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Evaluation of Torula yeast as a protein source in extruded feline diets

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Abstract

The objective of this work was to evaluate the use of a Torula yeast (TY) on diet processing, palatability, and total tract nutrient digestibility in extruded feline diets. Four dietary treatments were compared, differing by protein source: TY, pea protein concentrate (PP), soybean meal (SM), and chicken meal (CM). Diets were produced using a single-screw extruder under similar processing conditions. Palatability assessment was conducted as a split plate design where both first choice and intake ratio (IR) were determined. Apparent total tract digestibility (ATTD) of nutrients was estimated using Titanium dioxide as an indigestible marker. During diet production, specific mechanical energy of TY and SM (average of 187 kJ/kg) was greater ($P < 0.05$) than for PP (138 kJ/kg); however, CM was similar to all treatments (167 kJ/kg). Kibble diameter, piece volume, and sectional expansion ratio were greatest for TY ($P < 0.05$). Additionally, both bulk and piece density were lowest ($P < 0.05$) for TY. Kibble hardness was lower for TY and SM ($P < 0.05$; average of 2.10 Newtons) compared to CM and PP (average of 2.90 Newtons). During the palatability trial, TY was chosen first a greater number of times than CM ($P < 0.05$; 36 vs. 4, respectively), but differences were not found between TY and PP (25 vs. 15, respectively) or TY and SM (24 vs. 16, respectively). Cats had a greater IR ($P < 0.05$) of TY compared to CM and PP (0.88 and 0.73, respectively). However, there was no difference in preference between TY and SM. ATTD of dry matter (DM) and organic matter (OM) was greater ($P < 0.05$) for CM (87.43% and 91.34%, respectively) than other treatments. Both DM and OM ATTD of TY were similar ($P < 0.05$) to PP and SM (average of 86.20% and average of 89.76%, respectively). Ash ATTD was greater ($P < 0.05$) for cats fed TY and SM (average of 37.42%), intermediate for PP (32.79%), and lowest for CM (23.97%). Crude protein (CP) ATTD of TY was similar to all other treatments (average of 89.97%), but fat ATTD was lower ($P < 0.05$; 92.52%) than other treatments (93.76% to 94.82%). Gross energy ATTD was greater ($P < 0.05$) for CM than TY (90.97% vs. 90.18%, respectively); however, TY was similar to PP and SM (average of 90.22%). Total dietary fiber ATTD was similar between TY and CM (average of 66.20%) and greater ($P < 0.05$) than PP and SM (average of 58.70%). The TY used in this study facilitated diet formation, increased diet preference, and was highly digestible when fed to cats.

Lay Summary

In 2021, US$50 billion was spent on pet food and pet treats in the United States alone. Pet diets are largely sourced from animal proteins; however, the sustainability of these diets remains a major concern. Microbial proteins from microorganisms such as yeasts offer a sustainable protein alternative. Torula yeast (TY) is produced specifically for nutritional value and is grown on low-value woody waste materials. In this work, a commercially available TY product was evaluated in extruded feline diets and compared against soybean meal (SM), pea protein (PP), and chicken meal (CM). During diet manufacturing, the TY ingredient facilitated processing and forming of the final product. When comparing preference of dietary treatments, the diet containing the TY was preferred over that of the PP diet and the CM diet but was not different from the SM diet. Nutrient digestibility was similar or greater for TY compared with other protein ingredients, apart from a lower fat digestibility. Cats fed TY produced softer and less formed feces likely attributed to fiber composition. It was concluded here that TY could be safely included into feline diets, but inclusion level may be limited by fecal quality considerations.

Introduction

In 2021, over US$123 billion was spent on pets in the United States alone, with pet food and treat sales accounting for US$50 billion of this total expenditure (APPA, 2022). Consumers often demand new "high-quality" ingredients and generally prefer high protein formulated diets (Swanson et al., 2013). Traditionally these diets are sourced from animal proteins. However, the sustainability of these diets has been challenged as some of these formulations use ingredients that directly compete with human food systems or potentially contribute to a greater environmental footprint (Swanson et al., 2013; Acuff et al., 2021). The use of plant-based proteins including pulses, legumes, and tubers have been proposed as a cost effective, sustainable alternative to animal proteins (Reilly et al., 2020). However, plant-based ingredients come with their own liabilities, including limitations in essential amino acids, lower palatability, and negative perceptions by consumers to name a few. Alternatively, microbial proteins

produced from heterotopic microorganisms, such as yeasts, have been proposed. These microorganisms utilize the elementary components of waste materials, that would otherwise be inaccessible to higher organisms such as humans and pets and convert them into bioavailable high-quality proteins with minimal environmental impact (Matassa et al., 2016; Spiller et al, 2020).

Yeast and yeast derived products have been fed to animals for over a century (Stone, 2006). Brewer's yeast, whey yeast, and Torula yeast (TY) have been categorized as nutritional yeasts when fed as inactive microbial biomass principally for nutritional value (Shurson, 2018). Among these traditionally used in livestock nutrition, TY has been favored due to its flexible utilization of carbon sources and robust growth capabilities (Bekatorou et al., 2006; Buerth et al., 2016). TY has the ability to metabolize xylose and xylose oligomers (Yanai and Sato, 2001), allowing for growth on low-value cellulosic waste materials. Thus, representing an opportunity to produce large amounts of microbial protein from a sustainable and cost-effective growth medium. Furthermore, yeast production from cellulosic material has a lower carbon footprint compared to soybean meal (SM) and does not compete for resources with the human food system (Øverland and Skrede, 2016; Spiller et al, 2020).

In companion animal research, most work involving yeast products have primarily been focused on immune response and gastrointestinal microflora modulation in dogs (Swanson et al., 2002; Grieshop et al., 2004; Gouveia et al., 2006; Pawar et al 2017; Kroll et al., 2020; Van den Abbeele et al. 2020) and cats (Santos et al., 2018; Calabrò et al., 2020). Two previous reports found brewer's yeast and sugarcane yeast to be an adequate protein source in dog diets (Martins et al., 2013; Reilly et al., 2021). A recently developed proprietary TY product has been introduced into the animal food marketplace (Arbiom, 2021) and previously evaluated in weaning pig diets (Espinosa et al., 2020; Lagos and Stein, 2020). In these studies, the researchers concluded that TY could be included into weaning pig diets in exchange for fish meal and plasma protein. Additionally, a previous press release detailed the use of this TY in exchange for chicken meal (CM) in dog diets (Arbiom, 2019). Currently little has been published on the application of yeast products in feline diets and, to our knowledge, no data are available regarding the nutritional utilization of this type of yeast biomass as a protein source for felines. It is assumed here that this TY ingredient can provide an alternative high-quality protein source for commercial feline diets. Therefore, the objective of this work was to evaluate the use of a wood-effluent grown TY (SylPro; Arbiom Inc., Durham, NC) and its influence on diet processing and kibble formation, palatability, and nutrient utilization in extruded feline diets.

