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Evaluation of Raw Yeast Extract (*Saccharomyces cerevisiae*) as an Ingredient, Additive or Palatability Agent in Wet Diet for Cats

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Abstract

Three experiments were performed to evaluate the effects of strain-specific yeast extract (SSYE) as an ingredient, functional additive or palatability agent when supplemented in its raw form in wet cat food. *SSYE as ingredient* – SSYE was chemically characterized and its use evaluated through fourteen cats divided into two treatments: control diet (complete wet adult cat food) and control diet with 30 % replacement by SSYE. The results of apparent digestibility coefficient of SSYE were 71.64 % for dry matter, 72.55 % for organic matter, 50.78 % for ashes, 78.59 % for crude protein, 84.33 % for the energy gross and digestible and metabolizable energy value, respectively, of 4,247 and 4,163 kcal/kg, these results indicated that SSYE is comparable to other protein sources for cat's food. *SSYE as a functional additive* - twelve cats were distributed into two 6x6 latin squares (treatments; experimental periods), and the treatments were control diet and replacement levels ranged from 2 % to 10 % SSYE. The following parameters were evaluated: digestibility, energy utilization, nitrogen balance, serum urea and creatinine levels. No differences were found. *SSYE as palatability agent* – Were used twenty cats by comparing the control diet with 2 % replacement by SSYE. A significant difference ($P < 0.01$) was observed with a preference for control diet. SSYE is a potential protein source for cats; however, it is not effective as additive and may compromise palatability when supplemented in its raw form in complete wet cat food.

Keywords: digestibility, glutamic acid, nutrition, palatability, pet

1. Introduction

Yeast is a good source of nutrients and has a flavor-enhancing and immunostimulant effect (Sgarbieri, Alvim, Vilela, Baldini, & Bragagnolo, 1999). Hydrolysates are produced by enzymatic digestion and the centrifugation of them results in cell walls and yeast extract, which are among the main yeast derivatives (Santucci, Alvim, Faria, & Sgarbieri, 2003).

All soluble nutrients of the hydrolysate remain as part of the yeast extract, although this will comprise of higher protein levels as compared to intact or hydrolyzed yeast (Fegan, 2006). Furthermore, yeast extract is rich in glutamic acid and nucleotides (Tibbetts, 2002). Glutamic acid has a dietary flavor-enhancing effect and is a vital energy substrate for cells involved in rapid cell division, including intestinal cells (Lacey & Wilmore, 1990). In turn, dietary nucleotides may provide benefits, including modulating the immune system, supporting the growth and recovery of tissue by DNA synthesis, and promoting the development of the small intestine (Fegan, 2006). Therefore, the use of yeast extract as a food additive for non-ruminant animals has been effective for nutrient utilization (Carlson, Verum, & Turk, 2005; Jarmołowicz et al., 2013; Rutz et al., 2006).

The quality of protein sources is extremely important, especially for cats which are obligate carnivores, but studies that have evaluated the dietary use of yeast extract in cats are scarce. A synergistic effect between yeast extract and an acidifying agent has been observed in enhancinge digestibility of dry cat food (Ogoshi et al., 2014). However, studies assessing the nutrient utilization and/or suggesting an optimal level of yeast extract for use in various cat foods have not been conducted. In addition to digestibility, palatability is important in evaluation of cat food

ingredients because cats have facial nerves that are extremely sensitive to amino acids; therefore, they may be sensitive to diets containing yeast derivatives (Aquino et al., 2010).

This study aimed to evaluate the strain-specific yeast extract (*Saccharomyces cerevisiae*; SSYE) as an ingredient, functional food additive and palatability agent in wet cat food.

2. Method

Table 1. Nutrient composition of complete wet cat food (control diet) according to the manufacturers' labels

Nutrients	**As feed basis (%)[1,2]**	**Dry matter basis (%)**
Moisture (%)	80.00	-
Crude protein (%)	8.00	40.00
Ether extract (%)	3.00	15.00
Crude fibre (%)	1.50	7.50
Ashes (%)	2.50	12.50
Calcium (%)	0.40	2.00
Phosphorus (%)	0.20	1.00
Taurine (%)	0.05	0.40

[1]Basic composition: Water, chicken meat, beef entrails, giblets, pig offal, sodium chloride, carrageenan, vitamin and mineral premix, taurine.

