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Heat Processing Changes the Protein Quality of Canned Cat Foods as Measured with a Rat Bioassay¹

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ABSTRACT: The purpose of this study was to determine the influence of increasing heat treatment on the protein quality of a canned cat food. A standard recipe cat food was canned and heat-treated for different times in a standard laboratory autoclave to obtain experimental diets containing different lethality values. Estimates of the lethality value of the different diets were calculated using the temperature-time relationship recorded with a data logger positioned at the center of the can. The experimental diets were analyzed for crude protein, amino acids, and reactive lysine (fluorodinitrobenzene and *O*-

methylisourea) and were used in a rat bioassay for the determination of the true ileal digestibility of amino acids. The heat treatment of the cat food resulted in experimental diets with lethality values of 5.3, 8.6, 17.2, and 24.3 min. There was no decrease in the amino acid content of diet with increasing heat treatment. The reactive lysine content of the diets also showed no change with heat treatment. There were significant ($P < .05$) changes in the true ileal digestibility of all amino acids and amino acid nitrogen, and the digestibility of most amino acids decreased with increasing heat treatment.

Key Words: Heat Treatment, Protein Quality, Lysine, Digestibility, Cats

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Introduction

Diets for companion animals such as cats and dogs are extensively heat-processed to increase shelf life, achieve a desired physical form, and(or) increase palatability. The heat treatments most often used include expansion, extrusion, baking, pasteurization, and sterilization. There is a plethora of information concerning the influence of various heat treatments on the protein quality of human foods and on feeds for production animals (e.g., Bender, 1978; Somogyi and Müller, 1989; van Barneveld, 1993; van der Poel et al., 1993; Voragen et al., 1995). However, little information is available on the effects of heat treatment on the nutritive value of diets for companion animals.

Heat processing generally has a negative impact on the nutritive value of pet foods (NRC, 1986; Lewis et al., 1987; Heinicke, 1995). Loss of vitamins during the production of pet foods has been documented (Roche,

1981), along with clinical signs of deficiency (Baggs et al., 1978). Hickman et al. (1992) showed that heat processing of a canned cat food changed the taurine status of cats, which was shown later by Kim et al. (1996a) to be a result of microbial deconjugation of bile acids. To the authors' knowledge, there is no information available in the literature concerning the effects of heat processing on the protein quality of diets for cats.

The present work was undertaken, therefore, to determine the influence of heat treatment on the protein quality of a canned moist food for cats. The diet was autoclaved for different times, and protein quality was measured using *in vitro* assays and a rat ileal digestibility assay.

Materials and Methods

Heat Processing of Cat Food. A standard recipe cat food (Table 1) comprising 60% meat (from fish, mutton, beef, and poultry offals), 37% water, 1.3% soy protein concentrate, .6% gelling and stabilizing agents (carageenan, locust bean gum, and guar gum), .4% shell powder, and .7% minor ingredients such as K_2PO_4 , and vitamin premix was canned in 20 standard 700-g cans (diameter 83 × 143 mm). The cans were cold-filled to 705 to 710 g using steam flow

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Table 1. The nutrient composition of the unprocessed cat food

Nutrient	Concentration, g/kg dry matter
Crude protein	567.3
Lipid	288.1
Ash	77.5
Amino acids	
Arginine	35.1
Histidine	14.5
Isoleucine	18.6
Methionine	12.0
Leucine	44.0
Lysine	36.2
Phenylalanine	25.4
Threonine	24.2
Valine	28.3
Alanine	38.8
Aspartic acid	50.8
Glutamic acid	69.3
Proline	33.0
Glycine	48.1
Serine	26.2
Tyrosine	17.4

closure to give a vacuum of 17 to 34 kPa. Four cans were immediately frozen after canning, and the remaining 16 cans were heat-treated at $121 \pm 1^\circ\text{C}$ in a laboratory-scale autoclave in batches of four cans. One can in each batch contained a Tracksense data logger (ELLAB A/S, Roedovre, Denmark) that recorded the temperature at the center of the can at 5-min intervals. The autoclave temperature was monitored using a standard thermometer positioned directly above (.1 m) the cans being processed. The four batches of cans were heat-treated for different periods of time, ranging from approximately 80 to 120 min, so that the center of the can was exposed to a calculated lethality value of 5, 10, 15, or 25. Lethality value of a thermal process is designated by the symbol F_0 and represents the time equivalent (minutes) of a heating process to destroy microorganisms at the reference temperature of 121.1°C . After processing, the cans in each batch were cooled for 30 min in running water, and the data logger was recovered. A Tracksense interface station (ELLAB A/S) was used to read the stored temperature data, and the lethality value for each batch of cans was calculated using Tracksense PCSOFT92/PCLINK92 software (ELLAB A/S).

