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Determination of Carnosine Content and Antioxidant Activity in Rendered Poultry Meals

Principal Investigator: Paul Dawson, pdawson@clemson.edu
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Principal Investigator(s): Paul Dawson, Professor
pdawson@*clemson.edu*
Department of Food, Nutrition and Packaging Sciences
204 Poole Hall
Clemson University
Clemson, SC 29634
(864) 656-1138/(864) 656-0331 FAX

Collaborators: Paljinder Manhiani
pmanhia@*clemson.edu*
Department of Food, Nutrition and Packaging Sciences
Newman Hall
Clemson University
Clemson, SC 29634
(864) 656-4403/(864) 656-0331 FAX

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Lay Summary: This study found that a natural antioxidant and bioactive compounds found in meat can be recovered in the active form from poultry by-products and from rendered protein meal. Certain tissues such as the liver contained greater amounts of carnosine compared to other tissues such as the heart**.** Certain poultry protein meals also contained carnosine and these meals also displayed antioxidant activity. Live birds that were stressed prior to slaughter had higher concentrations of carnosine in their tissues compared to unstressed birds. The imadiazole ring contained in the structure of carnosine was found to be the major contributing factors in the antioxidant activity of carnosine. Finally, even though carnosine was recovered and quantitated in poultry tissues and in protein meals, it may not be the only contributing factor for the antioxidant activity of these materials.

Objectives:

1. Determine the carnosine content and antioxidant activity in poultry meals
2. Determine carnosine content and antioxidant activity extracts in raw poultry by-products.
3. Determine the effects of stress on carnosine content of poultry tissues
4. Determine the active components of carnosine.

Summary

Four separate studies were conducted examining carnosine in poultry co-products, in rendered poultry protein meal, in tissue from stressed or non-stressed chicken and constituents parts for antioxidant activity. In the first study, carnosine was extracted from poultry co-products such as head, liver, lungs, tail, gizzard, brain and heart. Liver showed the highest (102.29 mg/gm) and brain was the lowest carnosine (0.95 mg/gm) ($p \leq 0.05$). Except brain, all tissue ultrafiltrates (20.87-39.57%) and reconstituted dry powders (5.66-14.47%) showed TBARS inhibition. Head ultrafiltrate and reconstituted dry powder showed maximum while gizzard showed the minimum metal chelating activity ($p \leq 0.05$). Free radical scavenging activity of ultrafiltrate of all tissue samples ranged from 25.11 to 79.38% while this activity was higher (29.76 to 84.05%) in the reconstituted dry powder of all tissue samples. ORAC values were highest in liver ultrafiltrate and lowest in heart ($p \leq 0.05$). Results indicated that carnosine was present in all the tissue samples investigated and their ultrafiltrates as well as dry powders of tissue samples possess antioxidant properties.

In the second study examining poultry protein meal, carnosine content of Sample-G was almost 2.6 times higher (104.71 mg/100g of dry sample) than sample–A (40.28 mg/100g of dry sample) ($p \leq 0.05$). TBARS inhibition by Sample-G was 15.86% while Sample-A did not exhibit any TBARS inhibition. Metal chelating activity and free radical scavenging activities of sample-A and sample-G did not differ. ORAC values (μM TE /gm of dry sample) of sample-A (84.35) was greater than sample-G (68.44) ($p \leq 0.05$).

The third study determined carnosine levels in different tissues of broilers under stress versus non-stress conditions. Corticosterone levels of stressed broilers (24358.67 pg/ml) was 10 folds higher ($p=0.002$) than non-stressed broilers (2275.46 pg/ml). There was increase in carnosine content in breast of stressed birds (17.39 mg/gm) was 10 times ($p=0.005$) than non-stressed birds (1.85 mg/gm). Carnosine content in thigh of stressed birds (21.25 mg/gm) was approximately 2-fold higher ($p=0.001$) than non-stressed birds

(11.10 mg/gm). Carnosine content in brain of stressed birds did not differ (p=0.82) from that in non-stressed birds. Results indicated that carnosine may play significant role in muscles during short term stress. Finally, it was determined that TBARS inhibition and metal chelating activity of carnosine was due to the imidazole ring present in the histidine while free radical scavenging activity of carnosine was attributed to histidine amino acid.

Project Overview:
We found poultry meals had relatively high levels of carnosine remaining in the meals after rendering (Table 1).