[2]Enrichment/kg food: folic acid (2 mg), pantothenic acid (15 mg), copper (12 mg), choline (2.000 mg), iron (100 mg), iodine (2 mg), manganese (7.5 mg), niacin (60 mg), selenium (0.1 mg), vitamin A (12.500 IU), vitamin B1 (6 mg), vitamin B12 (25 mg), vitamin B2 (5 mg), vitamin B6 (6 mg), vitamin D3 (900 IU), vitamin E (100 mg), zinc (130 mg).

Table 2. Guaranteed levels per kilogram of strain-specific yeast extract (SSYE) on a dry matter basis according to the manufacturer

Moisture (%)	6.00	Ashes (%)	8.20
Ether extract (%)	0.20	Sulfur (%)	0.46
Carbohydrates (%)	22.20	Sodium (%)	1.68
Crude fibre (%)	0.40	Phosphorus (%)	1.53
Crude protein (%)	50.00	Potassium (%)	1.47
Total nucleic acids (%)	5.40	Magnesium (%)	0.32
Aspartic acid (%)	3.75	Calcium (%)	0.05
Glutamic acid (%)	5.10	Iron (ppm)	52.00
Alanine (%)	2.94	Copper (ppm)	3.00
Arginine (%)	1.88	Zinc (ppm)	160.00
Cystine (%)	0.40	Manganese (ppm)	9.00
Phenylalanine (%)	1.87	Chloride (ppm)	442.00
Glycine (%)	1.94	Niacin (mg/kg)	103.00
Histidine (%)	0.97	Biotin (mg/kg)	0.92
Isoleucine (%)	1.94	Pantothenic acid (mg/kg)	16.60
Leucine (%)	3.60	Vitamin B1(mg/kg)	35.00
Lysine (%)	2.60	Choline chloride(mg/kg)	3,800.00
Met+Cis (%)	1.14	Vitamin B2 (mg/kg)	23.60
Methionine (%)	0.74	Vitamin B6 (mg/kg)	5.95
Ornithine (%)	0.09	Vitamin B12 (mcg/kg)	6.21
Proline (%)	2.11	Vitamin E (mg/kg)	17.70
Serina (%)	1.94	Inositol (mg/kg)	12,500
Taurine (%)	0.09		
Tyrosine (%)	0.76		
Threonine (%)	1.94		
Tryptophan (%)	0.49		
Valine (%)	2.46		

The study was conducted at the Centre for the Study of Companion Animal Nutrition of the Animal Science Department in the Federal University of Lavras, located in Lavras, Minas Gerais, Brazil.

One commercial wet food popular brands in Brazil (control diet; Table 1) was used to compose the experimental diets.

The SSYE was a commercial product (Nupro®, Alltech, Araucária, Brazil; Table 2) produced by patented process of extracting the cellular content of the *Saccharomyces cerevisiae* yeast strain cultivated in sugarcane juice.

2.1 Evaluation of SSYE as an Ingredient in Wet Cat Food

Fourteen adult male mixed-breed cats with a mean weight of 3.68 ± 0.73kg were used. The cats were individually housed in suspended metabolic cages sized 60 x 70 x 50 cm during each experimental period and distributed in two treatments with seven replicates. The treatments consisted of the control diet and the control replaced on a dry matter basis with 30 % SSYE. The study was conducted over a ten-day period, with five days of adaptation and five days for collection of feces and urine.

The diets remained available to the animals for 23 hours and the quantity was calculated using the formula to estimate the energy requirements for adult cats according to National Research Council (2006), with the diets manually prepared by mixing the SSYE and control until a homogenous mass was produced. During the collection days sampling for assessment of nutrient digestibility followed the Association of American Feed Control Officials (AAFCO) (2006).

Bromatological analyses of the food and feces were conducted in the Animal Research Laboratory of the Department of Animal Science at Federal University of Lavras (Universidade Federal de Lavras - UFLA) and the analysis of crude protein, acid hydrolysed ether extract, moisture and ash were performed according to Association of Official Analytical Chemists (AOAC) (2006). The gross energy levels were assessed in a Parr 260 automatic bomb calorimeter (Parr Instruments, Moline, Illinois, USA).

The measuring of apparent digestibility coefficient (ADC) and energy utilization values of SSYE followed Matterson, Potter e Stutz (1965) as below:

$$\mathrm{ADC}_{\text{test ingredient}}\ (\%) = \mathrm{ADC}_{\text{control diet}} + \frac{(\mathrm{ADC}_{\text{control diet}} - \mathrm{ADC}_{\text{test diet}})\ \mathrm{x}\ 100}{\%\ \text{replacement}\ /100}$$

Where: % replacement = replacement of control diet by test ingredient on a dry matter basis.

