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Feline Trichobezoars: Composition and Degradation

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Abstract: Trichobezoars (TCBs) are an affliction of domesticated cats; they rarely are fatal but can cause undesirable social behavior. Bromelain, a cysteine protease found naturally in pineapple juice, has been suggested as a practical dietary treatment, but there are few data on its activity. Little is known about the chemical composition of TCBs; such data are needed in developing a treatment. Samples of TCBs and hair were obtained from cats in a commercial facility and analysed for amino acids and total protein concentrations. Amino acid concentrations varied dramatically among TCBs but not among hair samples, suggesting that the proportions of hair and non-hair components varied markedly among TCBs. TCBs were estimated to contain 42% hair on average, but there was substantial variation among samples. Bromelain was able to degrade N in TCBs more effectively at a pH of 7.0 than at a pH of 2.5, but differences were not large (37 at pH of 7.0 vs. 26 % at 2.5). Some TCBs were degraded less than 10%, while others were degraded more than 50%; the variation probably reflected amount of non-hair material. While bromelain did not degrade TCBs extensively or consistently; the degradation that did occur probably was sufficient to disrupt the structure of TCBs and promote passage. Testing is needed to establish in vivo efficacy.

Key words: Trichobezoar, Bromelain, Hair, Cat

Introduction

Gastric trichobezoars (TCB) can occur in companion animals such as rabbits and cats. TCBs occur in rabbits about 25 % of the time (Hartman, 1997) and can be fatal. In cats, TCBs are not considered to be life threatening because of the cat's ability to vomit, but decreasing the incidence could reduce health concerns and eliminate undesirable behavior. An effective, practical treatment for TCB has not been reported. This is complicated by an apparent lack of published data on the composition of TCB; such data are helpful in developing an effective treatment strategy.

Pineapple juice contains the enzyme bromelain; bromelain is a cysteine protease and has been suggested as a treatment for TCB. Carpenter et al. (1995) incubated rabbit hair in papaya juice, pineapple juice (which contains bromelain), Viokase-V (a proteolytic enzyme) and saline (pH 2.0) for 24, 48 or 72 h at 38C. There was no significant degradation for any treatment; Carpenter et al. (1995) concluded that bromelain could not degrade rabbit hair. Hotchkiss (1995) incubated a rabbit TCB with bromelain; there were no changes in physical appearance of the TCB. While these limited reports provide some initial information of the effectiveness of bromelain in degrading TCB, there are few quantitative data on enzymatic activity of bromelain and factors that affect its effectiveness. The objectives were to: (1) determine the composition of TCB, (2) evaluate the proteolytic activity of two bromelain sources, and (3) evaluate the ability of bromelain to degrade TCB.

Materials and Methods

Chemical Composition of Trichobezoars and Hair: Fifteen TCB samples were obtained from commercial cat facilities. Preliminary chemical analyses as well as direct observation made it apparent that the distribution of components (hair, feed particles, mucous, etc.) in TCB varied dramatically. This made it essentially impossible to obtain representative sub-samples of intact specimens. In addition, moisture content among specimens was variable. To minimise the effects of these biases, TCB samples were dried at 50 C for 48 hours, ground through a 1.0 mm screen and thoroughly mixed. In addition to TCB samples, five hair samples were obtained from cats at a commercial facility and ground through a 1.0 mm screen.

Dry matter concentrations of TCBs and hair samples were determined by drying overnight at 105 C. Nitrogen content was determined via thermal conductivity (Leco, 1994). Crude protein was estimated as nitrogen times 6.25. Amino acid concentrations of TCBs and hair were measured by high performance liquid chromatography (A.O.A.C., 1984).

Activity of Bromelains Against Gelatin and Soybean Meal Protein: Bromelain was obtained from two biochemical suppliers (B1 and B2). Enzyme concentration and enzymatic activity can vary with the manufacturer; therefore, the enzymatic activity of each bromelain was determined so that they could be compared on the same basis. The assay was a titrimetric quality control procedure (Sigma, 1998). Five grams of gelatin (300 bloom, porcine skin,

