Ruminal and Intestinal Protein and Amino Acid Digestibility of Feather Meal and Feather Meal with Blood Products

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Introduction

This project is a follow-up study of the initial feather meal product survey conducted to characterize nutrient profiles of feather meal and feather meal with blood produced throughout the United States. The current project determined ruminal and intestinal digestibility of protein and amino acids for a subset of feather meal samples obtained during the initial project.

Procedures

A 3-step *in vitro* procedure previously developed at the University of Minnesota (Calsamiglia and Stern, 1995) was used to determine ruminal and intestinal digestion of protein in the feather meal products. This method closely simulates the physiological conditions of digestion in cattle and provides a rapid, repeatable, and less expensive alternative to use of intestinally cannulated animals to determine digestibility. Briefly, a sample is subjected to:

1. 12 to 18 hours of *in situ* rumen incubation, followed by
2. pepsin-hydrochloric acid (HCl) digestion (to mimic abomasal digestion), followed by
3. buffered pancreatin digestion (to mimic intestinal digestion).

Additionally, the modification of using an ANKOM Daisy system of *in vitro* incubation was evaluated compared with the original *in situ* procedure (Gargallo et al., 2006). The analyses for this project were conducted at Cornell University in the laboratory of Dr. Mike Van Amburgh.

Samples

Thirteen samples of feather meal were selected for analysis. Samples were chosen to encompass the different products across the various hydrolysation parameters. There were 6 samples of feather meal without blood, 4 samples with blood added pre-hydrolysation and 3 samples with blood added post-hydrolysation. Hydrolysation parameters ranged from short (5 to 12 min) to long (60 to 150 min) time x temperature (190 to 325°F) x pressure (30 to 75 psi).

Validation of Procedures

Validation of the modified procedure used at Cornell compared with the original Calsamiglia and Stern (1995) method is evidenced by the similar values for total protein digestibility of hydrolyzed feather meal and soybean meal obtained by Van Amburgh in this trial compared with Calsamiglia and Stern (1995). Calsamiglia and Stern (1995) obtained nitrogen (N) digestibilities of 69.5% for feather meal and 89.8% for soybean meal; these values compare favorably to the 64 to 66% for feather meal and 93% for soybean meal obtained by Van Amburgh in this study.

Primary Results

1. The current 3-step methodology of Calsamiglia and Stern (1995) as modified by Gargallo et al. (2006) is a viable method of determining ruminal and intestinal digestion of protein and amino acids.
2. Intestinal digestion of nitrogen was highest for feather meal with blood versus without blood regardless of blood addition pre- or post- hydrolysation: Nitrogen digestion of 57.6% (no blood) versus 64.1% (blood pre-hydrolysation) or 66.6% (blood post-hydrolysation).
3. Highest intestinal amino acid digestion occurred in feather meal samples with highest total N digestion. In other words, intestinal digestion of amino acids was similar to total N digestibility.
4. Ruminal N digestibility varied across feather meal samples. The addition of blood pre-hydrolysation increased ruminal digestion of amino acids, within the extremes of hydrolysation parameters.
5. Buffer-insoluble residue amino acid profile was similar to the original sample. The nutritional significance is that the proteins/amino acids in feather meal do not differ in their rumen solubility. This allows for greater confidence in predicting the specific amino acid profile that reaches the small intestine of cattle.
6. Following ruminal and intestinal digestion, all samples had lower amino acid levels compared to the original sample. Again, this indicates the similar extent of digestion of all amino acids in feather meal indicating that there is no amino acid more or less easily digested.
7. These data indicate that the differences in amino acid digestibility in feather meal product are primarily due to the addition of blood or not, and whether blood is added pre- or post-hydrolysation.
8. Overall, the 3-step method for determination of protein and amino acid digestion is a viable means of estimating ruminal and intestinal digestion of feather meal product.

Implications

The 3-step procedure accurately measured ruminal and intestinal protein and amino acid digestibility for feather meal. Addition of blood improved the nitrogen and amino acid digestibility of feather meal. This limited sample set indicates that addition of blood pre-hydrolysis results in greater ruminal nitrogen digestion. These protein digestibility data, when combined with the previous compositional data, will allow nutritionists to optimize the use of feather meal products in ration formulations for ruminants and nonruminants.

INTRODUCTION

Currently, feather meal is an underutilized protein source in diets for dairy and beef cattle. A major reason for the lack of use of this product is that very little information exists regarding the nutrient content of the product. A review of the U. S. Poultry & Egg Association web site reveals little chemical composition information beyond crude protein, moisture, crude fiber, crude fat, and ash analyses. The Dairy and Beef NRC publications (1996; 2001) also contain incomplete information with only one entry for feather meal. Increasingly, nutritionists use ration formulation software (such as CPM Dairy®) for dairy diets that require complete chemical fractionation of the feed ingredient to be incorporated into the diet. Analyses required include protein fractions (for instance to estimate rumen degradability), carbohydrate fractions (structural and nonstructural), lipid and fatty acid content, minerals, and amino acids (total and neutral detergent insoluble protein).

Another practical concern is the extent of variability in the feed product among plants and within a plant over time. For a nutritionist to feel comfortable using an ingredient, an accurate and representative nutrient analysis must be provided for ration formulation. It is conceivable that, in the future, further restrictions may be placed on use of ruminant-derived byproducts. If this occurs, then having accurate and complete nutrient profiles on feather meal products would position them well to compete with other protein sources in dairy and beef diets.

