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Effects of yeast extract on diet digestibility and intestinal fermentation metabolites in dogs

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Abstract: Yeast extract is rich in nutrients, including amino acids, nucleic acids, and B vitamins, which can contribute to the intestinal health of dogs. This study aimed to evaluate the effects of yeast extract supplementation on diet digestibility, fecal characteristics, and intestinal fermentation metabolites in dogs. The analyses of diet digestibility, fecal concentrations of short-chain and branched-chain fatty acids and ammonia, fecal pH, score (1 = liquid and 5 = dry), dry matter (DM), and production were conducted using 12 adult Beagle dogs, randomly distributed into two diets containing 0% and 2% yeast extract (n = 6/treatment). The dogs were fed the experimental diets for 25 days of adaptation, followed by 5 days of total fecal collection. The 2% yeast extract diet reduced crude protein digestibility ($P < 0.05$) but did not influence other apparent digestibility coefficients ($P > 0.05$). However, the diet with the inclusion of 2% yeast extract increased fecal concentrations of acetate ($P < 0.05$) and improved fecal score ($P < 0.05$). In conclusion, dietary supplementation of 2% yeast extract improved fecal concentrations of acetate and fecal consistency, without affecting the digestibility of most diet fractions in dogs.
Keywords: Prebiotic, *Saccharomyces cerevisiae,* Short-chain fatty acids.

1. Introduction

Dogs are often exposed to stressful daily situations, such as sudden dietary changes, the onset of illnesses, and the administration of medications. These factors can negatively affect the gut microbiome and its metabolite composition. Consequently, potentially pathogenic bacteria proliferate, and protein fermentation metabolites are produced, which can impair diet digestibility, the immune system, and nutritional support for enterocytes (Masuoka et al., 2017; Chaitman et al., 2020). Functional additives, such as prebiotics, have been studied to mitigate these effects, demonstrating their ability to modulate the intestinal microbiota and its metabolites in dogs (Bastos et al., 2023).

Prebiotics can be defined as substrates that selectively stimulate the growth and/or activity of certain groups of bacteria in the gut (Gibson et al., 2017). In addition, prebiotics can contribute to increasing dietary utilization by reducing fecal nitrogen excretion (Perini et al., 2023). Among prebiotics, yeast-derived products are especially notable, as they can contribute to the intestinal functionality of dogs (Kaelle et al., 2022). In this context, yeast extracts from *Saccharomyces cerevisiae* are particularly relevant. These extracts are obtained by autolysis of yeast cells, followed by separation and concentration of soluble components, including cytoplasm and yeast cell wall soluble fractions (Borchani et al. 2014). The yeast cell wall contains 20-30% mannanoligosaccharides (MOS), which can modulate the intestinal microbiota through interaction with pathogens, reducing the formation of nitrogen fermentation compounds (Félix et al., 2009) and possibly increasing the production of short-chain fatty acids (SCFA) (Van den Abbeele et al. 2020; Kaelle et al., 2022). A study comparing a more soluble yeast cell wall preparation with conventional yeast cell wall preparation observed that the soluble fraction used resulted in greater fecal concentrations of fermentation metabolites in dogs (Theodoro et al., 2019). Thus, it is expected that yeast extract contributes to the improvement of digestibility and the production of fermentation metabolites in the gut. Therefore, this study aimed to evaluate the effects of *Saccharomyces cerevisiae* extract on diet digestibility, metabolizable energy, intestinal fermentation metabolites, and fecal characteristics of healthy dogs.

2. Materials and Methods

All animal procedures were approved by the Ethics Committee on Animal Use of the Agrarian Sciences Sector at the Federal University of Paraná, Curitiba, Paraná, Brazil, under protocol number. 054/2017. The study was conducted at the Research Laboratory in Canine Nutrition – LENUCAN in Curitiba, Paraná, Brazil (25º 25’ 40" S, 49º 16’ 23" W).