Materials and Methods

Ingredients and dietary treatments

Four dietary treatments were designed using concept five formulation software (Creative Formulation Concepts [CFC] Tech Services Inc., Pierz, MN) to meet AAFCO (AAFCO, 2019) minimum recommendations for "Growth and Reproduction" for cats (Table 1). Diets differed primarily by protein source and were formulated to be similar in terms of crude protein (CP), crude fat, and gross energy (GE). Test protein ingredients included a Torula dried yeast (SylPro; Arbiom Inc.,

Table 1. Ingredient composition of dietary treatments

Ingredient, %	Dietary treatment[1]			
	CM	PP	SM	TY
Rice, brewers	45.96	39.14	38.58	39.97
Chicken meal, low ash	43.09	28.26	28.42	26.60
Pea protein concentrate	—	20.00	—	—
Soybean meal, high protein	—	—	20.00	—
Torula yeast[2]	—	—	—	20.00
Beet pulp	3.00	3.00	3.00	3.00
Fish oil	1.00	1.00	1.00	1.00
Titanium dioxide	0.40	0.40	0.40	0.40
Salt	0.35	0.35	0.35	0.35
Choline chloride, 60% dry	0.20	0.20	0.20	0.20
Taurine	0.20	0.20	0.20	0.20
Vitamin premix[3]	0.15	0.15	0.15	0.15
Trace mineral premix[4]	0.10	0.10	0.10	0.10
Potassium chloride	0.30	0.10	0.10	0.10
DL-Methionine	0.10	0.10	0.10	0.14
Calcium carbonate	—	—	—	0.06
Chicken fat[5]	3.66	5.50	5.90	6.23
Dry flavor digest[5]	1.50	1.50	1.50	1.50

[1]Dietary treatments: Chicken meal (CM); Pea protein (PP); Soybean meal (SM); Torula yeast (TY).
[2]Torula yeast: SylPro.
[3]Vitamin E Supplement (79,887 IU*kg^{-1}), Niacin Supplement (64,736 mg*kg^{-1}), Calcium Pantothenate (12,186 mg*kg^{-1}), Vitamin A Supplement (17,162,998 IU*kg^{-1}), Thiamin Mononitrate (14,252 mg*kg^{-1}), Pyridoxine Hydrochloride (5,537 mg*kg^{-1}), Riboflavin Supplement (4,719 mg*kg^{-1}), Vitamin D3 Supplement (920,000 IU*kg^{-1}), Biotin (70 mg*kg^{-1}), Vitamin B12 Supplement (22 mg*kg^{-1}), Folic Acid (720 mg*kg^{-1}).
[4]Zinc Sulfate (88,000 mg*kg^{-1}), Ferrous Sulfate (38,910 mg*kg^{-1}), Copper Sulfate (11,234 mg*kg^{-1}), Manganous Oxide (5,842 mg*kg^{-1}), Sodium Selenite (310 mg*kg^{-1}), Calcium Iodate (1,584 mg*kg^{-1}).
[5]Surface applied to dry kibble.

Durham, NC), pea protein concentrate (PP; VITESSENCE Pulse 1550; Ingredion Inc., Westchester, IL), high-protein SM, and low-ash CM sourced from a local mill (Lortscher's Animal Nutrition Inc. Bern, KS). TY, PP, and SBM were included at 20% of their respective treatment formulas (TY, PP, and SM, respectively) to offset a portion of CM, whereas the last treatment contained only CM as its sole protein source. The remainder of the treatment formulas included brewers rice, beet pulp, fish oil, taurine, DL-methionine, vitamin, and mineral premixes. Each dietary treatment also included titanium dioxide (0.40%) as an indigestible marker to estimate digestibility. Dry ingredients were mixed, extruded, and kibbles were dried prior to the addition of topical chicken fat and dry flavor digest.

Digestibility assessment

Experimental diets were evaluated for apparent total tract nutrient digestibility (ATTD) at the Kansas State University. All experimental procedures were reviewed and approved by the Kansas State University Institutional Animal Care and Use Committee under protocol #4348 prior to beginning of study. A total of 12 healthy adult American shorthair cats (10 neutered males and 2 spayed females) with average age of 2.72 ± 1.52 yr (mean ± SD) and weight of 5.60 ± 1.27 kg (mean ± SD) was used in this experiment. Cats were fed over four 14-d periods which included 9 d of diet adaption followed by 5 d of

fecal collection. Stainless steel litter pans (12 in × 8 in × 4 in) fitted with rubber turf and elevated drainage mats were used for the collection of fecal and urine samples. All cats exclusively utilized liter pans for elimination during collection. Cats were randomized to treatment and period in a 4 × 4 replicated Latin square design according to Kim and Stein (2009). This design allows each cat to serve as its own control.

Initial food amounts offered were estimated based on the chemical composition of the diets and individual energy requirements of each cat to maintain body weight according to the National Research Council (NRC, 2006; Metabolizable energy, kcal*d^{-1} = 62*Body weight in kg). Daily food allowance was adjusted weekly, if necessary, to maintain body weight. Cats were fed twice daily (0900 and 1600 h) and excess food was collected. Water was provided ad libitum. Cats were kept in a temperature-controlled room (22 °C ± 1 °C) with a 12-h light cycle (lights automatically shut off from 1945 to 0745 daily). Each day was considered to start at 0900 h, coinciding with the first feeding. Cats were group housed with four cats to a room during adaptation (three rooms of four cats each) but were individually fed in stainless steel metabolic cages for 1 h. After the 1-h feeding, refused food was collected and weighed for intake calculation. To acclimate cats to metabolic cages, two phases were used during each adaptation. During phase 1 of adaptation (days 1 to 4), the cats were only kept in the cages during the two 1-h feeding periods (0900 to 1000 h and 1600 to 1700 h). During phase 2 of adaptation (days 5 to 9), cats were kept in individual cages from the beginning of the first feeding to the end of the last feeding (0900 to 1700 h). During fecal collection (days 10 to 14), cats were housed individually for the entire 120 h. During fecal collection, excess food was collected 1 h prior to the start of the next feeding day (0800 h).

Feces were collected prior to each meal and whenever observed throughout the day. Fecal samples were used to calculate ATTD of nutrients but also to characterize fecal scores, defecation frequency, dry and wet fecal output, and fecal pH. Upon collection, feces were scored subjectively according to a 5-point scale (1 runny to 5 hard, in 0.5 point increments; Carciofi et al., 2008) then stored in sterile polyethylene bags (Whirl-Pak; Nasco sampling, Madison, WI) and frozen at –20 °C for later analysis. Due to their qualitative measurements, fecal scores were evaluated based on frequency of occurrence rather than on average of aggregate scores. One fresh fecal sample (within 15 min of defecation) was also collected from each cat during each period and stored at –80 °C.