Rat Digestibility Study. Approval for this experiment was granted by the Animal Ethics Committee at Massey University, Palmerston North, New Zealand. Sprague Dawley male rats ($n = 36$) weighing 157 g (SE 4.0) were obtained from the Small Animal Production Unit at Massey University. The rats were randomly allocated to one of six diets such that there were six rats on each diet. The rats were housed individually in stainless steel wire-bottomed cages at 22°C (± 2.0) with a 12-h reverse light/dark cycle. A

Table 2. Ingredient composition (g/kg air-dry diet) of the cat food-based (CF) and enzymatically hydrolyzed casein-based (EHC) experimental diets

Ingredient	Diet	
	CF	EHC
Cat food ^a	185.8	—
Enzymatically hydrolyzed casein ^b	—	123.8
Wheat starch ^c	572.9	634.9
Sucrose	100.0	100.0
Purified cellulose ^d	50.0	50.0
Soybean oil	50.0	50.0
Vitamin-mineral mix ^e	36.3	36.3
Chromic oxide	5.0	5.0

^aProcessed to contain lethality values of 0, 5.3, 8.6, 17.2, and 24.2. Nutrient composition of the unprocessed cat food is given in Table 1.

^bNew Zealand Pharmaceuticals Ltd., Palmerston North, New Zealand.

^cFielders Wheaten Cornflour, Starch Australasia Ltd., Tamworth, Australia.

^dAhaki Chemical Industry Co Ltd., Osaka, Japan.

^eProvided (g/kg diet): Ca, 4.6; P, 3.5 ($\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$); K, 3.6 (K_2SO_4); Cl, 1.0; Na, .7 (NaCl); choline, .9 (choline chloride); Mg, .5 ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$); (mg/kg diet); Fe, 36 ($\text{FeSO}_4 \cdot \text{H}_2\text{O}$); all-*rac*- α -tocopherol, 33; Zn, 26 ($\text{ZnSO}_4 \cdot \text{H}_2\text{O}$); nicotinic acid, 21; Mn, 13 ($\text{MnSO}_4 \cdot \text{H}_2\text{O}$); pantothenate, 10; Cu, 6.5 ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$); pyridoxine, 6.0; thiamine, 4.0; riboflavin, 3.5; cholecalciferol, 1.7; folic acid, 1.0; phylloquinone, 1.0; retinol, .7; cobalamin, .5; I, .3 (CaIO_3); biotin, .2; and Se, .1 (Na_2SeO_4).

basal diet was formulated to exceed the requirements of growing rats (NRC, 1995) for all nutrients except protein. The protein source in the experimental diets was included at 10% and was supplied by the cat food processed to different lethality values. A diet based on enzymatically hydrolyzed casein (**EHC**) was also formulated to allow determination of endogenous ileal amino acid flows (Butts et al., 1991). The ingredient composition of the experimental diets is shown in Table 2.

The rats were acclimatized for 3 d before the start of the study, during which time they were fed a normal rat chow to appetite. The rats received their respective experimental diets during nine equal meals served hourly between 0800 and 1600. The hourly feeding regimen was employed to ensure a constant flow of material at the terminal ileum. At each mealtime the diets were available for a 10-min period. The rats were weighed on the first and final day of the study, and food intake was recorded hourly. Fresh water was available at all times.

On the 9th d of the study, from 6 to 8 h after the start of the hourly feeding regimen, the rats were asphyxiated in carbon dioxide gas and then decapitated. The body cavity of the rat was opened, and 20 cm of the ileum (approximately 20% of the length of the small intestine) immediately anterior to the ileocecal junction was dissected out. The dissected ileum was washed with distilled deionized water to remove any blood and hair and then carefully blotted

on an absorbent paper towel. The digesta were then gently flushed from the ileal section using a syringe with distilled deionized water. The digesta of the rats fed the diets containing cat food were freeze-dried, thoroughly mixed, and analyzed for dry matter, chromium, and amino acids. The digesta of the rats fed the EHC-based diet were adjusted to approximately pH 3 with 6 M HCl to minimize protease activity and then centrifuged and ultrafiltered according to the procedure of Butts et al. (1991) before chemical analyses.