2.1. Animals and facilities

Six male and six female adult dogs (2 years old), weighing 10.3 ± 1.07 kg, were used. The body condition score (BCS) of the dogs was analyzed at the beginning and end of the trial, on a scale of 1 to 9 (Laflamme 1997). The dogs had a mean BCS of 5.2 ± 0.1

and underwent clinical examination before and after the experimental period. The dogs were individually housed in brick kennels (5 m long x 2 m wide), which contained a bed and provided free access to fresh water.

During most of the experimental period, the dogs had free access to an outdoor area of 1,000 m^2 for 4 h/day for voluntary exercise and socialization. During the feces collection period, the dogs were individually housed in kennels. The facilities had side wall bars that allowed visual and limited interaction with neighboring dogs. Besides, the animals received extra attention and environmental enrichment inside the kennel during this period. The temperature ranged from 16 ºC to 28 ºC, with a 12 h light-dark cycle (light from 6:00 am to 6:00 pm).

2.2. Ingredients and experimental diets

Two diets were evaluated: a control diet (3 males and 3 females), with no yeast extract (0%), and a test diet (3 males and 3 females) with 2% yeast extract (Nupro®, Alltech Brasil Ltda, Maringá, Brazil). Yeast extract was added at the expense of the 0 g/kg diet formulation. The 0 and 2 g/kg diets were formulated according to the nutritional recommendations of the Association of American Feed Control Officials (AAFCO 2016) for adult dogs.

The yeast extract used consisted of dry brewer's yeast extract (*Saccharomyces cerevisiae*). The ingredients and chemical composition of the diets are presented in Table 1, and the chemical composition of the yeast extract is presented in Table 2. The ingredients were ground in a hammer mill equipped with a 1 mm sieve and extruded in a single screw extruder (Ferraz, E-130; Ribeirão Preto, SP, Brazil). After extrusion, diets were dried in a triple-deck drier (100–110 °C), sprayed with poultry fat, and cooled.

	Diets (g/kg yeast extract)	
Item	0	2%
Ingredients, g/kg		
Corn	356.5	347.7
Poultry offal meal	276.5	270.9
Soybean meal[1]	205	200.9
Poultry fat	50	50
Poultry hydrolysate	10	10
Corn gluten meal[2]	35.6	34.9
Fish meal	20	19.6
Wheat bran	20	19.6
Beet pulp	10	10
Sodium Chloride	4.3	4.3
Mineral-vitamin supplement[3]	4.4	4.4
Acidifier	2	2
Propionic acid	1.8	1.8
Sodium hexametaphosphate	1	1
Choline chloride	1	1
DL-methionine	1	1
Mycotoxin adsorbent	0.5	0.5
Antioxidant	0.4	0.4
Yeast extract	0	2
Chemical composition		
Dry matter	890.6	917
Crude protein	287.6	289.4
Acid-hydrolyzed ether extract	114.0	102.7
Ash	81.8	83
Crude fiber	30.8	28.3
Calcium	20.3	20.3
Phosphorus	13.4	12.8
Gross energy, kcal/kg	4868.7	4866

Table 1 – Ingredients and analyzed chemical composition (g/kg dry matter) of the experimental diets.
Note: [1]460 g crude protein/kg; [2]600 g crude protein/kg; [3]Enrichment per kg of product -1: vitamin A (retinol), 20,000 IU; vitamin D3, 2,000 IU; vitamin E (alpha-tocopherol), 48 mg; vitamin K3, 48 mg; vitamin B1, 4 mg; vitamin B2, 32 mg; pantothenic acid, 16 mg; niacin, 56 mg; choline, 800 mg; Zn as zinc oxide, 150 mg; Fe as ferrous sulphate, 100 mg; Cu as copper sulphate, 15 mg; I as potassium iodite, 1.5 mg; Mn as manganese oxide, 30 mg; Se as sodium selenite, 0.2 mg; antioxidant, 240 mg.

