ABSTRACT

FREEMAN, SHARON ROWE. Utilization of Poultry Byproducts as Protein Sources in Ruminant Diets. (Under the direction of Matthew H. Poore and Peter R. Ferket.)

Production of market-ready poultry products results in co-production of millions of tons of wastes. Some, such as poultry litter, poultry by-product meal, and feather meal, are well known and have high acceptance. Others, like spent laying hens and dissolved air flotation skimmings, are less well known and therefore may not be utilized to their full potential. Our research demonstrated that spent laying hens can be mechanically deboned to yield a soft tissue fraction (66% of carcass weight) which could be valuable as a monogastric feedstuff. It also produced a hard tissue fraction (largely feathers and bones and 34% of carcass weight) which could be further processed into a proteinaceous meal for ruminant feeds. Steam hydrolysis of the hard tissue improved pepsin digestibility from 74 to 85%. True amino acid digestibility in the hydrolyzed hard tissue averaged 77% (SEM = 1.9%). Subsequent keratinase enzyme addition did not improve either pepsin or true amino acid digestibility beyond levels achieved by steam hydrolysis ($P > 0.10$). Co-extrusion of the steam hydrolyzed hard tissue with soybean hulls resulted in feather-bone meal (FBM) which had handling characteristics similar to those of soybean hulls; contained 94% dry matter, 23% protein, 55% neutral detergent fiber, and 7% fat; and which was well accepted when incorporated into concentrate pellets for meat goats to provide up to 60% of added nitrogen. Animals which received FBM in place of a portion of the traditional protein source, soybean meal, had a tendency for higher dry matter intake (703, 710, 673, and 779 g/d intake for 0, 20, 40, or 60% FBM substitution, respectively; $P = 0.11$) and had higher nitrogen retention than control goats (2.2, 1.4, 2.1, 2.5g N/d for 0, 20, 40, and 60% FBM substitution,

respectively; $P = 0.06$). Dissolved air floatation skimmings (90% water, 6% fat, and 4% non-fat solids) are a product of waste water treatment in poultry processing facilities. Partial fat extraction and dehydration yields a proteinaceous meal called secondary protein nutrients (SPN; 5% moisture, 46.5% protein, 27.8% fat, 28,200 ppm Fe). Evaluation of secondary protein nutrients confirmed that it can be utilized in diets for meat goats and beef steers to supply up to 40% of supplemental protein. Inclusion rates above 50% in steer diets resulted in reduced dry matter intake (6.8, 7.3, 6.9, 60, and 5.2 kg DM/d for 0, 25, 50, 75, and 100% SPN substation, respectively; $P < 0.001$) and average daily gain (1.26, 1.21, 1.11, 0.94, and 0.67 kg/d for 0, 25, 50, 75, and 100% SPN substitution, respectively; $P < 0.01$). Reductions were likely the result of a combination of factors including reduced fiber degradation caused by inadequate supplies of ruminally degradable protein, increasing levels of fat in the diet, and elevated iron content. Goats receiving up to 40% of added N as SPN had similar N retention (g/d) to control animals ($P = 0.11$). Changes in ruminal parameters varied with species, but reflected the dietary addition of rumen undegradable protein and changes in fat and Fe content. Our research established that wastes from the poultry industry can successfully be converted into novel feedstuffs, offering the opportunity to recycle nutrients and reduce their release into the environment. The novel feedstuffs evaluated supported animal growth, supplying supplemental protein. Although detailed economic analysis was not conducted, we hypothesize that use of novel feedstuffs produced from wastes could reduce feed costs and increase the value of waste, thus improving the profitability of animal and poultry production.

Utilization of Poultry Byproducts as Protein Sources in Ruminant Diets

by

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DEDICATION

This work is dedicated first to my husband, Terry, who has patiently endured my preoccupation with class work, research trials, and writing for 8 years and who has encouraged me to carry on with confidence in my eventual success. It is also dedicated to the memories of my father, Wilfrid Rowe, Jr. (1930-1999), who by his example, inspired me to practice life-long learning and, of my sister-in-law, Jennifer Freeman (1962-2007), who modeled perseverant faith and who was certain that this degree would be used to bring glory to our Lord.