Sigma Chem. Co., St. Louis, MO) were dissolved in 100 ml deionized water, placed in water bath (80 C) for 20 minutes and cooled to 45 C. One gram of bromelain was dissolved in 100 ml of a 0.1 N sodium acetate/2.6 M sodium chloride buffer; pH was adjusted to 4.5 with 1.0 M sodium hydroxide. For each bromelain source, aliquots (25 ml) of gelatin solution were placed in four Erlenmeyer flasks (125 ml). Buffer solution (1.0 ml) was added to two flasks (B1 and B2); buffer without enzyme (1.0 ml) was added to two other flasks (controls). Contents were incubated for 20 min at 45 C. Three percent (v/v) hydrogen peroxide (0.1 ml) was added to all flasks to inactivate the bromelain, and an additional 1.0 ml of enzyme solution was added to each of the two control flasks. All flasks were adjusted to pH 6.9 with 0.05 N sodium hydroxide, followed by the addition of 10.0 ml of 37 % formaldehyde. Enzymatic activity was measured by titrating the contents of each flask to pH 7.8 with 0.05 N sodium hydroxide; the volume of titrant was recorded. Enzymatic activity was calculated as (Sigma, 1998):
Units/g enzyme = (V1 – V2) *(N) *(14) * (1000)/(mg enzyme/ l reaction mixture),
Where:
V1 = titration volume for unknown
V2 = titration volume for control
N = normality of sodium hydroxide used for titration
14 = molecular weight of N
1000 = milligrams/gram
One unit was defined as the quantity of enzyme that will hydrolyze 1.0 mg of amino nitrogen from gelatin in 20 min at a pH of 4.5 and at 45 C (EC 3.4.22.32, Sigma Chemical Co., St. Louis, MO).
TCBs are likely to contain significant amounts of dietary material (protein); it would be important to know if bromelain could degrade dietary proteins or if dietary proteins might interfere with activity of bromelain. Soybean meal was used to determine this possibility. Bromelain was dissolved in either distilled water or 0.1N HCl at concentrations of 0.0 (controls), 0.16, 0.32, 0.65, 1.30, 2.60 to 5.20 units/ml. Distilled water would not represent the acidic conditions of a cat's stomach, but it would be important to determine if activity of bromelain against SBM might differ under different pH conditions. For each bromelain concentration, 0.5 g of SBM was placed into eight Erlenmeyer flasks (125 ml). Fifty ml of the bromelain/distilled water solution were added to each of four flasks; 50 ml of the bromelain/0.1N HCl solution were added to each of the other four flasks. Starch can interfere with proteolysis (Tomankova and Kopecny, 1995). Therefore, effects of amylase on the proteolytic activity of bromelain were evaluated. One hundred fifty-five units of amylase (Amylase 3403, Sigma Chemical Co., St. Louis, MO) were added to two of the four flasks containing bromelain/distilled water solution and to two of the four flasks containing bromelain/0.1N HCl solution. Contents were incubated for 8 hours in a water bath (39 C). Flasks were removed and contents filtered immediately through pre-weighed filter paper (hardened and ashless). Filter papers and residues were dried overnight at 105 C and reweighed; dry matter disappearance (DMD) was calculated as:
DMD = (100-(residue weight/sample weight)*100).
Nitrogen content of residues was determined by thermal conductivity (Leco, 1994). Nitrogen disappearance (ND) was calculated as:
ND = (100-(residue nitrogen weight/sample nitrogen weight)*100).
The experiment was repeated three times.

Effect of pH on Degradation of SBM Protein by Bromelains: Effects of pH on activity of each bromelain source were determined using SBM as the substrate. Nine buffers (pH = 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0 or 5.5) were prepared by adjusting a 0.1N HCL solution with 1.0 M sodium bicarbonate; a tenth (pH = 7.0) contained only distilled water. Each buffer (~ 1.5 L) solution was divided into three 500 ml aliquots; B1 was added to one aliquot and B2 was added to the second, each to result in a final concentration of 1.3 units/ml (based on previous assays). Bromelain was not added to the remaining aliquot (control buffer). For each buffer, 0.5 g of SBM was added to each of 12 flasks. Fifty ml B1 (in buffer) were added to each of 4 flasks, and 50 ml of B2 (in buffer) were added to each of four more flasks; 50 ml of control buffer were added to the remaining 4 flasks (controls). Flasks and contents were placed in a water bath (39 C) for 8 hours. Flasks were removed, and contents filtered immediately through pre-weighed filter paper (hardened and ashless). Filter papers and residues were dried overnight at 105 C and reweighed; DMD and ND were calculated as described for the enzyme concentration experiment. This experiment was repeated three times.

Degradation of TCB at a Neutral pH (7.0): Fifteen TCBs were obtained from cats at commercial facilities; they were dried and ground as described earlier. Sub-samples (ranging from 0.3 to 0.9 g each) were placed in pairs of 125 ml Erlenmeyer flasks. For this and subsequent experiments, only one bromelain was used because activities of the two bromelains were very similar and because there was limited amount of TCB for substrate. Bromelain was added to distilled water (pH 7.0) to effect a concentration of 1.3 units/ml. Fifty ml of bromelain/distilled water

