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Alternative methods for disposal of spent laying hens: Evaluation of the efficacy of grinding, mechanical deboning, and of keratinase in the rendering process

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ABSTRACT

Besides the challenges of mortality and litter disposal, the poultry industry must find economical means of disposing of laying hens that have outlived their productive lives. Because spent hens have low market value and disposing of them by composting and burial is often infeasible, finding alternative disposal methods that are environmentally secure is prudent. The feasibility of grinding or mechanically deboning spent hens with and without prior mechanical picking was evaluated for the production of various proteinaceous by-product meals. The end products were analyzed for nutrient content and found to be high in protein (35.3–91.9% CP) and, with the exception of the feathers, high in fat (24.1–58.3%), making them potentially valuable protein and energy sources. After considering physical and economic feasibility, mechanical deboning was determined to be a logical first step for the conversion of spent hens into value-added by-product meals. Because the hard tissue fraction (primarily feathers, bones, and connective tissue) generated by mechanically deboning the hens presents the greatest challenge to their utilization as feedstuffs, attention was focused on technologies that could potentially improve the nutritional value of the hard tissue for use as a ruminant protein source. Traditional hydrolysis of this hard tissue fraction improved its pepsin digestibility from 74% to 85%; however, subsequent keratinase enzyme treatment for 1 h, 2 h, 4 h, or 20 h after steam hydrolysis failed to improve the pepsin or amino acid digestibility any further ($P > 0.10$). Enzyme hydrolysis did, however, increase the quantities of the more soluble protein fractions (A: 45.5, 46.6, 52.8, 51.6, and 55.8% of CP; B_1: 3.2, 9.8, 6.0, 4.6, and 4.1% of CP; B_2: 11.7, 18.1, 22.8, 29.6, and 22.0% of CP for 0, 1 h, 2 h, 4 h, and 20 h, respectively) and reduced quantities of the less soluble fractions (B_3: 30.2, 18.1, 10.8, 5.5, and 10.2% of CP; C: 9.4, 7.5, 7.6, 8.8, and 7.9% of CP for 0, 1 h, 2 h, 4 h, and 20 h, respectively). The protein digestibility of the steam hydrolyzed hard tissue fraction from the mechanical deboning of spent hens was found to be comparable to the digestibility of feather meal, but post-hydrolysis keratinase treatment did not improve feeding value for ruminants.

1. Introduction

Like many industries today, the poultry industry faces the challenge of producing high quality products in a manner which meets consumer expectations, satisfies environmental regulations, and that maximizes profitability. In the process of meeting these demands, it is estimated that over 144 million live, spent layers must be removed annually from production (C.A.S.T., 1995; Lyons and Vandepopulier, 1996). The yield of white meat per spent hen is only 166 g, which yields a value to processors that barely covers the cost of handling the birds (Middleton, 2000). Because the cost of removing spent laying hens from the farm often exceeds their value for meat (Aho, 1999), euthanasia followed by burial, composting, or incineration are often used for depopulating hen houses. These methods raise environmental concerns. Finding methods of disposal that minimize nutrient and biohazard emissions into the environment while yielding residual value to the poultry producer is a great challenge.

Converting proteinaceous animal wastes, such as spent hens, into feedstuffs is one of the most biologically efficient means for recycling nutrients. The objective of this project was to investigate the potential of adding value to spent fowl as a raw material for protein by-product meals by using novel applications of available technologies. The efficiency and feasibility of grinding, mechanical picking, and mechanical separation of meat (mechanically deboned meat, MDM, consists primarily of meat, skin, and viscera) from hard tissue (feathers and bones) were measured and the nutritive value of the various end products determined. The impact of keratinase hydrolysis on the availability of protein in the hard tissues was also evaluated.

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2. Methods

2.1. Evaluation of available technologies

All animal handling techniques were in accordance with protocol approved by the NCSU Institutional Animal Care and Use Committee. One hundred Leghorn-type spent laying hens were brought to the Animal and Poultry Waste Management Center at North Carolina State University (NCSU; Raleigh, NC) and euthanized by cervical dislocation. They were then divided into groups of 25 and weighed by group. Two of these groups of birds were selected at random to be mechanically picked (P). The selected birds were placed one group at a time in 63 °C water for 2–3 min. after which they were placed into a drum picker (Ashley Sure Pick, model SP-30, Ashley Machine Co., Greensberg, IN) for 3 min. Feathers from each group of birds were collected and the feather yield was determined by subtraction of group picked weight from group initial weight.