BIOGRAPHY

Sharon Anne Rowe arrived on a snowy January 2 in 1957, the first child of Wilfrid, Jr. and Jeanne B. Rowe. She was reared in Penfield, NY with a sister, Judy, and brother, John. Childhood was filled with school, viola lessons and orchestras, horseback riding, a love of drawing, and passion for the outdoors and things of nature. Summer jobs as a green bean and strawberry harvester, a stable hand, and a dairy milker influenced her career path and further developed a keen interest in animals, particularly ruminants. Following graduation from Penfield High School in 1975, Sharon attended the College of Agriculture and Life Sciences at Cornell University, Ithaca, NY, with hopes of attending veterinary school. When this dream could not become a reality, she moved to Raleigh, NC to pursue a Master of Science in nutrition at North Carolina State University (NCSU). Upon completion of this degree in 1982, she accepted the position of Research Operations Manager of the Metabolism Education Unit in the Field Laboratories Department at NCSU. This position allowed her to continue to express her interest in ruminants through in her daily work. She has enjoyed a 27-year career as a care-taker, instructor, and research assistant. While in this position, she met and later married Terry A. Freeman. In 1999, Sharon decided to go back to school to pursue a Doctor of Philosophy degree in nutrition, plans she had set aside in 1982. Upon completion of this degree, her plans are to remain with the University (at least until she is eligible to retire). She also hopes to apply her education to extension projects in developing areas of the US and world with hopes of improving the quality of life for the people she serves.

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Utilizing Poultry Byproducts as Protein Sources in Ruminant Diets: A Review of the Literature

INTRODUCTION

The word "byproduct" is generally taken to mean a product produced during the making of something else. This general interpretation may not be appropriate when applied to food production, however, since it implies a certain value to human consumers for the "byproduct". In fact, many "byproducts" of food production have little, if any, value to consumers and therefore could be more accurately designated as "waste materials" (Walker, 2000). Waste materials can become waste products and eventually byproducts, which have value, through processing. True byproducts are nutrient dense, highly digestible, and free of contaminants (Walker, 2000). They can be stored for extended periods of time, require little further processing, are easy to transport and handle, and if incorporated into feeds are cost effective. Byproducts are well accepted by the general public and they are legal. Waste materials don't meet many of these criteria and so the mentality regarding their disposal is that it should be done as inexpensively and conveniently as possible. They often end up in landfills or incinerators or they are land-applied (Westendorf, 2000).

Animal agriculture is generates millions of tons of waste materials annually. Things such as manure, hair, feathers, offal and inedible body parts, condemned animals, mortalities, and wastewater have little value for human consumption, and while some of them are easily disseminated, others pose considerable challenges to the agricultural

community. All of them must be handled in economically feasible and environmentally responsible manners.

The concept of nutrient recycling has led to the use of many agricultural waste materials by other phases of agriculture. An excellent example of nutrient recycling is the application of manure on to cropland as a source of fertilizer, allowing the crop to absorb the nutrients in the animal waste and incorporate them into plant products. If done properly, this practice provides livestock producers with a means of disposing of the manure waste material in a manner that reduces chemical fertilizer costs, adds organic matter back to the soil, keeps waste nutrients out of ground water, and prevents their build-up in soil.

Also for the purpose of recycling nutrients, the meat processing industry has developed a variety of feedstuffs for animal diets from the waste materials it produces. Rendering plants across the country produce meat meal, meat and bone meal, blood meal, lard and grease, feather meal, and other useful, nutrient-rich products from "waste materials". Many of these products are commercially available and so are byproducts in the truest sense. They provide nutritionists with alternatives to traditional diet ingredients, are considered reliable sources of high quality protein and energy for livestock and pet diets, and can supply essential nutrients not found in adequate quantities in traditional feedstuffs. They may offer reductions in feed costs and therefore have a great impact on the total cost of livestock production.