Item	
Dry matter	931.70
Crude protein	346.50
Acid-hydrolyzed ether extract	34.40
Ash	75.30
Crude fiber	7.60
Calcium	2.10
Phosphorus	10.80
Non-protein nitrogen	37.70
Gross energy, kcal/kg	5216.30
Essential amino acids*	
Histidine	8.30
Leucine	26.30
Isoleucine	16.10
Lysine	26.0
Methionine	5.70
Phenylalanine	15.30
Threonine	17.20
Tryptophan	4.20
Valine	19.0
Nonessential amino acids*	
Alanine	25.30
Aspartic Acid	39.30
Cysteine	3.30
Glutamic Acid	51.30
Glycine	21.10
Proline	17.80
Serine	20.90
Tyrosine	12.20
Arginine	23.50
Taurine	0.60
Nucleic acids*	5.51

Table 2 – Analyzed chemical composition (g/kg, dry matter) of yeast extract. Note: *Manufacturer's data

2.3. Digestibility test

The digestibility assay followed the total fecal collection method recommended by the Association of American Feed Control Officials (AAFCO 2016). The diets were offered during a 25-day adaptation period followed by 5 days of total fecal collection. The animals were fed twice a day (8:30 am and 6:30 pm) in sufficient amounts to supply the metabolizable energy (ME) requirement of adult dogs in maintenance as recommended by the National Research Council, NRC (NRC, 2006): ME (kcal/day) = 130 × body weight (kg)$^{0.75}$. Water was provided *ad libitum.*

Feces were collected and weighed twice a day, stored in individual plastic bags previously identified by animal and period, and stored in a freezer (-20 °C). At the end of each collection period, the feces were thawed at room temperature and homogenized separately to form a composite sample from each animal. The samples were then dried in a forced ventilation oven (320-SE, Fanem, São Paulo, Brazil) at 55 °C for 72 hours or until they reached a constant weight. After drying, the feces and the experimental diets were ground using a 1 mm sieve (Arthur H. Thomas Co., Philadelphia, PA, USA) and analyzed at 105 ºC for dry matter (DM105) for 12 hours. Nitrogen (N, method 954.01) and crude protein (CP) were calculated as N x 6.25, crude fiber (CF, method 962.10), acid-hydrolyzed ether extract (AHEE, method 954.02), and ash (method 942.05), according to the Association of the Official Analytical Chemists (AOAC 1995). Organic matter (OM) was obtained as 100% - ash. Gross energy (GE) was determined by an isoperibol calorimeter (IKA C2000 Basic, IKA-Werke, Staufen, Germany). Chemical analyses were conducted in duplicate and repeated when the results varied by more than 5%.

2.4. Fecal characteristics and intestinal fermentative metabolites

Fecal characteristics were evaluated during the collection period by total DM (DMf) content, fecal output, and fecal consistency by score. Ammonia, pH, SCFA, and branched-chain fatty acids (BCFA) were analyzed in feces collected up to 15 min after spontaneous defecation on day 30 of the study.

The DMf was calculated as: (DM at 55 °C × DM at 105 °C) /100. The fecal score was evaluated by the same researcher, considering a 5-point scale: 1 = pasty, shapeless feces; 2 = soft and unshaped feces; 3 = soft, shaped, and moist feces; 4 = well-formed and consistent feces; 5 = well-formed, hard, and dry feces (Carciofi et al. 2009). Fecal output was calculated as g faeces/g DM intake/day.

Fecal pH was measured with a digital pH meter (331, Politeste Instrumentos de Teste Ltda, São Paulo, Brazil) using 3 g of fresh feces diluted in 30 mL of distilled water. The ammonia concentration was determined according to Brito et al. (2010).