The experimental diets containing cat food were analyzed for dry matter, chromium, and amino acids. The cat foods with different lethality values were analyzed for dry matter, nitrogen, amino acids, and reactive lysine determined with the fluorodinitrobenzene and *O*-methylisourea method. The unprocessed cat food was also analyzed for crude fat and ash.

Chemical Analyses. Total nitrogen was determined in duplicate using the Kjeldahl method (AOAC, 1995), and crude protein was calculated by multiplying total nitrogen by 6.25. Dry matter was determined in duplicate by drying samples at 105°C to constant weight, and ash was determined by heating the samples at 550°C for 16 h. Lipid was determined by petroleum ether extraction of duplicate freeze-dried samples (AOAC, 1995). The chromium contents of the diet and ileal digesta samples were determined in duplicate on a GBC 902 AA absorption/emission spectrophotometer (GBC Scientific NZ Ltd, Auckland, New Zealand) following the method of Costigan and Ellis (1987).

Amino acids were determined on duplicate 5-mg samples by hydrolyzing with 1 mL of 6 M glass-distilled HCl (containing .1 g phenol/L) for 24 h at 110°C (\pm 2.1) in glass tubes, sealed under vacuum. The tubes were opened, norleucine was added to each tube as an internal standard, and the tubes were then dried under vacuum (Savant Speedvac Concentrator AS 290, Savant Instruments, Farmingdale, NY). Amino acids were dissolved in 2 mL of sodium citrate buffer (pH 2.2) and loaded onto a Waters ion-exchange HPLC system (Millipore, Milford, MA) employing postcolumn derivatization with ninhydrin and detection at 570 nm. Proline was detected at 440 nm. The chromatograms were integrated using dedicated software (Maxima 820, Waters, Millipore) with amino acids identified by retention time against a standard amino acid mixture (Pierce, Rockford, IL). Tryptophan and cysteine were not determined. No corrections were made for loss of amino acids during acid hydrolysis. Amino acid concentrations were corrected for recoveries of norleucine and converted to a weight basis using free amino acid molecular weights.

1-Fluoro-1, 4-dinitrobenzene (**FDNB**)-reactive lysine was determined according to the method of Carpenter (1960) using the modifications described by Booth (1971). The method involves reacting the lysine with FDNB in ethanol and NaHCO₃ at room

temperature for 2 h followed by hydrolysis of the formed dinitrophenyl (**DNP**)-protein in 5.8 M HCl for 16 h, extraction with diethyl ether to remove interfering components, and measurement of the DNP-lysine by absorbance at 435 nm. A blank value is obtained by treatment of a paired hydrolyzed sample with methoxycarbonyl chloride followed by extraction with diethyl ether. Correction factors for the loss of DNP-lysine during acid hydrolysis were determined according to the method of Booth (1971).

O-Methylisourea (**OMIU**)-reactive lysine was determined according to the method described by Rutherford et al. (1997). This involved incubation of the sample (5 mg) with .5 to 1.0 mL of .6 M OMIU in a shaking water bath at 21°C for 3 d. The formed homoarginine was measured in the dried sample according to the amino acid analysis procedure described previously.

Materials. The FDNB, DNP-lysine, and OMIU were obtained from Sigma Chemical (St. Louis, MO). Barium hydroxide octahydrate was obtained from BDH Laboratory Supplies (Poole, U.K.). Centriprep 10 disposable ultrafiltration devices were obtained from Amicon (Beverly, MA).

Data Analysis. Amino acid flows at the terminal ileum for each rat were calculated using the following equation (units are $\mu\text{g/g DMI}$): Ileal amino acid flow = amino acid concentration in ileal digesta \times (diet chromium/ileal chromium). True ileal amino acid digestibility was calculated using the following equation ($\mu\text{g/g DMI}$): True ileal amino acid (AA) digestibility (%) = [dietary AA intake - (ileal AA flow - endogenous ileal AA flow)]/dietary amino acid intake \times 100.

The true ileal digestibility coefficient for each amino acid was tested for homogeneity of variance using Bartlett's test (Snedecor and Cochran, 1980). When the variance was heterogeneous, the data were transformed (\log_{10}). True ileal amino acid digestibilities were subjected to ANOVA (SAS, 1985) with lethality value as the variable. The mean daily food intake data of the rats receiving the cat food experimental diets were subjected to repeated measures ANOVA (SAS, 1985) with diet as the variable and time (d) as the repeated factor.