2.2 SSYE Additive Effect in Wet Cat Food

Twelve adult cats, male and female, mixed-breed, weighing 3.10 ± 0.5kg were used. The animals were individually housed in metabolic cages distributed in two 6 x 6 latin squares (treatments; experimental periods) with six replicates per treatment. Each period lasted 15 days, with 8 days for adaptation, 6 days for the collection of total feces and urine and 1 day for blood collection. The treatments consisted of control diet and SSYE replacement levels of 2 %, 4 %, 6 %, 8 % and 10 % on a dry matter basis.

The preparation of diets, the quantity and time of exposure to cats, the protocols of chemical analysis were similar to that described in the first experiment.

Nitrogen balance was assessed based on the data of ingestion and fecal and urinary nitrogen excretion. Blood samples (5 ml) were collected from each animal, stored in a tube without anticoagulant and sent to the Santa Cecilia laboratory, Lavras, Brazil. The levels of serum urea and creatinine were assessed by the point kinetic method using commercial kits Kinetic Urea - K056 and Kinetic Creatinine - K067 (Bioclin Quibasa, Belo Horizonte, Brazil).

2.3 Palatability Evalutation of SSYE Supplemented Wet Cat Food

SSYE palatability was evaluated with 20 adult cats, male and female, mixed-breed, which had a mean weight of 3.71 ± 0.6kg. The animals were individually housed in metabolic cages for four days. Each animal received two dietary options once per day, the control diet and the control with 2 % SSYE replacement, following the Two-Bowl Test (Griffin, 2003), totally 80 observations. In this methodology two diets were placed in their respective bowls and presented simultaneously to the cats, wherein the intent was to determine whether the animal has a preference. The bowls were left with the animal for 30 min or until one of the bowls had been completely consumed. The amount of food offered in each bowl was been sufficient for the animals' daily caloric intake. Both dietary options were manually homogenized prior to feeding. In addition, the relative consumption was expressed as a proportion of the total food consumed by the formula:

$$\text{Relative consumption}\ (\%) = \frac{(\text{Food A intake})}{(\text{Food A intake} + \text{Food B intake})}\ \mathrm{X}\ 100$$

2.4 Statistical Analysis

Evaluation of SSYE as an ingredient in wet cat food -After calculating the apparent digestibility coefficients, digestible and metabolizable energy by the methodology of Matterson et al. (1965), data were represented by means of descriptive statistics for average and its respective standard error of the mean (n = 7).

SSYE additive effect in wet cat food - All of the data of this second experiment were evaluated using the statistical software Statistical Analysis System (SAS; 1996) and subjected to an analysis of variance (ANOVA) and regression analysis for the dietary replacement levels of SSYE.

Palatability evalutation of SSYE supplemented wet cat food - The data were evaluated using the PROC GLM procedure of the statistical software SAS (1996) and subjected to an analysis of covariance and F-test.

Statistical significance was based on $P < 0.05$.

3. Results

3.1 SSYE as Ingredient

The chemical composition of SSYE exhibited high protein and low crude fiber and fat levels as the main characteristics (Table 3).

The ADC and energy utilization values of SSYE for adult cats obtained by the methodology of Matterson et al. (1965) are outlined in Table 4.

Table 3. Chemical composition of strain-specific yeast extract (SSYE) on a dry matter basis

Nutrients	**%**
Dry matter[1]	95.00
Organic matter[1]	89.53
Ashes[1]	5.47
Crude protein[1]	46.55
Crude fibre [1]	2.19
Ether extract[1]	0.18
Nitrogen-free extract [2]	45.61

[1]Analyses performed at the Animal Research Laboratory of the Animal Science Department at Federal University of Lavras, Lavras, MG, Brazil.

[2]Calculated using the equation ENN=100-(crude protein (CP)+ether extract (EE)+crude fiber(CF)+ashes).

Table 4. Apparent digestibility and energy utilization of strain-specific yeast extract (SSYE) for adult cats obtained by the substitution method of diet control (wet food)

Parameters	**Medium (SD)**
ADC (%)	
Dry matter	71.64 ± 2.56
Ashes	50.78 ± 5.74
Organic matter	72.55 ± 2.44
Crude Protein	78.59 ± 5.49
Crude Energy	84.33 ± 3.14
ADE (kcal/kg)	4,247 ± 170
AME (kcal/kg)	4,163 ± 227

ADC - apparent digestibility coefficient (ADC) of nutrients (%); ADE - apparent digestible energy; AME - apparent metabolizable energy; SD - standard desviation.