For SCFA (acetate, butyrate, valerate, and propionate) and BCFA (isovalerate and isobutyrate) determination, 10 g of fecal sample was mixed with 30 mL of 16% formic acid. This solution was homogenized and stored at 4 °C for 3 to 5 days. Before the analysis, these solutions were centrifuged at 5000 rpm (2K15, Sigma, Osterode am Hans, Germany) for 15 min. After centrifugation, the supernatant was separated and subjected to further centrifugation. Each sample underwent three centrifugations and, at the end of the last one, part of the supernatant was transferred to an identified Eppendorf tube for subsequent freezing at −20 °C. Afterward, the samples were thawed and centrifuged again at 14,000 rpm for 15 min (Rotanta 460 Robotic, Hettich, Tuttlingen, Germany). Fecal SCFA and BCFA were analyzed by gas chromatography (Shimadzu®, model GC2014, Kyoto, Honshu, Japan) using a 30-mm-long and 0.32-mm-wide glass column (Agilent Technologies, HP INNO wax-19091 N, Santa Clara, USA). Nitrogen was used as the carrier gas at a flow rate of 3.18 mL/min. Working temperatures were 200 °C at injection, 240 °C at the column (at a 20 °C/min rate), and 250 °C at the flame ionization detector.

2.5. Calculation and statistical analysis

Based on the laboratory results, the ATTD and ME of diets were calculated according to the Association of American Feed Control Officials (AAFCO 2016): ATTD% = [(g nutrient intake – g nutrient excretion)/g nutrient intake] × 100. ME (kcal/g) = {kcal/g GE intake – kcal/g GE fecal excretion – [(g CP intake – g CP fecal excretion) × (1.25 kcal/g)]} /g feed intake. Data were analyzed using a completely randomized design with six replications per treatment. Data were analyzed using the SAS statistical package (version 8, SAS Institute Inc., Cary, USA). Normality was assessed using the Shapiro–Wilk test. When the data assumed a normal distribution, they were analyzed using the Student t-test ($P < 0.05$). Non-parametric data were analyzed by the Wilcoxon test ($P < 0.05$).

3. Results

All dogs remained healthy throughout the study. No episodes of food refusal, weight loss, vomiting, or diarrhea were observed, and all the animals consumed the total food offered throughout the period (mean intake: 0% yeast extract = 192.14 g/animal/day and 2% yeast extract = 195.62 g/animal/day).

The yeast extract diet presented a lower ATTD of CP ($P<0.05$, Table 3). The ATTD of other nutritional fractions and ME of the diets did not differ between treatments ($P>0.05$, Table 3). The yeast extract improved the dogs' fecal score ($P<0.05$, Table 3). The other fecal characteristics did not differ between treatments ($P>0.05$, Table 3).

	Yeast extract (g/kg)		SEM[1]	P-*value*
Item	0	2		
ATTD				
Dry matter	78.90	78.00	0.554	0.439
Organic matter	84.20	83.50	0.395	0.359
Crude protein	83.70	81.30	0.616	0.032
Ether extract	85.40	86.90	0.747	0.417
Gross Energy	84.00	83.00	0.395	0.702
ME	4067.00	4052.00	22.310	0.676
Fecal characteristics				
Dry matter (%)	33.65	35.08	0.004	0.143
Fecal output[2]	0.63	0.63	0.020	0.950
Ammonia (g/kg)[3]	0.05	0.07	-	0.227
pH[3]	6.66	6.58	-	0.492
Fecal Score	3.60	3.80	0.050	0.044

Table 3 – Means of apparent total tract digestibility (ATTD, %) and metabolizable energy (ME, kcal/kg) of diets containing (2%) or not (0%) yeast extract and fecal characteristics of dogs.
Note: [1]SEM: standard error of the mean; [2]Fecal output = g feces produced as-is/g dry matter consumed/day; [3]pH (range: 6.43 to 6.72) and ammonia (range: 0.03-0.09 g/kg) are presented as medians.

Dogs fed the diet containing yeast extract had higher fecal concentrations of acetate ($P<0.05$, Table 4). The other SCFA and BCFA did not differ between treatments ($P>0.05$, Table 4).