Table 1, Carnosine content of commercial poultry meals.

Sample	Carnosine content (mg/ gm of sample) Wet basis
Sample A - Pet Grade Poultry meal	11.01±0.37
Sample B- Feed Grade poultry meal	9.76±0.87
Sample C- Poultry meal	2.19±0.10
Sample D- Poultry meal	2.46±1.04

This result is encouraging since the rendering process did not destroy carnosine which is a dipeptide and could be susceptible to heat.

The meals were also found to possess strong antioxidant properties (Table 2) however; this needs further study since the meals obtained for this first round of research contained added antioxidants. To determine what antioxidant properties the additives were contributing, these were tested for their antioxidant properties at the same level used in the meals. While they did display strong free radical scavenging capacity, they did not posses any metal chelating ability which was found in the poultry meals. Metal chelating is very important in preventing the initiation of oxidation in feedstuffs and in the retention of flavor and nutrients. It was not known at the time of the report at what stage in the rendering the supplementary antioxidants were added to the protein meals and if meals could be obtained without these supplements.

Table 2. Antioxidant properties of commercial poultry meals.

Sample	Total Antioxidant Capacity (%)	Free Radical Scavenging Capacity (%)	Metal Chelating Activity (%)
Sample A - Pet Grade Poultry meal	70.75±13.64	77.67 ± 10.11	34.83±9.64
Sample B- Feed Grade poultry meal	2.05±3.32	85.67 ± 1.27	16.16±3.66
Sample C- Poultry meal	10.41±5.78	81.24±1.82	44.865± 10.64
Sample D-Poultry meal	14.24±4.63	82.48 ±3.79	45.135±9.82
Carnosine-10mM/ml	11.09±2.76	7.4163 ± 3.07	94.93±4.67
Ethoxyquin-400ppm	99±1	93.7±0.516	0
Petox-500ppm	99.5±0.5	93.92±0.34	0

Based on these results further study will be conducted on the pet grade meal. Composition of these meals are shown in Table 3.

Table 3. Composition of pet grade meals.

Sample	% Moisture	% crude protein Content	% Ash Content	% crude fat	% crude fiber	% Carnosine	Carnosine mg/gm
Sample B- Feed Grade poultry meal	5.50	65.00	12.00	12.50	2.50	0.9	9.76
Sample A - Pet Grade Poultry meal	5.16	62.37	10.00	11.85	2.20	1.1	11.01

Pet grade poultry protein meal samples (sample-A and sample-G) were obtained from two different rendering facilities without added stabilizers. The composition of these meals is shown in Table 4 and mineral concentration is shown in Table 5.

Table 4: Proximate Composition of Poultry Protein Meals

Sample	Moisture Content (%)	Protein Content (wb) [1] (%)	Protein content (db) (%)	Ash Content (%)	Carnosine Content (wb) [1, 3]	Carnosine Content (db)
Sample-A	4.96±0.06 [a]	67.6±1.48 [a]	70.1±1.48 [a]	13.28±0.16[a]	38.28±0.46 [a]	40.28±0.49[a]
Sample-G	2.16± 0.07 [b]	65.4±2.03 [b]	69.4±2.03[a]	12.76±0.20[a]	102.44±10.06[b]	104.71±10.28[b]

1: HPLC method was used for determination of carnosine content; carnosine content is expressed in mg/ 100 gm of original sample. Wb= wet basis and db= dry basis.

2: All values are Mean ± SEM (N=3)

3: Fisher's Least Significant Difference Test was used to compare mean values; [a-b] similar letters indicate that the means values are not significantly different ($p \geq 0.05$); while different letters indicate that the mean values are significantly different ($p \leq 0.05$).

Table 5: Mineral Composition of Poultry Protein Meals [1]

Sample	Mineral Composition (ppm)											
	P	**K**	**Ca**	**Mg**	**Zn**	**Cu**	**Mn**	**Fe**	**S**	**Na**	**B**	**Al**
Sample-A	546.29	1686.21	62.32	71.85	0.22	0.05	0.03	0.66	292.34	769.26	0.24	0.02
Sample-G	398.14	1247.28	42.28	47.85	0.08	0.10	0.02	0.55	221.67	751.99	0.16	0.18

1: Concentrations are parts per million (ppm) on dry basis.