Table 1: Amino acid and total protein content (% dry matter) of TCB samples

Item	TCB samples number 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Mean	CV1
Aspartic acid	2.75	2.25	2.68	2.75	3.28	2.65	2.34	2.99	1.64	2.73	3.24	2.43	3.16	2.76	3.54	2.74	17.2
Threonine	2.44	1.18	1.92	2.25	2.71	2.35	1.86	2.52	1.34	2.22	2.73	2.06	2.86	2.42	3.13	2.27	23.6
Serine	3.06	1.13	2.50	2.67	3.22	2.90	2.23	3.09	1.75	2.76	3.23	2.45	3.63	3.03	3.87	2.77	25.3
Glutamic acid	5.40	4.53	4.87	4.80	5.84	5.23	4.09	5.76	3.04	4.99	5.63	4.57	6.03	5.36	6.25	5.09	16.3
Proline	2.94	1.93	2.37	2.68	3.30	2.83	2.22	3.03	1.59	2.59	3.04	2.39	3.36	3.00	3.55	2.72	20.1
Glycine	2.51	1.76	2.51	2.55	3.19	2.44	1.92	2.69	1.46	2.53	2.79	2.01	3.02	2.75	3.19	2.49	20.4
Alanine	1.51	1.93	1.70	1.54	1.84	1.48	1.30	1.68	0.98	1.59	1.85	1.35	1.73	1.159	1.96	1.60	16.3
Cysteine	6.05	1.11	3.62	5.33	6.49	6.15	4.20	6.01	3.18	4.90	6.01	4.85	6.92	6.03	7.11	5.20	31.0
Valine	2.06	1.53	1.98	2.10	2.53	2.04	1.72	2.21	1.22	2.00	2.44	1.74	2.31	2.08	2.67	2.04	18.7
Methionine	0.43	0.58	0.52	0.44	0.52	0.41	0.39	0.50	0.26	0.48	0.55	0.42	0.49	0.45	0.55	0.47	17.3
Isoleucine	1.21	1.21	1.24	1.26	1.56	1.14	1.12	1.39	0.76	1.26	1.54	1.14	1.33	1.21	1.57	1.26	16.3
Leucine	2.81	3.02	2.80	2.88	3.42	2.81	2.40	3.23	1.64	2.82	3.33	2.55	3.37	3.04	3.69	2.92	16.9
Tyrosine	1.73	1.16	1.51	1.89	2.29	1.67	1.33	2.06	0.90	1.63	2.02	1.46	2.36	2.04	2.48	1.77	25.6
Phenylalanine	1.29	1.37	1.28	1.36	1.61	1.30	1.17	1.52	0.77	1.37	1.62	1.19	1.58	1.41	1.77	1.37	17.4
Histidine	0.67	0.64	0.67	0.69	0.81	0.68	0.57	0.77	0.38	0.68	0.82	0.61	0.80	0.71	0.86	0.69	17.3
Ornithine	0.13	0.05	0.12	0.07	0.10	0.12	0.05	0.09	0.07	0.07	0.08	0.07	0.08	0.05	0.07	0.08	29.3
Lysine	1.55	1.33	1.53	1.52	1.79	1.47	1.25	1.67	0.87	1.51	1.85	1.41	1.74	1.54	1.88	1.53	16.9
Arginine	3.47	1.73	2.55	3.13	3.87	3.37	2.47	3.45	1.76	3.06	3.57	2.84	4.08	3.47	4.36	3.15	24.4
Tryptophan	0.20	0.28	0.15	0.13	0.20	0.12	0.12	0.21	0.06	0.20	0.24	0.12	0.10	0.10	0.19	0.16	37.8
Total AA[2]	42.48	29.18	36.71	40.12	48.66	32.82	44.99	23.74	39.69	46.70	36.01	49.08	43.19	52.81	40.50	40.50[a]	19.4
Total protein[3]	43.85	31.59	41.73	44.35	53.72	45.58	36.02	51.89	26.72	43.30	52.21	42.86	56.13	48.61	60.75	45.21[b]	20.0

[ab]Total amino acid and total protein means differ (P+0.14); SE = 2.21

[1]Coefficients of variation [2]Amino acids [3]Total protein = N * 6.25

Table 2: Amino acid and total protein content (% dry matter) of hair samples

Item	Hair sample number 1	2	3	4	5	Mean	[1]CV
Aspartic acid	5.48	5.47	5.12	5.32	5.35	5.35	2.7
Threonine	4.50	4.79	4.44	4.41	4.44	4.52	3.5
Serine	5.21	6.53	5.28	5.11	6.05	5.64	11.1
Glutamic acid	11.66	12.69	11.40	12.01	12.62	12.08	4.7
Proline	5.71	6.11	5.54	5.48	5.37	5.64	4.4
Glycine	4.85	4.92	5.10	4.83	4.72	4.88	2.2
Alanine	3.44	2.99	3.24	3.33	3.04	3.21	5.9
Cysteine	11.86	13.26	11.75	11.59	11.10	11.9	6.8
Valine	4.16	3.99	3.92	3.99	3.73	3.96	3.9
Methionine	0.92	0.95	0.84	0.89	0.93	0.91	4.7
Isoleucine	2.48	2.40	2.25	2.38	2.23	2.35	4.5
Leucine	6.09	6.00	5.74	5.95	5.92	5.94	2.2
Tyrosine	3.96	3.95	4.11	3.79	3.87	3.94	3.0
Phenylalanine	2.70	2.56	2.53	2.56	2.51	2.57	2.9
Histidine	1.30	1.29	1.27	1.31	1.28	1.29	1.2
Ornithine	0.66	0.77	0.70	0.79	0.73	0.73	7.2
Lysine	3.17	3.19	3.10	3.19	3.22	3.17	1.4
Arginine	7.05	7.46	6.84	6.99	6.85	7.04	3.6
Tryptophan	0.20	0.23	0.21	0.22	0.26	0.22	10.3
Total amino acids	85.51	89.66	83.51	84.28	84.35	85.47	2.6
Total protein	91.34	91.46	89.89	88.99	86.10	89.56	2.2