Previous research conducted in 2004 at Miner Institute analyzed feather meal and feather meal with blood products from 18 plants representing approximately 85% of the feather meal produced in the US. Comprehensive nutrient profiles were developed for this sample set with the notable exception of ruminal degradation and intestinal amino acid availability. The current project is a follow-up to the original study designed to characterize the nutrient composition of feather meal within and across processing plants. Feather meal is a substantial source of rumen undegradable protein (RUP) and essential amino acids. The question remains as to the digestibility of the RUP of feather meal in the lower gut. Additionally, intestinal digestibility of amino acids is of great concern to nonruminant nutritionists as well. Previous research has shown the intestinal digestibility of feather meal to vary between 8.5 and 33.2% of the crude protein (England et. al., 1997). The variable intestinal digestibility has raised doubts about the value of feather meal as a source of RUP for ruminants and amino acids for nonruminants.

Recently, Calsamiglia and Stern (1995) have developed a method to assay for ruminal and intestinal digestion of protein and amino acids. Until now, much of the information used to predict both ruminal and intestinal digestion of proteins has been based on *in situ* digestion work, which is subject to high degree of variability. The Calsamiglia and Stern three-step protein degradation assay may be a more viable and repeatable alternative to *in situ* degradation for prediction of ruminal and intestinal digestion of protein. Use of this assay to determine the digestibility of feather meal needs to be validated.

With the given set of feather meal samples from the previous study, a sub-set was chosen for this project in attempts to more clearly define the range of intestinal digestibility among plants and products and also to allow for the investigation of processing methods that could maximize intestinal digestion of feather meal protein.

The value to the poultry industry would be that complete and accurate nutrient information would be known for feather meal products, the data would be incorporated into feed dictionaries for access by nutritionists, and use of the product would presumably increase as nutritionists have easy access to the data. This study will also demonstrate that a single plant can produce a uniform product (or if not, the study will identify where improvements need to be made).

OBJECTIVES

A. Determine the extent of ruminal and intestinal digestion of feather meal protein and amino acids using the three-step protein degradation assay of Calsamiglia and Stern (1995).
B. Examine the potential effects of various hydrolysation parameters on protein digestibility of feather meal products.
C. Determine the validity of the three-step method regarding protein and amino acid digestibility using both *in vitro* and *in situ* pre-incubations.

MATERIALS AND METHODS

Samples

In the aforementioned study, a representative sample of each day's production of feather meal was collected at each plant for five consecutive days. The 5 daily samples were sent to Miner Institute for analyses of moisture, protein, fat and ash to determine daily variation within the product by processing plant. These 5 samples were then composited at Miner Institute to create a single week's sample. This was done for three consecutive weeks to result in three composite samples (15 daily samples). The weekly composite samples (three total, or six if a plant produces both feather meal and feather meal with blood) from each plant were logged into the database, sub-sampled, dried (at 55^{o}C), ground with a Wiley mill (1-mm screen), and distributed to the cooperating laboratories. The daily samples collected at each plant weighed approximately ~0.5 kg (1 lb) of air-dry product, sealed in a plastic sample bag, and carefully labeled with date of collection, plant ID, type of sample (feather meal or feather and blood meal), and processing information (temperature, time and other pertinent information). The samples used in the current project for protein degradation were selected from the weekly composite samples.

Thirteen feather meal (FM) samples representing a range of processing parameters with and without added blood (Table 1) were chosen, representing 12 different processing plants. The FM samples were stored at -20ºC. Three soy products including a solvent-extracted soybean meal (SBM) and two processed soy products were analyzed as controls with the FM through all procedures. The soluble and precipitable true protein values of the feeds were determined according to Licitra et al., 1996 and Ross et al. (2006), respectively. The amino acid content of the whole feeds and the borate-phosphate buffer insoluble residues (Licitra et al., 1996) were also obtained. All analyses were conducted in duplicate. Dry matter was determined at 106ºC in a forced-air oven for 4 hr. Nitrogen was measured by macro Kjeldahl (ID number 984.13;

AOAC, 1990) modified to include distillation into boric acid (Pierce and Haenisch, 1940). Results were expressed as percent of N to avoid assuming all protein contains 16% N.

Table 1. Processing parameters of feather meal samples.

Sample ID	%Blood	Blood addition pre vs. post hydrolysation	Hydrolysation time (min)	Hydrolysation temp (°F)	Hydrolysation PSI
1450	0	NA	NA	NA	68
1454	0	NA	35	292	45
1459	0	NA	NA	325	75
1461	0	NA	5	260	60
1462	0	NA	30	190	40
1466	0	NA	15	NA	65
1443	10	pre	60	270	30
1451	32	pre	60	287	40
1452	38	pre	30	300	45
1455	30	pre	90	325	30
1453	12	post	12	300	67
1487	15	post	30	300	70
1488	10	post	150	270	30

NA, not-applicable

Nitrogen Disappearance

In vitro ruminal and intestinal digestions (*in vitro* dig) were performed in an ANKOM Daisy Incubator (Ankom Technology, Macedon, NY) using a modification of Gargallo et al. (2006) which is based on the three-step procedure of Calsamiglia and Stern (1995). Samples (0.25g) were placed in fiber bags (twelve bags per feed; 25 micron pore size; Ankom F57; Ankom Technology, Macedon, NY), and incubated with rumen fluid and buffer (Goering and Van Soest, 1970) in an ANKOM Daisy system for 18-h, removed and rinsed with tap water until runoff was clear. This was followed by *in vitro* intestinal digestion: incubated with 0.1 *N* HCl and pepsin for 1-h, rinsed; incubated with pancreatin in potassium phosphate buffer (pH 7.75) for 24-h and rinsed. Residues in bags were air dried for 3 h and placed in a 55ºC oven for 48-h.