Results

The measured lethality values for the differently processed batches of cat food, as calculated using the temperature data recorded with the data logger at the center of the can, were 5.3, 8.6, 17.2, and 24.2.

The rats consumed the experimental diets readily and appeared healthy throughout the study. The rats gained weight during the trial; they weighed 157 g (SE 4.0) on d 1 and 187 g (SE 4.3) on d 9. There was no difference ($P > .05$) in food intake of the rats receiving the different cat food diets as analyzed by

Table 3. Amino acid composition (g/16 g N) of a canned cat food heat-treated to contain different lethality values (min)

Amino acid	Lethality value				
	0	5.3	8.6	17.2	24.2
Arginine	6.2	6.3	6.5	6.5	6.4
Histidine	2.6	2.6	2.7	2.6	2.7
Isoleucine	3.3	3.4	3.5	3.5	3.5
Methionine	2.1	2.2	2.3	2.3	2.3
Leucine	7.8	7.9	8.1	8.1	8.1
Lysine	6.4	6.8	6.9	6.9	6.9
Phenylalanine	4.5	4.7	4.8	4.8	4.9
Threonine	4.3	4.3	4.5	4.4	4.5
Valine	5.0	5.1	5.2	5.2	5.2
Alanine	6.8	6.9	7.0	7.0	7.0
Aspartic acid	9.0	9.1	9.4	9.4	9.4
Glutamic acid	12.2	12.5	13.1	13.0	13.1
Proline	5.8	5.9	6.2	6.3	6.5
Glycine	8.5	8.8	9.0	8.8	9.0
Serine	4.6	4.8	4.9	4.9	4.9
Tyrosine	3.1	3.2	3.4	3.3	3.4

repeated measures analysis. There was an effect ($P < .001$) of time on the food intake of the rats; food intake increased as the study proceeded. The food intake of rats receiving the experimental diets containing cat food on the first (total of nine meals) and last (total of six meals) days of the study were 14.4 (SE, .39) and 11.7 g (SE, .50), respectively. The food intake of rats fed the EHC-based diet on the first (total of nine meals) and last (total of six meals) days were 12.7 (SE, .38) and 13.5 g (SE, .11), respectively. Feces were not detected in the gastric content at slaughter, indicating that coprophagy had not occurred.

The amino acid composition of the unprocessed (F_0) and processed ($F_0 = 5.3, 8.6, 17.2,$ or 24.2) cat food is shown in Table 3. There was no apparent decrease in the concentration of amino acids due to the processing of the cat food.

The crude protein and total and reactive lysine content of the cat food containing different lethality values is given in Table 4. The differences in crude protein, total lysine, and reactive lysine content among the five cat foods were small. The crude protein content varied between 563 ($F_0 = 8.6$) and 568 g/kg

dry matter ($F_0 = 17.2$), and the total lysine content varied from 36.2 ($F_0 = 0$) to 39.2 g/kg dry matter ($F_0 = 17.2$ and 24.2). The FDNB-reactive and OMIU-reactive lysine content of the cat foods with different lethality values, when expressed on a dry matter or crude protein basis, were consistently lower than the corresponding total lysine values. The differences between the FDNB-reactive lysine content and the OMIU-reactive lysine content were small. There was no significant change in the reactive lysine content due to the processing of the cat food.

The true ileal amino acid digestibility and amino acid nitrogen digestibility of the unprocessed cat food and the processed cat foods are shown in Table 5. There was an effect ($P < .05$) of lethality value on the true ileal digestibility of all amino acids, and the majority of the effects were significant at $P < .001$. In general, the true ileal amino acid digestibility coefficients were high in the unprocessed cat food and decreased with increasing lethality value. The true ileal digestibility of arginine, histidine, alanine, proline, and glycine, however, increased as a result of the first heat treatment ($F_0 = 5.3$), whereafter the

Table 4. The concentration (g/kg dry matter) of crude protein, total lysine, fluorodinitrobenzene (FDNB)- and O-methylisourea (OMIU)-reactive lysine of a cat food heat-treated to contain different lethality values (min)

Chemical component	Lethality value				
	0	5.3	8.6	17.2	24.2
Crude protein	567	564	563	568	565
Total lysine	36.2 (6.4) ^a	38.1 (6.7)	39.1 (6.9)	39.2 (6.9)	39.2 (6.9)
FDNB-reactive lysine ^b	32.9 (5.8)	31.2 (5.5)	32.3 (5.7)	34.1 (6.0)	32.4 (5.7)
OMIU-reactive lysine	31.9 (5.6)	32.9 (5.8)	34.1 (6.1)	32.7 (5.8)	32.4 (5.7)

^aThe value in parentheses is the percentage of the crude protein content.