Digestibility calculations

At the culmination of the feeding assay, fecal samples were placed in an aluminum pan, weighed, and dried in an oven (Cat 52755-20, Matheson Scientific, Morris Plains, NJ) at 55 °C for 72 h. Dried feces were later ground through a 1-mm screen in a fixed blade laboratory mill (Retsch, type ZM200, Haan, Germany). Both food and feces were analyzed for titanium concentration using an adaptation of the procedure described by Leone (1973). Briefly, 0.3 g of fecal sample or 0.6 g of food sample were incinerated overnight in muffle furnace at 450 °C and allowed to cool to room temperature. Next, 1.0 g of sodium sulfate and 5 mL of sulfuric acid were added to the incinerated samples and were digested on a hot plate at 280 °C for 25 min. After cooling to room temperature, samples were transferred to 50-mL centrifuge tubes and brought to 50 g with distilled water. The tubes were centri-

fuged at 1,000 × g for 10 min and allowed to rest for 24 h. The following day, 0.25 mL of each sample was pipetted, in duplicate, into a 96-well plate. Then 30 µL of 30% hydrogen peroxide solution was added to each well and the plate was allowed to rest for at least 15 min. Absorbance values were measured at 410 nm using a microplate reader (Synergy H1, Biotek, Winooski, VT). ATTD was calculated using Titanium Dioxide (TiO$_2$) as an indigestible marker, using the following equations:

$$\text{ATTD} \quad (\%) = \left[1 - \left[\left(\frac{\% \text{ TD}}{\% \text{ TF}} \right) * \left(\frac{\% \text{ NF}}{\% \text{ ND}} \right) \right] \right] *100$$

herein %ND is the percent nutrient in the diet, %NF is the percent nutrient in the feces, %TD is the percent Titanium in the diet, and %TF is the percent Titanium in feces.

Nutrient analysis

Test ingredients, experimental diets, and dried fecal samples were analyzed using Association of Official Analytical Collaboration (AOAC) approved methods for dry matter (DM; AOAC 930.15), organic matter (OM; AOAC 942.05), ash (inorganic matter calculated by difference), CP (AOAC 990.03), crude fat by acid hydrolysis (AHF; AOAC modified 954.02), and total dietary fiber (TDF; AOAC 991.43; TDF kit, K-TDFR-200A, Megazyme Ltd., Bray, Ireland), according to AOAC international approved analytical methodologies. All nutrients were reported on a DM-basis. GE was determined by bomb calorimetry (model 6200, Parr Instrument Company, Moline, IL). Additionally, diets were also analyzed for crude fiber (AOAC Ba 6a-05), insoluble dietary fiber (AOAC 991.43; TDF kit, K-TDFR-200A, Megazyme Ltd., Bray, Ireland), and soluble dietary fiber by difference between TDF and insoluble dietary fiber.

Palatability testing

Experimental diets were evaluated for palatability at a commercial kennel (Summit Ridge Farms, Susquehanna, PA). The cattery facility is registered with the USDA No. 23-R-0126 under the Animal Welfare Act. Palatability tests were conducted as a split plate design (Griffin, 2003). A total of 20 healthy adult cats (6 neutered males and 14 spayed females) with average age of 9.57 ± 3.25 yr (mean ± SD) were used in this experiment. Three split plate tests were conducted: TY versus CM (Protocol: KSUPALF00120), TY versus SM (Protocol: KSUPALF00220), and TY versus PP (Protocol: KSUPALF00320). Since the purpose of this palatability testing regimen was to compare the novel TY protein to currently utilized protein ingredients, the other treatments (CM, SM, and PP) were not directly compared to one another. During a two-bowl test, two stainless steel bowls each containing ~100 g of a single test diet were presented once daily for up to 4 h. Each test was evaluated over a 2-d period with bowl placement being switched (left–right) between days; thus, the three tests were completed over a total of 6 d. If one experimental diet was completely consumed prior to the end of the 4-h feeding window both bowls were removed. Both first choice (first diet consumed) and intake ratio (IR) were reported for the present study. IR was determined using to the formula below:

$$\text{IR} = \frac{\text{intake of diet A}}{\text{intake of diet A} + \text{diet B}}$$

Diet production

Diets were produced using a pilot-scale single screw extruder (Wenger single screw X-20, Wenger Manufacturing, Sabetha, KS), with a screw diameter of 82.55 mm and a length to diameter ratio of 10. The extruder screw profile is presented in Figure 1. The die was a 4-mm single opening diameter (resulting in die open area of 12.6 mm) and was fitted with six short, hard blades. Raw material was fed into the preconditioner (PC) at a rate of 88.5 kg/h. Material in PC was hydrated to form a dough by water injection that fluctuated between 9.2 and 9.3 kg/h. Thermal energy was applied to dough in the PC via steam injection that fluctuated between 8.0 and 8.2 kg/h, resulting in discharge temperatures between 88 and 92 °C. Extruder screw speed was set at 398 rpm for all treatments. Water was injected into the extruder at a rate of 7.0 to 7.7 kg/h; slight adjustments were made during processing to achieve a target bulk density of 300 to 350 g/L. No additional thermal energy was applied to the extruder.

All diets were produced on a single day in sequential order. Once processing stability was achieved, treatment order began with CM, followed by SM, then PP, and last for TY. Treatments were switched once target product amounts were reached, and collection of the following treatment began after allowing extruder to clear out for 30 min. After extrusion, kibbles were dried in a double pass forced-air oven (Series 4800, Wenger Manufacturing, Sabetha, KS) at 121 °C for ~5 min each pass, to achieve a target moisture content of ~7%, followed by a subsequent ~5 min cooling prior to bagging. Chicken fat and dry flavor digest were surface applied to the kibble in a rotating barrel mixer at a later date. Processing parameters and samples were collected in triplicate at three equally spaced time intervals (~45 min apart) during the production of each treatment. Recorded processing parameters included feed rate (kg/h), PC water flow (kg/h), PC steam flow (kg/h), PC discharge temperature (°C), extruder screw speed (rpm), extruder water flow (kg/h), die pressure (psi), and die temperature (°C). Additionally, extruder mass flow rate was measured at the end of each experimental treatment by collecting material out of the extruder into a bucket for 1 min, then weighed for mass per unit time (kg/min). At each observation time, samples were collected from the PC, extruder and dryer and stored at –20 °C for further analysis. Specific mechanical energy (SME) was calculated using the following equation:

$$\text{SME}\left(\frac{\text{kJ}}{\text{kg}}\right) = \frac{\left(\frac{\tau - \tau_0}{100}\right) * \left(\frac{N}{N_r}\right) * P_r}{m}$$

where τ is the % torque, or motor load, τ_0 is the no-load torque (34%), N is the screw speed in rpm, N_r is the rated screw speed (508 rpm), P_r is the rated motor power (37.3 kW), and m is the total mass flow in kg/s. In-barrel moisture (IBM) was calculate as described below:

$$\text{IBM (\%)} = \left(\frac{\text{mf} * \text{Xf} + \text{mps} + \text{mpw} + \text{mes} + \text{mew}}{\text{mf} + \text{mps} + \text{mpw} + \text{mes} + \text{mew}}\right) * 100$$

where mf is the dry feed rate, Xf is moisture content of the feed material, mps is the steam injection rate in the PC (kg/h), mpw is water injection rate in the PC (kg/h), mes is the steam injection rate in the extruder (kg/h), and mew is the rate of water injected in the extruder (kg/h).