***In situ* Study**

To test *in situ* versus *in vitro* ruminal digestion an *in situ* (IS) study was conducted with 6 FM and the 3 soy products. The ruminal incubation procedure of Gargallo et al. (2006) was followed using 12- and 18-h time points. *In situ* bags (5 x 10 cm nitrogen-free polyester with 50 ± 15 micron pore size; Ankom R-510; Ankom Technology, Macedon, NY) containing 5 g sample were placed in mesh bags and inserted into the rumen of two lactating cows (224 DIM, 630 kg BW consuming a 14 % CP, 47 % corn silage based diet). Both time periods (12 and 18 h) were inserted at the same time and removed at the appropriate time. Residues in bags were rinsed in tap water until runoff was clear and dried in a 55ºC oven for 48-h. After weighing, residue (2 g) was placed in a 5 cm x 5 cm *in situ* bag and placed in an ANKOM Daisy incubator for determination of *in vitro* (IV) intestinal digestibility per Gargallo et al (2006). Further, the *in situ* residues were also subjected to the three-step procedure (TSP) of Calsamiglia and Stern (1995) to determine N digestion; however, amino acid contents of the residues were not determined.

Amino Acid Hydrolysis

For the determination of amino acid (AA) in a sample, three hydrolysis procedures (HCl, performic acid (PA) preoxidation prior to HCl hydrolysis, and alkaline hydrolysis with barium hydroxide ($Ba(OH)_2$)) were performed in duplicate on each sample using acid washed (50% nitric acid) glassware. Samples were defatted with hexane after hydrolysis. For the three hydrolysis procedures, an aliquot of feed or buffer insoluble residue containing approximately 3 mg N was weighed into Teflon-lined screw top culture tubes (20 x 125 mm; 16 x 125 mm for alkaline hydrolysis). Norleucine was added as an internal standard to each tube to yield 125 n*M*/mL or 250 n*M*/mL in the analyzed sample for the HCl and PA or $Ba(OH)_2$ samples, respectively, when diluted, which is in the approximate range of AA expected at this retention time. For acid hydrolysis, 6 *M* HCl was added, the mixture flushed with nitrogen, loosely capped and placed in boiling water for 10 min to remove oxygen. Upon removal from the water, the cap was tightened and the tube placed in a heating block at 110ºC for 21 h. At the end of hydrolysis the tube was cooled slightly and the contents were transferred quantitatively and filtered through a Whatman 541 filter paper into a 50-mL volumetric flask. An aliquot of the filtrate was evaporated at 65ºC as rapidly as possible using a stream of N gas to prevent further loss of serine, threonine and tyrosine (Gehrke et al., 1985). The residue was rinsed with distilled water and evaporated until no HCl was detectable. Sample buffer (0.05 *M* lithium hydroxide, 0.1415 *M* lithium chloride, 0.0457 *M* citric acid and 0.1% phenol, pH 2.8) was added to the residue and the mixture filtered through a 0.2 µm nylon filter into a sample vial, covered with a septa lined cap and frozen at -20ºC until analyzed.

Cystine and methionine, were pre-oxidized with PA (Moore, 1963) and analyzed as cysteic acid and methionine sulfone, respectively. The PA was prepared according to Mason et al. (1980) with the following modification: to produce 5 mL PA, 4.5 mL 88% formic acid, 0.5 mL 30% hydrogen peroxide and 25 mg phenol were combined, incubated for 1 h at room temperature and moved to an ice bath at 4ºC for 15 min. The tubes containing the 3 mg samples of N were placed in an ultrasonic bath filled with ice and 1.5 mL of the PA solution was added to each. The contents of the tubes were sonicated for 15 min and transferred to an ice bath at 4ºC for 16 h. The oxidizing reaction was stopped and the excess PA reduced with 0.3 mL concentrated HCl. After standing for 15 min at room temperature the tubes were placed under vacuum using a water aspirator (Elkin and Griffith, 1985) to remove the residual PA and HCl. The pre-oxidized samples were then subjected to the HCl hydrolysis procedure as described previously.

Tryptophan was determined using a barium hydroxide hydrolysis. A 3 mg sample of N was added to a screw top culture tube (16 x 125 mm) containing 1.2 g barium hydroxide ($Ba(OH)_2 \cdot 8H_2O$). Two mL water was added to each tube (1.90 *M* barium hydroxide) and the tube flushed for 10 seconds with N gas, loosely capped and placed in boiling water for 15 min to remove the oxygen. Upon removal from the water, the cap was tightened and the tube placed in a heating block at 110ºC for 16 h. At the end of hydrolysis the tubes were removed, cooled and the contents transferred quantitatively to a 16 x 100 mm tube with a small amount of concentrated HCl. The pH was adjusted to 2.8 with additional HCl. The barium was precipitated with the addition of 2 mL of 18.2% sodium sulfate and the volume adjusted to 10 mL with water. An aliquot was centrifuged in a microfuge (Eppendorf 5465C; Brinkman Instruments, Inc; Westbury, NY 11590) at 12,000 x *g* for 10 min at room temperature. The

supernatant was filtered through a 0.2 µm nylon filter into a sample vial, covered with a septa lined cap and frozen at -20ºC until analyzed.