[1]CV = coefficient of variation; SE = 1.04 [ab]Means differ (P<0.05)

solution were added to one flask of each pair (bromelain). Fifty ml of distilled water (pH 7.0) were added to the other flask (control). Contents were incubated in a water bath (39 C) for 8 hours, removed and filtered immediately through pre-weighed filter paper (hardened and ashless). Filter papers and residues were dried overnight at 105 C and reweighed; DMD and ND were calculated as described for the enzyme concentration experiment.

Degradation of TCB at a Low pH (2.5): Thirty-three TCBs were obtained, dried and ground as described previously. Sub-samples (ranging from 0.3 to 1.1 g) were placed in pairs of 125 ml Erlenmeyer flasks.

A 0.1N HCL buffer was prepared by diluting conc. HCL with distilled water; bromelain was added to half of the HCL buffer (pH 2.5) to result in a final concentration of 1.3 units/ml. The other half contained no bromelain (only

Table 3: Estimates of hair content of TCBs based on cysteine and glutamic acid concentrations of diets.

	% Hair in TCB 1 when			
	Dietary cysteine concentration was:		Dietary glutamic acid concentration was:	
Sample Number	33%	0.46%	4.05%	4.62%
1	49.4	48.8	16.8	10.5
2	6.7	5.7	6.1	-1.2
3	28.4	27.6	10.2	3.4
4	43.2	42.5	9.3	2.4
5	53.2	52.7	22.3	16.4
6	50.3	49.7	14.7	8.2
7	33.4	32.7	0.5	-7.1
8	49.1	48.5	21.3	15.3
9	24.6	23.8	-12.6	-21.2
10	39.5	38.8	11.7	5.1
11	49.1	48.5	19.7	13.5
12	39.1	38.3	6.5	-0.7
13	56.9	56.4	24.7	18.9
14	49.2	48.6	16.3	9.9
15	58.5	58.1	27.4	21.8
Means	42.1	41.4	13.1	6.3

[1]Hair was assumed to contain 11.91% cysteine and 12.08 % glutamic acid

Table 4: Dry matter (DMD) and N (ND) degradation[1] of soybean meal at increasing pHs

pH	DMD[2]			ND[3]		
	Control	B1[2]	B2[4]	Control	B1[2]	B2[4]
1.5	57.29	58.54	58.16	36.74	38.29	38.38
2.0	41.78	50.65	49.74	4.72	27.09	22.98
2.5	43.46	54.48	62.49	7.79	60.35	55.42
3.0	44.48	69.85	65.60	10.49	73.24	64.79
3.5	47.21	71.72	66.73	16.14	76.08	67.63
4.0	46.09	69.88	66.09	12.64	70.20	65.84
4.5	45.37	71.67	68.83	11.97	76.36	70.48
5.0	47.86	73.68	71.16	17.82	79.50	75.52
5.5	51.37	77.58	74.85	26.64	88.66	85.70
7.0	53.30	72.91	72.55	28.93	74.96	74.47

[ab]Within main effect (DMD or ND), mean within same row and with unlike letters differ ($P<0.05$)
[1]%Dry matter or N loss at 8 h [2]SE=0.70 [3]SE = 1.13
[4]B1 and B2 = Bromelain 1 and 2

0.1N HCL). Fifty ml of the bromelain/HCl solution were added to one flask of each pair (bromelain). Fifty ml of the 0.1N HCl solution (pH 2.5) were added to the other flask (control). Flasks were incubated in a water bath (39 C) for 8 hours and removed; contents were filtered immediately through pre-weighed filter paper (hardened and ashless). Filter papers and residues were dried overnight at 105 C and reweighed; DMD and ND were calculated as described for the enzyme concentration experiment.

