ANALYSIS, PURCHASING, AND UTILIZATION OF ANIMAL BY-PRODUCTS: ASSESSING THE VALUE OF ANIMAL BY-PRODUCTS

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Animal derived protein meals (animal by-products) such as meat and bone meal (MBM), meat meal (MM), poultry by-product meal (PBM), hydrolyzed feather meal (FM) and to a lesser extent blood meal (BM) and fish meal are all important feedstuffs for poultry diets. Traditionally, the poultry industry has utilized approximately 37% of the total animal protein meal produced, making the poultry industry the overall largest user (Pearl, 2004). Poultry can effectively utilize animal by-products and MBM and PBM comprise the largest quantities of those used; however, animal by-products are one of the most variable feed ingredients used in swine and poultry diets. Animal by-products contribute many nutrients to the overall diet composition, but in particular they provide three important classes of nutrients which are the most costly in formulation; amino acids (i.e., crude protein), energy (fats) and phosphorus (available phosphorus) and each of these areas will be discussed in greater detail.

AAFCO DEFINITIONS

The American Association of Feed Control Officials (AAFCO) defines the composition for rendered animal by-products as well as all feed ingredients. The 2007 AAFCO Ingredient Manual references some 125+ individual animal by-products, and thus there can be confusion about the definitions. The AAFCO definition is important, because it describes the materials which may be used to manufacture rendered animal by-products and, more importantly, the raw materials that cannot be used. The raw materials used affects the levels and digestibility of nutrients as does how the product was processed. The primary animal by-products used by the poultry industry are meat and bone meal, poultry by-product meal, and hydrolyzed feather meal and their AAFCO definitions are below.

Meat and bone meal is the rendered product from mammal tissues, including bone, exclusive of any added blood, hair, hoof, horn, hide trimmings, manure, stomach and rumen contents, except in such amounts as may occur unavoidably in good processing practices. It shall not contain added extraneous materials not provided for in this definition. It shall contain a minimum of 4% phosphorus and the calcium level shall not be more than 2.2 times the actual phosphorus (P) level. It shall not contain more than 12% pepsin indigestible residue and not more than 9% of the crude protein in the product shall be pepsin indigestible. The label shall include guarantees for minimum crude protein, minimum crude fat, maximum crude fiber, minimum phosphorus, and minimum and maximum calcium.

Poultry by-product meal consists of the ground, rendered, clean parts of the carcass of slaughtered poultry, such as necks, feet, undeveloped eggs, and intestines, exclusive of feathers, except in such amounts as might occur unavoidably in good processing practices. The label shall include guarantees for minimum crude protein, minimum crude fat, maximum crude fiber, minimum phosphorus, and minimum and maximum calcium. The Calcium level shall not exceed the actual level of P by more than 2.2 times.

Hydrolyzed poultry feathers are the product resulting from the treatment under pressure of clean, undecomposed feathers from slaughtered poultry, free of additives and/or accelerators. Not less than 75% of its crude protein content must be digestible by the pepsin method.

NUTRIENT CONTENT AND ASSESSMENT

Typical nutrient composition of the three most commonly used animal by-products are shown in Table 1.

Table 1. Nutrient Composition of Animal By-Products

	Meat and Bone Meal		Poultry By-Product Meal		Hydrolyzed Feather Meal
Item[1]	Low Quality	High Quality	Low Quality	High Quality	
Crude protein, %	50.4	51.0	54.5	56.9	8.5
Fat, %	8.5		14.0		4.0
Calcium, %	9.2		5.0		0.20
Available Phosphorus, %	4.7		2.7		0.70
TME_n, kcal/kg	2,530		3,040		2,880
Total Amino Acids[2], % (Digestibility Coefficients)					
Lysine	2.52 (76)	2.74 (85)	2.92 (76)	3.07 (83)	2.06 (65)
Methionine	0.64 (80)	0.71 (88)	0.88 (82)	0.95 (89)	0.60 (74)
Cystine	0.55 (60)	0.58 (67)	1.16 (63)	1.11 (73)	4.43 (61)
Met + Cystine *(weighted)*	1.19 (71)	1.29 (79)	2.04 (71)	2.06 (80)	5.03 (63)
Threonine	1.60 (76)	1.75 (84)	2.31 (76)	2.36 (84)	3.90 (73)
Isoleucine	1.38 (79)	1.45 (87)	2.21 (83)	2.21 (88)	3.97 (86)
Tryptophan	0.32 (77)	0.32 (82)	0.43 (76)	0.41 (80)	0.54 (83)
Arginine	3.33 (81)	3.68 (89)	3.95 (86)	3.92 (90)	5.84 (83)
Valine	2.15 (78)	2.27 (86)	3.04 (81)	2.98 (86)	6.12 (82)

[1] Proximate composition values from Feedstuffs ingredient analysis table (Dale and Batal, 2007).
[2] Crude protein and amino acid values are from the Ajinomoto Heartland Tables, Revision 7.05 (2007).

Animal by-products are good sources of protein (amino acids), available phosphorus and energy, the most expensive nutrients in a poultry diet. Meat and bone meal and PBM are higher in protein than soybean meal and other plant proteins (Pearl, 2004). Even though animal by-products are good sources of amino acids, the amino acid (AA) digestibility coefficients (DC) and hence digestible amino acid levels can vary greatly as shown above, especially when compared to soybean meal. The amino acid and DC values above are from the Ajinomoto Heartland LLC Amino Acid Digestibility Table (2007), but there are several other sources of coefficients available to the nutritionists. The phosphorus content in MBM is ~ seven-fold that found in SBM and is in a form that is highly available to livestock and poultry.

A primary issue of consideration for the nutritionist in utilizing animal by-products (MBM, PBM, etc.) efficiently is in determining the appropriate nutrient content, to maximize value. Thus, the question arises of how one adequately measures the nutrient levels and thus, value of their animal by-products. These points will be discussed in more detail below.

ASSESSMENT OF ENERGY CONTENT

One of the concerns expressed when using animal by-products is the determination of the metabolizable energy content (Firman *et al.*, 2004). The metabolizable energy (ME) of MBM and PBM listed by the NRC (1994) which was determined several decades ago appears to be too low (Dale, 1997) because of the confounding effect of high levels of calcium and phosphorus on the assay results. Due to variation in the composition of animal by-products, table values can be of limited use in describing the nutritional value of animal by-products from a new supplier. Analytical testing should be done to establish reliable nutrient parameters.