One group of P hens and one group of whole birds (W, i.e., hens with feathers) were ground in separate runs of a commercial meat grinder (Buffalo Grinder Model 78-BG, John E. Smith's Sons Co., Buffalo, NY) with a 5 mm die screen in place. The resulting ground materials (PG or WG, respectively) were collected, weighed, and sub-sampled. Samples were placed into a −23 °C freezer for storage until they could be further processed.

A second group of *P* hens and a second group of W birds were fractionated using a commercial deboning device (Beehive Model RSTD06, Sandy, UT). The deboning separation process yielded two products from each group of birds, a hard tissue fraction (PH or WH), containing primarily bones, connective tissues, and feathers (if present), and a soft, MDM meat fraction (PS or WS) containing meat, fat, and entrails. Each fraction was weighed, subsampled, and frozen as described earlier.

In preparation for freeze drying for laboratory analysis, all samples were thawed and similar samples were pooled. The particle size of the hard tissue samples was reduced to facilitate freeze drying and analysis by alternately processing in a food cutter (Model #FC19, Blakeslee and Co., Cicero, IL) and grinding with a hand-crank meat grinder (Back to Basics Products, Inc., Draper, UT) using initially a 10 mm die screen and subsequently a 4 mm die screen. Because their initial particle size was smaller, the soft tissues were prepared for freeze drying by grinding with the hand grinder through the 4 mm screen. All sample types were subdivided into tared, zipper-locking plastic bags, refrozen, weighed, freeze dried, and then re-weighed for calculation of DM percentage. Following freeze drying, the samples were ground in a food processor (Little Oskar, Sunbeam Appliance Co., Boca Raton, FL) for subsequent laboratory analysis.

2.2. Evaluation of the addition of enzymatic hydrolysis to the rendering process

An additional 1000 spent hens from the same flock were euthanized by cervical dislocation and fractionated through the deboning device. The hard tissues were frozen at −23 °C prior to further processing. The soft tissue fraction was directed to another project for further study. To create a product with sufficient moisture for traditional hydrolysis, 29.2 kg of thawed hard tissues were mixed with 16.3 kg water to create a 30% DM product. A subsample of this material was frozen for later analysis (designated E-1). The remaining water and hard tissue mixture was hydrolyzed as the first replication of the hydrolysis process (rep 1) using a pilot-sized (133.1 L capacity) hydrolyser (Anco-Eaglin, Inc., Greensboro, NC) by holding the pressure in the unit at 10.8 torr for 45 min to approximate the cooking conditions in commercial rendering facilities. The product was agitated constantly during cooking. A maximum cooking temperature of 124 °C was achieved and maintained for the last 10 min of cooking time.

After the hydrolysis process was completed and atmospheric pressure was reestablished inside the hydrolyzer, the product was placed into an improvised thermal container (plastic drum wrapped in six layers of 2.54 cm diam. bubble-wrap) to reduce the rate of cooling. Sub-samples of the hydrolyzed material were frozen at −23 °C for analysis (0 h enzyme hydrolysis, E 0). Keratinase enzyme (300,000 U/g activity, laboratory of Dr. Jason Shih, NC State Univ., Raleigh, NC) produced from *Bacillus licheniformis* PWD-1 (12.5% wt/vol stock solution) was added to the hydrolyzed material to give a final enzyme concentration of 1%. The enzyme solution was added after the temperature of the hydrolyzed material had cooled to 63 °C, the optimum temperature suggested by the supplying laboratory. The material was sampled at multiple times during the enzymatic digestion process (1 h, 2 h, 4 h, and 20 h, designated E 1, E 2, E 4, and E 20) and samples frozen and stored at −23 °C.