^bThe correction factor used for the FDNB method was 1.05.

Table 5. Mean true ileal amino acid digestibility coefficients (%) of a canned cat food heat-treated to contain different lethality values (min)^a

Amino acid	Lethality value					Overall SE	P <
	0	5.3	8.6	17.2	24.2		
Arginine	87.8	90.0	86.9	84.8	84.5	.81	.001
Histidine	69.4	75.7	67.0	69.6	64.2	1.95	.01
Isoleucine	85.2	80.8	76.5	73.5	72.0	1.31	.001
Methionine	85.9	81.9	78.2	75.2	75.0	1.12	.001
Leucine	87.7	83.2	79.7	77.6	76.7	1.04	.001
Lysine	84.2	84.1	81.1	78.3	77.4	.94	.001
Phenylalanine	83.4	79.2	75.9	73.8	71.6	1.45	.001
Threonine	77.1	75.3	70.5	66.9	64.5	1.61	.001
Valine	84.2	80.2	75.4	73.1	71.9	1.34	.001
Alanine	77.8	80.0	75.7	71.9	71.7	1.25	.001
Aspartic acid	78.3	58.7	49.0	43.0	40.2	2.43	.001
Glutamic acid	81.3	80.2	76.1	72.5	71.6	1.25	.001
Proline	64.2	72.4	69.2	66.2	64.7	1.89	.05
Glycine	44.8	62.3	52.9	46.4	45.0	2.78	.001
Serine	75.7	74.2	69.8	64.7	62.6	1.62	.001
Tyrosine	87.6	80.9	77.1	74.5	73.2	1.62	.001
Amino acid nitrogen	76.7	77.4	72.5	69.3	67.9	1.37	.001

^aValues are means for six rats.

digestibilities of these amino acids decreased similarly to the other amino acids.

Discussion

Animal models are often used for ethical or practical reasons because direct measurements on the animal species of interest may be difficult to obtain. The rat is a convenient animal model, and is often used for determination of protein quality in human diets (FAO/WHO, 1990). Recently the rat was shown to be an accurate animal model for estimating the ileal digestibility of amino acids of protein sources used in diets for pigs (Donkoh et al., 1994; Pearson et al., 1998) and chinook salmon (Wright, 1996). In the present study, besides various *in vitro* assays, an *in vivo* rat ileal digestibility assay was used to measure protein quality. The laboratory rat, however, has not been validated as an animal model for the digestion of protein in diets for domestic cats. An indication of the suitability of the rat as an animal model for the digestion of protein in cats can be obtained from the anatomy of the digestive tract of both animals. The ratios of mucosal to serosal area of the intestine of cats have been found to be as follows: jejunum 15:1, ileum 12:1, and colon 1:1, and these values for rats are 6:1, 4:1, and 1:1, respectively (Wood, 1944). Even though the mucosal area to serosal area is greater in cats, the ratios of mucosal area of the entire small intestine to body weight (absorbable surface area of amino acids per unit body weight) in cats and rats are almost identical (Wood, 1944). Gross anatomy of the digestive tract, therefore, indicates that the rat may be a suitable animal model for the digestion of protein in

cats. However, many other factors also determine the *in vivo* digestibility of protein, and the rat as an animal model for cats, will need to be validated experimentally.