The antioxidant activity of the two pet grade poultry is shown in Table 6. Results in the present study, indicates the presence of antioxidant active carnosine in poultry protein meals. Carnosine extraction from poultry meal using hot water could have a relatively low manufacturing cost while also possessing excellent solubility in pet food and easy digestion in animals. Moreover, there is an increasing demand of producing

novel functional foods containing bioactive peptides such as carnosine, anserine and L-carnitine. Therefore, it can be concluded that the presence or extraction of carnosine from poultry meal may have a positive economical impact for the rendering industry. By carnosine incorporation, potential therapeutic pet food applications could benefit animal health.

Table 6: Antioxidant Activity Tests

Sample	Antioxidant Activity Test			
	TBARS Inhibition (%) [1]	**Metal Chelation (%) [2]**	**Free Radical Scavenging (%) [3]**	**ORAC Values [4]**
Sample-A	No activity detected	64.16±5.12[a]	81.41±0.19[a]	84.35±0.34[a]
Sample-G	15.86±2.01 [a]	63.78±4.53[a]	84.17±0.50 [a]	68.44±1.36[b]
Carnosine-10Mm	11.09±0.98 [a]	94.93±1.65[b]	8.93±1.75 [b]	Not Determined

All values are Mean ± SEM (N=4); ND= Not determined

[1]% TBARS Inhibition= {(MDA without extract-MDA with extract) / MDA without extract} x100

[2]% Metal chelating activity= {1- absorbance of the sample at 562nm/ absorbance of control at 562nm} x 100

[3]% Free radical scavenging was calculated as {(Absorbance of control at 517 – Absorbance of sample at 517)/Absorbance of control at 517} x 100

[4]ORAC Assay (Oxygen Radical Absorbing Capacity); Values are expressed in Trolox equivalents (TE) per gram of original sample (dry basis).

Fisher's Least Significant Difference Test was used to compare mean values; [a-b] similar letters indicate that the means values are not significantly different ($p \geq 0.05$); while different letters indicate that the mean values are significantly different ($p \leq 0.05$). Carnosine was used as standard for comparisons.

Impacts and Significance: Carnosine is a high value product currently being sold as a nutritional supplement for animals and humans with many additional potential high value applications. Recovery of this compound from poultry by-products could increase the profitability of raw rendered materials and profits for renders handling these products. According the USDA slaughtering data for 2004 the annual byproducts and mortality volume from the poultry industry is 16.71 billion lbs and 0.34 billion lbs, respectively, making a total of 17.06 billion lbs. The domestic use of meat and bone meal from poultry industry is 2.4 million tons *(C. Ross Hamilton et al, 2006)*.

The world poultry industry is very active according to a recent report released by USDA *(October, 2008)*. In 2008 the world poultry meat demand is increased from 2007 and the forecasted increase in 2009 is 1% more than in 2008. The world production of poultry meat in 2008 was 169 billion lbs. There are approximately 250 rendering plants in United States which process 100 million lbs of meat industry waste every day, resulting in 50 billion lbs of processed co-products annually. This total includes the waste from the cattle industry, road kill animals, restaurant spoiled meat etc. According to USDA; 3.4 billion lbs of poultry waste is processed annually.

The potential application of this research will be the recovery of carnosine from poultry co-products. The potential recovery of carnosine from 3.4 billion pounds poultry co-products, without considering organic nature and purity has been estimated to generate approximately $3.07 million annually.

Publications:

Manhiani, P.S., J.K. Northcutt, I. Han, W.C. Bridges, T.R. Scott, P.L. Dawson. 2011. Effect of stress on carnosine levels in brain, breast and thigh of broilers. Poultry Science. Submitted. accepted.

Manhiani, P.S. and Dawson, P.L. 2011. Carnosine presence and antioxidant activity in poultry protein meals. Feed Technology. Submitted 2-2011.

Manhiani, P.S., J.K. Northcutt, I. Han, W.C. Bridges, P.L. Dawson. 2011. Carnosine content and antioxidant activity in poultry tissues Journal of Food Chemistry. Submitted 2-2011.

Manhiani, P.S. and Dawson, P.L. 2011. Carnosine properties, functions, and applications in food- A Mini Review. Open Food Science Journal. Submitted 3-2011.

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Future Work:
Study the antioxidant activity found in various protein meals.

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