Amino Acid Analysis

Amino acids were separated on a lithium cation exchange column (4 x 100 mm, P/N 0354100, Pickering Laboratories, Mountain View, CA) using a three-buffer step gradient (Li292, Li365 and Li375, Pickering Laboratories, Mountain View, CA) and a column temperature gradient (33, 42, 60 and 70ºC). Detection was at 560 nm following ninhydrin post column derivation on an HPLC System Gold with 32 Karat software (Beckman-Coulter, Inc., Fullerton, CA). Standards (250 n*M*/mL) for aspartic acid (Asp), threonine (Thr), serine (Ser), glutamic acid (Glu), glycine (Gly), alanine (Ala), valine (Val), methionine (Met), isoleucine (Ile), leucine (Leu), tyrosine (Tyr), phenylalanine (Phe), ammonia (NH_3), lysine (Lys), histidine (His), arginine (Arg) and cysteine (Cys) (125 n*M*/mL) were prepared by diluting a purchased stock (Amino acid standard H, #20088; Pierce Chemical; Rockford, IL 61105) with the sample buffer. Standards (250 n*M*/mL) for cysteic acid, Met sulfone, norleucine and tryptophan (Trp) were prepared in sample buffer and combined with the others. The volume of samples and standards loaded on the column was 50 µL.

Statistical Analysis

The GLM procedure of SAS was used to perform the analysis of variance on the EAA content of the three fractions (whole, buffer insoluble residue (BIR) and undigested by Ankom (IV)-18) and undigested residues (IV18, IS12 and IS18). Means were compared using Tukey's method when significance was observed in the analysis of variance test. Orthogonal contrasts were used to evaluate the addition of blood and when the blood was added (pre or post hydrolysation) on the amino acid content of the whole, buffer insoluble and IV18 indigestible residues. Additional contrasts were used to assess significant differences in amino acid digestibility between the degradation treatments: *in situ* vs. *in vitro* and length of rumen degradation: 12-h vs. 18-h. Means were considered to be different when ($P < 0.05$).

RESULTS

An overall description of the processing parameters (i.e., percentage blood, when blood was added relative to hydrolysation, hydrolysation time, temperature and pressure) of the feather meals is provided in Table 1 with means (± SD) of these parameters in Table 2. The N fractions and fat composition of the feather meals, grouped by when blood was added, are presented in Table 3 with means (± SD) given after each group.

***In Vitro* Digestibility**

In vitro total N digestion was highest in the FM samples with blood added post hydrolysation (66.6 ± 2.4%), compared to those when blood was added pre hydrolysation or no blood added (64.1 ± 16.6% and 57.6 ± 9.8% respectively), Table 3. Due to the large standard deviations, no significant differences in digestibility between the FM were observed.

Table 2. Processing parameters of feather meal samples grouped by when blood was added. Values represent means ± SD.

Blood Added	% Blood	Hydrolysation Time (min)	Hydrolysation Temp* (°F)	Hydrolysation psi
None	0	14.2	201.2	58.8
		15.3	111.0	13.6
pre	27.5	60.0	295.5	36.3
	12.2	24.5	23.2	7.5
post	12.3	64.0	290.0	55.7
	2.5	75.0	17.3	22.3

*Assumed hydrolysation conducted at room temperature of 70ºF for feeds with no temperature listed.

Table 3. Nitrogen fractions, fat content and *in vitro* N digestibility of individual feather meals with mean ± SD of when blood added. Values represent percent dry matter, percent N or percent total N digested.

ID	Blood Added*	TN** % DM	Sol N % TN	PTP %Sol N	% Fat	Total N Dig
1450	NA	12.78	1.50	100	15.30	69.61
1454	NA	13.52	1.04	100	11.20	59.54
1459	NA	13.69	1.53	100	9.10	65.57
1461	NA	13.36	0.35	100	10.50	43.02
1462	NA	14.49	3.95	100	5.20	57.87
1466	NA	14.19	5.04	100	8.30	50.19
	mean	**13.67**	**2.23**		**9.93**	**57.64**
	± SD	**0.61**	**1.84**		**3.36**	**9.79**
1443	Pre	11.13	3.93	100	21.40	62.51
1451	Pre	13.81	1.76	100	9.40	76.74
1452	Pre	13.93	4.67	100	6.80	84.69
1455	Pre	13.81	2.39	100	8.70	46.21
	mean	**13.17**	**3.19**		**11.58**	**64.07**
	± SD	**1.36**	**1.34**		**6.64**	**16.58**
1453	Post	13.79	5.02	100	9.80	63.47
1487	Post	13.79	4.58	100	10.40	67.99
1488	Post	12.94	1.40	100	12.40	67.21
	mean	**13.51**	**3.67**		**10.87**	**66.62**
	± SD	**0.49**	**1.97**		**1.36**	**2.42**

* Blood added pre or post hydrolysation; NA, not applicable as no blood added.
** TN, total N in whole feed; Sol N, soluble N; PTP, precipitable true protein; Fat, ether extract (Miner Institute); N Dig, fraction of feed N digested in the *in vitro* digestion trial.