Statistical Analyses: Analytical data for TCB and hair samples were not replicated because of lack of sufficient sample; means and coefficients of variation (CVs) were determined for each amino acid across TCBs (SAS, 1989). Mean total amino acids and crude protein data were analysed across TCBs using a simple block design (SAS, 1989); means were compared by least significant difference (LSD). The DMD and ND data for SBM were replicated and were analysed as a two way factorial with pH and enzyme treatments as main effects; means were compared

Table 5: Dry matter (DMD) and N degradability (ND) of TCBs at a pH of 7

Sample No	DMD1,2		ND1,3	
	Control	Bromelain	Control	Bromelain
1	26.22	29.48	27.53	30.16
2	31.60	31.55	17.02	17.91
3	28.96	30.23	22.74	27.06
4	28.78	30.53	11.86	17.23
5	32.38	30.96	24.38	28.18
6	29.68	30.31	14.88	19.37
7	28.15	26.41	20.63	19.88
8	31.51	30.82	21.04	28.93
9	23.36	23.63	4.03	22.29
10	29.93	30.09	20.81	24.44
11	33.97	54.07	32.55	53.11
12	28.17	27.49	17.04	16.23
13	29.69	30.87	24.96	30.37
14	26.91	26.39	22.07	24.28
15	20.69	23.39	22.07	24.28
Means	28.67[a]	23.39[b]	19.71[c]	25.03[d]
CV[4]	11.89	23.32	35.11	37.16

[1]%loss of dry matter or N in 8 h [2]SE = 1.43 [3]SE = 2.12
[4]CV = coefficient of variation [ab]Means differ at P<0.05 [cd]Means differ at P<0.10

Table 6: Dry matter (DMD) and N degradability (ND) of TCBs at a pH of 2.5

Sample No	DMD[1,2]		ND[1,3]	
	Control	Bromelain	Control	Bromelain
1	28.76	26.04	11.79	16.45
2	25.00	23.94	11.46	18.55
3	20.66	20.04	24.43	26.12
4	28.17	28.03	31.84	29.06
5	22.26	21.58	35.87	38.46
6	24.29	23.95	21.01	27.54
7	38.22	30.22	15.24	28.2
8	20.91	21.60	83.51	25.93
9	20.11	18.69	-2.37	1.60
10	16.94	17.62	4.39	8.24
11	21.68	22.01	27.25	27.05
12	23.32	24.55	40.01	42.98
13	25.31	25.47	56.21	59.55
14	28.44	28.47	42.21	43.06
15	29.51	31.14	54.73	64.76
16	10.70	11.39	2.82	11.16
17	25.25	24.26	7.52	9.23
18	23.51	22.64	23.05	30.69
19	24.91	28.01	26.15	28.65
20	30.34	30.29	16.34	17.19
21	27.22	28.42	21.38	25.44
22	27.03	28.70	11.15	16.20
23	30.11	28.79	22.67	26.21
24	28.49	29.10	14.28	18.60
25	26.74	25.08	19.60	18.89
26	29.93	29.26	19.99	27.48
27	22.89	23.16	3.95	21.84
28	27.54	27.68	19.15	22.48

29	31.59	50.29	30.27	49.39
30	26.76	26.12	16.19	15.43
31	28.50	29.64	23.96	29.16
32	26.09	25.60	23.55	21.41
33	19.24	21.75	13.19	14.87
Means	25.49	25.87	21.15[a]	26.12[b]
CV[4]	19.38	23.76	64.52	52.83

[1]% loss of dry matter or N in 8 h
[2]SE = 0.97 [3]SE = 2.39 [4]CV = coefficient of variation [ab]Means differ at P = 0.15

June 26, 2004by LSD when effects were significant. The DMD and ND data for TCB were not replicated because of lack of sufficient sample; data were analysed as a simple block design, and mean values across TCBs were compared by LSD (SAS, 1989).

Results

Chemical Composition of TCB and Hair: Amino acid concentrations of TCB samples are presented in Table 1. There was considerable variation in amino acid concentrations among samples. For example, cysteine concentration ranged from 1.11 % (TCB #1) to 7.11 % (#15). The coefficients of variation (CVs) varied from ~16 to ~ 38 %; the standard error was 2.21. Because TCB can consist of differing proportions of hair, mucous, food residues, etc., such variation was not surprising. The mean for sum of total amino acids (across samples) was 40.50 %, (Table 1). This was significantly less than total crude protein (45.21 %, = total N * 6.25). The discrepancy between the two means could have been due to presence of non-protein nitrogen compounds (such as urea from saliva, etc.) in the total protein determination, which is unable to discriminate between protein and non-protein N sources.

Amino acid concentrations of hair samples (Table 2) had less variation than TCB; CVs for hair samples ranged from 1.42 to 11.06 %. Most amino acid concentrations had coefficients of variation less than 5 %. As in the TCB samples, the mean for sum of total amino acids (85.47 %) was significantly lower than crude protein (89.56 %). The amino acid concentrations in hair generally were about twice those of TCBs, indicating that much of the material in TCB was hair (Fig. 1).

