Increasing nutrient availability of feather meal for ruminants and non-ruminants using an ammonia pressurisation/depressurisation process

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Abstract: An ammonia pressurisation/depressurisation process (PDA) was evaluated for efficacy in enhancing solubility and digestibility of feather meal (FM) protein. Commercial FM was processed for 5 min with variable ammonia loadings (0.5–2 g g^{-1} DM), moisture contents (10–50%) and temperatures (75–90 °C). Dry matter and protein solubility were determined in a 0.15 M NaCl solution (6 h, 39 °C) and soluble protein content was determined by the Lowry method. Ruminal solubility was determined by the Kjeldahl method as protein lost in the rumen (1 min) of a cannulated steer. Protein digestibility (crude protein by Kjeldahl analysis) was determined using the *in situ* dacron bag and pepsin techniques. Protein solubility in the untreated FM was low (13.3 and 7.2%, in NaCl and in rumen fluid, respectively) and increased ($p < 0.05$) to 39.4 and 23.0%, respectively, in the treated FM at the optimal conditions (50% moisture, 2 g ammonia g^{-1} dry FM and 90 °C). Ruminal and pepsin protein digestibilities increased ($p < 0.05$) with increasing moisture content, ammonia loading and temperature. Protein digested in the rumen at 12 h increased from 21.9 (untreated) to 43.0%. Highest digestibility value at 48 h was 54.8%. Pepsin protein digestibility increased ($p < 0.05$) from 35.4% in the untreated FM to 89.3% in the optimal treatment. Protein solubility in NaCl correlated very well (0.98) with pepsin digestibility. The 3-fold increase in solubility and 2-fold increase in digestibility could improve nutrient availability of FM and increase its value as a protein source for both ruminants and non-ruminants.
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INTRODUCTION

Production and consumption of poultry in the US is steadily increasing and the Commerce Department is predicting a 5–7% growth in poultry production in each of the next few years.[1] Currently, approximately 1×10^6 t of feathers are generated per year.[2] Feathers represent a substantial (5–7%) fraction of the mature weight of birds, but are a keratinous source of protein of low nutritional value in their native state for both ruminants and non-ruminants with an amino acid imbalance, mainly of lysine.[3] On the other hand, sulphur amino acids, mainly cysteine, are in relatively high levels compared to common protein sources such as cereals, soybean meal and blood meal. Feather meal usually has protein levels higher than 75%. In addition, feeding FM during the finishing period has proven to be an effective method of reducing abdominal fat content in broilers[4,5] and swine,[6] although the effect has been attributed to FM being a non-specific nitrogen source. Processing methods which include steam and pressure and strong alkali or acid are currently used with the disadvantage of destroying nutritionally important amino acids and causing a substantial fraction to escape digestion in the whole animal.[7,8] As a result, other animal proteins such as blood (rich in lysine) may need to be added to enhance the nutritional quality of the product.[9,10] Even with processing, it is priced similar to other feedstuffs, ie soybean meal, with half the protein level. Low solubility and amino acid imbalance limits its use in non-ruminants in poultry[5] and swine.[6] In spite of its advantage as an escape protein[11] its low solubility

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limits its use in ruminants if protein in the diet does not supply sufficient ruminal ammonia for proper fibre digestion.[12,13]

The potential exists to make the protein in feathers more nutritionally available to animals, but the treatments investigated to date have been extensive and uneconomical. A novel, relatively inexpensive, process investigated in our laboratories, pressurisation and depressurisation with ammonia (PDA) could significantly increase the availability of feather meal protein. Preliminary results showed that solubility of the protein in 0.1 M NaCl was slightly increased (24%) with an ammonia treatment.[14] Enhancing solubility of protein should increase its digestibility, allowing the protein source a wider range of applications and enhancing its potential as a protein source for both ruminants and non-ruminants. On the other hand, when an increase in protein solubility has been achieved with severe treatments (long times, high temperatures), the amino acid bioavailability has decreased.[3] The objective of this work was to develop novel ammonia reactor processing conditions to treat feather meal which will enhance the use of feather meal protein products for animal feeding.