Feather meals were ranked by total nitrogen digestion in Table 4. This ranking indicates that, in general, feather meal with blood has higher total N digestion than feather meal without blood. However, there is no clear indication that certain hydrolysation parameters affect protein digestion given this small sample set. Also, Table 4 gives the amino acids projected to be digested by a cow fed one kg/d of feather meal DM. Variation in the digestion of individual AA was great although the highest digestion usually occurred in the feather meals with the highest

total N degradation. The thermal processing of feather meal, which is high in nitrogen and low in reducing substances such as carbohydrates, results in different types of cross-linking reactions (Papadopoulos et al., 1985) and formation of new amino acids, lanthionine from cystine and lysinoalanine from reactions of the ε-amino group of lysine. Although no lanthionine was detected in these feather meals, the cystine contents of the various samples varied greatly as did the cystine content of the undigested residues. Lysinoalanine, which is difficult to separate from Trp in the instrumentation used, may have contributed to the Trp values.

The amino acid profiles of the whole feather meals and the amino acid composition of the buffer insoluble residues are found in Tables 5 and 6, respectively. No significant differences were observed between the amino acid contents of the whole and borate-phosphate buffer insoluble residues. The amino acid content of the undigested residues following an 18-hr *in vitro* ruminal and intestinal degradations with all amino acid values were significantly ($\underline{P} < 0.05$) lower than the whole (Appendix A). The orthogonal contrasts for the effect of added blood on the amino acid content of the whole, buffer insoluble and IV18 undigested residues were significant ($\underline{P} < 0.05$) for all except Met, Phe and Trp. The orthogonal contrasts for when the blood was added (pre or post- hydrolysation) were significant for Met, Met + Cys, Lys, His and Pro. These data indicate some of the differences among the feather meals are due to the presence of added blood and when the blood is added in relation to the thermal processing steps.

Table 4. Amino acids (g) digested per day from a kg feather meal DM following *in vitro* intestinal digestion (Gargallo et al., 2006) of *in vitro* ruminal residues. Feather meals ranked by total nitrogen digestibility (highest to lowest).

Rank	ID	Blood	Rum N dig*	Total N dig**	Met	Met+ Cys	Thr	Val	Ile	Leu	Phe	Phe+ Tyr	Trp	Lys	His	Arg	Pro
1	1452	Pre	0.362	0.847	5.96	25.40	32.08	46.49	27.58	55.77	32.92	52.49	4.00	19.51	9.01	45.18	60.52
2	1451	Pre	0.298	0.767	5.80	27.46	27.15	41.36	24.12	46.78	30.77	47.55	5.58	15.02	7.01	39.59	57.09
3	1450	None	0.245	0.696	4.57	27.69	22.96	37.45	24.27	39.58	11.93	22.20	9.91	11.41	1.37	28.20	49.22
4	1487	Post	0.240	0.680	5.86	24.61	25.75	35.14	21.99	46.51	27.84	44.49	3.75	17.26	5.87	37.97	51.64
5	1488	Post	0.269	0.672	5.31	26.36	23.57	30.01	20.14	36.65	23.43	37.10	3.52	13.83	4.67	33.42	50.34
6	1459	None	0.206	0.656	8.38	33.14	36.45	45.34	23.43	38.59	26.65	40.65	1.71	7.11	2.00	33.85	63.13
7	1453	Post	0.188	0.635	7.64	33.29	24.42	23.52	17.93	37.93	23.71	37.61	4.50	15.83	5.79	31.18	41.33
8	1443	Pre	0.312	0.625	7.37	11.04	18.95	18.50	13.71	26.70	17.12	28.47	2.58	18.86	8.00	26.52	26.63
9	1454	None	0.225	0.595	8.20	36.27	20.30	34.81	26.23	38.82	27.94	35.51	3.03	9.60	6.61	26.88	56.69
10	1462	None	0.214	0.579	3.89	1.17	24.39	37.17	24.76	44.33	20.00	30.77	3.17	12.90	4.55	37.08	43.66
11	1466	None	0.176	0.502	0.00	0.00	19.07	28.58	19.47	25.41	18.08	30.23	1.59	10.96	6.14	31.40	36.40
12	1455	Pre	0.144	0.462	5.21	18.02	20.39	23.22	14.64	33.27	19.85	31.86	0.31	8.04	3.28	27.25	43.80
13	1461	None	0.128	0.430	1.98	1.98	9.65	28.37	17.72	23.09	6.07	12.05	1.00	6.51	2.60	19.91	21.92

*Ruminal N digestion, g/g DM.

**Total N digestion, ruminal and intestinal, g/g DM.

Table 5. Amino acid profiles of whole feather meal. Values are expressed as g AA/kg DM.