The concentrations of glutamic acid and cysteine were not determined for the diets of animals from which the TCBs were collected; however, cysteine and glutamine concentrations of diets fed at these facilities typically range from 0.33 to 0.46 % and 4.05 to 4.62 %, respectively. Assuming that the concentrations of cysteine and glutamic acid in hair were 11.91 and 12.08 % (Table 2), respectively, the proportion of hair in each TCB sample was estimated as:

AA conc. in TCB = 100*{X*(AA conc. in hair) + 1-X * (AA conc. in diet)}

Where:

AA = % cysteine or % glutamic acid

X = proportion of hair

1-X = non-hair components

The contribution of amino acids from non-hair (endogenous) components, such as mucous and sloughed cells, probably was small; it was ignored in these estimates because of lack of analytical data. Estimates of the hair content of TCBs, using cysteine and glutamic acid as indicators, are presented in Table 3. Estimates of hair content based on cysteine ranged from 40 to 50 %. There was considerable variation among TCBs; one sample had estimates of less than 10 %. Estimates based on glutamic acid were smaller in magnitude (less than 30 %) and were considerably more variable than those based on cysteine; some estimates were negative.

The greater accuracy of cysteine as an indicator of hair content of TCBs may be related to relative concentrations in constituents. The cysteine concentration of soybean meal (0.83 %, NRC, 1982) was much lower than that of hair (11.10 to 13.26 %, Table 2), while the glutamic acid content of soybean meal (10.2 to 11.5%) was similar to that of hair (12.08 %). A change in proportion of dietary protein, such as soybean meal, would impact the cysteine content of the TCB much more than the glutamic acid content. Therefore, cysteine is more sensitive than glutamic acid for estimating hair and non-hair contents of TCBs. Using cysteine as an indicator, estimates of hair content of TCB ranged from ~ 6 to ~ 58 % (average of ~ 42 %); non-hair components varied from ~ 42 to ~ 94 % (average of ~ 58 %). To our knowledge, this is the first report on the detailed composition of TCB in the cat.

Activity of Bromelains Against Gelatin and Soybean Meal Protein: Using gelatin as a substrate, enzymatic activities varied from 882 units/g for B2 to 1596 units/g for B1, which were lower than the manufacturers' activities (2000-4000 units/g for B1; 240,000 units/g for B2). The discrepancy may be due to how activity was defined. In this paper, one unit was defined as the quantity of enzyme that will hydrolyze 1.0 mg of amino nitrogen from gelatin

