

Protein Quality of Whole Spent Hens Co-Extruded with Corn or Wheat Compared with Soybean Meal or Meat and Bone Meal

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INTRODUCTION

Two of the most pressing problems facing the commercial egg industry are the disposition of mortality and the utilization of spent hens (Christmas et al., (1996). Several authors including McLaurin (1991) and Brown (1994) have emphasized the need for creating a demand for fowl through new innovative technologies.

Animal-byproduct meals such as meat and bone meal (MBM) and poultry byproduct meal (PBPM) are excellent sources of protein and phosphorus and as such are used extensively in poultry diets (Johnson and Parsons, 1997). The incorporation of rendered hens into sources of byproduct protein and the efficacy of these products in practical diets have been documented in numerous reports (Haque et al., 1991; Lyons and Vandepopuliers, 1995; Olejnik, 1995; Christmas et al., 1996; William and Damron, 1998). Christmas et al. (1996) incorporated various levels of rendered whole-hen meal into practical diets for commercial broilers and concluded that the product was comparable to soybean meal (SBM) as a source of protein.

While animal byproducts meals are high in crude protein, protein quality can vary greatly (Johnston and Coon, 1979). Some of the more common bioassays used to evaluate protein quality include Protein Efficiency Ratio (PER), Net Protein Ratio (NPR), and Net Protein Utilization (NPU). These are often combined with such chemical tests as the Carpenter Assay for available lysine and pepsin digestibility. Escalona et al., (1986) concluded that compared with other procedures, the PER assay allowed for the most accurate discrimination of protein quality between ingredients, especially at lower levels of dietary inclusion. They further stated that it was simple to determine, was correlated with other methods, and offered a simple and reproducible procedure for evaluating protein quality. This observation was later confirmed by Johnson and Parsons (1997). They concluded that while NPR values were higher than PER values for comparable protein sources, the ranking and statistical differences among the sources were almost identical so there was no clear advantage to using NPR over PER.

Amino acid (AA) requirements are most often based on the total amino acid concentrations in feed ingredients and diets. This can lead to variability in actual requirement estimates, however, due to the proportion of dietary total AA which are actually digested and absorbed by test animals (NRC, 1994). Several methods can be

used to estimate AA digestibility in poultry, including analysis of ileal digesta or excreta from intact or ceaeotomised birds. A major criticism of using intact birds is the extent to which caecal microflora would alter the amino acid composition of the excreta (Austic, 1983; Thomas and Crissey, 1983; Papadopoulos, 1985; Van Weerden, 1989; Whitacre and Tanner, 1989; Low, 1990; Ten Doeschale et al., 1993). While caeectomy would greatly reduce hindgut microbial activity, Whitacre and Tanner (1989) doubted it could be totally eliminated. Sakata (1987) had suggested that caeectomy might also lead to digestive disturbances in the upper GI tract. Other authors have recommended the use of ileal digesta as a more preferable means of determining AA digestibility in poultry (Kadim and Moughan, 1997; Ravindran et al., 1999).

The objective of the studies reported herein was to determine, in turkey poult, the protein quality of an ingredient resulting from the co-extrusion of whole spent hens with either corn or wheat. In the first experiments, the corn- and wheat-based products were studied alone whereas in the latter experiments, they were compared with soybean meal (SBM) and meat and bone meal (MBM).

Materials and Methods

In all the experiments, commercial turkey poult were purchased from Cuddy Farms (Danville, OH) at a day of age. They were wing-banded and brooded in Petersime battery brooders and fed the standard OARDC turkey starter diet for the first 7 to 10 days post-hatch, depending upon experiment. At the start of each experiment, each poult was individually weighed and body weights were stratified within a pen so there were no significant differences in pen weight within or between treatments. There were four poult per pen at the start of each experiment and pen was the experimental unit. Proximate analysis and AA composition of the corn- and wheat-based protein sources was determined along with all test diets.

Data were analyzed by analysis of variance using the General Linear Model of SAS (SAS Institute, 1986). The main effects tested were protein source, level of protein, and the interaction of source and level of protein. Significant main effects and interactions were further tested using Duncan's Multiple Range test ($P < 0.05$).