Prior to replicate 2 (rep 2) of the hydrolysis process, a cooling jacket was added to the hydrolyser which allowed cooked material to be cooled before its removal from the hydrolyzer. As a result of the use of the cooling jacket, rep 2 hydrolyzed hard tissue was removed from the hydrolyser at 43 °C. The material was placed into a smaller thermal container than was used in rep 1, which facilitated mixing and sampling. A sample was taken upon placement of the material in the thermal container and keratinase enzyme addition followed immediately. Samples were taken and stored as previously described. No sample of the raw material (E-1) was taken during rep 2.

2.3. Laboratory analysis of raw hen fractions

Dry matter, Kjeldahl nitrogen, NDF, ADF, and ash were determined according to AOAC procedures (1995). Protein fractions were determined according to the procedure of Licitra et al. (1996). Trichloroacetic acid was used to determine non-protein N in this process. The samples were subjected to ether extraction for fat determination (Labconco extraction apparatus, Labconco Corp., Kansas City, MO). The amino acid content of the fractions was determined by AOAC procedures (AOAC, 2000) at the laboratory of Novus International (St. Charles, MO).

2.4. Laboratory analysis of hydrolyzed hard tissues

Protein fractions were determined on the material from rep 2 of the hydrolysis process according to the procedure of Licitra et al. (1996) as described earlier and fat content was determined by Dairy One Laboratory (Ithaca, NY).

The 0.02% pepsin digestibility (PEPD) and CP content of samples from each enzyme hydrolysis time (E-1, E 0, E 1, E 2, E 4, and E 20) were determined according to AOAC procedures (1995) modified for use with a nitrogen combustion apparatus by Woodsen–Tenent Laboratory (Goldston, NC). To further evaluate protein digestibility, sub-samples from rep 2 of the hydrolysis process were sent to the laboratory of Dr. Carl Parsons at the University of Illinois at Urbana-Champlain for determination of true amino acid digestibility (TAAD) by means of caecectomized cockerels (Parsons, 1986; three birds per treatment). The DM, CP, and PEPD results were analyzed using PROC GLM ($n = 25$, 3 samples from session 1 + 2 samples from session 2 × 5 sampling times; SAS, Cary, NC) with session, time, and time(session) included in the model. The TAAD were also evaluated with PROC GLM with hydrolysis time and TAAD included in the model ($n = 15$, 3 birds per sample × 5 sample times). Protein fractions were analyzed with PROC GLM with time as the independent variable ($n = 12$, 6 sample times × 2 samples from each time). Orthogonal contrasts were made to look for relationships between

enzymatic hydrolysis time and DM content, CP content, PEPD, TAAD, and protein fraction proportions.

3. Results

3.1. Evaluation of available technologies

The feathers removed by mechanically picking the hens during this trial represented 3.7% of carcass weight (8.4% of DM). Recovery rates for the processing of both W and P spent hens in the grinder and deboner averaged 94.02 and 98.67%, for the two processes, respectively. Separation of the hard from MDM fractions by the deboner yielded the fraction quantities and proportions given in Table 1. About two thirds of the hen carcasses were MDM and the remaining one third hard tissues.

Laboratory analysis of the fractions from both the grinding and deboning processes for whole and mechanically picked birds is presented in Table 2. All the materials analyzed were relatively high in protein and fat. The hard tissue fractions also contained considerable ash.

The amino acid content of the various fractions as a percent of CP are presented in Table 3.

Table 1
Proportions of fractions produced by deboning spent hens with and without feathers.

Fraction	Weight (kg)	% of recovered material
Whole, hard	13.56	33.2
Whole, soft	27.30	66.8
Picked, hard	13.29	31.8
Picked, soft	28.45	68.2

Table 2
Chemical composition of raw ground hens and their deboning fractions.