Concern over the safety of using byproduct ingredients in livestock diets dramatically increased during the mid-1980s with the discovery that the prion which causes Bovine Spongiform Encephalopathy (**BSE**) in cattle could be transmitted to humans and

induce new variant-Creutzfeldt-Jakob syndrome. It was deduced that this fatal, neurological disorder resulted from the ingestion by humans of neural tissue from BSE-infected beef. Presumably, the cattle contracted the bovine form of the disease after eating meat and bone meal produced from inadequately rendered beef or mutton waste products coming from the processing of cattle carrying BSE or sheep carrying scrapie, the ovine version of BSE. In the wake of these discoveries, the U.S. Food and Drug Administration drafted Title 21 in the Code of Regulations, Part 589.2000, which prohibits the feeding of any ruminant-derived protein product to ruminants. This regulation took effect in August, 1997 and has limited the use of traditional rendered, ruminant-derived products in feeds for ruminants. Unaffected by this regulation, however, are protein products derived from poultry, swine, or fish.

Following an outbreak of Foot and Mouth Disease in Europe in 2001, additional restrictions were placed on animal-derived protein feed ingredients for ruminants by the Food and Drug Administration further complicating the recycling of nutrients in wastes as animal feedstuffs. Increased scrutiny was placed particularly on the practice of feeding restaurant waste to livestock. Additional regulations were applied to the importation of meat-derived byproducts. Again, however, the regulations did not impact poultry byproducts or waste materials.

Poultry byproducts and waste materials are in abundant supply, pose no risk of spreading BSE or Foot and Mouth disease, and are not impacted by legislation addressing concerns about mammalian-derived products. They therefore warrant renewed consideration as protein sources for ruminant diets. The familiar uses for conventional

byproducts should be revisited and paths that lead to new products produced from poultry waste materials should be explored. It is the purpose of this review to examine the role of poultry byproducts in ruminant nutrition and to discuss both familiar and novel sources of nutrients for ruminants which might offer the poultry industry means for disposing of waste materials.

NUTRITIVE VALUE OF POULTRY BYPRODUCTS FOR RUMINANTS

It is clear from the abundance of research which has been published about the usefulness of byproducts in ruminant diets that they are acceptable in the eyes of livestock producers. As new waste products are introduced (waste products are intermediary between waste material and byproduct status), investigative research has evaluated their abilities to substitute for standard feeds in diets. If animal production can be maintained and be accompanied by economic advantage, a waste product moves closer to becoming classified as a byproduct and being widely utilized by producers, particularly those in the geographic area of its origin where transportation costs are minimal.

Research to evaluate poultry-derived byproduct feedstuffs generally centers on replacing traditional, costly sources of supplemental protein because poultry byproducts are typically high in crude protein (**CP**) and are less expensive. Many are also high in fat and therefore serve as good sources of energy. As a step in the byproduct evaluation process, the amino acid (**AA**) profile is often determined and compared to the needs of the animals being fed. Its ability to compliment the AA in other feed ingredients can influence its value as a feedstuff. Table 1 lists the AA present in many of the byproducts to be discussed in this review along with those of muscle and casein, key protein components produced by animals

from the nutrients in the feed. A simple means of evaluation is to compare the proportion of each AA in the feed to its proportion in the product the animals produce. Knowledge of the amino acid profile of a feed is only one criteria of feed evaluation and other characteristics are also important. Information about digestibility, rumen escape qualities, palatability, and presence of anti-nutritional factors are of paramount importance.

Determination of amino acid requirements in ruminant species presents a special challenge because of the ability of the rumen microflora to alter the amino acid profile of feed through the fermentation process. In typical grass and grain-based ruminant diets, methionine or lysine is usually considered the first limiting amino acid (NRC, 2001), although results of such determinations vary. For example, Kim et al. (2000) determined that histidine was first limiting in lactating dairy cattle fed grass silage and cereal-based supplements, with methionine, lysine, and tryptophan following in that order. As these authors conceded, however, the exact order of limitation varies and will be determined by dietary components, level of intake, and production status. Gibb et al. (1992) suggested that not only are the absolute amounts of amino acids important, the ratios of the amino acids to each other may also have a role in determining how effectively they are metabolized, making the establishment of ruminant amino acid requirements highly elusive.

The ability of dietary protein to escape fermentation will impact its ability to supply limiting amino acids to the host animal and every feedstuff contains proteins of various types which supply AA of different fermentation susceptibilities. For example, according to NRC (1996), 35% of the protein in soybean meal (**SBM**) is rumen-undegradable protein (**RUP**). Conversely, 65% of the protein in SBM will be ruminally degraded and provide

amino acids that may differ from those in the original feed. In contrast, hydrolyzed feather meal (**FM**) supplies protein which is 70% RUP (NRC, 1996) leaving only 30% to be altered by ruminal fermentation. Other poultry byproducts fall in between SBM and FM. Protein which escapes ruminal degradation and which is able to be digested by the host animal in its lower gastrointestinal tract can supply a more predictable quantity and quality of amino acids and may therefore be more useful in meeting the host's requirements.