Kibble characteristics

Kibble samples were collected out of the dryer during each replicate to evaluate the macrostructure characteristics of the final product. From each time point, length, diameter, and weight were measured from 15 kibbles for calculation of piece volume (V), piece density (ρ), sectional expansion index (SEI), and specific length (l_{sp}) as follows:

$$V = \frac{\pi * l_e * d_e^2}{4}$$

$$\rho = \frac{m_e}{V}$$

$$\text{SEI} = \frac{d_e^2}{d_d^2}$$

$$l_{sp} = \frac{l_e}{m_e}$$

where V is the volume in cm³, l_e is the kibble length in mm, d_e is the average of two measurements of the kibble diameter in mm, ρ is the piece density in g*cm⁻³, m_e is the kibble mass in g, SEI is the sectional expansion index, and d_d is the die hole diameter in mm. Additionally, bulk density and true density (gas displacement) were measured for each treatment at each collection time point. Bulk density was measured during production both off the extruder and out of the dryer in duplicate, collected using a 1-L steel cup. True density was later measured in triplicate per time point using a Helium gas pycnometer (Ultrapyc 1200e, Quantachrome Instruments, Boynton Beach, FL).

Texture analysis

Texture analysis was performed using a texture analyzer (model TA-XT2, Texture Technology Corp., Scarsdale, NJ)

Figure 1. Schematic of extruder screw profile. Inlet starting on the left to discharge ending on the right. Screw element 1: inlet screw, single flight full pitch; 2: single flight, full pitch screw; 3: small steam lock; 4: single flight full pitch screw; 5: small steam lock; 6: Single flight, full pitch screw; 7: medium steam lock; 8: double flight, ½ pitch screw; 9: Large steam lock; 10: double flight, ½ pitch, cut cone screw.

equipped with a 30-kg load cell. A cylindrical probe (25-mm diameter) was used to compress 30 kibbles in triplicate from each collection time point for each diet (30 kibbles × triplicate × 3 time points). Prior to texture analysis, kibbles were dried in a convection oven at 55 °C for 48 h to equilibrate samples; after drying, samples were removed and placed in a desiccator (airtight with SiO_2 desiccant) at room temperature for an additional 48 h to stabilize dry samples. The pretest speed was 2 mm*s^{-1}, test speed was 1 mm*s^{-1}, and a posttest speed was 10 mm*s^{-1} (adapted from Dogan and Kokini, 2007). Strain level was set at 50%. Kibble hardness (Newtons) was considered to be the peak force of the first major kibble breakage. The average values of 30 kibbles for hardness was used as the experimental unit for statistical analysis to help account for variation among individual kibbles.

Statistical analysis

The digestibility experiment was performed as a replicated 4 × 4 Latin square design, where cat and period served as blocking factors. Diet was considered the fixed effect, whereas square, period, and cat nested within square were considered as random effects. ATTD, daily food intake, defecation rate, wet and dry fecal output, percent fecal DM, fecal pH, and urine pH were dependent variables. For kibble measurements and texture analysis, diet was considered the fixed effect and replication nested within diet was considered as the random effect. Kibble length, diameter, weight, volume, piece density, true density, SEI, bulk densities out of the extruder and dryer, and hardness were all dependent variables. Data were analyzed using statistical software via the general linear mixed models procedure (GLIMMIX in SAS; v. 9.4). Least square means were considered significant at $P < 0.05$ and multiple comparisons were adjusted using tukey posthoc method. Fecal scores of each treatment were also separately analyzed using the GLIMMIX procedure with cat and period as random effects. Fecal score frequencies were then determined using the frequency procedure (PROC FREQ in SAS; v. 9.4). For palatability testing, first choice and IR were evaluated using a chi-square test and 2-way ANOVA, respectively. Differences were also considered significant at a $P < 0.05$ for both tests.

Results

Ingredients and dietary treatments

The nutrient composition of experiment protein ingredients is presented in Table 2 to provide context to dietary differences. The nutrient composition of the TY ingredient was relatively similar to that of PP and SM in terms of CP (average of 50.92%), AHF (average of 4.45%), TDF (average of 17.14%), and GE content (average of 4798 kcal/kg). CM had much greater concentrations of CP (70.66%), AHF (15.02%), and GE (5853 kcal/kg) and lower amounts of TDF (6.49%). When comparing dietary treatments (Table 3), CP was similar across treatments (38.35%) but AHF content was slightly greater for TY (13.50% vs. average of 12.61%) and GE was slightly lower for CM (5236 kcal/kg vs. average of 5399 kcal/kg) compared to other treatments. TDF was greatest for TY (11.32%) compared to other treatments. Additionally, TY had greater relative proportions of soluble fiber content (42% of TDF). Both PP and SM had intermediate TDF values (average of 9.61%) which both primarily consisted of insoluble fiber (>99% and 94% of TDF, respectively). The TDF of CM was lower than the other treatments (7.01%), but had a larger

Table 2. Nutrient composition of protein sources

Composition	Experimental ingredient			
	Chicken meal	Pea protein	Soybean meal	Torula yeast
Dry matter, %	92.88	92.39	88.02	96.33
	Dry matter basis			
Ash, %	8.09	5.67	8.06	8.41
Crude protein, %	70.66	50.45	47.74	54.58
Acid-hydrolyzed fat, %	15.02	4.71	2.44	6.21
Total dietary fiber, %	6.49	15.30	17.71	18.42
Gross energy, kcal/kg	5852.92	4826.11	4720.30	4846.70

relative proportion (27% of TDF) of soluble fiber compared PP and SM. Crude fiber content was low in all treatments but was slightly greater for SM (1.71%) compared to other treatments (average of 1.07%).