Component	WG[a]	WH[a]	WS[a]	PG[a]	PH[a]	PS[a]	Feathers
DM (%)	41.8	46.8	40.9	39.3	42.2	40.7	94.7
OM (% of DM)	89.2	83.2	96.1	90.3	80.1	96.8	98.0
CP (% of DM)	42.8	58.0	37.1	42.6	52.3	35.3	91.9
EE (% of DM)	45.8	24.1	57.0	48.5	26.1	58.3	5.2
NDF (% of DM)	45.6	53.3	47.4	53.4	50.2	48.8	82.6
ADF (% of DM)	23.8	3.4	4.3	18.8	15.0	4.5	9.9
Ash (% of DM)	10.8	16.8	3.9	9.7	19.9	3.2	2.0
Protein fractions				% of CP			
A	14.5	7.2	11.9	17.4	8.0	11.3	1.5
B_1	12.4	2.1	8.4	3.3	2.9	9.6	1.3
B_2	42.3	56.7	53.1	40.8	56.6	51.3	10.4
B_3	5.4	17.8	20.2	36.4	24.7	26.3	17.4
C	25.5	16.2	6.5	2.1	7.8	1.4	69.3

[a] WG = whole hen, ground; WH = whole hen, hard tissues; WS = whole hen, soft tissues; PG = mechanically picked hen, ground; PH = mechanically picked hen, hard tissues; PS = mechanically picked hen, soft tissues.

3.2. Evaluation of the addition of enzymatic hydrolysis to the rendering process

Hydrolysis replicate had an impact on DM content but not on CP content of the hydrolyzed hen tissues (Table 4). The LS means DM for reps 1 and 2 were 34.0% and 39.9%, respectively (SE = 0.008, $P < 0.01$). The CP content of the samples averaged 53.8% and did not differ between reps 1 and 2 ($P = 0.17$). Hydrolysis time had linear, quadratic, and cubic effects on both DM and CP contents of the material ($P < 0.01$).

Rep and time both impacted PEPD ($P < 0.01$) and the relationship between these parameters is illustrated in Fig. 1. The data from rep 1 included a sample of the raw material (Fig. 1, time = −1 h) which was evidently less digestible than the hydrolyzed samples. An orthogonal contrast based on duplicate analyzes of samples from this rep shows this to be the case ($P < 0.01$).

When enzymatic hydrolysis results from both sessions were combined, the linear and cubic relationships between time and pepsin digestibility were significant ($P < 0.01$ for linear and cubic relationships; $P = 0.20$ for quadratic relationship). The results of partitioning the enzymatically hydrolyzed hen hard tissue from the second replicate into protein fractions are given in Table 5. Equivalent results for the unhydrolyzed material ($t = -1$ h) are included but these data were not included in our statistical model. No statistical comparison between reps was possible because inadequate material from rep 1 remained after PEPD analysis to complete protein fraction analysis so protein fraction determinations were only made on material from rep 2. Enzymatic hydrolysis seemed to increase the solubility of the protein in the material in rep 2. Linear increases ($P = 0.01$) in the A (non-protein N) fraction

Table 3
Amino acid content of raw, ground hens and their deboning fractions.

Content as % of CP	WG[a]	WH[a]	WS[a]	PG[a]	PH[a]	PS[a]	Feathers
Arginine[b]	6.38	6.53	6.07	6.99	6.61	6.44	6.92
Histidine[b]	2.18	1.35	2.98	3.14	1.61	3.32	0.51
Isoleucine[b]	3.95	3.48	4.69	4.88	3.26	5.02	4.53
Leucine[b]	6.78	6.38	7.89	8.46	6.03	8.46	9.01
Lycine[b]	5.41	3.94	7.33	8.29	4.83	8.24	1.20
Methionine[b]	1.86	1.37	2.44	2.74	1.59	2.80	0.63
Phenylalanine[b]	4.71	3.53	3.99	4.31	3.35	4.25	4.94
Threonine[b]	3.42	3.15	3.71	3.96	3.12	4.06	4.52
Tryptophan[b]	0.81	0.51	0.93	0.95	0.56	1.16	0.29
Valine[b]	5.14	5.13	5.45	5.61	4.36	5.86	7.48
Alanine[c]	5.73	5.86	5.53	6.51	6.23	5.86	4.71
Aspartate[c]	7.75	6.80	8.51	9.73	6.84	8.51	6.27
Cystine[c]	1.56	2.64	1.49	1.41	1.45	1.54	6.95
Glutamate[c]	12.46	11.47	12.87	16.02	10.93	13.84	10.43
Glycine[c]	8.02	10.42	5.25	6.80	10.62	4.96	8.53
Proline[c]	6.27	7.71	4.38	5.26	6.69	4.22	9.92
Serine[c]	3.69	4.42	2.89	3.06	3.57	3.12	11.78
Taurine[c]	0.46	0.27	0.59	0.62	0.37	0.58	0.01
Tyrosine[c]	2.26	2.07	3.09	2.95	2.15	3.12	2.49

[a] WG = whole hen, ground; WH = whole hen, hard tissues; WS = whole hen, soft tissues; PG = mechanically picked hen, ground; PH = mechanically picked hen, hard tissues; PS = mechanically picked hen, soft tissues.
[b] Amino acid considered essential to ruminants.
[c] Amino acid considered non-essential to ruminants.