While protein sources such as FM can supply AA to the intestines for absorption because the protein is undegradable in the rumen, they also supply limited amounts of degradable protein which is required by microflora. Reducing protein degradation by ruminal microorganisms can be accompanied by reductions in ruminal NH_3 concentrations (Lu et al., 1990; Cunningham et al., 1994; Cozzi et al., 1995; Bohnert et al., 1998; Huntington et al., 2001). These reductions are not necessarily deleterious to the host animal; however, they can become so if ruminal NH_3 becomes low enough to limit ruminal fiber fermentation. The minimum level of ruminal NH_3 required for optimal fermentation varies in the literature and is in the range of 5 to 24mg/dL (Satter and Slyter, 1974; Mehrez et al., 1977; Van Soest, 1994a; Boucher et al., 2007). It likely varies with species and diet. Caution will be needed when utilizing sources high in RUP that ruminal use of other nutrients is not impaired by low NH_3 levels. Addition of urea to diets is a simple means of insuring adequate NH_3 is present.

Supplying protein to the intestines does not guarantee that it will be digested and absorbed. Just as ruminal degradability varies among feedstuff proteins, so does intestinal digestibility. Using *in vitro* techniques, Piepenbrink and Schingoethe (1996) estimated that

intestinally absorbable dietary protein was 29, 47, 70, and 77% of CP for canola meal, FM, blood meal, and corn gluten meal, respectively. Intestinal digestibilities of RUP varied from 73% for canola meal to 94% for corn gluten meal with blood meal (77%) and FM (83%) having intermediate intestinal digestibilities. By monitoring AA profiles at various stages of digestion, these authors also determined that even though canola meal provided less protein to the intestine for digestion, the AA balance more closely matched that of milk protein and so potentially would be of more value to lactating dairy cattle than the proteins from the other sources (Piepenbrink and Schingoethe, 1996). This trial and others like it (Erasmus et al., 1994; Maiga et al., 1996) demonstrated that meeting the AA needs of producing ruminants is a complex issue with many facets to be considered and that multiple, complimentary sources of supplemental protein fed in concert will likely be required to obtain optimum results.

Palatability, while a simple concept, is paramount. No protein-rich feedstuff can meet nutrient needs if animals will not consume it in adequate quantities to receive the benefits it offers. Likewise, if a feed is palatable and readily consumed, but poorly digested, it is of little use. Anti-nutritional factors in a feedstuff can negate its potential benefits for the animal and the potential of carry-over of these factors to the end product might warrant consideration as well. One such anti-nutritional factor is biogenic amines. These compounds are the result of bacterial degradation of AA. They have been shown to be associated with reduced intake, dry matter (**DM**) digestibility, and rumen motility in dairy cattle (Phuntsok et al., 1998) and with gizzard lesions and increased mortality in chicks (Shalaby, 1996). They have been implicated in numerous human poisonings worldwide

(Shalaby, 1996) and given their wide impact are worthy of consideration in feedstuff evaluation. Additional factors that may impact feed palatability or digestibility include fat, minerals, heat damage, and mold. These and other factors which might limit intake or digestion of a byproduct feedstuff should be taken into advisement.

Additionally, the nutritionist must consider how the feedstuff impacts the economics of livestock production to allow producers maximum profitability. Price of individual ingredients must be considered, as must the efficiency with which they are utilized for production. Hence, combinations of byproducts, which may be less expensive than traditional ingredients and which may offer a better supply of RUP and thus essential AA, should be considered. Klemesrud et al. (1998) demonstrated this principle when they found that diets for calves containing combinations of FM and poultry byproduct meal elicited greater rates of gain than diets containing urea and that utilization of protein was most efficient when FM, with the highest level of RUP, was offered alone (not in combination with PBM). Gibb et al. (1992) had similar results when meat and bone meal and FM combinations were fed to growing steers. Goedeken et al. (1990b) observed a positive associative effect between FM and blood meal (**BM**) and suggested it was due to the complimentarity of the amino acids supplied by these sources of RUP since BM supplies lysine which is lacking in FM and FM is rich in sulfur amino acids. Clearly the ruminant nutritionist has many options for providing diets with protein sources which meet the needs of the host animal in a manner that maximizes profitability and minimizes negative environmental impacts.