Digestibility assessment and palatability

During the digestibility experiment, cats fed TY had a daily food intake similar ($P > 0.05$; average of 76.53 g*day^{-1}) to all other treatments (Table 4). Defecation frequency of cats fed TY was similar to those fed CM and PP ($P > 0.05$; average of 0.61 defecations*day^{-1}) but was lower ($P < 0.05$) than SM (0.53 vs. 0.73 defecations*day^{-1}). Cats fed TY had similar total fecal output to CM (average of 23.65 g*day^{-1}) but was lower ($P < 0.05$) than those fed PP (30.3 g*day^{-1}) and SM (33.4 g*day^{-1}). However, on a DM-basis, TY had similar fecal output to all other treatments (average of 9.47 g*day^{-1}). Fresh fecal pH of TY was also similar ($P > 0.05$; average of 5.49) to all other treatments. Cats feed TY had a greater frequency of lower fecal scores compared to the other treatments ($P < 0.05$; Figure 2). Additionally, the urine pH of cats fed TY was lower ($P < 0.05$; 6.55) than the other dietary treatments (6.87 to 7.41).

Cats fed TY had similar ($P > 0.05$; Table 5) DM, OM, and GE ATTD to PP and SM (averages of 86.20%, 89.76%, and 90.22%, respectively). However, CM had greater ($P < 0.05$) DM, OM, and GE ATTD (87.43%, 91.34%, 90.97%, respectively) compared to all other treatments. Ash digestibility of TY was similar to SM (average of 37.42%) but was greater ($P < 0.05$) than PP (32.79%) and much greater than CM (23.97%). For cats fed TY, CP digestibility was similar to all other treatments ($P > 0.05$; average of 89.97%); however, AHF digestibility was lower ($P < 0.05$; 92.52%) than all other treatments (93.76% to 94.82%). TDF digestibility of TY was similar to CM ($P > 0.05$ average of 66.20%) but greater ($P < 0.05$) than PP and SM (average of 58.70%).

Out of the 40 observations (20 cats × 2 d) during the palatability trial, TY was chosen first ($P < 0.05$; Table 6) over CM (36 vs. 4, respectively), but differences were not found between TY and PP (25 vs. 15, respectively) or TY and SM (24 vs. 16, respectively). When comparing preference, TY had a greater IR ($P < 0.05$) when compared to CM and PP (0.88 and 0.73, respectively). However, the IR of TY (0.59) was not different when compared to SM.

Diet production

During diet production, dry feed rate, water and stream injection into PC, and extruder screw speed were held constant

Table 3. Nutrient composition of dietary treatments

Composition	Dietary treatment[1]			
	CM	PP	SM	TY
Dry matter, %	96.43	93.38	93.88	93.92
		Dry matter basis		
Ash, %	5.79	5.32	6.01	6.00
Crude protein, %	38.70	38.13	38.20	38.35
Acid-hydrolyzed fat, %	12.26	12.60	12.97	13.50
Crude fiber, %	1.04	1.05	1.71	1.13
Total dietary fiber, %	7.01	9.39	9.63	11.32
Insoluble, %	5.12	8.83	9.59	6.55
Soluble, %	1.89	0.56	0.04	4.77
Gross energy, kcal/kg	5236	5409	5378	5410

[1]Dietary treatments: Chicken meal (CM); Pea protein (PP); Soybean meal (SM); Torula yeast (TY).

Table 4. Food intake and fecal characteristics of experimental diets

	Dietary treatment[1]				SEM	*P*-value
	CM	PP	SM	TY		
Food intake						
g*day^{-1} (DM)	78.1[a]	76.2[a,b]	75.4[b]	76.4[a,b]	4.81	0.0329
Fecal characteristics						
Defecations*day^{-1}	0.62[ab]	0.67[a,b]	0.73[a]	0.53[b]	0.072	0.0015
Fecal output (as-is), g*day^{-1}	22.6[b]	30.3[a]	33.4[a]	24.7[b]	2.52	<0.0001
Fecal dry matter, %	38.8[a]	33.1[b,c]	31.6[c]	36.4[a,b]	1.24	<0.0001
Fecal output (DM), g*day^{-1}	8.64[b]	9.83[a,b]	10.47[a]	8.94[a,b]	0.776	0.0269
Fecal pH	5.75[a]	5.30[b]	5.37[b]	5.54[a,b]	0.090	<0.0001
Urine pH	6.87[b]	7.05[b]	7.41[a]	6.55[c]	0.076	<0.0001

[1]Dietary treatments: Chicken meal (CM); Pea protein (PP); Soybean meal (SM); Torula yeast (TY).
[a,b,c]Means with unlike superscripts differ ($P < 0.05$).

across treatments with minor fluctuations (Table 7). Water injection was adjusted between treatments and ranged from 7.0 to 7.7 kg/h. Die pressure was greatest ($P < 0.05$) for TY (358 vs. average of 294 psi; Table 8) but die temperature was similar ($P > 0.05$) between TY and the other treatments. IBM differed among all treatments ($P < 0.05$) and was greatest for SM, followed by CM, PP, and TY (29.3%, 29.2%, 28.3%, and 27.8%, respectively). SME of TY was similar ($P > 0.05$) to SM and CM (average of 180 kJ/kg) but was greater ($P < 0.05$) than PP (138 kJ/kg).

Kibble characteristics

Bulk density out of the extruder and out of the dryer were lowest for TY ($P < 0.05$; Table 8). Kibble diameter and piece volume were greatest for TY ($P < 0.05$), whereas kibble length was greatest for SM ($P < 0.05$). Piece density was lowest for TY ($P < 0.05$), intermediate for SM, and greatest for CM and PP (0.382, 0.431, and average of 0.497 g*cm^{-3}, respectively).

Kibble hardness of TY was similar to that of SM (average of 2.10 Newtons), which were lower that CM and PP ($P < 0.05$; average of 2.90 Newtons).

Discussion

The TY used here had a DM content similar to that of several previous reports (93.70% to 95.63%; Ringrose, 1949; Olvera-Novoa et al., 2002; Øverland et al., 2013; Lagos and Stein, 2020). Figueroa et al. (1990) and Ringrose (1949) reported similar ash (8.4% and 8.0%, respectively) but lower CP values (44.9% and 48.5%, respectively), whereas Olvera-Novoa et al. (2002) reported greater ash (10.2%) and lower CP (46.11%). Øverland et al. (2013) reported lower ash (5.4%), similar CP (56.0%), and greater GE (5110 kcal*kg^{-1}) concentrations. On a DM basis, CP (54.16%), GE (4810 kcal*kg^{-1}), and TDF (21.5%) reported by Lagos and Stein (2020) were relatively similar to that observed here; however, those authors reported greater ash content (13.16%). Interestingly, fat content of the TY used in the current work was higher than all other reports (0.9% to 3.37%; Ringrose, 1949; Olvera-Novoa et al., 2002; Øverland et al., 2013; Lagos and Stein, 2020), and almost double the next leading amount (3.37% DM basis; Lagos and Stein, 2020). Differences in the nutrient composition of TYs are not surprising, as they have been grown under a wide range of conditions (Buerth et al., 2016) and cultivated on a variety of different substrates including beet pulp (Athar et al., 2009), distillery waste (García et al., 2014; Hosken et al., 2015), pineapple effluent (Nigam, 1998), and wheat bran (Yunus et al., 2015) among others; whereas, the particular yeast used in the present experiment was grown on forestry byproducts from the timber industry.