MATERIALS AND METHODS

Ammonia processing

A laboratory-scale ammonia reactor unit consisting of a 4 L reactor with appropriate support equipment was used for the treatment of feather meal. A batch hydrolysed FM obtained from a local processor in Texas was used in these studies. Water and liquid anhydrous ammonia were added to 60 g dry samples and the temperature was rapidly raised to the desired value. After treatment time, pressure was suddenly released and samples allowed to air-dry overnight. Two treatments (A and B) were carried out at the intitial moisture content of FM (10%), using 0.5 and 1.0 g ammonia g^{-1} dry FM at 75 °C. Moisture was increased to 50% in the remaining treatments. Treatment C was conducted with an ammonia loading level of 1.0 g g^{-1} at 75 °C to allow for examination of moisture effects. Treatment D was carried out at a higher temperature (90 °C) and treatment E was conducted with an ammonia loading level of 2 g g^{-1}, to investigate the influence of altering the ammonia loading levels (from 1.0 to 2.0 g g^{-1}) at the higher moisture level (50%) and temperature (90 °C).

Analytical procedures

Proximate composition of untreated feather meal was determined using Association of Official Analytical Chemists methods.[15] Dry matter solubility was determined in 0.15 M NaCl after a 6 h incubation at 39 °C.[16] Protein solubility in NaCl was determined by measuring protein in the filtrate by the Lowry method as modified by Sigma.[17] *In situ* ruminal solubility (1 min in the rumen) and digestibility (3, 6, 12, 24 and 48 h in the rumen of a cannulated steer) were determined by the dacron bag method.[18] Pepsin digestibility was determined after a 24 h incubation at 50 °C[15] with 0.002% pepsin as recommended by Parsons[19] for feather meal. Egg white, soybean meal and ground feathers were used as controls. The Kjeldahl method was used for crude protein determination in the solids from the digestibility studies. All methods were carried out in triplicate, except pepsin digestibility, which was performed in duplicate samples. Treatments were labelled as ammonia loading (g NH_3 g^{-1} dry FM)-moisture content (%, wet basis)-temperature (°C).

Statistical analyses

The results of protein solubility and digestibility for untreated and PDA-treated samples were analysed using GLM procedures of SAS.[20] An analysis of variance was applied to each of the studies and differences among treatments ($p < 0.05$) were investigated by Tukey's mean comparison.[21]

RESULTS AND DISCUSSION

FM had 10% moisture content (wet basis), 880 g kg^{-1} crude protein, 14 g kg^{-1} crude fat and 62 g kg^{-1} ash (dry basis). Untreated FM dry matter solubility in 0.15 M NaCl was 133 g kg^{-1} and did not increase with PDA treatments at 10% moisture and 75 °C, independent of ammonia loading (Table 1). Increasing moisture content to 50%, however, caused a significant increase ($p < 0.05$) in solubility (upto 281–394 g kg^{-1}). In addition, increasing temperature from 75 to 90 °C and ammonia loading from 1.0 to 2.0 g g^{-1} dry FM increased 0.5 M NaCl protein solubility ($p < 0.05$) within the 50% moisture samples (treatment E versus C). Protein recovery was determined in the solution obtained by filtration after incubation of FM with 0.15 M NaCl, and it was found that increasing ammonia loading increased ($p < 0.05$) protein recovery for the PDA treatments held at 75 °C (treatment B versus A and untreated FM). High moisture (50%) treatment combinations (treatments C, D, and E), however, considerably increased protein recovered in the filtrate (32 g kg^{-1} in untreated FM versus a 3-fold increase in protein recovered for treatments C, D and E, Table 1). There were no significant differences among the 50% moisture treatments (C, D and E). Limited solubility has been inferred as a major cause for nutrient limitation of FM,[22] therefore the increase in FM solubility observed with PDA processing may enhance the potential use of FM as a feed. Ammonia is a weak alkali and is expected to break sulphur bridges between cysteine moieties, opening the compact and unavailable structure. Compared to other treatments applied to feathers, ammonia treatments are expected to cause potentially less damage to feather meal protein since the reaction takes place within 5 min and oxidation reactions are avoided because there is no oxygen in the reactor. When pressure is released after the treatment, temperature declines rapidly, avoiding further heating

Table 1. NaCl solubility, protein recovered and *in situ* ruminal solubility of untreated and PDA treated feathermeal (FM) protein