Experiment 1

A total of 96 poult were used in this experiment. The objective of the experiment was to test a range of dietary protein levels that would allow for incremental increases in body weight over a 10-day test period. Diets were formulated to contain 12%, 15%, or 18% CP with either the corn- or wheat-based hen meals (CHM; WHM) serving as the only sources of dietary protein (Table 1). This resulted in a 2 X 3 factorial arrangement of treatments with four replicates per source and level of protein. Feed and water were

available *ad libitum* throughout the 7-day experimental period. The feeders did not have screens placed in them at the beginning of the study, hence there was considerable feed wastage, so feed intake was not recorded. The poultlets were exposed to a 24 h photoperiod throughout the experiment and all poultlets were individually weighed after 3, 7, and 10 days on the experimental diets.

Table 1. Dietary content and Analysis (Experiment 1)

Ingredient	CHM 12	CHM 15	CHM 18	WHM 12	WHM 15	WHM 18
Glucose	29.0	16.5	4.0	35.6	24.9	14.2
Solka Floc	14.0	14.2	14.8	13.3	13.4	13.6
Dical	3.8	4.5	3.8	3.8	3.8	3.8
Vit Premix	2.0	2.0	2.0	2.0	2.0	2.0
Limestone	0.7	---	---	1.2	0.9	0.6
Salt	0.5	0.3	0.4	0.5	0.5	0.4
Corn-Hen	50.0	62.5	75.0	---	---	---
Wheat-Hen	---	---	---	43.6	54.5	65.4

The analysis of all diets resulted in CP levels that were close to formulated values.

Experiment 2

This experiment was similar to Experiment 1 with the exception that a nitrogen-free diet was formulated and fed to an extra replicate of pens along with the same diets used in Experiment 1. In addition, screens were placed in each feed trough at the beginning of the study so that accurate feed intake data could be recorded. The experimental period was 7 days. In this study, dietary protein consumption for each treatment was calculated after dietary analysis and used for the determination of Protein Efficiency Ratio (PER) as shown below. The body weight loss resulting from the N-free diet combined with the positive weight gain from the other experimental diets allowed for the determination of Net Protein Ratio (NPR) as reported below. The body weight loss associated with the N-free diet is assumed to be an adjustment for maintenance protein requirements.

Formulas

Formulas used in the calculations of PER, NPR and AA digestibility were:

$$\text{PER} = \frac{\text{Body weight gain (gm)}}{\text{Protein consumed (gm)}}$$

$$\text{NPR} = \frac{\text{Body weight gain (gm)} + \text{loss in body weight (nitrogen-free diet)}}{\text{Protein consumed (gm)}}$$

Experiment 3

Poult s were fed diets containing 12%, 15% and 18% CP from soybean meal (SBM) and meat and bone meal (MBM) along with the diets containing CHM and WHM (Table 2). These diets also contained 1.5% Celite, a source of acid insoluble ash (AIA) which was added as an indigestible marker for the determination of AA bioavailability.

This experiment began when all poult s were 10 d of age such that older birds could be used for ileal digesta collection at the end of the study. The experimental period was 8 days with individual poult weights and feed intake determined at day 4 and 8. At the end of the experiment, poult s fed the diets containing 18% CP from the MBM, WHM, CHM diets were retained for ileal digesta collection (AA availability). The numbers of birds per pen were reduced from 4 to 2 and all poult s were switched to a 12 L:12 D photoperiod. After several days of adjustment to the 12 L:12 D photoperiod, poult s were asphyxiated with carbon dioxide gas 3 to 4 h after the beginning of the 12 h light cycle. Digesta was collected from the distal end of the small intestine, from meckels diverticulum down to the ileo-cecal junction. Digesta samples from individual poult s were pooled by pen within a treatment so as to have adequate material for chemical analysis. The samples were dried at 60 C for 24hr after which they were cooled and finely ground with mortar and pestle. The ground excreta samples were stored at -20 C prior to analysis. Samples of all diets and duplicate excreta samples from the CHM, WHM, and MBM treatments were analyzed for AIA and AA (University of Missouri Analytical Laboratory). Digestible AA were calculated according to the following formula:

$$\text{Apparent AA digestibility} = \frac{(\text{AA/AIA}) \text{ feed} - (\text{AA/AIA}) \text{ ileum}}{(\text{AA/AIA}) \text{ feed}}$$

Table 2. Experimental diets containing four sources of CP (Experiment 2).