While fiber components were not equalized across treatments, they are still an important constituent of the diet. The TY used here had a TDF concentration over 18%, slightly less than that reported by Lagos and Stein (21.5% DM-basis; 2020). Those authors also identified about 80% of the total fraction as being soluble. Considering that 10% to 30% of the yeast cell consists of cell wall (Lipke and Ovalle, 1998; Nguyen et al., 1998), which is composed largely of β(1,3)/(1,6)-glucans, a large proportion of soluble fiber is not surprising. The diets containing legume proteins had more moderate fiber content but were almost completely insoluble when evaluated by the TDF assay. However, SM and PP also contain significant portions of raffinose family oligosaccharides (Tosh and Yada, 2010), which are soluble low molecular weight compounds that do not appear in the TDF analysis (Fahey et al., 2019). Crude fiber was also reported for diets because it is required as part of the guaranteed analysis for pet food by the American Association of Feed Control Officials (AAFCO, 2019); however, this measurement mainly includes cellulose, only small portions of hemicellulose and lignin, and no soluble components (Fahey et al., 2019). Despite labeling requirements, this measurement accounts for a small portion of the true fiber content and holds little nutritional relevance for monogastric animals.

The greater total fecal output of cats fed SM and PP was largely attributed to a greater moisture content in the feces, whereas dry fecal output was only different between SM and CM. Previous work (Clapper et al., 2001; Carciofi et al., 2009; Menniti et al., 2014) has shown an increase in total fecal output and fecal moisture when SBM is substituted for poultry meal or poultry byproduct meal. Irrespective of fecal
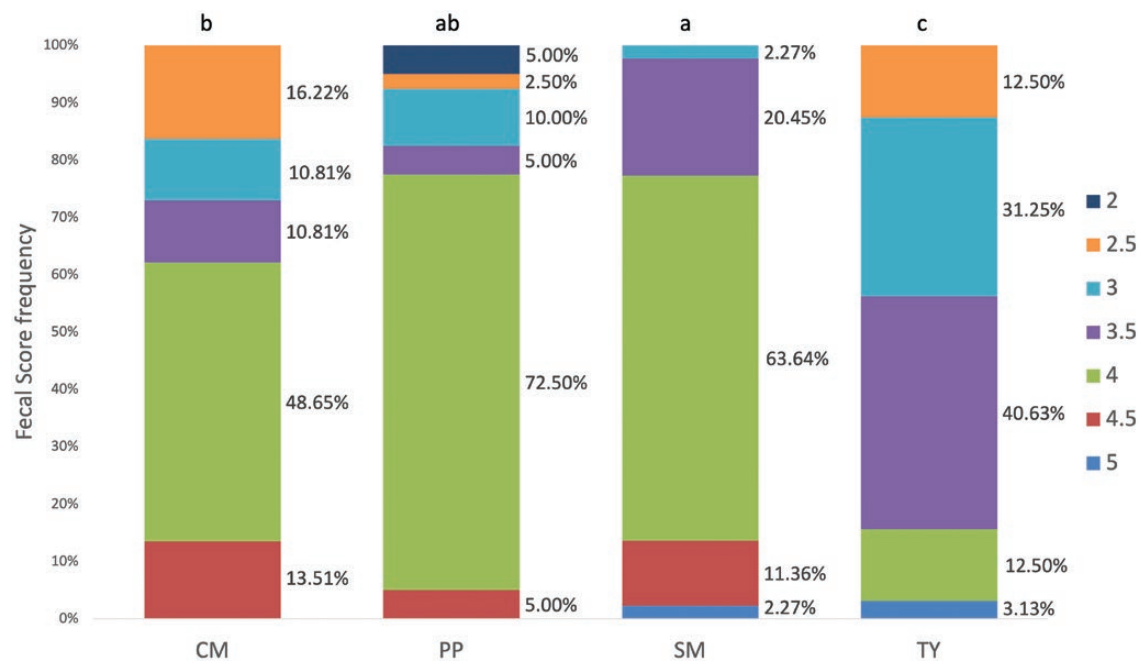
Figure 2. Fecal score frequency of cats fed experimental diets. Fecal scores based on a 1 to 5-point scale in 0.5-point increments (1—liquid stools, 5—hard dry feces). Dietary treatments: Chicken meal (CM); Pea protein (PP); Soybean meal (SM); Torula yeast (TY). [a,b,c]Bars with unlike superscripts differ (*P* < 0.05); letters appearing first alphabetically represent greater frequency of higher fecal scores.

Table 5. Apparent total tract digestibility of cats fed dietary treatments estimated by use of indigestible marker TiO_2

Digestibility, %	Dietary treatment[1]				SEM	*P*-value
	CM	PP	SM	TY		
Dry matter	87.43[a]	86.54[b]	85.66[c]	86.39[b,c]	0.422	<0.0001
Organic matter	91.34[a]	89.71[b]	89.82[b]	89.75[b]	0.414	<0.0001
Ash	23.97[c]	32.79[b]	38.25[a]	36.58[a]	0.631	<0.0001
Crude protein	90.26[a]	90.32[a]	89.35[b]	89.90[a,b]	0.396	0.0200
Acid-hydrolyzed fat	93.76[b]	94.82[a]	93.91[b]	92.52[c]	0.264	<0.0001
Gross energy	90.97[a]	90.30[a,b]	90.18[b]	90.18[b]	0.386	0.0154
Total dietary fiber	64.92[a]	57.52[b]	59.87[b]	67.48[a]	1.710	<0.0001

[1]Dietary treatments: Chicken meal (CM); Pea protein (PP); Soybean meal (SM); Torula yeast (TY).
[a,b,c]Means with unlike superscripts differ (*P* < 0.05).