ID	Met	Met + Cys	Thr	Val	Ile	Leu	Phe	Phe + Tyr	Trp	Lys	His	Arg	Pro
1450	6.27	47.34	35.21	55.88	33.45	59.84	23.16	38.78	11.84	14.96	2.41	46.17	77.70
1454	10.31	64.52	36.99	59.70	39.96	68.01	43.40	58.46	4.84	14.85	8.13	50.08	95.37
1459	10.82	61.85	50.00	64.52	34.14	61.18	38.81	58.68	3.30	10.99	2.76	51.19	94.83
1461	9.91	45.19	32.01	62.30	35.66	62.15	28.30	47.12	4.58	13.28	4.32	47.95	75.57
1462	7.74	40.01	41.01	61.90	37.96	72.19	36.66	55.66	5.09	18.27	6.63	53.57	79.34
1466	6.52	44.79	41.48	59.66	38.17	63.10	39.62	63.41	3.84	17.44	7.65	59.53	87.89
1443	9.76	29.67	31.30	37.57	23.67	50.15	29.52	47.18	5.04	24.32	10.24	43.43	53.21
1451	7.31	40.37	37.18	57.60	32.14	65.16	41.34	63.67	7.08	19.36	8.72	52.53	78.72
1452	7.24	33.26	39.07	56.98	32.35	68.41	39.49	62.62	5.36	24.24	11.08	52.76	72.91
1455	8.36	40.95	40.84	54.23	31.62	70.34	39.96	63.34	4.94	18.44	7.27	52.73	83.91
1453	9.78	67.38	39.69	47.33	29.97	64.86	38.26	59.59	8.23	20.50	7.19	53.06	77.03
1487	7.87	50.82	38.94	55.56	33.24	68.72	40.27	63.15	5.64	22.34	7.38	55.94	80.78
1488	8.97	53.46	36.49	48.20	29.98	31.24	34.84	54.70	6.18	19.01	6.37	50.11	77.94

Table 6. Amino acid profiles of the buffer insoluble residues of feather meals. Values are expressed as g AA/kg DM.

ID	Met	Met + Cys	Thr	Val	Ile	Leu	Phe	Phe + Tyr	Trp	Lys	His	Arg	Pro
1450	6.97	59.84	36.73	50.35	35.42	67.26	32.64	49.09	9.65	13.20	3.84	49.63	77.89
1454	6.30	51.82	40.62	55.61	34.78	64.00	38.67	59.93	4.75	14.82	6.02	54.91	86.51
1459	8.39	51.90	39.85	55.46	32.77	64.03	39.32	70.73	3.01	14.46	5.42	56.50	84.62
1461	6.88	45.27	38.79	58.11	34.26	68.47	38.48	59.48	5.06	15.79	5.13	51.89	88.05
1462	6.93	41.03	38.49	58.97	37.05	66.87	39.74	58.45	3.66	18.77	7.62	56.55	84.68
1466	6.12	49.45	39.71	65.95	36.79	72.89	39.80	63.01	3.54	16.97	5.06	61.00	88.99
1443	6.76	29.45	26.29	35.49	22.17	46.64	27.05	43.05	4.91	23.21	10.43	37.99	46.16
1451	6.25	39.40	37.72	56.76	32.85	68.73	39.94	61.52	4.77	19.49	8.00	53.18	75.64
1452	5.96	31.08	33.92	51.76	28.72	62.82	37.31	58.08	5.55	20.46	8.54	49.12	67.17
1455	7.20	45.22	41.40	58.64	35.22	68.09	40.22	61.32	6.34	19.54	7.25	55.48	75.59
1453	10.21	65.78	38.77	49.60	31.32	63.67	39.02	60.70	9.02	19.38	6.91	51.40	77.54
1487	9.15	53.02	35.83	52.84	35.48	63.25	37.04	58.11	5.78	19.74	7.79	50.70	74.19
1488	11.55	60.16	36.56	49.37	31.22	58.75	35.12	54.35	6.59	17.46	6.37	50.73	74.07

***In Situ* Digestibility**

Mean (± SD) nitrogen digestibility of the 6 selected feather meals and the 3 soy products were very similar among all treatments: 73.5 (±12.8%), 73.4 (±15.7%), 72.2 (±15.2%), 76.0 (±18.5%), 75.0 (±15.1%) for the IV18, TSP12, TSP18, IS12, IS18, respectively. The N digestibility from all treatments were positively correlated ($\underline{P} = 0.001$). The *in vitro* intestinal procedure of Gargallo et al. (2006) using either *in situ* or *in vitro* ruminal residues and the three-step procedure (TSP) intestinal digestions of *in situ* residues yielded similar N degradations for the feeds tested.

The undigested residue amino acid contents of selected feather meals following a 12-h or 18-h rumen incubation and an *in vitro* intestinal digestion are found in appendix B and C, respectively. Appendix D presents the percentages of amino acids digested in the selected feather meals following *in vitro* intestinal digestion of *in situ* or *in vitro* rumen residues. Met appeared to be 20% less digestible from the *in vitro* ruminally degradation than the *in situ*. Trp appeared to be indigestible when incubated *in situ* but lysinoalanine, an AA resulting from a dehydroalanine residue reacting with the ε-amino group of lysine, may have contributed to the higher Trp contents of the undigested residues. The addition of blood to the feather meals affected the digestibility of particular amino acids, such as His ($\underline{P} < 0.07$) and Met. Rumen exposure for 18-h resulted in ten percent more proline being degraded compared to the 12-h incubation. Proline, a hydrophobic amino acid, which due to its imino group produces a very rigid secondary structure in a protein, is hard to degrade under anaerobic conditions.

Three soy products were analyzed through all procedures as positive controls. Nitrogen digestion (98.2 to 99.3%) was similar among the three digestibility treatments -- *in vitro* intestinal digestion of the *in situ* and *in vitro* ruminal residues. Estimated small intestine amino acid digestion was higher in the IS18 (99.2 ± 0.4 %) than the IV18 (98.0 ± 1.9 %) and IS12 (97.8 ± 1.5 %). These values are consistent with previous data for these feeds and methods.