3.2 SSYE as Functional Food Additive

The chemical composition of the diets used in experiment for to evaluate SSYE as food additive is presented in Table 5.

Table 5. Some nutritional levels of experimental diets on a dry matter basis prepared by replacing wet cat food (control) with up to 10 % strain-specific yeast extract (SSYE)

Component (%)[1]	Diets (SSYE replacement levels)					
	Control	2	4	6	8	10
Crude protein	38.24	38.41	38.57	38.74	38.90	39.07
Ashes	9.91	9.82	9.64	9.64	9.55	9.47
Crude fibre	0.22	0.26	0.34	0.34	0.38	0.42
Ether extract	23.10	22.64	21.72	21.72	21.27	20.81

[1]Analyses performed at the Animal Research Laboratory of the Animal Science Department at Federal University of Lavras, Lavras, MG, Brazil.

Differences were not observed (P > 0.05) in nutrient utilization, energy utilization, nitrogen balance and blood parameters when the complete wet cat food was replaced by up to 10 % SSYE (Table 6).

Table 6. Nutrient digestibility, energy utilization, nitrogen balance and blood parameters of cats fed a complete wet food (control) with replacement by strain-specific yeast extract (SSYE)

Variable	Levels replacement by SSYE (%)						SEM
	Control	2	4	6	8	10	
ADC (%)							
Dry matter	80.76	81.09	82.40	81.89	81.62	82.20	0.95
Organic matter	84.69	84.96	86.19	85.32	85.14	85.61	0.96
Ashes	45.09	45.60	47.27	49.80	48.32	47.85	2.80
Crude Protein	82.48	83.84	84.90	84.61	83.90	84.28	0.99
Crude Energy	83.36	84.10	85.20	84.06	84.23	84.88	1.16
ADE (kcal/kg)	4,795	4,825	4,875	4,797	4,794	4,818	66.28
AME (kcal/kg)	4,488	4,539	4,595	4,504	4,516	4,518	72.26
Nitrogen (g/cat/day)							
Consumed	3.06	3.22	3.20	3.22	3.10	3.38	0.18
Excreted in feces	0.51	0.52	0.48	0.49	0.51	0.52	0.03
Excreted in urine	0.30	0.31	0.30	0.32	0.27	0.33	0.03
Absorbed	2.54	2.70	2.72	2.72	2.59	2.85	0.16
Retained	2.24	2.39	2.41	2.40	2.32	2.51	0.14
Plasma urea (mg/dL)	51.00	49.58	50.33	51.75	49.66	52.16	2.40
Plasma creatinine (mg/dL)	1.16	1.11	1.11	1.06	1.05	1.07	0.04

ADC - apparent digestibility coefficient; ADE - apparent digestible energy; AME - apparent metabolizable energy; SEM: standard error of mean .

P > 0.05 by simple polynomial regression.

3.3 SSYE as Palatability Agent

A significant difference (P = 0.0074) was found in the intake of diets used in palatability test, with preference to the control diet over 2 % SSYE (Table 7). In addition, the relative consumption results showed that 56.75 % of cats preferred the control, 33.75 % preferred the diet containing, 2 % SSYE and 10 % presented ambiguity with regard to the palatability of the two diets.

Table 7. Intake of wet food (control) or control with 2 % replacement by strain-specific yeast extract (SSYE) by adult cats in a two-bowl palatability test

Diets	Intake (g dry matter/day)
Control	33.63 a
2 % SSYE	26.58 b
SEM	3.44

Means followed by a different letter in rows were different according to the F-test ($P < 0.05$).

4. Discussion

The chemical composition of SSYE was similar to the values found by TESHIMA et al. (2007). However, the protein level was lower than those recorded by Silva, Amoroso, Fukayama, Dourado e Moraes (2009), who observed a value of 51.95%. It is known that chemical composition of the yeast extract was dependent on the raw materials and methods used in its preparation; the extracts may have been derived from breweries or cane distilleries, and the processing may have included drying using the spray dry method or rotary cylinder method (Silva et al. 2009). Thus, significant differences may have occurred in the chemical composition of the SSYE.