WASTE PRODUCT AND BYPRODUCT FEEDSTUFFS

Poultry Excreta

When the average person thinks of animal waste products, they will likely think first of manure. Manure from poultry operations comes in two forms. The first is manure with no incorporated bedding. This product is most commonly produced on caged laying hen operations and, when processed for utilization as a feed ingredient, is known as dehydrated poultry excreta (**DPE**). It is often sanitized by either heat treatment or ammoniation (Zinn et al., 1996). The second form of manure is mixed with bedding material, most commonly wood shavings, wheat straw, or peanut hulls, and is known as poultry litter or recycled poultry bedding (**RPB**). Either DPE or RPB may be fed to ruminants as a source of supplemental N.

Dehydrated poultry excreta contain 4.0 to 5.0% N (26 to 32.5% CP; Zinn et al., 1996; Rossi et al., 1999; Jordan et al., 2002). Of the N, about 25% is true protein (Zinn et al., 1996), 50% is uric acid (Jordan et al., 2002), and the remainder is other forms of non-protein N (**NPN**), including ammonium ($\mathbf{NH_4^+}$). When included in ruminant diets, the N in DPE is readily digestible. Zinn et al. (1996) estimated the true digestibility of N in this product to be 84%, which was in agreement with estimates presented by Bhattacharya and Fontenot (1965) of 81 to 88%. More specifically, Oltjen et al. (1968), in examining the comparative degradability of N from different NPN sources in diets for cattle, found that the N in uric acid was highly degradable but disappeared at a slower rate than the N in urea.

The high percentage uric acid in DPE might allow poultry excreta to perform better than urea in ruminant diets containing slowly degradable carbohydrate sources, such as low quality forage.

Production parameters in ruminants fed DPE are favorable when compared to the same parameters in livestock fed traditional sources of N. Jordan et al. (2002) saw no significant difference in the gains of beef cows fed supplement containing SBM as compared to cows receiving pelleted DPE as a supplement for low quality pasture. Both SBM and DPE cow groups gained more than cows consuming supplement containing urea (which degrades very quickly in the rumen) as the sole source of supplemental N. Likewise, Rossi et al. (1999) found that gestating and lactating beef cattle had similar weight changes and body condition scores when poultry manure replaced SBM in their diets. Sheep consuming DPE had similar N retention (g/d) to control sheep (Smith and Cavert, 1976). Feed to gain ratios and DM digestibility were also similar among control and DPE-fed sheep.

While DPE is a good source of N for ruminants, it is also typically high and variable in ash content. Bhattacharya and Taylor (1975) gave a range of 26 to 30% ash for DPE. Zinn et al. (1996) found that ash contributed 42% of dry matter (**DM**) in the DPE utilized in their trial while Smith and Calvert (1976) list ash as 24% of DM in their DPE. High ash content, such as reported by Zinn et al. (1996), has an energy-diluting effect and results in lower DE values for diets. The ash provides, however, a source of available Ca and P. Bull and Reid (1971) found that the Ca in DPE was 9.3 % of DM and 95% available when the

DPE was fed as the sole source of N in diets for beef cattle. The P was 1.5 % of DM and 75% available.

The ash may have additional impacts on ruminants as well, some positive and others negative. Smith and Calvert (1976) suggested that the ash in the DPE provided buffering capacity to the rumen which allowed greater concentrations of volatile fatty acids to be produced. The ash in DPE often includes high levels of copper and has been shown to create copper toxicity in sheep when fed on a long-term basis (McCaskey and Anthony, 1979). Urinary calculi have not been reported in animals consuming DPE and should not be a problem as long as proper Ca and P levels are maintained.