From this study, the total digestibility of the amino acids in feather meals using an *in vitro* intestinal degradation of *in vitro* rumen residues varied. Blood added to the feather meal had an effect on the quantity of Met+Cys, Thr, Val, Ile, Leu, Lys, His, Arg and Pro digested. Further, when blood was added relative to hydrolysation, pre vs. post, appeared to have an effect on His and Met digestibility. *In vitro* versus *in situ* ruminal digestion appeared to yield significant differences for Met; however, Met + Cys were very similar across treatments. The IV18 digestion yielded higher Thr, Leu, Phe, Lys, His, Arg and Pro digestibilities than did either *in situ* time point while the IS18 yielded higher Met, Val and Ile degradations.

CONCLUSIONS

The current 3-step methodology of Calsamiglia and Stern (1995) as modified by Gargallo et al. (2006) is a viable method for determining ruminal and intestinal digestion of protein and amino acids. The results of the soy products included in this trial match those of Gargallo et al. (2006) providing confidence in the repeatability of the method and the validity of the digestibility values obtained for the feather meal products. The results of this trial support the notion of this method as an accurate means of predicting protein and amino acid ruminal and intestinal digestibility of various feedstuffs. This will allow for improved precision of predicting amino acid digestibility

of RUP in ruminant feeds as well as total tract digestibility in monogastrics. The dairy ration balancing program CPM 3.0 uses a default value of 80% intestinal digestibility of RUP for all feedstuffs. As evidenced here, that value should be 98% for SBM and between 58-65% for feather meal products. In spite of the observed protein digestibility value being lower than the CPM default value, it is much better than what nutritionists have believed it to be based on previous literature values of 8.5-33.2% (England, 1997).

The intestinal digestion of nitrogen was highest for feather meal with blood versus without blood regardless of blood addition pre- or post- hydrolysation: nitrogen digestion of 57.6% (no blood) versus 64.1% (blood pre-hydrolysation) or 66.6% (blood post-hydrolysation). The highest intestinal amino acid digestion occurred in feather meal samples with highest total N digestion. In other words, intestinal digestion of amino acids was similar to total N digestibility. Ruminal N digestibility varied across feather meal samples. The addition of blood pre-hydrolysation increased ruminal digestion of amino acids, within the extremes of hyrdolyzation parameters. Buffer-insoluble residue amino acid profiles were similar to the original samples. The nutritional significance is that the proteins/amino acids in feather meal do not differ in their rumen solubility. This allows for greater confidence in predicting the specific amino acid profile that reaches the small intestine of cattle. Following ruminal and intestinal digestion, all samples had lower amino acid levels compared to the original sample. Again, this indicates the similar extent of digestion of all amino acids in feather meal indicating that there is no amino acid more or less easily digested.

These data indicate that the differences in amino acid digestibility in feather meal product are primarily due to the addition of blood or not, and whether blood is added pre- or post-hydrolysation. Unfortunately, there was no clear indication of how hydrolysation parameters affect ruminal or total N digestion given this small sample set. It would be worth examining the effects of varying hydrolysation parameters under controlled conditions to better determine how digestibility is affected. This might allow for means of improving amino acid digestibility above the current 65% as determined in this study. This could benefit both ruminant and non-ruminant usage of feather meal product. Overall, the 3-step method for determination of protein and amino acid digestion is a viable means of estimating ruminal and intestinal digestion of feather meal product.

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ABBREVIATIONS

AA: amino acid
Ala: alanine
AOAC: Association of Analytical Communities
Arg: arginine
Asp: aspartate
$Ba(OH)_2$: barium hydroxide
BIR: buffer insoluble residue
BW: body weight
ºC: degree Celsius
cm: centimeter
CP: crude protein
Cys: cysteine
DIM: days in milk
DM: dry matter
EAA: essential amino acids
ºF: degree Fahrenheit
FM: feather meal
g: gravity
g: gram
Gly: glycine
Glu: glutamic acid
h: hour
HCl: hydrochloric acid
His: histidine
HPLC: high pressure liquid chromatography
Ile: isoleucine
IS: *in situ*
IV: *in vitro*
kg: kilogram
lb: pound
Leu: leucine
Lys: lysine
M: molar (concentration)
Met: methionine
mg: milligram
min: minute
mL: milliliter
mm: millimeter
N: normal (concentration)
N: nitrogen
N Dig: nitrogen digestion
NA: not applicable
nmol: nanomoles (mass)
NRC: National Research Council
PA: performic acid
Phe: phenylalanine
Pro: proline
psi: pounds per square inch
PTP: precipitable true protein
RUP: rumen-undegradable protein
SAS: Statistical Analysis System
SBM: soybean meal
SD: standard deviation
Ser: serine
Sol N: soluble nitrogen
Thr: threonine
TN: total nitrogen
Trp: tryptophan
TSP: three step procedure
Tyr: tyrosine
US: Unites States
Val: valine

Appendix A

Amino acid contents of feather meal residues following *in vitro* 18-h (IV18) digestibility and 24-h *in vitro* intestinal digestion. Values represent the undigested residue amino acids expressed as g AA/kg DM.