Source	CHM			WHM			SBM			MBM		
	12	15	18	12	15	18	12	15	18	12	15	18
Glucose	29.0	16.5	4.0	35.6	24.9	14.2	48.8	43.0	39.0	55.6	51.7	49.5
SolkaFlock	12.5	12.7	13.3	11.8	11.9	12.1	10.3	9.2	6.5	14.7	12.5	9.7
Dical	3.8	4.5	3.8	3.8	3.8	3.8	3.7	3.6	3.7	-----	-----	-----
Limestone	----	----	----	1.2	0.9	0.6	2.0	2.0	2.0	----	----	----
Salt	0.7	0.3	0.4	0.5	0.5	0.4	0.5	0.5	0.5	----	----	----
Celite	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Vit/TM	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
CHM	50.0	62.5	75.0	----	----	----	----	----	----	----	----	----
WHM	----	----	----	43.6	54.5	65.4	----	----	----	----	----	----
SBM	----	----	----	----	----	----	25.2	31.6	38.5	----	----	----
MBM	----	----	----	----	----	----	----	----	----	22.2	27.8	33.3
A/V Fat	----	----	----	----	----	----	6.0	6.6	6.3	4.0	4.5	4.0
Analyzed Nutrient Content												
CP, %	13.9	15.4	18.3	13.4	15.2	19.3	13.1	16.0	20.2	14.3	15.9	21.5
AIA, %	1.65	1.65	1.54	1.37	1.56	1.83	1.78	1.69	1.87	1.49	1.79	1.66
Lysine	0.64	0.80	0.90	0.69	0.79	0.95	0.74	0.92	1.25	0.69	0.78	0.96
Methionine	0.24	0.31	0.35	0.25	0.28	0.33	0.17	0.21	0.27	0.21	0.24	0.27
Cysteine	0.23	0.30	0.35	0.23	0.30	0.33	0.20	0.24	0.31	0.19	0.19	0.28
Threonine	0.44	0.57	0.63	0.45	0.54	0.63	0.46	0.57	0.75	0.48	0.53	0.63
Arginine	0.80	0.92	1.11	0.81	0.99	1.09	0.86	1.06	1.44	1.0	1.13	1.38
OH-proline	0.20	0.21	0.30	0.20	0.27	0.26	0.02	0.02	0.02	0.65	0.75	0.93

Statistical Analysis

The data collected in all the experiments was analyzed by analysis of variance using the General Linear Model (GLM) of SAS (SAS Institute, 1990). The main effects were sources of protein, level of protein and their interaction. Duncan's Multiple Range test was used to separate treatment means ($p < 0.05$).

RESULTS & DISCUSSION

Experiment 1

As can be seen from the results in Table 3, there was no significant differences in BW due to source of protein. There was a significant increase in BW associated with level of CP, however.

Table 3. The effect of source of protein in poult body weight.

<u>Source</u>	<u>Level</u>	<u>Body Weight Gain</u>
WHM	12	119 ± 7.8
	15	132.7 ± 4.8
	18	174.3 ± 36.2
CHM	12	113.7 ± 5.6
	15	147.1 ± 20.6
	18	185.6 ± 12.4

Analysis of Variation

Source of CP	$P < .3746$
Level of CP	$P < .0001$
Source X level	$P < .5272$

Experiment 2

The results from this experiment are shown in Table 4. Feed intake in the CHM-15 diet was actually lower than that of CHM-12 and this had an effect on body weight gain in the CHM-15 diet and resulted in a significant source X level interaction ($P < .0262$). At the highest level of CP (18%), however, feed intake and gain were not too dissimilar and overall, there were no source effects on either PER or NPR. This is the

beauty of these two bioassays, they take into consideration variations in feed intake and adjust body weight responses according to actual levels of protein consumed. This data also supports the results of Johnson and Parsons(1997) who concluded there is no clear advantage of using NPR over PER.

Table 4. The effect of source and level of protein on growth efficiency.

Source	Level	Intake	Gain	PER	NPR
CHM	12	187.9 ± 7.9	51.2 ± 10.0	2.26 ± .36	3.83 ± .31
	15	183.8 ± 7.9	49.8 ± 10.3	1.80 ± .21	3.09 ± .15
	18	224.3 ± 18.1	94.4 ± 7.5	2.35 ± .32	3.23 ± .38
WHM	12	170.4 ± 18.8	36.3 ± 4.5	1.77 ± .11	3.52 ± .22
	15	193.7 ± 15.0	65.1 ± 4.9	2.25 ± .22	3.48 ± .29
	18	205.1 ± 9.7	88.5 ± 10.2	2.39 ± .25	3.36 ± .25
Analysis of Variance		Probability			
Source		.079	.0058	.7116	.4836
Level		.0012	.0001	.0281	.0506
Source X Level		.421	.0262	.0137	.1162

Experiment 3

There were no significant differences in body weight gain between diets containing SBM, CHM, or WHM after 4 days on the experimental diets and all resulted in significantly improved performance compared with diets containing MBM (Table 5). After 8 days, poults fed the diets containing CHM were the heaviest followed by those fed the SBM and WHM. Similar to what was observed at 4 days, the MBM diets resulted in the poorest growth responses. As would be expected, there was a significant increase in gain with increasing levels of protein. Feed intake was greatest in poults fed diets containing CHM followed by SBM and WHM, somewhat similar to what was observed for body weight (Table 6). Poults fed the MBM diets had significantly lower feed consumption which contributed to their poorer growth responses.