Table 6. Palatability comparison of experimental diets assessed by cats

Diet comparison[1] (A vs. B)	First choice, *n*[2]	Intake ratio[3]
TY vs. CM	36[*]	0.88[*]
TY vs. PP	25	0.73[*]
TY vs. SM	24	0.59

[1]Dietary treatments: Chicken meal (CM); Pea protein (PP); Soybean meal (SM); Torula yeast (TY).
[2]First choice: number of first choices for Diet A (40 observations).
[3]Intake ratio: Diet A/(Diet A+B)
[*]Comparisons differ (*P* < 0.05).

moisture content, the TY ingredient tended to produce softer stools in cats. Thus, the inclusion of TY may need to be limited to 20% in the cat diet for fecal quality concerns or offset with a fecal bulking ingredient. Martins et al. (2013) found that the inclusion of sugarcane yeast at the expense of poultry byproduct meal led to an increase in fecal moisture content and less structure formation when fed to dogs. Zentek et al. (2002) also reported a decrease in fecal consistency in dogs supplemented with yeast cell wall components. Softer fecal formation is likely due to the partially soluble polysaccharide structure forming a loose gel-like structure when interacting with water (Selvendran et al., 1987). On the contrary, the higher portion of insoluble fiber in the legume proteins may have provided a bulking effect that could trap moisture while still maintaining a firmer structure. The differences in fiber structure are also likely responsible for the greater defecation frequency of SM compared to TY. It has been shown that insoluble fiber has a laxation effect in cats (Loureiro et al., 2017), whereas soluble fiber can slow gastric emptying and the movement of digesta through the GI tract (Schneeman, 1999).

Fecal output, fecal DM, and fecal pH indirectly reflect the colonic environment in response to the structural and nonstructural polysaccharide content in the diets. Carbohydrate fermentation in the colon leads to the production of lactate and short chain fatty acids, which decrease pH and increase luminal osmolarity (Binder, 2010). In canine diets containing soybean protein, total fecal output, and fecal moisture decrease with the removal of oligosaccharides (Wiernusz et al., 1995; Clapper et al., 2001), which would otherwise be rapidly fermented in then colon. The structural β-glucans of yeast cells are also known to be susceptible to fermentation and are likely responsible for the greater apparent TDF digestibility of TY. However, the more complex polysaccharide structure prolongs the fermentation process in the colon, producing organic acids at a rate in which the colonocytes

can readily absorb and blunting osmolarity and pH changes (Binder, 2010). The higher portion of nonfermentable structural fibers intrinsic to the legume proteins would result in depressed apparent TDF digestibility as these would pass the gastrointestinal tract largely intact. Additionally, since the soluble oligosaccharides present in the legume proteins are undetectable by the TDF analysis, their disappearance would be invisible to the methods used here.

The elevated fiber levels in diets containing TY and legume proteins are likely responsible for the reduced DM and OM digestibility. In a recent review comparing SM to poultry byproduct meal in extruded dog diets, SM tended to reduce the digestibility coefficients of DM, OM, AHF, and GE in the majority of papers examined (Vanelli et al., 2021). In the present study, TY seemed to lower apparent fat digestibility. Theodoro et al. (2019) reported that the inclusion of a soluble yeast cell wall reduced the coefficient of fat digestibility in an extruded dog diet without affecting any other nutrient digestibility. These authors attributed the reduction in apparent fat digestibility to the higher water solubility of yeast cell wall, possibly interfering with fat absorption. The increased ash digestibility of TY may reflect better mineral digestibility compared to PP and CM. It has been reported that phosphorus digestibility of bone tissue is lower than other sources (Sulabo and Stein, 2013). Lagos and Stein (2020) demonstrated that a diet containing TY had improved phosphorus digestibility compared to high ash fish meal when phosphorus intake was similar. However, even though phosphorus intake was not different, the fish meal diet had a much larger calcium concentration compared to the TY diet, which possibly could interfere with phosphorus digestion (Pastoor et al., 1994). On the contrary, Kim et al. (2014) showed that among diets with similar Ca:P ratios, ethanol and brewers' yeasts had improved phosphorus digestibility compared to SBM and fish meal.

In the present work, urinary pH was elevated in cats fed CM, PP, and SM. Particularly, SM exhibited alkalinity over normal pH values (5.5 to 7) expected of cat urine (Knight and Leitsberger, 2016). Additionally, CM, PP, and SM had pH values above that recommended for the prevention of struvite uroliths (6.0–6.5; Kopecny et al., 2021). Plant-based diets have been suspected to be a potential cause of urinary alkalinization, possibly attributed to lower proportions of acidic amino acids; however, further supporting research is needed (Knight and Leitsberger, 2016; Dodd et al., 2021). Additionally, this would not explain the elevated urine pH observed in CM, which was composed only of animal proteins. One

Table 7. Extrusion processing parameters during production of dietary treatments

Processing parameters	Dietary treatment[1]			
	CM	PP	SM	TY
Raw material				
Feed rate, kg/h	88.5	88.5	88.5	88.5
Preconditioner				
Water injection, kg/h	9.3	9.2	9.2	9.2
Steam injection, kg/h	8.2	8.0	8.1	8.1
Discharge temperature, °C	88.0	90.7	89.7	92.0
Extruder				
Screw speed, rpm	398	398	398	398
Water injection, kg/h	7.7	7.4	7.6	7.0

[1]Dietary treatments: Chicken meal (CM); Pea protein (PP); Soybean meal (SM); Torula yeast (TY).

Table 8. Production outputs and kibble characteristics of extruded dietary treatments

	Dietary treatment[1]				SEM	*P*-value
	CM	PP	SM	TY		
Production outputs						
Die pressure, psi	300[b]	300[b]	283[b]	358[a]	9.3	0.0022
Die temperature, °C	110[a]	105[b]	104[b]	107[a,b]	0.7	0.0015
Mass flow rate, kg/min	1.72	1.74	1.61	1.56	0.038	0.0798
IBM, %	29.2[b]	28.3[c]	29.3[a]	27.8[d]	0.03	<0.0001
SME, kJ/kg	167[a,b]	138[b]	182[a]	191[a]	7.1	0.0034
Kibble characteristics						
Bulk density OE[2], g*L^{-1}	450[a]	431[b]	410[c]	365[d]	4.0	<0.0001
Bulk density OD[3], g*L^{-1}	390[a]	379[a]	350[b]	324[c]	4.6	<0.0001
Length, mm	4.76[c]	4.87[b,c]	5.38[a]	5.02[b]	0.056	0.0002
l_{sp}, cm*g^{-1}	4.70[c]	4.82[b,c]	5.29[a]	4.95[b]	0.051	<0.0001
Diameter, mm	7.47[b]	7.26[c]	7.50[b]	8.24[a]	0.047	<0.0001
SEI	3.49[b,c]	3.29[c]	3.52[b]	4.24[a]	0.047	<0.0001
Weight, g	0.101	0.101	0.102	0.102	0.0017	0.9907
V, cm^3	0.209[c]	0.202[c]	0.238[b]	0.268[a]	0.0052	<0.0001
ρ, g*cm^{-3}	0.488[a]	0.505[a]	0.431[b]	0.382[c]	0.0058	<0.0001
True density, g*cm^{-3}	1.35[b]	1.36[a]	1.36[a]	1.36[a]	0.001	0.0003
Hardness, *N*	2.94[a]	2.86[a]	2.21[b]	1.98[b]	0.101	0.0003

[1]Dietary treatments: Chicken meal (CM); Pea protein (PP); Soybean meal (SM); Torula yeast (TY).
[2]Out of the extruder.
[3]Out of dryer.
[a,b,c]Means with unlike superscripts differ (*P* < 0.05).

explanation for the lower urinary pH of cats fed TY is the slightly higher methionine inclusion, which is considered an acidifying agent of urine (Knight and Leitsberger, 2016; Queau, 2019; Dodd et al., 2021).