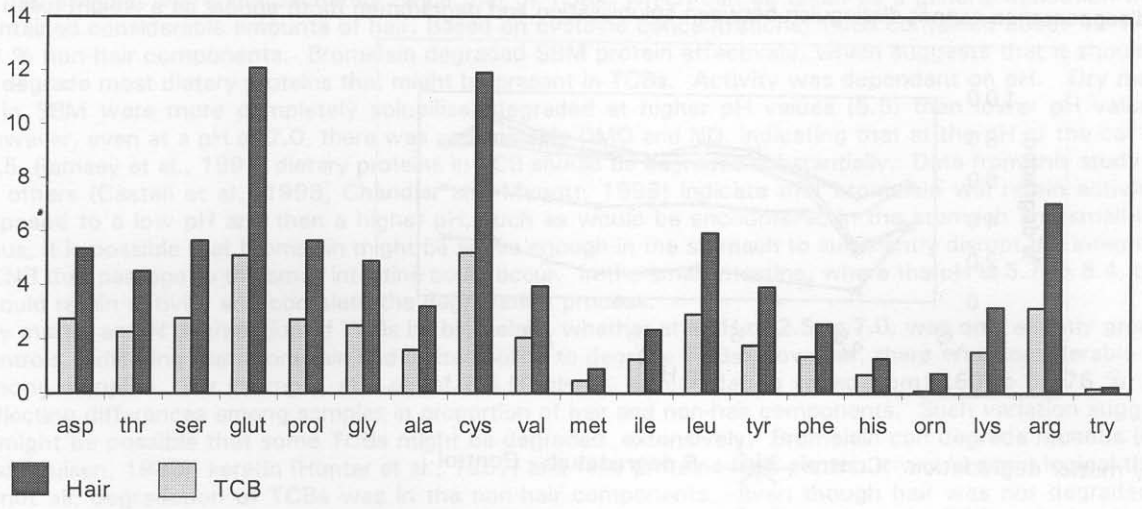

Fig. 1: Comparison of amino acids in TCB vs. hair

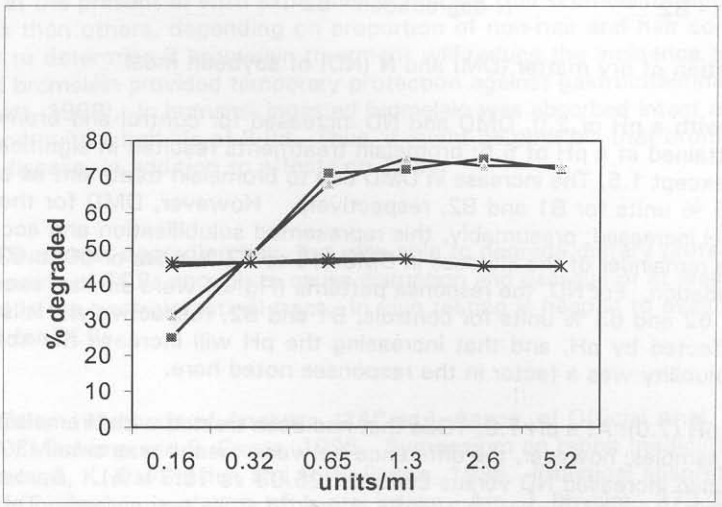

Fig 2: Effect of bromelain concentration on degradation of soybean meal

in 20 minutes at pH 4.5 at 45 C. Manufacturers used different substrates, pH values, temperatures, degradation times, and methods of measurement, making it difficult to compare activities across sources.

Optimum activity of the bromelains when SBM was used as the substrate depended on pH. When distilled water (pH 7.0) was used as the buffer, disappearance of N from SBM increased as bromelain concentration was increased from 0 to 1.3 units per ml of buffer; at concentrations higher than 1.3 units per ml, activity was unchanged or slightly declined (Fig. 2). When 0.10 N HCL was used as the buffer, activity ceased. The pH of 0.1N HCL is approximately 1.0, indicating that bromelain was inactive at this pH. Amylase had no effect on activity of bromelain.

Effect of pH on Degradation of Soybean Meal Protein by Bromelains: Dry matter degradation of SBM was not affected by bromelain source, but it was affected significantly by pH (Table 4). At a pH of 1.5, about 58 % of dry matter and 38 % of N disappeared. Because DMD and ND for the bromelain treatments were not different from controls and because bromelain is not active at this pH (Fig. 2), these disappearances most likely were due to solubilisation (possibly the result of hydrolysis) rather than degradation. The gravimetric method used to determine dry matter disappearance cannot distinguish between solubilisation and degradation (both appear as a weight loss).

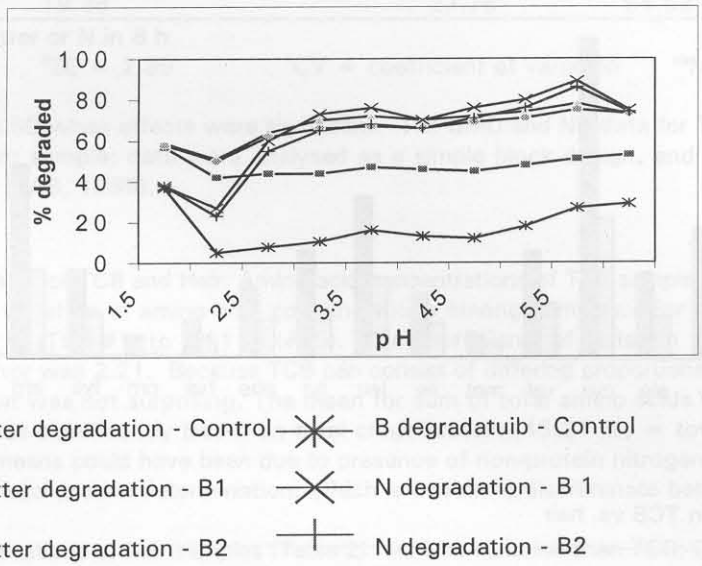

Fig. 3: Effects of pH on degradation of dry matter (DM) and N (ND) of soybean meal

As pH was increased, starting with a pH of 2.0, DMD and ND increased for control and bromelain treatments. Maximum DMD and ND were attained at a pH of 5.5; bromelain treatments resulted in significantly higher DMD and ND than controls at all pHs except 1.5. The increase in DMD due to bromelain treatment as pH was increased from 2.0 to 5.5 was 27 and 25 % units for B1 and B2, respectively. However, DMD for the control samples increased about 10 % units as pH increased; presumably, this represented solubilisation and accounted for about 37 to 40 % of the increase. The remainder of the increase in DMD (15 to 17 % units or 60 to 63 %, respectively) was assumed to be due to degradation. For ND, the response patterns (Fig. 3) were similar, except the increases were of greater magnitude (24, 62 and 63 % units for controls, B1 and B2, respectively). It is well known that solubility of feed proteins is affected by pH, and that increasing the pH will increase the solubility of certain proteins. It seems likely that solubility was a factor in the responses noted here.