There are various methods that can be used for measuring/calculating the energy value of animal by-products; TME_n assay using precision-fed roosters, AME assays based on ileal or excreta collections and the use of prediction equations based on proximate composition. Recent work by Firman *et al.* (2004) examined a variety of methodologies that have been used over the years to determine the energy content of feedstuffs (Table 2). While some differences occurred because of methodology, in most cases the methods provided similar energy estimations for MBM. Table 3 shows a listing of various prediction equations for MBM and PBM based on proximate composition. It has been reported that the use of most of these equations underestimate the metabolizable energy value of PBM samples (Dozier and Dale, 2005), thus the best way to determine the metabolizable energy value of an animal by-product is to run an *in vivo* assay (either TME_n or AME_n). The ash and fat content appear to have the largest impact on the metabolizable energy content of animal by-products; however, as can be seen in Table 4 these two parameters alone do not fully explain the differences in energy values.

Table 2. Mean Metabolizable Energy (ME) Values for Each Assay Method of Each MBM Product (kcal/kg)[1]

	MBM - 2		MBM - 3		MBM - 5		MBM - 7		MBM - 8		MBM - 9	
Sample	Mean[2]	SE[3]	Mean[2]	SE[3]	Mean[2]	SE[3]	Mean[2]	SE[3]	Mean[2]	SE[3]	Mean[2]	SE[3]
Rooster TME_n	2,240[bc]	90	2,469	74	3,026[a]	61	3,329	131	2,547	86	3,356[a]	97
Turkey TME_n	2,528[ab]	81	2,517	74	2,600[bc]	79	3,103	131	2,585	86	2,669[b]	97
Chick Digesta AME_n	2,135[c]	81	2,436	74	2,555[c]	61	2,705	131	2,401	97	2,858[b]	86
Chick Excreta AME_n	2,508[ab]	81	2,577	74	2,751[abc]	61	3,038	131	2,552	86	3,003[ab]	86
Chick Excreta $aAME_n$	2,475[abc]	81	2,614	74	2,786[abc]	61	3,081	131	2,594	86	3,040[ab]	86
Poult Digesta AME_n	2,722[a]	90	2,454	74	2,882[ab]	69	2,863	131	2,581	97	2,946[ab]	137
Poult Excreta AME_n	2,586[ab]	90	2,510	74	2,975[a]	61	2,888	131	2,503	86	2,822[b]	86
Poult Excreta $aAME_n$	2,611[ab]	90	2,534	74	3,004[a]	61	3,103	131	2,503	86	2,851[b]	86
Significance	0.007		None		<0.001		None		None		0.0017	

[1] Pearl, 2004.
[2] Means with no common letters are significantly different.
[3] Pooled standard error (SE) differs due to unequal number of experimental units.
TME_n = true metabolizable energy nitrogen corrected; AME_n = apparent metabolizable energy nitrogen corrected; $aAME_n$ = apparent metabolizable energy nitrogen correct and adjusted for endogenous loss.

Table 3. Prediction equations; Estimating the Energy Value (kcal/kg) of Various Animal By-Products from Proximate Composition

Ingredient	Prediction Equation	Reference
Meat and bone meal	$ME_n = 33.94 \times DM - 45.77 \times ash + 59.99 \times EE$	Janseen, 1989
Meat and bone meal	$ME = 240.8 - 75.9 \times CHO + 47.8 \times CP$	Firman *et al.*, (2004)
Meat and bone meal	$ME_n = -978 - 59.3 \times CHO + 0.9 \times GE$	Firman *et al.*, (2004)
Poultry By-product meal	$TME_n = -725 + 0.841 \times GE$ (kcal/kg DM)	Pesti *et al.*, 1986
Poultry By-product meal	$TME_n = 4{,}070 - 142 \times calcium$	Pesti *et al.*, 1986
Poultry By-product meal	$TME_n = 4{,}330 - 61 \times ash$	Pesti *et al.*, 1986
Poultry By-product meal	$TME_n = 5{,}060 - 263 \times ash + 491 \times calcium$	Pesti *et al.*, 1986
Poultry By-product meal	$TME_n = 479 + 89 \times CP - 1{,}094 \times phosphorus$	Pesti *et al.*, 1986
Poultry By-product meal	$TME_n = 11{,}340 - 103 \times CP - 327 \times calcium$	Pesti *et al.*, 1986
Poultry By-product meal	$TME_n = 934 - 69 \times CP - 110 \times ash$	Pesti *et al.*, 1986
Poultry By-product meal	$TME_n = 561 - 154 \times calcium - 622 \times phosphorus$	Pesti *et al.*, 1986
Poultry By-product meal	$TME_n = 556 - 63 \times ash - 506 \times phosphorus$	Pesti *et al.*, 1986
Poultry offal meal	$TME_n = 2904 + 65.1 \times EE - 54.1 \times ash$	Dale *et al.*,, 1993
Poultry offal meal	$ME_n = 31.02 \times CP + 74.23 \times EE$	Janseen 1989
Feather meal (pepsin dig>80%)	$ME_n = 33.2 \times CP + 57.33 \times EE$	Janssen, 1989

Note: Abbreviations used above are as follows: GE = gross energy; ME = metabolizable energy; ME_n = nitrogen-corrected metabolizable energy; TME_n = nitrogen corrected true metabolizable energy; CP = % crude protein; EE = % ether extract; CF = % crude fiber, DM = % dry matter, CHO = carbohydrate.

Table 4. Composition and TME_n content of poultry offal meal samples[2]

Samples	CP, %	Fat, %	Ash, %	TME, (kcal/kg)	Predicted TME, (kcal/kg)	Difference in predicted and actual TME
1	65.6	23.9	2.8	4261	4308	1.11
2	59.5	28.6	4.8	4697	4506	4.15
3	67.6	18.3	5.2	3626	3814	5.05
4	50.5	35.6	5.6	4772	4919	3.03
5	47.5	33.8	5.6	4495	4801	6.59
6	53.5	34.8	5.9	4853	48/50	0.06
7	47.8	36.5	5.9	4785	4961	3.61
8	57.1	28.3	6.0	4508	4422	1.93
9	47.8	39.4	6.2	5247	5134	2.19
10	55.8	27.2	6.3	4475	4334	3.20
11	48.8	36.4	6.4	4739	4927	3.90
12	48.6	37.7	6.6	5146	5001	2.85
13	47.1	35.4	6.7	4728	4846	2.47
14	49.8	35.2	6.8	5001	4828	3.53
15	48.4	32.7	7.1	4884	4649	4.94
16	47.9	34.9	7.1	4902	4792	2.27
17	47.4	36.5	8.1	5148	4842	6.13
18	47.6	36.0	9.6	4631	4728	2.08
19	53.4	29.5	9.8	4486	4294	4.37
20	54.9	25.0	10.2	4204	3980	5.48
21	48.4	32.1	11.4	4330	4377	1.08
22	48.3	30.5	14.5	3901	4105	5.10
Average	52.0	32.2	7.2	4629	4429	3.41
High	67.6	39.4	14.5	5247	5134	6.59
Low	47.1	18.3	2.8	3626	3814	0.06

[1] Dale *et al.*, 1993.
[2] All data were adjusted to 92% dry matter.