	Treatment conditions			Response		
	Ammonia loading (g g^{-1} dry FM)	*Moisture level (% wet basis)*	*Temperature during process (°C)*	*NaCl solubility (% dry matter)*	*Protein recovered (% dry matter)*	*In situ ruminal solubility (% dry matter)*
Untreated FM	NA*	NA	NA	13.34 ± 1.89a	3.22 ± 0.60a	7.18 ± 2.25a
PDA-treated FM						
Treatment A	0.5	10	75	12.77 ± 0.78a	3.95 ± 0.92a	10.18 ± 1.96a
Treatment B	1.0	10	75	15.93 ± 0.33a	5.95 ± 0.90b	8.38 ± 1.96a
Treatment C	1.0	50	75	28.08 ± 2.78b	9.60 ± 0.53c	17.05 ± 2.12b
Treatment D	1.0	50	90	32.22 ± 0.48bc	9.50 ± 0.55c	22.09 ± 1.92c
Treatment E	2.0	50	90	39.44 ± 2.12cd	10.02 ± 0.69c	23.04 ± 1.03c

* NA, not applicable.

a–d Means in the same column with different letters following differ significantly. Each mean represents triplicate determinations.

of the biomass. The disruptive effect caused by the sudden depressurisation also helps in denaturing the protein structure.

When untreated FM was exposed to ruminal fluid (Table 1), only 72 g kg^{-1} of the protein became solubilised. It was found that high moisture (50%) treatment combinations (treatments C, D and E) considerably increased ($p < 0.05$) *in situ* ruminal solubility when compared with either untreated FM or PDA treatments A and B (Table 1). In addition, increasing temperature from 75 to 90 °C increased solubility ($p < 0.05$) within the 50% moisture samples (PDA treatments D and E versus C). PDA treatment affected ruminal protein solubility in a similar way as solubility in NaCl ($r = 0.92$), which is in agreement with results found in many feeds, mainly forages and grains.[16] Therefore, if ammonia-treated FM (at optimal conditions) is fed to a ruminant, more than 200 g kg^{-1} of the protein could be potentially available immediately for the ruminal micro-organisms.

Low solubility of untreated FM indicates that ammonia nitrogen concentration may be below the optimal for maximal rumen digestion, as reported by Church *et al*[12]. Mehrez *et al*[23] and Ricke and Schaefer[24] showed that optimal ammonia concentrations in the rumen are different for substrate fermentation rates and bacterial protein yields. Lower ammonia concentrations would first affect fibre digestion and this was in agreement with Church *et al*.[12] Goedeken *et al*[25] however, did not find negative effects in growing calves fed hydrolysed feather meal as a protein replacement for soybean. If protein content in the diet is high (160 g kg^{-1} or there is an additional source of ruminally available protein, low solubility of feather meal would not be critical.[13,26] FM could be a good source of escape protein, however, increasing flow of non-ammonia nitrogen to the small intestine and supplying sulphur amino acids among others.[11,27] Limited digestibility has also been indicated in FM with *in vivo* studies,[28] but FM PDA-treated at 50% moisture content (Fig 1) meets the requirement of supplying both a substantial and, at the same time, constant amount of ammonia in the rumen so that it does not become limiting and fibre digestion is optimised. At 12 h of digestion, protein disappearances of FM treated at 1-50-75, 1-50-90 and 2-50-90 processing conditions were 311, 371 and 430 g kg^{-1}, respectively, and were much higher ($p < 0.05$) than FM PDA-treated at 10% moisture and the control (equal to or lower than 236 g kg^{-1}). Protein disappearance was rapid during the first hours, then it increased at a much lower rate. Significant effects were found for temperature and ammonia loading ($p < 0.05$). These results show that pressurised ammoniation can be used to increase the digestibility of FM protein. The highest digestibility at 48 h for treated samples was 54.8% (2-50-90 treatment) whereas digestibility of the control was 34%. Depending on the demand for nitrogen in the rumen, a variety of processing conditions can be chosen. It is possible that total protein disappearance (rumen + small intestine) is similar for FM PDA-treated at different conditions, therefore the demand for bypass protein will play a major role in choosing processing conditions. The remainder of the protein after 12 h in the rumen is currently considered as a measure of amount of escape protein and is well correlated with *in vivo* digestibilities.[10] PDA-treated FM with a protein disappearance of up to 430 g kg^{-1}, which would indicate a low escape