Table 4
DM and CP contents of raw hen samples and with traditional and enzymatic hydrolysis.

Enzymatic hydrolysis time (h)	−1	0	1	2	4	20	SE	LFF	Q[a]	C[a]
Replicate 1 (% of DM[b])	30.1	42.2	32.4	33.0	32.4	33.7				
Replicate 2 (% of DM[b])	ND[c]	39.0	39.0	40.0	40.5	40.7				
Overall (% of DM[d])	*NE*[e]	*40.6*	*35.7*	*36.5*	*36.5*	*37.2*	*0.001*	*<0.01*	*<0.01*	*<0.01*
Replicate 1 (% of CP[b])	54.7	48.6	54.7	55.2	56.6	55.0				
Replicate 2 (% of CP[b])	ND[c]	54.4	54.6	53.4	52.5	53.5				
Overall (% CP[d])	*NE*[e]	*51.5*	*54.6*	*54.3*	*54.5*	*54.3*	*0.24*	*<0.01*	*<0.01*	*<0.01*

[a] *L*, *Q*, *C*: *P*-values for linear, quadratic, and cubic relationships between hydrolysis time and protein fractions, respectively.
[b] Arithmetic mean.
[c] Not determined: no sample taken.
[d] LS mean ($n = 25$).
[e] Not estimable due to missing data point.

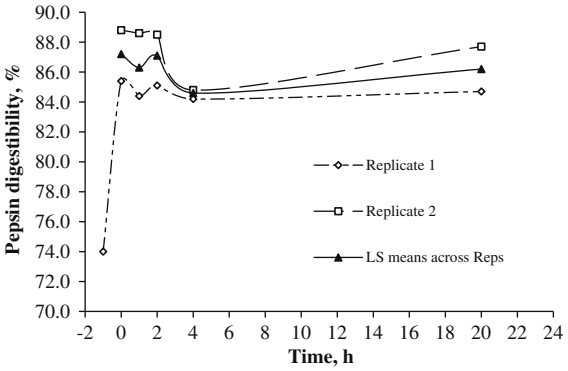

Fig. 1. Relationship between hydrolysis time and 0.02% pepsin digestibility of hen hard tissues. For LS means: $n = 25$, SE = 0.34, $P < 0.01$ for linear relationship and cubic relationship, $P = 0.20$ for quadratic relationship.

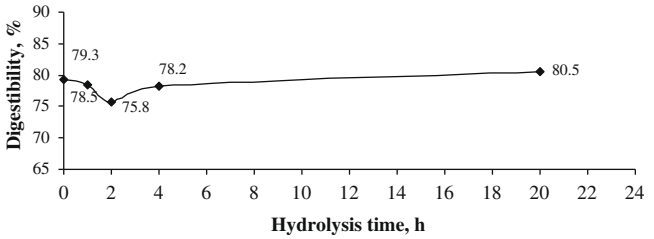

Fig. 2. Average true amino acid digestibility of hen hard tissue as a function of enzymatic hydrolysis time ($n = 15$, SE = 1.9, $P = 0.53$).

were observed. The highly soluble, B_1 fraction was increased by a cubic function ($P < 0.01$), peaking at 1 h of hydrolysis. The moderately soluble B_2 fraction increased by a quadratic function ($P = 0.05$), peaking at 4 h of hydrolysis. The protein present as B_3 fraction, which has moderate to low solubility, declined quadratically ($P < 0.01$) with a minimum observed at 4 h of hydrolysis. Finally, the protein in the insoluble C fraction declined according to a cubic function ($P = 0.02$) with a minimum observed approximately 1 h after enzyme addition.