Recycled poultry bedding, the second form of poultry excreta, tends to be somewhat more variable in quality than DPE due mainly to variation in the amount and type of bedding material which is mixed with the excreta. Storage time and conditions can also affect product quality, as does the amount of soil mixed with the RPB when it is removed from the poultry house. Stephenson et al. (1990) evaluated the variability and value of RPB from 106 sources in Alabama. The bedding in their samples consisted of wood shavings, sawdust, or peanut hulls. Some of the RPB was fresh and other samples came from stacks that had been stored for as much as 90 days (**d**). A partial summary of the qualities of their samples is given in Table 2.

Significant variation in N content resulted from differences in storage time, with the stacked RPB being higher in N than fresh litter. Acid detergent insoluble N (**ADIN**), which

is largely unavailable to the ruminant consuming it, also increased with storage time. Storing RPB for 30 to 90 d resulted in the best combination of high total N with low ADIN. Type of bedding material did not influence quantity of N or ADIN in the final product, a result which was in agreement with Bhattacharya and Fontenot (1966).

As is the case for DPE, RPB is also a significant source of supplemental N when it is added to ruminant diets. Crude protein content averaged 25% (Stephenson et al., 1990) with true protein contributing approximately 45% of total N and uric acid an additional 30% (Bhattacharya and Fontenot, 1965; Bhattacharya and Fontenot, 1966). According to Hopkins and Poore (2001), fractionation of the CP based on solubility and classification of the fractions as described by VanSoest (1994b) resulted in 40.5% of the CP in RPB appearing as A fraction (NPN), 4.3% as B_1 fraction (buffer soluble, true protein), 31.8% as B_2 fraction (neutral detergent soluble protein), 7.4% as B_3 fraction (neutral detergent insoluble protein), and 16.1% as C protein (acid detergent insoluble protein). These data suggest that 83.9%, all but the C fraction, would be degraded in the rumen (A, B_1, and B_2) or digested in the small intestine (B_3). This is similar to the apparent digestibility for the N in RPB of 77.8% obtained by Brugman et al. (1964) and also agrees with digestibilities reported by Bhattacharya and Fontenot (1966).

When RPB replaced wheat straw in diets for gestating and early lactation beef cows, Brosh et al. (1993) found that N digestibility of the diet was directly related to the amount of litter in the diets and was 40.7, 44.6 and 53.5% for diets containing 15, 30, and 45% litter, respectively. This contradicted data presented by Bhattacharya and Fontenot (1965), who

showed that N digestibility declined as litter increased in the diet. The conflict in the data may be explained by the relative digestibilities of the N in the ingredients which the litter replaced in the rations. Brosh et al. (1993) replaced a less digestible source of N (wheat straw) with a more digestible source of N (litter) while Bhattacharya and Fontenot (1965) did the opposite, replacing a more digestible source of N (isolated soybean protein) with a less digestible source of N (RPB).

Unlike DPE, recycled poultry bedding is an excellent source of fiber and energy for ruminants. It tends to have digestible energy values in the range of 2440kcal/kg for sheep (Bhattacharya and Taylor, 1975), similar to that of alfalfa hay. As was the case for the CP in the litter, changing the bedding material had little effect on the DE content (Bhattacharya and Fontenot, 1966). Fiber digestibility values in the literature ranged from 70% (Bhattacharya and Fontenot, 1965; Bhattacharya and Fontenot, 1966) to 91% (Brugman et al., 1964). Brosh et al. (1993) demonstrated decreasing fiber digestibility as poultry litter increased in the diets of cattle. The decrease was attributed to reduced ruminal retention time as a result of increased levels of intake. Smith and Cavert (1976), on the other hand, found that digestibility of DM, fiber, and N did not vary as RPB increased from 0 to 14% of DM in diets for sheep. Differences in results are likely attributable to differences in species and dietary constituents. Beef cows receiving 30 or 45% poultry litter in their diets had higher DM intakes and were heavier after calving than those receiving 15% litter in their diets. Calves from the 3 treatment groups gained at similar rates.

Like DPE, recycled bedding is high in ash and provides animals that consume it with copious amounts of minerals, including Ca and P. Stephenson et al. (1990) drew attention to the Cu and Fe content of RPB (Table 2). While both Cu and Fe are necessary for animal health, excessive amounts of Cu can result in toxicity, particularly in sheep, and excess Fe can create a Cu deficiency (Humphries et al., 1985). The amounts of these minerals necessary to cause toxic reactions vary and depend on total mineral content as well as the species consuming the feed. Toxic concentrations are not absolute, making it clear that if litter is to be a component of ruminant diets, thorough analysis of the product would be necessary before it was incorporated into feeds. Since the minerals are largely contributed by the excreta, similar mineral analysis would also be recommended for DPE before utilizing it as a feedstuff.