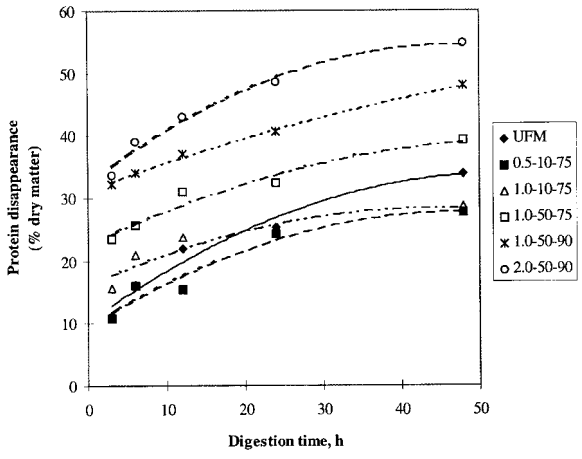

Figure 1. *In situ* ruminal solubility of untreated (UFM and PDA-treated feather meal protein. Treatments: ammonia loading (g g^{-1} dry FM)-moisture (% wet basis)-temperature (°C). SEM = 1.1.

protein value of 570 g kg^{-1}, would appear as a low escape protein when compared with untreated (770 g kg^{-1}). A 570 g kg^{-1} escape protein value, however, is above that (430 g kg^{-1}) recommended for high milk yielding cows.[13] It is not clear how much FM protein is actually indigestible, but it is very likely that total protein digestibility is higher in PDA-treated FM than in untreated FM. An increase in undegraded protein entering the small intestine is necessary, but not a sufficient condition for improved protein status. This undegraded dietary protein must be digestible.[16] Pepsin digestibility data can be associated with digestibility at the small intestine.

It is apparent that high moisture is required as well as relatively high temperature to substantially increase protein solubility and digestibility of FM. Moisture may be required to enhance ammonia penetration, since ammonia readily dissolves in water. Experiments carried out on cereal straws[29] also indicated the need for moisture. Temperature also enhances the chemical action of ammonia by promoting diffusion, lowering viscosity of the media, and weakening sulphur bridges and other chemical interactions in the structure of FM protein. In addition, pressure developed in the reactor (150–300 psi) guarantees ammonia penetration on FM tissues. Ammonia could also produce plasticisation of protein polymers, which would enhance the disrupting action of the sudden depressurisation applied at the end of the ammonia treatment.

Many researchers have reported high pepsin digestibility values for FM,[30] however amino acid content and availability, and *in vivo* studies, did not support this response.[3,8,31] Parsons[19] showed that the current standard pepsin digestibility method[15] does not correlate well with actual digestibility in poultry ($r = 0.23$ for lys digestibility). When pepsin concentration is decreased from 0.2 (standard) to 0.002%, however, pepsin digestibility can be used to predict true digestibility ($r = 0.68$). With the adjusted pepsin concentration, common pepsin digestibilities of FM of 70–81% declined to 17–49%,[31] which are more closely related to *in vivo* digestibility. Untreated FM (Fig 2) had a digestibility of 35.4%, which is within the range reported by Han and Parsons,[31] but much higher than the digestibility of ground feathers (13.6%). PDA treatments, however, even at the least effective processing condition, 0.5-10-75, greatly increased protein digestibility ($p < 0.05$, Fig 2). Differences in pepsin digestibility among the PDA treatments were greater than differences among the ruminal digestibility. Ammonia loading, in addition to moisture and temperature, as observed with ruminal data, appeared to enhance effects of the treatment. With PDA treatment at 2-50-90, FM reached a protein digestibility of 89.3%, which was even higher than that of soybean meal protein (82.3%). Digestibility of egg white protein was very high (98.5%), as expected. With a digestibility such as this, FM would double its potential as a protein source for non-ruminants, and suggests a high total digestibility in ruminants. Cupo and Cartwright[5] have reported that the calorie/protein ratio in broiler diets using FM should be low (around