Enzymatic hydrolysis time had no impact on the TAAD of individual amino acids tested ($P > 0.10$) so these data are not reported. Average true amino acid digestibility before keratinase addition was 79.3 (SE = 1.9). The relationship between hydrolysis time and TAAD is illustrated in Fig. 2.

4. Discussion

4.1. Evaluation of available technologies

Both the whole and mechanically picked spent hens were successfully processed through the grinder and the deboning device. While the yield results demonstrate that each of these processing techniques can be used with minimal material loss or waste, yields were lower for the grinding process due to some material remaining in the grinder after the ground material stopped flowing. This yield loss would become negligible for larger size processing runs.

Roughly two thirds of bird mass (Table 2) was partitioned into the MDM fraction with the remaining material being separated out as the hard tissue fraction. Based on observation of the hard tissue fraction, it contained bones, connective tissue, feet, the exterior of the eyes, combs, and feathers (if they were present on the carcasses). It also contained a small amount of soft tissue as residue of the deboning process. The soft tissues were pulverized during the deboning process and could not be distinguished, but this fraction would logically contain muscles, fat, nervous tissue, and the viscera with its contents. Either fraction could be further processed to yield useful feed ingredients. The more digestible soft tissues would likely be directed to monogastric diets and the less digestible hard tissues to ruminant diets.

The nutrient composition of the WG hen (Table 2) was similar to that seen in other trials (Haque et al., 1991; Lyons and Vandepopulier, 1996; and Kim and Patterson, 2000). The WH and WS materials from our trial are similar to the mechanically deboned hen fractions described by Lyons and Vandepopuliere (1997). Nutrient analysis of all the fractions (Table 2) confirms that each has potential value of as a source of both protein and energy, since they are at least 35% CP and, with the exception of the feather fraction, all the products contained more than 24% fat (Table 2, EE).

Table 5
Protein fractions of hen hard tissue hydrolyzed with and without keratinase ($n = 12$).

Enzyme hydrolysis time	(% of CP)									
	−1 h (raw)	0 h	1 h	2 h	4 h	20 h	SE	LFF	Q[a]	C[a]
Protein fraction										
A	7.2	45.5	46.6	52.8	51.6	55.8	0.02	0.01	0.90	0.96
B_1	2.1	3.2	9.8	6.0	4.6	4.1	0.01	0.10	<0.01	<0.01
B_2	56.7	11.7	18.1	22.8	29.6	22.0	0.03	0.01	0.05	0.22
B_3	17.8	30.2	18.1	10.8	5.5	10.2	0.02	<0.01	<0.01	0.49
C	16.2	9.4	7.5	7.6	8.8	7.9	0.01	0.24	0.11	0.02

[a] *L*, *Q*, *C*: *P*-values for linear, quadratic, and cubic relationships between hydrolysis time and protein fractions, respectively.

The high fat content of the hen fractions could make incorporating these products into ruminant feeds a particular challenge. Ruminant diets should not contain more than 5 to 6% fat (DM basis; Hess et al., 2008). Greater concentrations of fat can lead to decreased fiber digestibility and reduced efficiency of use of the energy available in the feed. Since the hen fractions would have to be cooked prior to use as feed ingredients, one solution to the high fat challenge would be removal of a portion of the fat after cooking. Screw press technology is currently applied after cooking in the rendering process to manufacture poultry oil (Ockerman and Hansen, 2000). Similar technology could likely be used to remove the oil from hen fractions during processing as well. An alternative method would be to dilute the fat with a low-fat, highly digestible and palatable byproduct (such as soybean hulls) thus reducing the total fat content to acceptable levels. The dilution technique would also reduce energy requirements for drying the final product to acceptable moisture levels for storage, assuming a dry diluent was utilized.

Another consideration for high-fat feed ingredients is oxidative rancidity. Rancid fat does not seem to impact feed intake or fat utilization by ruminants to any great extent (Zinn, 1995); however, it can reduce milk fat and protein production in lactating animals (Heinrichs et al., 2005). Oxidative damage could also reduce vitamin activity over time and result in vitamin deficiencies. Addition of an anti-oxidant (such as ethoxiquin) might be efficacious when considering the use of hen products as feed ingredients.