The PER and NPR assays are designed to correct for differences in protein intake due to source of protein. While the diets containing CHM, WHM, and SBM had varying effects on intake and body weight gain, there were only small but inconsistent differences between these three sources of protein when the data from all protein levels was considered (Table 7). All three proteins gave far superior PER and NPR responses when compared with MBM.

Table 5. Body weight gain in poult fed different sources of dietary protein.

SOURCES	Days on Diet	% CP		
		12	15	18
MBM b ¹	0-4	19.1 b ²	21.9 b	29.5 a
SBM a	0-4	41.9 b	51.2 b	84.1 a
CHM a	0-4	56.7 b	57.1 b	79.3 a
WHM a	0-4	45.5 b	51.9 b	57.0 a
MBM c ¹	0-8	19.3 b ²	35.5 a	51.9 a
SBM ab	0-8	77.9 b	102.7 b	212.6 a
CHM a	0-8	140.4 b	140.4 b	172.0 a
WHM b	0-8	108.2 b	123.3 b	143.3 a

¹ Different letters within a column represent significant differences within sources of protein.

² Different letters within a row represent significant differences within levels of protein.

Table 6. The effects of different protein sources on feed intake.

SOURCES	Days on Diet	% CP		
		12	15	18
MBM b ¹	0-4	68.2	71.9	78.1
SBM a	0-4	100.3	89.8	121.2
CHM a	0-4	108.4	106.9	128.2
WHM a	0-4	101.6	104.9	101.5
MBM c ¹	0-8	150.4 b ²	162.4ab	176.5 a
SBM b	0-8	219.1 b	225.4ab	333.0 a
CHM a	0-8	309.3 b	292.3 ab	323.7 a
WHM ab	0-8	267.2 b	290.8 ab	269.6 a

¹ Different letters within a column represent significant differences within sources of protein.

² Different letters within a row represent significant differences within levels of protein.

Table 7. The effect of source of protein on PER and NPR

SOURCES	PER % CP			SOURCES	NPR % CP		
	12	15	18		12	15	18
MBM c ¹	0.90	1.35	1.37	MBM b	1.60	1.93	1.76
SBM ab	2.71	2.85	3.16	SBM a	3.23	3.26	3.38
CHM a	3.27	3.12	2.90	CHM a	3.62	3.45	3.15
WHM b	3.03	2.80	2.75	WHM a	3.46	3.14	3.08

¹ Different letters within a column represent significant differences within sources of protein.

Table 8. Dietary Amino Acid Composition: Animal byproduct, Soy

<u>Dietary AA</u>	<u>MBM 12</u>	<u>MBM 15</u>	<u>MBM 18</u>	<u>SBM 12</u>	<u>SBM 15</u>	<u>SBM 18</u>
OH-Proline	0.2	0.75	0.93	0.02	0.02	0.02
OH-Lysine	0.06	0.07	0.08	0	0	0
Threonine	0.48	0.53	0.63	0.46	0.57	0.75
Serine	0.55	0.61	0.71	0.51	0.65	0.82
Lanthionine	0.08	0.08	0.09	0	0	0
Cysteine	0.19	0.19	0.28	0.2	0.24	0.31
Methionine	0.21	0.24	0.27	0.17	0.21	0.27
Valine	0.63	0.69	0.83	0.62	0.75	0.98
Isoleucine	0.42	0.46	0.56	0.56	0.7	0.93
Leucine	0.89	1	1.2	0.94	1.14	1.53
Tyrosine	0.3	0.34	0.41	0.38	0.47	0.65
Phenylalanine	0.47	0.53	0.64	0.62	0.77	1.03
Histidine	0.27	0.3	0.36	0.33	0.4	0.55
Lysine	0.69	0.78	0.96	0.74	0.92	1.25
Arginine	1	1.13	1.38	0.86	1.06	1.44
Tryptophan	0.1	0.12	0.13	0.2	0.23	0.28