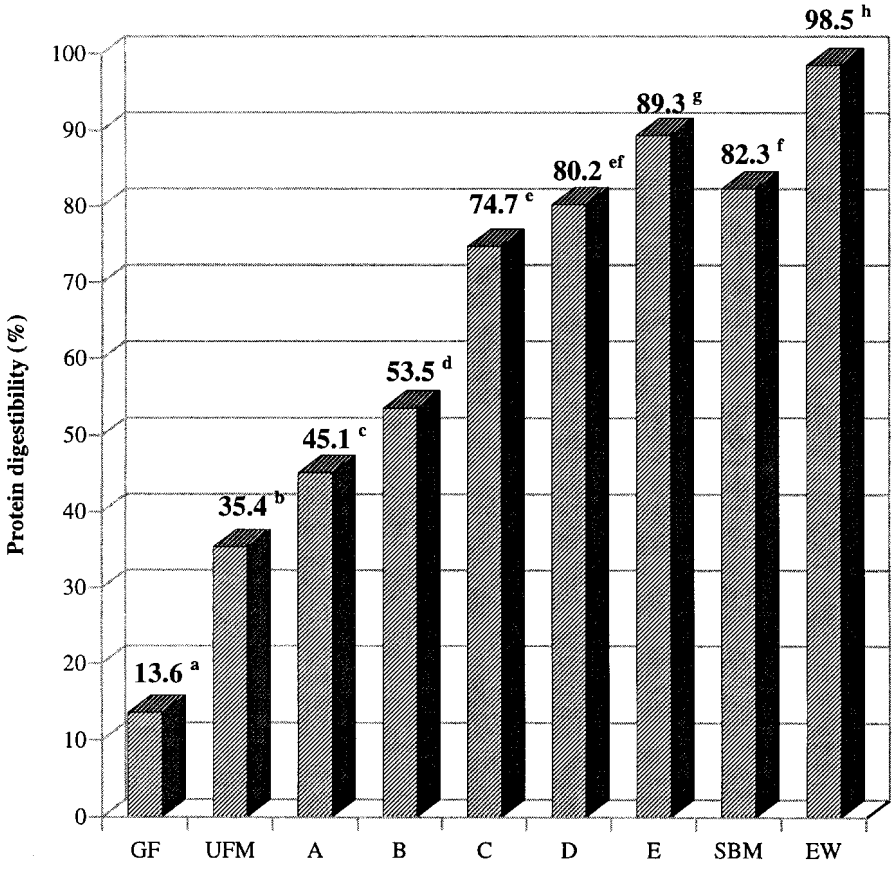

Figure 2. Pepsin digestibility of PDA-treated feather meal protein. Treatments: ammonia loading (g g^{-1} dry FM)-moisture (% wet basis)-temperature (°C); GF, ground feathers; UFM, untreated feathermeal; SBM, soybean meal; EW, egg white, were used as controls. SEM = 2.2 A = 0.5-10-75; B = 1.0-10-75; C = 1.0-50-75; D = 1.0-50-90; E = 2-50-90. a–h, means with different superscripts differ significantly ($p < 0.05$).

1.6) for optimal growth. A higher digestibility protein would be expected to improve performance in isocaloric diets.

It is necessary to develop protein solubility assays that can be used as indicators of protein digestibility for non-ruminants such as poultry. Solubility in NaCl has been primarily used to predict ruminal solubility. Protein solubility in NaCl, however, correlated very well with pepsin digestibility in this work ($r = 0.98$) and suggests its utility as a predictor of pepsin digestibility (a time-consuming assay) in PDA-treated feather meal. Pepsin digestibility also correlated well with ruminal solubility ($r = 0.96$).

Based on results presented in this work, it is apparent that PDA treatment increases availability of feather meal protein, since solubility increased almost 3-fold and digestibility more than 2-fold. This increased availability would be meaningful for both ruminants and non-ruminants. PDA treatment conditions (ammonia loading, moisture and temperature) greatly affect performance of the treatment. An ammonia loading of 1 g g^{-1} dry FM appears sufficient to attain significant increases in solubility and digestibility, although greatest values were achieved with an ammonia loading of 2 combined with 50% moisture at 90 °C. A 5 min treatment time was sufficient, and it could be decreased if processing conditions are optimised.

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