The protein fractions in the hen products (described as A, B_1, B_2, B_3, and C by VanSoest, 1994), are determined by chemical solubility and indicate how the protein might be utilized if incorporated into a ruminant ration. The A and all B fractions are largely available for use by ruminants and only the C fraction is indigestible. Results from the unhydrolyzed samples (Table 3) indicate that the products that do not contain feathers would be readily digested by ruminant animals, even in the absence of further processing, as evidenced by the low content of C protein. The elevated C fraction values for the feathers and for the bird products containing feathers indicate that, as expected, the feathers cause a large reduction in feeding value and would require more aggressive further processing to enhance digestibility.

These tests were run on the raw material and for any of these bird fractions to be used as feed ingredients, further processing would be a necessity to insure feed safety. The processing involved would likely improve the availability of the protein in all the fractions, but particularly in those containing feathers. The amino acid content of WG hens presented (Table 4) is similar to those presented by Douglas et al. (1997) and Kersey et al. (1997) for rendered spent hen meals. The amino acid composition we found in feathers was similar to that reported by Han and Parsons (1991) for seven batches of feather meal and those for WH and WS fractions are in agreement with data in Lyons and Vandepopuliere (1997). No data regarding the other hen fractions was available for comparison. Amino acid compositions should likely be considered in conjunction with the 0.02% pepsin digestibility and the true amino acid digestibility data illustrated in Fig. 1 and 2 since it is ultimately the availability of the amino acids that determines the feeding value of a protein source.

Lysine and methionine are often the first and second limiting amino acids in traditional corn and soybean meal-based animal diets (NRC, 1994; NRC, 1998; and NRC, 2001, respectively). The hen soft tissue (Table 4) would potentially be a better source of lysine than soybean meal, which is listed as having a lysine content of 6.6% of CP (NRC, 1998). Lysine in the hard tissue had a true amino acid digestibility of 72.6% after traditional hydrolysis and it would therefore likely be at least as available in the soft tissue fraction. With the absence of feathers, any of the hen products would supply more methionine than soybean meal (1.2% of CP as methionine). Feathers are high in cysteine, however, and so their inclusion raises the cysteine proportion (Table 4). Since cysteine can spare methionine, the inclusion of the feathers as a cysteine source might balance their diluting effect on methionine content. The true amino acid digestibility of methionine in the hydrolyzed hard tissue was 81.3% and that of cysteine was 72.0% suggesting that these amino acids would also be fairly well utilized from any rendered hen products.

For the production of any product to proceed on a large scale, it must prove to be economically feasible. The fractionation of spent hens by the methods employed in this project proved to be physically possible, but when the economics of production are considered, it seems logical that working with the whole birds would be more economical than picking them. Since whole birds include feathers and feather feeding value in non-ruminant diets is limited, economics would seem to dictate that the best way to handle the birds would be to mechanically debone them into the WH and WS fractions described earlier. This would result in the soft tissues being feather-free and available for further processing into highly digestible, more profitable monogastric feeds. It would also allow for the subsequent hydrolysis of the hard tissue fraction to improve its digestibility. Based on our work, the hard tissues hold potential value and can be processed under normal conditions successfully.

4.2. Evaluation of the addition of enzymatic hydrolysis to the rendering process as applied to hard tissues

Differences in dry matter content between reps 1 and 2 (Table 4) were attributable to the release of more steam during the course of the second run than during the first in order to maintain constant pressure within the cooking chamber. The high DM value and low CP proportion for E 0 in rep 1 as compared to the other samples in that replicate was the result of sampling error. The discrepancy seen in this one data point was likely the cause of the cubic relationship seen between these parameters and hydrolysis time.

The PEPD of the hard tissue fraction appears to be improved by traditional hydrolysis (Fig. 1, rep 1). This is supported by the results of Lee et al. (1991). Enzymatic hydrolysis, however, did little to alter the PEPD of this fraction (Fig. 1) in amounts that would be significant to animals consuming the final product. While there was a statistically significant relationship between PEPD and hydrolysis time, it was at time points that would be beyond the scale of normal production practices. The cubic aspect of the relationship suggested that the keratinase enzyme worked slowly but steadily and that it broke down the proteins most susceptible to pepsin digestion first, causing a slight drop in PEPD, before it began working on the proteins which were also less susceptible to pepsin. As the keratinase continued to work, PEPD then improved slightly.