In addition to toxic levels of minerals, concern has been raised that products containing poultry excreta may contain concentrated levels of antibiotics, arsenic, other poultry feed additives, and naturally occurring hormones. Any of these compounds could potentially impact livestock consuming the excreta and might even make their way into the human food supply. Brugman et al. (1968) looked for the carry over of amprolium and arsenic from poultry litter to the tissues of sheep that consumed the litter. Neither amprolium nor arsenic was detected in the sheep. Bhattacharya and Taylor (1975) reviewed the subject of drug and hormone carry over and concluded that human food could be kept safe by removing excreta from ruminant diets 5 d prior to slaughter.

Excreta can also contain pathogenic bacteria and viruses which are known to be zoonotic and a threat to human health. *Salmonella sp.* are of primary concern. Sanitization of excreta products is possible and when done properly, sanitized excreta products are safe for consumption by livestock and pose little risk to human health and well being. Deep stacking RPB for at least 21 d after it is removed from the poultry house causes it to go through a natural heating cycle and is accompanied by release of ammonia. These conditions effectively kill pathogens (Bush et al., 2007). Dehydration processes used for excreta with no added bedding, such as oven or drum drying, apply adequate heat to kill pathogens (*Salmonella spp.* are killed in less than 10 seconds at 71^{o} C; Juneja et al., 2003).

Hydrolyzed feather meal

Feathers represent 5 to 7% of the body weight of most domestic fowl. While they afford many benefits to birds, they become a waste product during poultry processing. Feathers, which are comprised of about 90% keratinaceous protein, have poor digestibility characteristics and deficiencies of several essential amino acids (Onifade et al., 1998) including lysine (2.5% of CP in feathers), methionine (0.6% of CP in feathers), and histidine (1.1% of CP in feathers). Despite the challenges they present, feathers can be washed, hydrolyzed to improve digestibility, dried, and ground to yield hydrolyzed feather meal (Ockerman and Hansen, 2000). Part or all of the blood from the processing facility may be mixed with the feathers and may contribute up to 10% of the DM in the final product.

Feather meal is particularly appealing for ruminant diets because a large portion of the protein is RUP and is available for digestion in the lower gastrointestinal tract. *In situ*

experiments indicated that about 70% of the CP in FM escapes degradation as compared to only 25% of the CP in SBM (Goedeken et al., 1990a; Klemesrud et al., 1998; Klemesrud et al., 2000). Total tract digestibility of the CP in FM was approximately 85% (Klemesrud et al., 1998; Klemesrud et al., 2000). When FM was utilized as a supplemental protein source in place of all or a portion of SBM in diets for lambs, weight gains increased linearly as FM increased (Thomas et al., 1994) and steers fed FM or other protein sources gained weight faster than steers fed a diet containing urea as a source of supplemental N (Goedeken et al., 1990). Cozzi et al. (1995) observed similar weight gains and feed to gain ratios (**F:G**) among growing and finishing lambs fed diets containing SBM or a mixture of 56% SBM, 22% FM, and 22% BM. Feather meal also improved daily gains when it was added to steer diets containing meat and bone meal (Klemesrud et al., 2000); however, additions of ruminally-protected methionine to the combination further increased gains, supporting the notion that FM is deficient in this essential amino acid.

Feather meal can also be utilized in dairy rations. A FM and BM mix added to diets for lactating dairy cows increased milk production, milk protein, and solids-corrected milk yield when the diets contained 17.6% CP (Grant and Haddad, 1998) but resulted in decreased production when diets contained 19.6% CP. These authors offered no explanation for the reduction in milk production observed at the higher protein level and it seemed to defy their finding that efficiency of use of metabolizable protein improved with the addition of FM at both protein levels.

Lactating dairy goats responded well to the inclusion of a FM and BM mix which supplied 30% of supplemental N in their diets (Andrighetto and Bailoni, 1994). Digestion

parameters were similar in does fed FM and BM to those of does fed meat meal and milk yield was not compromised. Milk yield and body weight were also maintained in a trial conducted by Lu et al. (1990).