During the palatability assessment TY was only chosen first over CM, which would primarily be associated with an animal's response to aroma characteristics (Aldrich and Koppel, 2015). This may have been due to the fact that CM had a smaller amount of surface applied fat. The remaining diets had similar amounts of surface applied fat, which would have greatly enhanced their initial appeal (Koppel et al., 2015). Although it is known that nutrient composition can also influence preference in cats (Rutherford, 2004), it may have been more appropriate to test diets without surface coatings of fat or flavor in order to determine the true undiluted effect of the experimental ingredients. Based on IR, cats had preference along some attributes of the TY ingredient; however, what those attributes are is unclear. It is possible that the TY had a more appealing flavor compared to that of CM and PP, but it is surprising that it was not favored over SM as well. Alternatively, TY kibbles were better expanded and lower in hardness compared to CM and PP, but were similar to SM. Thus, there may have been textural influences contributing to the palatability results.

To our knowledge, palatability of TYs has not previously been explored in either feline or canine diets. What little research that has been conducted using yeast or yeast products in the companion animal space has focused on *Saccharomyces cerevisiae* (brewer's yeast, baker's yeast, sugarcane yeast, etc.) and favored canine research. For example, Lin et al. (2019) stated that a 0.2% inclusion of a fermentation product from *S. cerevisiae* resulted in increased palatability relative to a control in a two-bowl dog study. However, those authors only reported a total consumption ratio (1.93:1) in leu of an IR, commonly used in the split plate method (Griffin, 2003). An animal is usually considered to have a clear preference when consuming at least double the amount of one diet over another, exceeding a consumption ratio of 2:1 or an IR of 0.67 (Griffin, 2003; Aldrich and Koppel, 2015). Using an IR helps to reduce statistical bias that may result from appetite or size effects of the animal, thus equally representing each test animal. Conversely, when reporting only mean consumption animal differences such as body size and appetite can arbitrarily skew the results. Martins et al. (2013) also evaluated the use of *S. cerevisiae* in canine diets, reporting greater average IRs (>0.67) for diets containing 7.5% sugarcane yeast relative to a control. In cat diets, de Oliveira et al. (2016) evaluated the palatability of extruded kibbles supplemented with yeast extract from *S. cerevisiae* and reported a greater IR for a combination of yeast extract and sodium pyrophosphate; however, these ingredients individually did not increase palatability.

Final product formation is a function of diet processing conditions and the ingredient composition of the formula. Despite similar water and steam injection into the PC, TY had a slightly elevated discharge temperature which may indicate better endothermic capacity of the TY ingredient. This would improve material softening within the PC and may be the reason for less water needed in the extruder barrel. During extrusion, TY generated a highly viscous melt, evident by greater die pressure and better expansion properties (Pasqualone et al., 2020). This may be related to the emulsion-like properties that have been reported for yeast cell proteins (Vélez-Erazo

et al., 2021) with the extruder applying the energy input necessary to produce a highly viscous system (Quek et al., 2015). The Torual yeast ingredient used here demonstrated good ingredient functionality which aided in diet processing and kibble formation. This novel protein exhibited superior expansion properties under similar processing parameters and did not require adjustments in water or energy input. It is generally recognized that plant proteins provide better ingredient functionality compared that of animal origin; however, it appears this particular yeast biomass may be a competitive ingredient in this regard. Thus, this ingredient could potentially be used to promote expansion in an extruded diet.

As mentioned, the TY ingredient used here was comprised of entire yeast cells containing complex cellular components such as β-glucans and glycoproteins which could potentially hinder digestibility if not processed adequately. It is well-understood that extrusion promotes starch gelatinization wherein, under proper hydration and energy input, native starch granules swell and lose their crystalline structure which dramatically improves the availability of starch to digestion (Riaz, 2000). Similarly, extrusion improves protein digestibility via denaturization of the native structure and has also been shown to increase ß-glucan solubility in oats and barley (Gaosong and Vansanthan, 2000; Sharma and Gujral, 2012). Thus, it is likely that the extrusion cooking process was largely responsible for the high digestibility coefficients observed for all dietary treatments.

While many interesting observations were made through this work, there were some limitations identified in the present study. Diet processing was a point of interest in this work, but the primary goal here was to evaluate apparent digestibility. The greater expansion properties of the novel Torula ingredient was an interesting observation and could be of great relevance to researchers and industry professionals alike. Future work should further characterize the properties of ingredient functionality and investigate graded inclusion levels of TY on final product characteristics. It is expected that ingredient preference was afforded by the TY ingredient, but results were confounded by surface coated fat and kibble texture. The greater TDF disappearance and fecal characteristics for cats fed TY were attributed to colonic fermentation; unfortunately, postbiotic analyses were not taken during this study. Additionally, it was noted that diets containing the legume proteins and CM resulted in alkaline urine pH. While urinary characteristics were not a major emphasis in this work, urinary health is a significant point of concern in felines and urine pH largely influencing the development of urolithiasis. Future work should investigate the role protein source may play on urinary parameter in felines and acid-base balance in particular. Lastly, it was assumed here that the greater ash disappearance may have been attributed to improved phosphorus digestibility. However, evidence to confirm this would require a mineral balance study, which was beyond the present scope.

Conclusions

In summary, the TY used in this study was highly digestible when fed to cats, increased diet preference, and aided diet processing and kibble formation. Cats fed TY produced feces that were soft and less formed which may be attributed to fiber composition. Apparent nutrient digestibility was similar or greater for TY compared with other protein ingredients,

with the exception of fat digestibility. Preference was observed for TY over PP and CM; however, TY was not found to be preferred over SM. Under similar processing conditions, the TY ingredient resulted in a more highly expanded product, particularly in the radial direction, which resulted in the lowest density and hardness. It was determined that TY can be safely included into feline diets; however, inclusion levels may need to be limited for fecal quality considerations. Further work should be conducted to evaluate postbiotic analysis, mechanisms of ingredient functionality, and implications of protein ingredients on urinary health in cats.

Acknowledgments

Conflict of Interest Statement

The authors declare no real or perceived conflicts of interest.

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