Value of the apparent protein digestibility coefficient of SSYE was higher than result found by Teshima et al. (2007) in dogs (72.44 %). Limited information is available on feline digestibility of protein sources, especially SSYE. The present study is the first to evaluate SSYE as a protein source for cats; thus, a broader discussion of the results is precluded. However, the protein digestibility of SSYE was similar to the values cited by Carciofi (2007) for ingredients commonly used in the formulation of cat food, including meat and bone meal and soybean meal. The same author cited metabolizable energy values for such ingredients (2,262 and 2,823 kcal/kg dry matter, respectively) that were considerably lower than the values for SSYE. These results characterize SSYE as a potential protein ingredient for cats.

Inclusion of SSYE at 10 % had no changes observed on apparent digestibility coefficient (Table 6). With respect to the energy use, no differences are probably explained by to maintenance of the digestibility of all nutrients. In contrast, SSYE when evaluated as functional additive in other studies have showed positive effects on nutrient utilization in chickens during the first week of life (Rutz et al., 2006) and in piglets (Tibbets, 2002; Maribo, 2003). The benefits in those studies were attributed to the increased intestinal villus:crypt. It is known that yeast extract is rich in nucleotides that are responsible to support mitotic division in the intestine, as well as provides glutamic acid which is known as an important energy source for the metabolism of intestinal cells (Fegan, 1006; Lacey & Wilmore, 1990).

Other researchers, Menten e Miyada (1995) reported that the use of complex diets, low sanitary challenges, and appropriate management and environments reduced the positive benefits associated with the use of additives in animal nutrition. In present study, the control diet used was a high-quality diet which may have influenced the results.

The NB in the present study was positive regardless of the level of supplementation with SSYE. Similar results were observed by Green et al. (2008) in cats that ingested diets with high protein levels from which supplementation or food source. Regarding the biochemical parameters, creatinine increases during renal failure or situations of muscle protein catabolism; whereas it is rapidly cleared from the blood and excreted under normal conditions (González & Scheffer, 2003). In turn, the value of serum urea may be elevated in catabolic reactions or when there is high protein intake (González & Scheffer, 2003). In the present study, supplementation with up to 10 % SSYE did not influence the NB and biochemical parameters evaluated. It is suggest that the control diet with a lower protein value and/or challenged adult animals should be use in further studies assessing SSYE in cats.

One of the most common methods for pet food palatability evaluation is a two-bowl forced choice evaluation wherein the intent is to determine whether the animal has a preference (Aldrich e Koppel, 2015). The no preference of 2% SSYE in contrast of control diet by the cats is probably explained by the presence of substances that intensify flavours of the food. A flavor enhancing effect that stimulates dietary intake has been attributed to glutamate and the umami taste it generates upon supplementation with SSYE of up to 5 % in poultry (Rutz et al., 2006; Silva et al., 2009), pig (Carlson et al., 2005) and dog food (Teshima et al., 2007). However, other studies have reported a negative effect on preference and feed intake upon supplementation with SSYE and other yeast derivatives (Andrade et al., 2011; Aquino et al., 2010; Ogoshi et al., 2014). One hypothesis for these controversial results is the threshold of supplementation with substances that have a umami taste and contain monosodium glutamate because an excess may reduce palatability (Halpern, 2000). Shi e Tang (2003) reported that thermal

processing may modify certain flavor enhancers and decrease their flavor-enhancing effect, which would increase the threshold of SSYE supplementation. This infact may explain, for example, why Teshima et al. (2007) observed improved palatability upon supplementation with 2 % SSYE with thermal processing (extrusion) in dogs, whereas similarity of the presente study, Ogoshi et al. (2014) recorded decreased palatability upon supplementation with 1.5 % raw SSYE in cats.

Another hypothesis is related to a particularity of cats. Studies have shown that cats reject amino acids that are considered "bitter" for humans, including leucine, arginine, isoleucine, phenylalanine and tryptophan (Zaghini & Biagi, 2005). In this case, the bitter taste resulting from leucine, a predominant amino acid in yeast extract, may has been enhanced by the umami taste, thus reducing the preference for the diet with 2 % SSYE.

Thus, the results on SSYE palatability recorded in this experiment are applicable to unprocessed cat food that is similar to natural diets. This study indicates that SSYE should be evaluated with thermal processing, which is common in the production of cat food, especially during the extrusion process.

SSYE is a potential protein ingredient for cats, although it shows no effectiveness as a functional food additive and palatability agent upon supplementation in its raw form in wet cat food. Further experiments should be performed to evaluate the effects of thermal processing on the flavor-enhancing potential of SSYE.

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