Diets	SCFA (µmol/g)				BCFA (µmol/g)		Total SCFA	Total BCFA
	Acetate	Propionate	Butirate	Valerate	Isobutyrate	Isovalerate		
0%	29.08	15.42	4.37	0.36	0.62	0.83	49.19	1.82
2%	33.30	17.51	4.43	0.41	0.53	0.70	53.94	1.77
SEM[1]	1.669	1.164	0.363	0.065	0.045	0.084	2.924	0.197
P-*value*	0.016	0.131	0.902	0.430	0.134	0.230	0.161	0.943

Table 4 – Means of fecal concentrations of short-chain (SCFA) and branched-chain (BCFA) fatty acids of dogs fed diets containing (2%) or not (0%) yeast extract.
Note: [1]SEM: standard error of the mean.

4. Discussion

Yeast products can be considered functional ingredients in dog and cat nutrition (Santos et al., 2018; Lin et al., 2019). Although these products have shown positive effects on intestinal functionality, their impact on nutrient digestibility remains contradictory (Maturana et al., 2023), and studies specifically evaluating yeast extract's effects on nutrient digestibility in dogs are scarce. Generally, most studies using yeast products have observed reductions in the ATTD of CP (Swanson et al., 2002; Zentek, Marquart, and Pietrzak, 2002; Middelbos, Fastinger, and Fahey, 2007; Reilly et al., 2021; Kaelle et al., 2022), findings similar to those of the current study. However, this reduction in ATTD of CP may be due to the high non-protein nitrogen (around 5.5% nucleic acids) content in yeast extract, which may underestimate the true CP digestibility in diets containing yeast products (Kaelle et al., 2022).

Other results supporting this hypothesis included the lack of differences in fecal concentrations of ammonia and BCFA, as well as fecal pH. Diets containing lower CP digestibility may result in greater fecal concentrations of metabolites from nitrogen fermentation. These compounds in excess may be toxic to the gut mucosa and increase inflammation (Bastos et al., 2023). Other studies evaluating yeast extract (Kaelle et al., 2022) and yeast cell wall components with different solubilities (Theodoro et al., 2019) also observed similar results in dogs, with no differences in fecal concentrations of ammonia and total BCFA and DMf.

Regarding SCFA, we observed an increase in acetate concentration in the feces of dogs fed yeast extract. The production of this metabolite may be related to the greater fecal score observed concentrations in dogs fed the yeast extract. Acetate and other SCFAs are rapidly absorbed as non-dissociated acid through the luminal membrane by passive diffusion. This process, which involves the exchange of sodium and hydrogen ions through the membrane, causes the large intestine to absorb more water from the fecal bulk (Hume, 1997). Additionally, acetate is both an intermediate and a final product of gut microbial fermentation and, along with other SCFA, plays a vital role in the gut functionality of dogs, contributing to intestinal motility, mucosal barrier integrity, and controlling inflammatory processes (Pilla and Suchodolski, 2020).

Yeast extract can contribute to gut microbiome fermentation, especially by *Lactobacillus* spp., which primarily produces lactic acid but also acetate (Leroy and De Vuyst, 2004). This effect is enhanced by the presence of MOS in the extract, which can modulate the intestinal microbiota, reducing, through direct binding, the presence of potentially pathogenic microorganisms such as *Salmonella* spp. and *Escherichia coli*. This may contribute to the growth of beneficial microorganisms, such as *Lactobacillus* spp. and *Bifidobacterium* spp. (Strickling et al. 2000). Other studies evaluating yeast extract and yeast cell wall components have also observed higher fecal concentrations of SCFA in dogs (Theodoro et al., 2019; Kaelle et al., 2022).

5. Conclusion

The dietary inclusion of 2% yeast extract reduced the apparent digestibility of crude protein. However, the yeast extract improved fecal consistency and increased fecal concentrations of acetate, suggesting an enhancement of intestinal functionality in dogs.

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