Work by Dale (1997) suggested that for practical diet formulation, energy values of 2,450 kcal/kg should be employed for MBM's of beef origin and 2,800 kcal/kg for pork. For mixed meals of uncertain origin, an intermediate value can be estimated on the basis of fat, ash and gross energy. More recent work by Dozier and Dale (2005) reported TME_n values for feed-grade PBM ranged from 2,775 to 3,555 kcal/kg; whereas, pet food-grade PBM varied from 3,316 to 3,401 kcal/kg. Although there was no difference in the metabolizable energy value of the pet food-grade or feed-grade PBM samples, the pet food-grade samples were of a much more consistent composition.

ASSESSMENT OF PHOSPHORUS CONTENT

Meat and bone meal and PBM are valuable components of poultry diets and while they might be primarily considered as protein sources, these animal by-products also contain substantial quantities of highly available phosphorus (Mendez and Dale, 1998) and thus can be a good replacement for inorganic P sources. Since it is generally assumed that the P in animal by-products is close to 100% available, as it contains very little phytate phosphorus, a measurement of the total P in animal by-products is usually adequate. Studies have shown no significant differences in the relative biological availability of P in animal by-products vs. feed grade mono-dicalcium phosphorus (Waldroup and Adams, 1994). However, variation in the levels of P between individual samples of MBM and PBM can cause problems in feed formulation. Shutze and Benoff (1982) reported P values in MBM ranging from 2 to 6%, this variation could be critical when formulating a broiler diet. Waldroup and Adams *et al.* (1994) did not find as much variation in the MBM samples that they tested (2.72 to 4.57, Table 5), however they did find significant variation in the PBM samples they tested (1.71 to 3.19, Table 6).

Table 5. Nutrient Composition of samples of Meat and Bone Meal[1]

Sample	ME, kcal/kg[2]	CP, %	Ash, %	Calcium, %	Phosphorus, %
1	2481	47.44	28.61	8.31	4.06
2	2859	50.45	26.39	8.42	4.23
3	3349	50.66	20.37	5.33	2.81
4	2387	51.41	31.05	9.76	4.57
5	2607	50.46	28.00	8.34	3.98
6	3325	50.40	19.98	5.37	2.72
7	2981	45.50	25.47	7.85	3.82
8	2679	51.78	25.33	5.44	2.65
9	2549	54.24	25.54	7.45	3.60
10	2806	46.96	27.07	7.13	3.42
11	2795	55.19	23.51	7.23	3.71

[1] Waldroup and Adams *et al.*, 1994.
[2] Calculated from proximate composition based on the NRC (1994) equations.

If a broiler diet contained 5% PBM which was 3.19% P (sample 6, Table 6), this ingredient alone would supply 35% of the available P requirement. However, if the PBM contained only 1.71% P (sample 5, Table 6) at the same inclusion level, this ingredient would only supply 19% of the available P requirement. If this were not fully accounted for during formulation, the diet would be deficient in P and chick performance would be severely comprised. Thus, consistent and predictable levels of P in MBM and PBM are crucial for adequate diet formulation and animal performance.

Table 6. Nutrient Composition of samples of Poultry By-Product Meal[1]

Sample	ME, kcal/kg[2]	CP, %	Ash, %	Calcium, %	Phosphorus, %
1	3239	62.86	12.44	2.78	1.78
2	2833	65.93	15.78	3.84	2.28
3	2609	57.66	19.59	7.48	1.81
4	3005	68.48	12.11	2.81	1.81
5	3061	69.18	11.99	2.58	1.71
6	2672	53.57	24.87	6.35	3.19

[1] Waldroup and Adams *et al.*, 1994.
[2] Calculated from proximate composition based on the NRC (1994) equations.

ASSESSMENT OF PROTEIN/AMINO ACID QUALITY

The variability in protein (amino acid) quality of MBM and PBM is perhaps the most important consideration and often limits their use in poultry and livestock rations (Parsons, *et al.*, 1997). A number of factors can affect the protein/amino acid quality of animal by-products. Processing temperature and pressure have been shown to significantly impact AA digestibility (Wang and Parsons, 1998[a], Shirley and Parson, 2000). Ash content has also been shown to influence protein quality and amino acid digestibility of animal by-products (Shirley and Parsons, 2001). Animal by-products that contain higher ash are generally considered to be of lower protein quality. Wang and Parsons (1998[a]) and Shirley and Parsons (2001) found that protein quality of MBM and PBM does indeed decrease with increasing ash content but the reduction is due to a poorer balance of total AA and not decreased AA digestibility.

Because of the many factors that can influence protein and AA quality of animal by-products it is of great importance that nutritionists be able to predict the protein or AA quality of the animal by-products they use. Many different types of laboratory assay have been evaluated, but only a few have been found to be useful in evaluating the protein or AA quality of animal by-products. Some of the most common *in vitro* and *in vivo* methods of assessing protein quality of animal by-products are the Protein Efficiency Ratio (PER), pepsin digestibility, Near Infrared Reflectance Spectroscopy (NIRS), and Immobilized Digestive Enzyme Assay (IDEA). However, perhaps the best and most direct method is to determine the AA DC either using the precision-fed rooster assay or ileal digestibility method. Other in-vitro tests, such as, protein solubility in potassium hydroxide or multi-enzyme pH change have little correlation with true lysine digestibility, 0.08 and 0.10, respectively (Parsons *et al.*, 1997). Thus, these tests are of limited value when measuring the AA or protein quality of animal by-products.

Wang et al. (1997) reported that the order of AA limitation in MBM and PBM for chicks is cystine, tryptophan, and threonine (with phenylalanine and tyrosine being fourth) with cystine and tryptophan being much more deficient than the others (Wang *et al.*, 1997). The observed order of amino acid limitation of MBM and PBM is show in Table 7, although this may differ depending upon the individual product and amino acid levels targeted in formulation.