Table 8a. Amino Acid Composition: Corn-and Wheat-Hen Meal

<u>Dietary AA</u>	<u>CHM 12</u>	<u>CHM 15</u>	<u>CHM 18</u>	<u>WHM 12</u>	<u>WHM 15</u>	<u>WHM 18</u>
OH-Proline	0.2	0.21	0.3	0.2	0.27	0.26
OH-Lysine	0.02	0.02	0.03	0.02	0.03	0.02
Threonine	0.44	0.57	0.63	0.45	0.54	0.63
Serine	0.49	0.62	0.7	0.5	0.61	0.69
Lanthionine	0.01	0	0	0	0	0
Cysteine	0.23	0.3	0.35	0.23	0.3	0.33
Methionine	0.24	0.31	0.35	0.25	0.28	0.33
Valine	0.6	0.76	0.64	0.63	0.75	0.85
Isoleucine	0.46	0.57	0.64	0.47	0.56	0.64
Leucine	0.92	1.15	1.28	0.89	1.06	1.21
Tyrosine	0.32	0.38	0.48	0.31	0.39	0.44
Phenylalanine	0.51	0.63	0.73	0.52	0.62	.71
Histidine	0.33	0.4	0.44	0.33	0.38	0.45
Lysine	0.64	0.8	0.9	0.69	0.79	0.95
Arginine	0.8	0.92	1.11	0.81	0.99	1.09
Tryptophan	0.16	0.15	0.2	0.13	0.15	0.18

Table 9. Amino Acid Digestibility of 18% CP Diets

<u>Dietary AA</u>	<u>MBM DIG</u>	<u>CHM DIG</u>	<u>WHM DIG</u>
OH-Proline	0.63	0.65	0.54
OH-Lysine	0.48	0.59	0.54
Threonine	0.51	0.69	0.70
Serine	0.53	0.66	0.68
Cysteine	0.39	0.54	0.51
Methionine	0.63	0.84	0.83
Valine	0.60	0.64	0.75
Isoleucine	0.58	0.76	0.77
Leucine	0.64	0.78	0.78
Tyrosine	0.60	0.80	0.77
Phenylalanine	0.63	0.78	0.78
Histidine	0.59	0.80	0.80
Lysine	0.58	0.81	0.82
Arginine	0.68	0.81	0.81
Tryptophan	0.63	0.80	0.77

The results from these studies provide strong evidence that the extruded corn- and wheat-hen diets are good sources of dietary protein for turkeys. As invariably happens when one feeds relatively low protein, purified diets to turkeys, the results of particular treatments may be influenced by variability within particular pens and this would appear to be the case in Experiment 2, Table 4. The low PER values for the CHM-15 and WHM-12 diets resulted in a source by level interaction, but this is experimental variability, not truly treatment effects.

In Experiment 3, PER values across all sources and levels of protein suggest that the CHM and WHM diets were of similar protein quality to the diets containing soybean meal. The NPR values take into consideration differences in feed intake and its relationship with growth and it also shows no differences between these three sources of protein. In all aspects measured, the meat and bone meal resulted in the poorest performance and this was supported by the amino acid digestibility data. The amino acid concentration of all experimental diets is shown in Tables 8 and 8a and the digestibility coefficients for the 18% CP CHM, WHM, and MBM diets are shown in Table 9. The samples of digesta from the SBM diets were lost, hence the lack of digestibility values for that ingredient. The most notable characteristic of the diets containing MBM are the relatively high concentrations of OH-proline compared with the other experimental diets. This amino acid is specific to collagen and the increased level in MBM is due to the high proportion of bone in this product. Collagen is not a particularly well digested protein and this probably contributes to the significantly lower gain, PER, and NPR responses in poult fed the MBM diets when compared with the other sources of protein. It should be noted that the amino acid digestibility values for the following amino acids in MBM corresponded closely to the ileal digestibility

values reported for broilers by Ravindran et al. (1999) as published Br. Poultry Science 40:266-274:

<u>Amino Acid</u>	<u>Broiler</u>	<u>Turkey Experiment</u>
Threonine	.46	.51
Valine	.63	.60
Methionine	.63	.63
Arginine	.64	.68
Lysine	.53	.58
Histidine	.54	.59
Leucine	.60	.64

SUMMARY

The extruded corn- and wheat-hen diets were of similar protein quality to soybean meal when compared in an *in vivo* bioassay and were significantly better than meat and bone meal. Amino acid digestibility was approximately 80% for the indispensable amino acids of greatest interest (methionine, lysine, tryptophan, arginine) but was only 70% for threonine.

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