While the pepsin digestibility assay is useful in assessing the feeding value of substances, it is not a bioassay and so may not yield results that are indicative of what live animal performance would be. To better assess the value of the protein in the hard tissue fraction after keratinase exposure, TAAD were also determined for the products from the second hydrolysis replicate. Enzymatic hydrolysis had no impact ($P < 0.10$) on the true digestibility of any of the 20 individual amino acids evaluated across time (Fig. 2), which validates our results from the pepsin digestibility assay. Digestibility of similar magnitude (77%) was reported for feathers by Lee et al. (1991) who also reported that keratinase improved the digestibility of feathers in diets for chicks and roosters when it was incorporated into the feed rather than being used as a feather processing aid. Carter and Shih (1997) observed linear improvements in *in vitro* digestibility as keratinase additions to FM increased. Keratinase was also found to be more effective when the FM had been de-fatted (Carter and Shih, 1997) and when pH was adjusted from 5.9 to 7.5. The lack of keratinase effects in our trial may have been caused by the high

fat levels in our product or to non-optimum pH. We did not measure the pH of our product. Additionally, our hard tissue product included material other than feathers. If the enzyme attacked the non-feather materials in preference to the feathers, the result would be little improvement in digestibility if the non-feather material was already readily digestible.

The changes seen in the protein fractions of the cooked hard tissue as a result of enzymatic hydrolysis (Table 5) suggested that the enzyme was indeed acting on the proteins. The enzyme appeared to be hydrolyzing less soluble protein (B_3) into more soluble amino acids, peptides, and proteins (A, B_1, and B_2). The cubic effect of hydrolysis time on the B_1 fraction suggested that it is initially increased by the attack of keratinase on the less soluble B_2 and B_3 protein fractions but is then reduced by its degradation to the even more soluble A fraction as the keratinase continued to work. The cubic effect of hydrolysis time on the C protein fraction was more difficult to explain and may have been the result of the fact that protein fractions are calculated as proportions of the total protein. The increase in the proportion of C protein observed after 4 h of hydrolysis may have been due to the fact that the % CP at this time point is lower than that at either 2 h or 20 h. If the absolute amount of C protein in the sample was similar to that of the other hydrolysis times, it would have represented a greater proportion of the smaller total amount of protein. Maximum changes were again seen, however, at times that would likely be beyond what would be reasonable in a commercial setting.

The apparent impact of enzymatic action on the protein in the hard tissues could affect its utilization by ruminant animals. The C fraction was largely unaffected by the enzymatic digestion process. The fact that it did not vary greatly as a result of enzymatic hydrolysis (Table 5) suggested that this treatment is not improving the overall availability of the protein for ruminants and supported the finding that TAAD was not altered by enzymatic hydrolysis. Increasing the solubility of the available protein (increasing levels of A, B_1, and B_2 while B_3 decreased) suggested that more of the protein would be degraded by the microorganisms of the rumen and less would pass on to the lower gastrointestinal tract for direct digestion by the host animal. Since the microorganisms tend to alter the amino acid profile of the protein they degrade, enzymatic hydrolysis would likely impact the ability of this product to supply amino acids needed by the host, but whether this impact would be positive or negative can not be determined from our data and would require additional evaluation.

5. Conclusions

We have demonstrated the processes of grinding and mechanical deboning can be readily applied to the further processing of spent hens with or without feathers. Based on economic considerations, mechanical deboning without prior mechanical picking would be the most feasible of these technologies and would yield hard and soft tissue fractions, which both appear to be potentially valuable sources of protein and energy. The value of each of these fractions would be enhanced by further processing using conventional rendering techniques. Adding keratinase enzyme to the processing regime for the hard tissues did not improve the digestibility of the protein in the final product. The next logical step in the evaluation of the hard tissue as a potential protein source would be to utilize it in a feeding trial. Determination of its palatability and *in vivo* digestibility would aid in discerning whether this product could be used on a commercial basis, providing an economical and environmentally responsible means of disposal for spent hens.

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