Table 7. Order of Amino Acid Limitation in MBM and PBM for Chicks[1]

Amino Acid	Meat and Bone Meal	Poultry By-Product Meal
Cystine	1	1
Tryptophan	1	2
Threonine	3	3
Isoleucine	4	6
Phe + Tyr	4	3
Methionine	6	5
Lysine	7	6
Valine	8	
Histidine	8	

[1] Wang *et al.*, 1997.

The protein efficiency rate (PER) assay has been used primarily for predicting protein quality of food for humans, and to a lesser extent to evaluate various feed ingredients for poultry (Johnson and Parsons, 1997). The PER assay consists of feeding the test ingredient as the sole source of dietary protein in a diet containing only 9 to 10% CP and is subsequently calculated as the amount of weight gain per until of protein consumed. Johnson and Parsons (1997), Johnson *et al.*, (1998) and Parsons *et al.* (1997) reported that ash was inversely correlated to the PER (R-square = -0.80) and crude protein was positively correlated to the PER (R-square = 0.68). However, PER value does not appear to be a good indicator of the amino acid digestibility of PBM (Table 8, 9) or MBM (Table 9) and as such might have limited value as a quality assessment.

Table 8. Composition and Protein Quality for Random Samples of Poultry By-Product meal (%)[1]

Sample	CP (%)	Ash (%)	Fat (%)	LYS digest. (%)	THR digest. (%)	PER
1	62	15	16	72	75	2.1
2	59	23	16	88	86	2.4
3	63	16	14	81	81	2.8
4	63	14	16	86	83	2.8
5	60	17	11	75	75	2.8
6	61	20	14	89	84	2.8
7	68	9	13	76	79	2.9
8	65	16	13	83	84	2.9
9	63	15	12	83	83	3.3
10	63	15	14	76	80	3.3

[1] Parsons *et al.* (unpublished).

Table 9. Digestibility of Total Essential Amino Acids (TEAA) and Protein Efficiency Ratio (PER) of Meat and Bone Meals (MBM) and Poultry By-product Meals (PBM) Varying in Ash Content

Meal	Ash (%)	Digest. Coeff. For TEAA (%)	PER
MBM	24	73	1.43
MBM	35	78	0.68
PBM	7	75	2.35
PBM	16	78	2.10

The pepsin digestibility assay is one of the most commonly used tests for monitoring protein quality of animal by-products (Parsons *et al.*, 1997). Using the AOAC (2007) standard reference pepsin digestibility test (0.2 percent pepsin) it was shown that pepsin digestibility correlated poorly with lysine digestibility in broilers (Pearl, 2004). Using a more dilute concentration of pepsin (0.002 or 0.0002 percent) improved the correlation (Parsons, *et al.*, 1997), but still accounted for less than 50 percent of the variation in digestibility of amino acids in MBM. Table 10 illustrates the correlation of the pepsin *in-vitro* analysis compared to lysine digestibility determined *in vivo*. Although there was a positive correlation between pepsin value and lysine digestibility, the correlations did not exceed 0.70 and there were no significant correlations between pepsin values and other protein quality measurements (such as, PER or sulfur AA digestibility). Some reports have concluded that pepsin digestibility can be somewhat useful in predicting the protein quality of animal by-products (particular MBM), if the level of pepsin is reduced from 0.2 to 0.002 or 0.0002%, however there is a continued need for an *in vitro* assay that is a better predictor of *in vivo* quality (Parsons *et al.*, 1997),

Table 10. Correlation Coefficients of Pepsin and Lysine Digestibility for 14 Meat and Bone Meals[1]

Assay	R Value
0.2% pepsin vs. Lys digestibility	0.25
0.002% pepsin vs. Lys digestibility	0.69*
0.0002% pepsin vs. Lys digestibility	0.62*

[1]Parsons *et al.*, 1997.
* r value is significant ($P<0.001$).

Near Infrared Reflectance Spectroscopy (NIRS) may be a quick, reliable, and easy to use method to determine the total AA concentration and AA digestibility of animal by-products. In this technique, the absorbance of light in the near infrared region (1100 to 2500 nm) is related by powerful statistical methods to parameters in the materials that one wants to predict, such as protein content, AA content, and digestibility. NIRS is a common quality control tool in the feed industry. Typically, NIRS is used for predicting the CP content of feed ingredients, but work by van Leeuwen *et al.* (1991) and van Kempen and Bodin (1998) have shown that it can also be used to predict total and digestible AA. While NIRS has been adopted by some labs as the method for determining total amino acid levels of common feed ingredients, the practical use of NIRS to predict either total AA levels or AA digestibility of animal by-products needs a great deal of additional evaluation, before it is widely accepted.

The in-vivo determination of AA digestibility coefficients appears to be the best indicator of AA quality of animal by-products. The variation in AA digestibility among samples of MBM is substantial (Table 11) and there is considerable differences in digestibility among specific individual amino acids within a sample.

Table 11. Variation in Amino Acid Digestibility Coefficients (%) for Different Samples of Meat and Bone Meal [1]

Sample	Lysine	Cystine	Threonine	Methionine
5	88	72	86	89
12	69	37	72	81
13	86	68	86	91
16	77	55	79	84

[1] Parsons, *et al.*, 1997.

The development of the precision-fed cecectomized rooster assay has resulted in a large increase in digestible AA data being available for animal by-products. This procedure provides a means of obtaining AA digestibility data within a reasonable amount of time and at a reasonable cost. However, the use of ileal digestibility method is becoming popular and has recently been agreed upon by a University consortium group as the reference standard (Feedstuffs, 2007). A concise description of these various methods was presented by Batal (2006). Whichever method one chooses to use, the determination of AA digestibility of their animal by-products is the best indicator of AA or protein quality and either method can be employed successfully. The use of digestible amino acid formulation is a significant improvement over one based on total amino acid levels (Creswell and Swick, 2001; Waldroup, 2001; Dudley-Cash, 2002; Burnham, 2007) and should be the method employed for any ingredient with a high amount of variability, such as animal by-products.

A method to rapidly test AA digestibility of animal proteins that uses immobilized enzymes has been developed by Dr. Chuck Schasteeen's group at NOVUS International and the method is known as the Immobilized Digestive Enzyme Assay (IDEA) (Schasteen *et al.*, 2002). The IDEA assay developed for MBM and PBM (called Poultry Complete) produces AA digestibility values that are highly correlated with cecectomzied rooster digestibility values. Research suggests that the IDEA system represents a rapid, robust, and inexpensive predictor of amino acid digestibility in several important feed ingredients. While this is an indirect method of determining amino acid digestibility coefficients, it has one distinct advantage over any *in vivo* method in the quick turn-around time in which results can be obtained (~ 2 hours).