ID	Met	Met + Cys	Thr	Val	Ile	Leu	Phe	Phe + Tyr	Trp	Lys	His	Arg	Pro
1450	1.70	19.64	12.25	18.44	9.19	20.26	11.24	16.58	1.93	3.55	1.04	17.97	28.49
1454	2.11	28.26	16.69	24.90	13.72	29.19	15.46	22.96	1.82	5.25	1.51	23.20	38.68
1459	2.44	28.71	13.55	19.18	10.70	22.59	12.16	18.03	1.59	3.88	0.76	17.33	31.69
1461	7.93	64.07	22.37	33.93	17.95	39.06	22.23	35.08	3.58	6.77	1.72	28.04	53.65
1462	4.97	38.84	16.62	24.72	13.20	27.86	16.65	24.88	1.91	5.36	2.08	16.49	35.68
1466	6.75	56.87	22.41	31.09	18.69	37.69	21.54	33.18	2.24	6.48	1.51	28.13	51.49
1443	2.39	18.63	12.35	19.06	9.96	23.45	12.40	18.71	2.45	5.46	2.25	16.91	26.59
1451	1.51	12.91	10.03	16.24	8.02	18.37	10.57	16.12	1.49	4.34	1.71	12.94	21.64
1452	1.28	7.86	6.99	10.49	4.77	12.64	6.57	10.13	1.36	4.73	2.06	7.58	12.39
1455	3.15	22.93	20.45	31.01	16.98	37.06	20.11	31.48	5.25	10.40	3.99	25.48	40.11
1453	2.14	34.09	15.26	23.81	12.04	26.93	14.54	21.98	3.73	4.67	1.40	21.88	35.70
1487	2.01	26.21	13.18	20.42	11.24	22.21	12.43	18.66	1.89	124.70	1.50	17.97	29.14
1488	3.67	27.10	12.92	18.19	9.83	20.68	11.41	17.60	2.65	5.18	1.70	16.70	27.61

Appendix B

Undigested amino acids of selected feather meals following 12-h *in situ* digestion and 24-h *in vitro* intestinal digestion (Gargallo et al., 2006). Values are expressed as g AA/kg DM.

ID	Met	Met + Cys	Thr	Val	Ile	Leu	Phe	Phe + Tyr	Trp	Lys	His	Arg	Pro
1450	1.63	26.31	13.21	16.51	10.78	23.16	14.70	22.60	5.40	4.46	0.53	20.07	37.97
1462	0.82	23.94	18.87	22.38	13.55	34.22	19.88	29.02	4.55	5.62	1.63	25.59	47.64
1451	0.98	16.51	11.18	13.72	7.08	18.16	11.27	16.71	4.87	4.23	1.86	13.72	26.62
1455	2.14	35.80	27.67	32.15	19.80	45.39	26.56	38.25	8.66	11.23	4.12	36.07	59.62
1487	1.33	27.48	18.71	21.02	13.58	31.72	18.81	27.63	8.50	6.54	2.47	24.31	46.91
1488	0.78	23.17	11.58	13.04	8.23	22.95	11.54	17.71	4.30	5.04	2.73	17.45	20.69

Appendix C

Undigested amino acids of selected feather meals following 18-h *in situ* digestion and 24-h *in vitro* intestinal digestion (Gargallo et al., 2006). Values are expressed as g AA/kg DM.

ID	Met	Met + Cys	Thr	Val	Ile	Leu	Phe	Phe + Tyr	Trp	Lys	His	Arg	Pro
1450	0.50	24.87	13.92	20.62	10.71	25.08	15.66	23.03	3.93	4.25	0.76	19.58	36.17
1462	2.18	27.32	18.12	22.75	13.21	32.46	19.75	30.41	8.35	5.47	1.74	24.59	48.79
1451	0.27	13.53	8.79	8.86	6.66	17.32	10.98	14.81	4.79	2.58	1.21	11.69	22.56
1455	2.51	27.82	20.09	22.41	15.34	32.82	18.56	30.50	5.86	8.98	3.70	24.48	40.89
1487	0.69	39.27	17.14	15.55	11.42	25.97	13.85	19.89	7.57	6.56	1.68	22.74	34.89
1488	0.42	23.26	10.66	10.52	8.19	23.45	7.73	10.22	6.27	4.20	1.20	16.68	23.21

Appendix D

Amino acid digestibility of selected feather meal feeds following *in vitro* intestinal digestion of *in situ* or *in vitro* rumen residues (Gargallo et al., 2006). Values represent percentages of the whole feed amino acid content.

	Percent digested		
Amino Acid	**IV18***	**IS12**	**IS18**
Met	64.01[a]	84.40[b]	87.97[b]
Met + Cys	45.20	46.83	45.27
Thr	63.06	54.85	60.61
Val	61.20	67.38	72.09
Ile	65.42	66.71	70.14
Leu	63.04	54.66	59.36
Phe	61.09	47.90	55.96
Phe + Tyr	62.63	50.81	57.80
Trp	57.05[a]	0[b]	0[b]
Lys	69.68	57.42	63.93
His	67.31	53.77	62.76
Arg	65.32	54.78	60.69
Pro	61.93	51.93	59.00

*IV18, *in vitro* ruminal 18-h digestibility; IS12, *in situ* ruminal 12-h; IS18, *in situ* 18-h; all treatments received *in vitro* intestinal digestion.
[a,b]Means in same row with different letters are significant ($P < 0.05$).