The effectiveness of a heating process in achieving product sterility can be evaluated by determination of the lethality value (F_0) of the process. The F_0 represents the time equivalent of a heating process to destroy microorganisms at the reference temperature of 121.1°C, and serves as a standard to compare sterilization values for different processes. A product processed to an F_0 of 10, theoretically, gives 100% sterilization, because this is equivalent to processing for 10 min at 121.1°C (Ball and Olson, 1957). Rather than measuring the external thermal energy applied to the product, F_0 provides a measure of the effectiveness of the heating applied at the coldest site within the product. The lethality value of a process can be calculated using heat transfer equations with the appropriate boundary conditions or can be determined from the relationship between time and temperature (Holdsworth, 1985). The data logger used in the present study recorded the temperature at the center of the can at 5-min intervals and provided accurate information on the time-temperature relationship at the coldest point of the can during processing. The F_0 values calculated using the time-temperature relationship for the various batches (5.3, 8.6, 17.2, and 24.2) agreed closely with the targeted F_0 values of 5, 10, 15, and 25 (calculated based on previous heat penetration data), although one batch was slightly underprocessed ($F_0 = 8.6$) and another batch was slightly overprocessed ($F_0 = 17.2$). Canned pet foods are normally heat-processed to an F_0 value of 12 to 14, and a value of 8 is accepted as the minimum. Heat processing the

center of a can to an F_0 value lower than 8 to 10 increases the risk of spoilage of the product by microorganisms during storage and may ultimately result in cans exploding due to a build-up of pressure.

Heat processing of the canned cat food in the present study did not alter the amino acid content of the diet. These results indicate that heat treatment of canned cat foods does not result in destruction of amino acids. The side chain of lysine readily combines with reducing sugars (Maillard reaction), oxidizing lipids, and polyphenolic acids during processing and storage to give covalent complexes or oxidation products (Hurrell, 1989). It has been suggested (NRC, 1986; Morris and Rogers, 1994; Heinicke, 1995) that lysine in diets for companion animals may undergo Maillard-type reactions during the heat-processing step in their manufacturing, thereby reducing lysine availability. In the present study, the total lysine content (expressed both on a dry matter and 16-g N basis) was slightly higher in the heat-treated samples than in the unheated sample, indicating that there was no significant destruction of lysine as a result of heat processing. Additionally, heat treatment did not result in a reduced reactive lysine content. The FDNB-reactive and OMIU-reactive lysine content remained relatively constant with increasing heat treatment of the diet. These results indicate that the ϵ -amino group of lysine did not react with other dietary components during heat processing of the moist cat food. This was unexpected, because lysine has been shown to undergo chemical reactions with other compounds present in a complex feed and feed ingredients under mild heating conditions (Rutherford and Moughan, 1997; Rutherford et al., 1997). Some lysine in the diet, however, was chemically unreactive, because the FDNB- and OMIU-reactive lysine contents were approximately 85% of the total lysine content. This unreactive or bound lysine, which reverted back under the conditions of acid hydrolysis, likely originated from the ingredients and/or a heat processing step used in the formulation of the diet, because the reactive lysine content in the unprocessed diet was also lower than the total lysine content.

Meat offals contain relatively large amounts of connective tissue that consist of collagen and elastin (Davey and Winger, 1979). Collagen naturally contains covalent cross-links involving lysine to maintain the native three-dimensional structure of the protein (Asghar and Henrickson, 1982; Singh, 1991). Collagen is, therefore, a natural source of bound lysine, which most likely explains the results found in the present study. The diet did not contain any blood meal or meat and bone meal, two ingredients that have been shown to contain bound lysine (Rutherford et al., 1997).

Heat treatment affected the true digestibility of all amino acids as measured at the terminal ileum of the rats, with a general tendency for true ileal amino acid

digestibility coefficients to be high in the unprocessed cat food and to decrease with increasing lethality value. The digestibility coefficient of aspartic acid showed the largest change, decreasing approximately 38 percentage units. For glycine and proline there was a marked increase in digestibility due to the first heat treatment ($F_0 = 5.3$). As mentioned previously, meat offals contain relatively large amounts of connective tissue that consist of collagen and elastin, two types of protein high in glycine and proline (Asghar and Henrickson, 1982). Upon heat treatment, collagen is gelatinized (Hamm, 1974; Asghar and Henrickson, 1982) and becomes more digestible. The relatively mild heat treatment employed to obtain the diet with an F_0 value of 5.3 is likely to have gelatinized the connective tissue, making it more digestible and, as a result, increased the digestibility especially of glycine and proline.