PRACTICAL APPLICATION / IMPLEMENTATION

Due to the variation in amino acid digestibility of ingredients, and the need to more closely meet the animals' nutrient requirements, formulation of diets containing animal by-product meals on an available or digestible AA basis should be superior to formulation on a total AA basis. This fact has been documented by several researchers and some of these papers will be discussed in more detail below. Perhaps the classic research trial examining the value of formulating diets on a digestible amino acid basis, versus a total amino acid basis, was published more than a dozen years ago (Rostagno *et. al.*, 1995).

The conclusion from this paper stated, "This result demonstrates the potential benefit of switching diet formulation from total to digestible amino acids". Formulating broiler diets based on digestible amino acids gives a better prediction of dietary protein quality and bird performance than total amino acids.

In regards to animal by-products, Wang and Parsons (1998[b]) evaluated the response in broilers when a low or high quality MBM at 10 or 20% inclusion was formulated on a either a total, digestible or bioavailable amino acid basis (Table 12). Feeding a low quality MBM at 20% of the diet required additional amino acid supplementation to reach equal performance of a corn soybean meal diet. They reported that formulating feeds on a digestible amino acid basis is superior to formulating on a total amino acid basis, particularly for diets which contain a low quality meat and bone meal.

Table 12. Low and High Quality MBM on Broiler Performance

Meat & Bone Level	Bodyweight gain (grams)	Feed Intake (grams)	Feed per Gain
Control (0%)	326[abc]	473[b]	1.449[a]
Total Amino Acid Values Used in Formulation			
10 % Low Quality	313[def]	487[ab]	1.553[de]
20 % Low Quality	288[g]	484[ab]	1.681[f]
10 % High Quality	322[bcd]	492[a]	1.524[cd]
20 % High Quality	310[ef]	475[b]	1.531[cde]
Digestible Amino Acid Values Used in Formulation			
10 % Low Quality	323[bcd]	482[ab]	1.495[b]
20 % Low Quality	304[f]	475[b]	1.560[e]
10 % High Quality	330[ab]	478[ab]	1.447[a]
20 % High Quality	319[cde]	485[ab]	1.520[bc]
Bioavailable Amino Acid Values Used in Formulation			
10 % High Quality	335[a]	486[ab]	1.453[a]
20 % High Quality	332[ab]	486[ab]	1.466[a]

In a three part series, Creswell and Swick (2001) discussed the concept and application of implementing digestible amino acids in broiler formulation. In part one, the advantages of digestible amino acid formulation over total amino acid formulation was stressed, citing several published papers which examined the inclusion of by-products into broiler feeds and how through the use of digestible values, these ingredients could be most efficiently utilized. In part two of this series of articles, the source of digestibility coefficients, or digestible amino acid values for ingredients was discussed. It was noted that while coefficients can be generated from a variety of methods (fecal or ileal, true or apparent, roosters or broilers, for examples), the slight differences which are noted should not prevent a nutritionist from adopting digestible amino acid formulation. To quote, "The question is not whether the values are absolutely correct, but that whether ingredients are being correctly ranked relative to one another". The values which they provided in a table were apparent ileal digestibility values, which tend to provide slightly lower estimates of digestibility than those of a true ileal determination. In this same article, digestible amino acid requirement values as well as ideal amino acid ratios relative to lysine were discussed and it was noted that lysine levels need to be higher than NRC (1994) based upon current research and the needs of the modern high meat yielding broiler strains in the marketplace today. A simple relationship for converting from total lysine to digestible lysine was to use a digestibility factor of 85%, but this might be overly simplistic, particularly if animal by-products are being utilized in the basal diet from which the total lysine content was determined. In the third article from this series, a demonstration of least cost formulations was presented, showing differences between those formulated on a total basis, versus those formulated on a digestible basis. A diet was formulated on a total lysine basis and subsequently two digestible amino acid formulated diets were made; the first one using an 85%

digestibility factor for the lysine minimum as noted above, and the second one using the calculated digestible lysine level, determined from the total diet. This gave a targeted minimum total lysine of 1.2% in the total diet, an 85% digestible lysine of 1.02% and a calculated digestible lysine of 0.996% in the two digestible amino acid diets. The 85% lysine diet showed an increase in feed cost as the total lysine went from 1.20% to 1.23%; however, it was noted that this diet should provide better performance since it is formulated to more closely meet the animal's needs and contains a higher percentage of usable amino acids. The formulation scenarios were repeated as more animal by-products were offered and it was noted that the cost differential could be reduced to below that of the total amino acid formulated diet. Obviously, whether one uses total or digestible amino acid values will greatly impact the shadow cost of animal by-products, especially those with digestibility coefficients lower than that of typically used ingredients (such as, corn and soybean meal). Ideally, digestible amino acid values should be used in formulation as they not only allow for more accurate formulation, based on the birds needs, but they will also give a more accurate assessment of the value of any animal by-product.

Dudley-Cash (2002) discussed methods to transition from formulating on a total amino acid basis to one on a digestible amino acid basis. The use of digestible amino acid matrix values and determined requirements, via a back-calculation method from the total levels using ideal amino acid ratios was proposed. The true opportunity or shadow cost of an ingredient was shown to be quite different if calculated on a total versus a digestible amino acid basis. It was stressed that the true shadow cost is determined using the digestible amino acid approach and that the shadow costs determined from diets formulated on a total basis overestimate the value of ingredients. This is particularly true for animal by-products, such as MBM, which typically have low to medium digestibility coefficients, relative to corn and soybean meal. In addition, it was stressed that additional costs occur from formulating on a total AA basis in lost performance from the animal fed such a diet. It was concluded, "In today's market of high-priced and sometimes scarce ingredients, the use of digestible amino acid values is an essential part of a successful least cost feed formulation".

Current formulation practices are demanding that more exact nutrient composition values be available. Formulation models using either the cecectomized rooster or ileal digestible amino acid levels, while maintaining established ideal protein ratios, are recommended. Ideal amino acid ratios establish amino acid levels in the formula based on a specific ratio to one set nutrient level (usually lysine). Lysine is often chosen as the reference because it is easy to assay for, is the second limiting amino acid in poultry diets, is primarily involved in protein accretion and is not a co-factor in other reactions and doesn't interact metabolically with other amino acids. In order to accomplish precision in formulation, research must provide the targeted amino acid requirements and analytical technology must provide an economical and accurate method to determine the nutrient composition of rendered animal by-products (Pearl, 2004). The available methods of determining nutrient composition have been discussed above. Emphasis will now be placed upon implementation of the use of digestible amino acids (using DC's), ideal amino acid ratios and using techniques to predict shadow-cost of an animal by-product meal.