Degradation of TCB at a Neutral pH (7.0): At a pH 7.0, TCBs that had been treated with bromelain had significantly higher DMD values than control samples; however, the difference between means was small (30.41 vs 28.67 %, Table 5). Bromelain treatment also increased ND versus controls (25.03 vs 19.71 %). Across TCBs, variation in DMD was smaller than ND (CVs were 11.9 and 23.2 vs 35.1 and 37.2 %, respectively, Table 5). The range for ND was from ~ 4 to ~ 30 %. For some TCBs (i.e., nos. 7 and 12), DMDs and NDs were slightly higher when incubated in buffer than when incubated with bromelain. The reason for this is not apparent, but may be due to sampling or analytical errors. The variation in the DMD and ND data probably reflects the heterogeneous nature of TCBs.

Degradation of TCB at a Low pH (2.5): When incubated with bromelain at a pH of 2.5, DMDs of TCBs were not different from those of controls (25.49 vs 25.87 %, Table 6); ND was greater for the bromelain treatment (21.15 vs. 26.12 %), but the significance level was not high ($P=0.15$). Across TCBs, there was a lot of variation in DMDs (CVs = 19.4 and 23.7 % for controls and bromelain) and NDs (CVs = 64.5 and 52.8 %). For some TCBs, DMDs and NDs of controls were greater than those exposed to bromelain treatment; the reason for this is not apparent, but could have been due to either sampling or analytical errors.

Discussion

TCBs contained about 42 % protein (as N * 6.25) on average. However, there was considerable variation; one TCB contained 26 % protein, while another contained 61 %. Amino acids accounted for 91 % of the total protein, suggesting that TCBs may contain a small amount of non-protein nitrogen, such as urea, amines, amides, etc..

It is interesting that, in four TCBs, total amino acids exceeded protein content; for example, TCB#7 contained 44.99 % total amino acids compared to 36.02 % protein (Table 1). The reason for this discrepancy is not apparent.

Hair samples contained about 90 % protein; about 95 % could be accounted for by amino acids. Variation in amino acid concentrations across samples was small, compared to TCBs. The concentrations of most amino acids in hair samples were approximately twice that of TCBs, which can be taken as a general indication that TCBs contained considerable amounts of hair. Based on cysteine concentrations, TCBs contained about 42 % hair and 58 % non-hair components. Bromelain degraded SBM protein effectively, which suggests that it should be able to degrade most dietary proteins that might be present in TCBs. Activity was dependent on pH. Dry matter and N in SBM were more completely solubilised/degraded at higher pH values (5.5) than lower pH values (2.0). However, even at a pH of 2.0, there was considerable DMD and ND, indicating that at the pH of the cat stomach (2.5, Ramsey et al., 1999) dietary proteins in TCB should be degraded substantially. Data from this study and that of others (Castell et al., 1998; Chandler and Mynott, 1998) indicate that bromelain will retain activity if first exposed to a low pH and then a higher pH, such as would be encountered in the stomach and small intestine. Thus, it is possible that bromelain might be active enough in the stomach to sufficiently disrupt the integrity of the TCNB that passage to the small intestine could occur. In the small intestine, where the pH is 5.7 to 6.4, bromelain should retain activity and complete the degradation process.

Dry matter and N degradation of TCBs by bromelain, whether at a pH of 2.5 or 7.0, was only slightly greater than controls, indicating that bromelain had limited ability to degrade TCBs. However, there was considerable variation among samples. For example, at a pH of 2.5 (Table 6), N degradation varied from 1.60 to 64.76 %, probably reflecting differences among samples in proportion of hair and non-hair components. Such variation suggests that it might be possible that some TCBs might be degraded extensively. Bromelain can degrade mucous (Ingerslev and Poulsen, 1980), keratin (Hunter et al., 1957) and feed proteins (this paper). It would seem logical that most, if not all, degradation of TCBs was in the non-hair components. Even though hair was not degraded or was degraded very little, degradation of non-hair materials could be sufficient to disrupt the TCB such that it would be dislodged and would pass through the gastrointestinal tract.

The data obtained in the present in vitro experiments indicate that bromelain treatment might be more effective against some TCBs than others, depending on proportion of non-hair and hair components. In vivo follow up studies are needed to determine if bromelain treatment will reduce the incidence and/or severity of TCB in cats. It is interesting that bromelain provided temporary protection against gastrointestinal disorders in pigs and rabbits (Chandler and Mynott, 1998). In humans, ingested bromelain was absorbed intact across the gut mucosal cell and appeared in the blood, with a half-life of 6-9d. Thus, it might be possible that bromelain could provide temporary protection against disease, in addition to effects on TCBs.

Conclusions

Bromelain was ineffective at degrading hair, but was able to degrade dietary proteins. Bromelain might degrade the non-hair components of TCBs enough to cause disruption and passage of at least some TCBs. Bromelain was active at pHs found in the gastrointestinal tract. In vivo testing is needed to evaluate efficacy of bromelain as a practical treatment for TCBs.

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