There are several possible explanations for the reduction in amino acid digestibility with increasing heat treatment. The food intake of rats on the different diets was similar, indicating that the differences in amino acid digestibility seen in the present study were not caused by differences in food intake. Changes in digesta viscosity have been shown to affect the digestibility of nitrogen and amino acids in other animals, such as chickens (Smits et al., 1997) and pigs (van Barneveld et al., 1995). Guar gum, a soluble nonstarch polysaccharide (NSP), is a common ingredient in canned cat foods and has been shown to decrease the digestibility of protein in diets for cats (Harper and Siever-Kelly, 1997). The diet used in the present study was formulated using guar gum, locust bean gum, and carrageenan; all are soluble NSP sources. It is possible, therefore, that the different heat treatments affected the viscosity of the diet, thereby causing differences in digesta viscosity and affecting amino acid digestibility. The viscosity of the five diets, when analyzed using a cone-plate viscometer (Smits et al., 1997), was similar (data not shown), suggesting that the reduction in amino acid digestibility with increasing heat treatment was caused by differences other than digesta viscosity.

A likely cause of the decrease in amino acid digestibility with increasing heat treatment may have been the formation of cross-linkages between various amino acids within and between proteins. Cross-linking reduces the rate of protein digestion by preventing enzyme penetration or by masking the sites of enzyme attack (Hurrell and Finot, 1985) and can occur between many amino acids. However, lysine, cysteine, and phosphoserine seem to be the most susceptible (Bender, 1978; Singh, 1991). Wiseman et al. (1991) subjected commercially processed fish meal to additional heating at 130°C for 3 h or 160°C for 1.5 h and measured the ileal digestibility of amino acids in pigs. There was no effect of the additional heating on the level of amino acids (including lysine) in the

fish meal. However, the 2,4,6-trinitrobenzene sulphonic acid reactive lysine level and the ileal digestibility of all amino acids decreased with increasing heat treatment, indicating that cross-linking had occurred. In the present study, the reactive lysine content did not decrease with increasing heat treatment of the diet, indicating that cross-linkages involving lysine were not formed. The latter casts doubt on cross-linking being the mechanism responsible for the reduction in ileal digestibility of amino acids in the present study. However, other amino acids such as cysteine may have formed cross-linkages, thereby reducing the digestibility of amino acids.

Another possible mechanism to explain the decrease in true ileal digestibility of amino acids with increasing heat treatment is increased gut endogenous amino acid excretions with increasing heat treatment. The EHC/ultrafiltration method used in the present study determines the endogenous amino acid losses, which are due to the ingestion of the N-free basal mixture plus protein source (Boisen and Moughan, 1996). It is possible that other components that may have formed in increasing amounts with increasing heat treatment in the cat food caused "additional" endogenous amino acid excretions. Because these additional endogenous amino acid losses would not have been measured in the present study, these increased losses may have lowered the digestibility of the amino acids. However, in order to explain the reduction in aspartic acid digestibility, the endogenous excretions of the rats fed the experimental diet containing cat food with an F_0 value of 24.2 must have been increased by a factor of five.

In addition to the changes in protein quality found in the present study, other effects of heat processing on moist canned cat foods have been observed. Heinicke (1995) noted that a longer processing time negatively affects the palatability of canned cat foods, an observation that we have also seen. Furthermore, processing of canned cat foods has been shown to increase the endogenous taurine loss from the gastrointestinal tract of cats (Hickman et al., 1992). Kim et al. (1996a) showed that the cause of the increased endogenous taurine loss was due to the increased deconjugation of bile acids by microorganisms. These authors hypothesized that a greater level of available substrate at the lower intestine is responsible for the increase in microbial activity and, subsequently, causes the increased deconjugation of bile acids in cats (Kim et al., 1996b). The present study supports this hypothesis; it was shown that the ileal amino acid digestibility of a canned cat food decreases with increasing heat treatment, resulting in more undigested amino acids at the terminal ileum.

Heat treatment of canned cat foods results in changes in the digestibility of amino acids, and most amino acids show a decrease in digestibility. The likely cause of this decrease in digestibility is at

present unknown, and further studies are required. Although the digestibility of lysine is reduced due to the heat treatment of canned cat foods, the ϵ -amino group of lysine does not seem to react with other dietary components. The heat treatment of canned cat foods, furthermore, does not result in destruction of amino acids.

Implications

The processing of canned moist diets for cats results in changes in the digestibility of amino acids, and the majority of amino acids show a decrease in digestibility as heat processing time increases. Overprocessing above the minimum time required for sterilization of the product results, therefore, in an avoidable loss of amino acids. Accurate control on heat processing conditions of canned moist diets for cats is important to minimize the reduction in amino acid digestibility, to maintain a high palatability, and potentially to maintain adequate taurine status.

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