VALUE ASSESSMENT FOR PURCHASING

Formulation examples using a High Quality (HQ) and Low Quality (LQ) MBM in a broiler grower diet, along with an assessment of shadow cost was made. In Table 12 below, are the total and digestible amino acid values and DC's of two MBM's recently analyzed using the cecectomized rooster assay. These products were obtained from the same supplier within the same month and as shown, not only are the total amino acid values quite different, so are the digestibility coefficients, which ultimately leads to drastic differences in the calculated digestible amino acid values.

Table 13. Amino Acid and Crude Protein Values of High and Low MBM used in Formula Example

Amino Acid	High Quality Meat & Bone Meal Total (%)	Digestibility Coefficient	Digestible (%)	Low Quality Meat & Bone Meal Total (%)	Digestibility Coefficient	Digestible (%)
Lysine	3.30	87.20	2.88	2.43	79.70	1.94
Methionine	1.00	94.64	0.95	0.60	90.49	0.55
Cystine	0.64	75.55	0.48	0.48	63.65	0.31
TSAA (weighted)	1.64	87.22	1.43	1.09	78.55	0.85
Threonine	2.00	88.06	1.76	1.50	80.52	1.20
Valine	2.32	87.79	2.04	1.87	83.83	1.57
Isoleucine	1.92	89.40	1.71	1.27	84.28	1.07
Arginine	3.88	88.77	3.44	3.74	83.49	3.12
Tryptophan	*0.31*	*80.8*	*0.25*	*0.33*	*73.8*	*0.24*
Crude Protein, %		58.75			51.70	

Tryptophan levels were not determined on the samples; instead, values used were taken from the Ajinomoto Heartland LLC Revision 7.05 table of amino acid and digestibility coefficients (2007). The nutrient levels as shown were used in the formulations below, to examine the impact of nutrient content upon overall value within a basic corn - soybean meal - MBM broiler grower diet. Identical metabolizable energy (2,530 Kcal/Kg) values and mineral (Calcium (9.2%), Available Phosphorus (4.7%) and Sodium (0.73%)) content (Dale and Batal, Feedstuffs, 2006) were applied to these two products, so only the impact of amino acid levels will be examined. In addition, identical ingredient prices were given to these two ingredients in the formulas shown below (Table 14), despite them having obvious nutritional differences. While this is a somewhat simplified approach to analyzing these products, it nonetheless will provide some indications as to how quality differences between two MBM products ultimately influence the value of that product. The broiler grower diets below were formulated on a digestible amino acid basis, using a minimum digestible lysine content of 1.08% and minimum digestible amino acid ratios relative to digestible lysine, set as follows: Methionine (45), Methionine + Cystine (TSAA) (76), Threonine (67), Valine (77), Isoleucine (68), Tryptophan (16) and Arginine (105). By setting these seven amino acids relative to digestible lysine and offering DL-Methionine, L-Lysine HCl and L-Threonine to the diet, the fourth limiting amino acid become the constraint which held crude protein up to an acceptable level (19.3% (HQ) versus 18.7% (LQ)).

Table 14. Broiler Grower Formulas with HQ or LQ MBM

Broiler Grower Diet	With HQ M&B Meal	With LQ M&B Meal	Difference (%)
Cost / Ton	*$182.57*	*$184.77*	*+$2.20*
Corn	1385	1373.4	-0.84%
Soybean Meal	447.9	479.9	7.14%
HQ *or* LQ M&B Meal	100 (max allowed)	60.6	-39.40%
Poultry Fat	20.8	27.6	32.69%
Salt	11.8	11.1	-5.93%
Calcium Carbonate	11.6	12.3	6.03%
Defluorinated P	8.4	18.4	119.05%
DL-Methionine	5.2	5.8	11.54%
L-Lysine HCl	4.8	5.9	22.92%
L-Threonine	1.5	2	33.33%
VTM	3	3	0.00%
TOTAL =>	2,000.0	2,000.0	

As can be seen, when these two MBM products were priced the same, diet cost increased by $2.20 per ton of complete feed for the feed containing the LQ MBM. The quantity of MBM used was reduced by over 39% when a LQ product was used, versus a HQ MBM, and this was compensated for with additional soybean meal and amino acid supplementation. Similar changes had been noted by the researchers cited above when LQ or HQ MBM were offered. However, as the overall ingredient changes are minimal and the diets are based on digestible amino acids, it is highly likely and expected that equal bird performance would be obtained between these two feeds, although the extra cost per ton would be un-justified. If we look at the actual opportunity / shadow cost (the price where an ingredient will just come into the diet) for these two products, determined using digestible amino acid formulation and levels, we see that the high quality MBM is actually worth more ($362.20 for the HQ versus $310.00 for the LQ, or 16.8% more for HQ). At the $300.00 price which was used in the scenario above, the HQ MBM would have been a bargain; whereas the use of the LQ product would have barely saved any money. This is also noted in the level of product which was pulled into the two examples as the HQ product hit the maximum 5% allowed and the LQ product came in at only ~ 60% of the maximum allowed. If these two MBM's had been evaluated on a total amino acid basis, their relative values would have been over-estimated.

