclusion. Specifically, the increased kibble toughness observed with the 10C and 15C treatments may have limited palatability in dogs. Regardless, dogs willingly consumed all treatments and no refusals were observed.

4.6 Application of CFP in the pet food industry

Several studies have raised concerns about the nutritional adequacy of vegetarian diets for companion animals, specifically insufficient amino acids. A future study exchanging CFP for an animal-based ingredient would be interesting. However, it is important to remember that animals require specific nutrients, rather than specific ingredients. Therefore, both dogs and cats can subsist on vegetation diets if adequate levels of nutrients are met. Like all pet food, special care is required when formulating vegetarian diets. Specifically, vegetarian diets for cats will need additional supplementation, such as taurine, as cats are unable to meet their nutrient requirements when fed exclusively plant-based ingredients.

5 Conclusion

In conclusion, acceptable processing parameters, stool quality, nutrient digestibility, and palatability indicate that CFP can be utilized as a plant-based alternative protein source for dogs. Surprisingly, many parameters evaluated in this study resulted in a quadratic or cubic relationship as CFP increased, rather than an exclusive linear response as expected. The quadratic relationships may indicate an optimum inclusion level of CFP for specific parameters, whereas the cubic relationships could reveal other factors that may have affected the results, such as processing conditions and physical characteristics of kibble, not CFP inclusion.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was reviewed and approved by Kansas State University Institutional Animal Care and Use Committee (IACUC) Protocol #4097.

Author contributions

CA and LK-K formulated the diets and designed the study. LK-K collected samples and performed sample analysis. LK-K performed statistical analysis and primary writing. CA provided editing and interpretation. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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