While the shadow cost approach is often used to quickly determine whether an ingredient will be pulled into a formula at a given set of ingredient prices, it is only a one snapshot picture. As competing commodity prices change, the shadow cost of animal by-products also changes. A concept will be proposed, which while using shadow cost, doesn't require access to a least cost program once it is generated, but rather just a simple equation set-up in a spreadsheet. This generated equation could then be used by the nutritionist or the purchasing agent within a company to quickly determine the shadow cost of an ingredient at any time, dependent upon changing commodity prices. As least-cost programs are "linear" in nature, the creation of such a prediction equation is fairly straight-forward and easy to accomplish. The equation is generated through regression analysis of prices for the key ingredients used in formulation, which could impact MBM usage and hence its' opportunity price, upon the shadow price of the product of interest. As MBM is a good source of energy, phosphorus and amino acids (i.e., protein), the logical inputs might be viewed as: Corn, Soybean Meal, Fat and the offered amino acids. This equation would have to be worked out by the nutritionist involved in formulating a given set of feeds as the generated equation must be based on the ingredient matrix employed as well as the nutrient constraints which are used for a given set of diets. The shadow cost of MBM will vary somewhat between the starter, grower and finisher feeds, so either the feed which shows the lowest shadow cost for MBM or the feed which is consumed the most, would be the logical choice on which to base the MBM prediction equation. If the nutrient matrix or formulation specifications change, then a new prediction equation should be generated. To generate the data needed to create the prediction equation, MBM should either be priced out or better yet, set as out of stock, so it will not be pulled into the formula. A table of multiple price points, for each commodity to be considered for incorporation into the prediction, should be set-up initially and the shadow cost determined at each of these scenarios (Table 15). Using the buffer or the parametrics option on pricing, which is available in many of the formulation packages today, these various formulas and determinations of shadow cost can be run relatively quickly. The table below (Table 15) shows a condensed version of the data used to generate the prediction equation for the HQ MBM in the broiler feed noted above. A similar table was set-up in order to generate the equation for predicting the opportunity cost for the LQ MBM. The low and high price range used for each ingredient is shown with two intermediate points, although the range of prices for each commodity which was used started at the low price and then increased by the increments noted up to the high price. As such, there were at least eight (8) price points for each ingredient used in the determination of the regression equation. While the same commodity prices were used for both the HQ and the LQ MBM products, the generated shadow cost differed for each formula, as noted above.

The Analytical Software, Statistix 8.1 (2003) was used to run stepwise linear regression on the data in order to generate the prediction equation for each MBM. The step-wise regression approach only includes those variables which have a significant p-value and thus improve the prediction equation. For example, in the HQ meat MBM the price of L-Lysine HCL and L-Threonine did not improve the prediction and so they were not included in the model. For the LQ MBM, the prices of L-Lysine HCL and L-Threonine did significantly improve the prediction equation and thus were included; whereas the DL-Methionine price did not, and so it was omitted from the equation.

Linear regression can also be run within the Excel spreadsheet, under Tools, Data Analysis, Regression and setting the constant to Zero, to generate the identical equation. At least two steps are required in Excel; the first includes using all of the variables in the x-range to generate an initial equation and then after omitting the variables which have p-values greater than desired (0.05 was used in this case), re-running the routine. The use of either software approach will lead to identical results. The equations generated, based on the particular nutrient matrix values used on the ingredients offered, along with the unique ingredient and nutrient constraints used in this broiler grower diets were as follows:

HQ MBM shadow cost = SBM x 1.1024 + Fat x 0.23564 + DeflP x 0.25 – Corn x 0.58763 + DL-Met x 0.00242. The R-square was 1.00 with a mean square error of 0.34446.

LQ MBM shadow cost = SBM x 0.94994 + Fat x 0.21106 + DeflP x 0.24823 – Corn x 0.38552 - LYS x 0.00777 - THR x 0.00569. The R-square was 1.00 with a mean square error of 2.95358.

SBM = price of Soybean Meal per ton, Fat = price of poultry fat per ton, DeflP = price of Defluorinated Phosphate per ton, Corn = price of Corn per ton, DL-Met = price of DL-Methionine per ton, LYS = price of L-Lysine HCl per ton and THR = price of L-Threonine per ton.

The equation can be set up in a spreadsheet so only the prices for the commodities which added significantly to the prediction equation need be entered to generate the shadow cost of MBM. This is easy to do by setting one column as the coefficients and one as ingredient pricing and then writing an equation multiplying the related coefficient and price together and summing them up. Attention must be taken in regards to the positive or negative value of the coefficient.

Meat and bone meal and other animal by-products can be used effectively in animal feeds. The keys to optimizing their utilization relies upon having a good assessment of the nutrient content, particularly metabolizable energy, mineral levels (especially phosphorus) and amino acid profile (totals and digestibility coefficients, from which digestible amino acids can be determined). Knowing the content of all three of these classes of nutrients is crucial to properly assessing the value of any animal by-product, relative to animal performance. Formulating diets on a full range of essential digestible amino acid ratios will give one the most accurate assessment of the value of an animal by-product. Product consistency is an extremely important part of the process of maximizing economic value from the animal by-products which you use in formulation. As such, perhaps the most important aspect of all in obtaining the greatest value from the animal by-products you use in formulation, is by working closely with your supplier to assure a consistent product.

Table 15. Partial Ingredient Pricing Table used in Determination of HQ MBM Shadow Cost Prediction Equation.

75	20	25	150	125	100	10	<=Increment
$480	$275	$230	$2,530	$1,800	$2,050	$135	<= Base $.$$/ton
Fat	DeflP	SBM	DL-Met	Lysine	Threonine	Corn	MBM Shadow $
480	275	230	2530	1800	2050	135	362.20
180	275	230	2530	1800	2050	135	295.60
405	275	230	2530	1800	2050	135	344.20
555	275	230	2530	1800	2050	135	380.00
780	275	230	2530	1800	2050	135	433.80
480	195	230	2530	1800	2050	135	342.00
480	255	230	2530	1800	2050	135	357.20
480	295	230	2530	1800	2050	135	367.20
480	355	230	2530	1800	2050	135	382.20
480	275	130	2530	1800	2050	135	252.60
480	275	205	2530	1800	2050	135	334.60
480	275	255	2530	1800	2050	135	389.80
480	275	330	2530	1800	2050	135	472.60
480	275	230	1930	1800	2050	135	360.60
480	275	230	2380	1800	2050	135	361.80
480	275	230	2680	1800	2050	135	362.60
480	275	230	3130	1800	2050	135	363.80
480	275	230	2530	1300	2050	135	362.40
480	275	230	2530	1675	2050	135	362.20
480	275	230	2530	1925	2050	135	362.00
480	275	230	2530	2300	2050	135	361.80
480	275	230	2530	1800	1650	135	362.20
480	275	230	2530	1800	1950	135	362.20
480	275	230	2530	1800	2150	135	362.00
480	275	230	2530	1800	2450	135	362.00
480	275	230	2530	1800	2050	95	386.00
480	275	230	2530	1800	2050	125	368.00
480	275	230	2530	1800	2050	145	356.20
480	275	230	2530	1800	2050	175	338.20

LITERATURE CITED

AAFCO. 2007. Official Publication, Association of American Feed Control Officials Incorporated.

Ajinomoto Heartland LLC. 2007. True Digestibility of Essential Amino Acids for Poultry. Revision 7.05. Excel Spreadsheet.

AOAC. 2007. AOAC Official Method 971.09 Pepsin Digestibility of Animal Protein Feeds. Section 4.4.04. Revised March 1999.

Batal, Amy B. 2006. Comparison of Methods to Determine Amino Acid Digestibility For Poultry. Proceedings of the 42nd Eastern Nutrition Conference. University of Guelph, Guelph, Ontario Canada. May 11th-12th. Pages 59-80.

Burnham, David. 2007. Digestible Amino Acid Formulation of Poultry Feeds: Practical Considerations. SYMPOSIUM: Non-Ruminant Nutrition: Lessons and Logistics of Application of Digestible Amino Acids in Diet Formulation" in Abstracts from Poultry Science Association Annual Meeting, July 10th. Abstract 479. Page 396.

Creswell, D and R. A. Swick. 2001. Formulating with Digestible Amino Acids. Asian Poultry Magazine. Three part Series: January/February, March/ April and May/June.

Dale, N. M., B. Fancher, M. Zumbado, and A. Villacres. 1993. Metabolizable energy content of poultry offal meal. Journal of Applied Poultry Research 2:40-42.

Dale, N. 1997. Metabolizable energy of meat and bone meal. Journal of Applied Poultry Research 6:169-173.

Dale, N. and A. B. Batal. 2007. Ingredient analysis table: 2007 edition. Feedstuffs. Vol. 78, No. 38, p. 16, September 13.

Dozier, W. A. and N. M. Dale. 2005. Metabolizable energy of feed-grade and pet food-grade poultry by-product meals. Journal of Applied Poultry Research 14:349-351.

Dudley-Cash, William. 2002. Using Amino Acid Digestibility Values in Feed Formulations. American Soybean Association.

Feedstuffs. 2007. Issue 19, Volume 79. May 7th.

Firman, Jeffre D., David Robbins and Gary G. Pearl. 2004. Poultry Rations. Render. August pages 12-41. www.rendermagazine.com

Janssen, W. M. M. A., ed. 1989. European Table of Energy Values for Poultry Feedstuffs. 3rd ed. Beekbergen, Netherlands: Spelderholt Center for Poultry Research and Information Services.

Johnson, M. L. and C.M. Parsons. 1997. Effects of raw material source, ash content, and assay length on protein efficiency ratio and net protein values for animal protein meals. Poultry Science 76:1722-1727

Johnson, M. L., C. M. Parsons, G. C. Fahey, Jr., N. R. Merchen, and C. G. Aldrich. 1998. Effect of species raw material source, ash content, and processing temperature on amino acid digestibility of animal by-product meals by cecectomized roosters and ileally cannulated dogs. Journal of Animal Science 76:1112-1122.

Mendez, A. and N. Dale. 1998. Rapid assay to estimate calcium and phosphorus in meat and bone meal. Journal of Applied Poultry Research 7:309-312.

Microsoft Office Excel. 2003. http://office.microsoft.com/en-us/excel

NRC, National Research Council. 1994. Nutrient Requirements of Poultry. 9th revised edition. National Academy Press, Washington, DC.

Parsons, C.M., F. Castanon and Y. Han. 1997. Protein and Amino Acid Quality of Meat and Bone Meal. Poultry Science 76:361-368.

Pearl, Gary G. 2004. Meat and Bone Meal usage in Modern Swine Diets. Render April. Pages 50-57. www.rendermagazine.com.

Pesti, G. M, L. O. Faust, H. L. Fuller, N. M. Dale, and F. H. Benoff. 1986. The nutritive value of poultry by-product meal. 1. Metabolizable energy. Poultry Science 65:2258.

Rostagno, H.S, J.M.R. Pupa and M. Pack. 1995. Diet Formulation for Broilers Based on Total versus Digestible Amino Acids. Journal of Applied Poultry Science Research. 4: 293-299.

Schasteen, Check, Jennifer Wu, Mike Schulz and Carl Parsons. 2002. An Enzyme-Based Protein Digestibility Assay for Poultry Diets. Proceedings of the Multi-State Poultry Meeting. May14th-16th.

Shirley, R.B. and C.M. Parsons. 2000. Effect of Processing on Amino Acid Digestibility of Meat and Bone Meal for Poultry. Poultry Science 79:1775-1781.

Shirley, R.B. and C.M. Parsons. 2001. Effect of Ash Content on Protein Quality of Meat and Bone Meal. Poultry Science 80:626-632.

Shutze, J. V. and F. H. Benoff. 1982. Samples needed for laboratory analysis: Statistical evaluation of feed ingredient variation and procedures for determining number of samples needed for laboratory analysis. Pages 134-136 in: Proc. Georgia Nutrition Conference, Atlanta, GA.

Statistix 8 Analytical Software. 2003. Tallahassee, FL. www.statistix.com

van Kempen, T. A. T. G. and J. C. Bodin. 1998. Near-infrared re-flectance spectroscopy appears to be superior to nitrogen-based regression as a rapid tool in predicting the poultry digestible amino acid content of commonly used feedstuffs. Animal and Feed Science Technology 76:139-147.

van Leeuwen, P., M. W. A. Verstegen, H. J. van Lonkhuijsen, and G. J. M. van Kempen. 1991. Near infrared reflectance (NIR) spectroscopy to estimate the apparent ileal digestibility of protein in feedstuffs. Pages 260-265 in Proc. 5th Int. Symp. Digestive Physiol. Pigs. EAAP Publication. Doorwerth, The Netherlands.

Waldroup, P.W. and M. H. Adams. 1994. Evaluation of the phosphorus provided by animal proteins in the diet of broiler chickens. Journal of Applied Poultry Research 2:209-218.

Waldroup, Park W. 2001. Present Status of the Use of Digestible Amino Acid Values in Formulation of Broiler Diets: Opportunities and Obstacles. ASA Technical Bulletin Vol. P046-2001. MITA (P) No. 271/10/2000.

Wang, Xincheng, Fructuoso Castanon an Carl M. Parsons. 1997. Order of Amino Acid Limitation in Meat and Bone Meal. Poultry Science 76:54-58.

Wang, X and C.M. Parsons. 1998[a]. Bioavailability of the Digestible Lysine and Total Sulfur Amino Acids in Meat and Bone Meal Varying in Protein Quality. Poultry Science 77: 1003-1009

Wang, X and C.M. Parsons. 1998[b]. Dietary Formulation with Meat and Bone Meal on a Total versus a Digestible or Bioavailable Amino Acid Basis. Poultry Science 77: 1010-1015.125