Poultry byproduct meal

Upon removal of the edible material from poultry carcasses, processing plants are left with a variety of products which are not used for human food. These include heads, offal, blood, condemned birds, and the skeletal racks from deboning operations. Any or all of these may be incorporated into a commodity known as poultry byproduct meal (**PBM**). Feathers are permitted in this byproduct in only the limited quantities that arise from normal processing.

Poultry byproduct meal can be processed by either wet or dry rendering, both of which involve cooking, drying, expelling a portion of the fat, grinding, and screening out large particles (Ockerman and Hansen, 2000). It typically contains 60 to 70% CP and 12 to 15% fat depending on how much ash the final product contains (11 to 20%, Simmons Protein, 2007). Poultry byproduct meal contains approximately 2.4% lysine, 1.0% methionine, and 1.5% histidine (Feedstuffs, 2006), giving it a better amino acid balance than FM, which is deficient in methionine.

Poultry byproduct meal ranks between SBM and FM for ruminal protein degradability. It has both readily degradable (45%) and undegradable fractions (55%) as determined by *in situ* incubation (Bohnert et al., 1998). The true protein in PBM is

approximately 90% digestible (Klemesrud et al., 1998). Steers fed corn silage and cottonseed hull-based diets that contained either PBM or SBM gained similarly (Bohnert et al., 1998; Klemesrud et al., 1998) and there were no significant differences seen in final weight or F:G.

Lallo and Garci (1994) replaced SBM with graded levels of PBM in sugar cane-based diets for growing hair sheep and saw improved weight gains (141.9g/d with 100% SBM vs. 161.3g/d with 100% PBM). Feed to gain ratios improved significantly, suggesting that the animals utilized the protein in PBM more efficiently than that supplied by SBM. It is possible that the protein from PBM escaping ruminal degradation was supplying an amino acid which was limiting growth with the SBM diets.

Nitrogen flow to the duodenum was higher in dually-cannulated steers fed diets containing PBM in place of SBM (Bohnert et al., 1998). Nitrogen reaching the duodenum from SBM and PBM had similar disappearance rates, suggesting that amino acids from PBM would be available for use by the steers and supporting the theory that PBM proteins which escape ruminal degradation may be able to supply essential amino acids which would otherwise be lacking.

WASTE MATERIALS: POTENTIAL SOURCES OF FEEDSTUFFS

Poultry hatchery byproduct

An estimated 140,000 t of waste are produced annually by hatcheries that generate commercial broilers, laying hens, and turkeys (Das et al., 2002). Included in this material are infertile eggs, dead embryos, egg shells from hatchlings, and unsalable chicks.

Traditional means of disposal for this material have been landfills or broadcast land application as fertilizer. These methods have become less acceptable as the volume of the waste and its concentration in location increased. As the volume of waste grew larger, the land mass required for proper application also increased, raising fuel expenditures for transportation to application sites, making land application increasingly less economical. Landfills in the vicinities of the hatcheries began rejecting the larger quantities of material without the assessment of large tipping fees. Finding practical techniques for recycling the nutrients in hatchery waste has become a high priority.

Co-extrusion with SBM is one means of processing hatchery waste (Lilburn et al., 1997). Lactic acid fermentation followed by extrusion or drying and grinding is another (Deshmukh and Patterson, 1997). If hatchery waste is cooked, dried, and ground, the resulting meal is known as poultry hatchery byproduct (**PHB**). Research has shown that all the resulting products can be successfully incorporated into diets for both laying fowl and broilers (Wisman, 1964; Vandepopuliere et al., 1977; Deshmukn and Patterson, 1997; Lilburn et al., 1997). In addition to supplying energy and protein, PHB and similar products also supply Ca. The mineral availability of PHB was similar to bone meal or limestone (Lilburn et al., 1997) and it resulted in good egg shell quality in laying hens (Vandepopulier et al., 1977). These same qualities might make it useful in diets for lactating livestock or in any diet in need of Ca and protein supplementation; however, research in this area has yet to be documented.

Mortalities

An estimated 455,000 t of poultry mortality is disposed of each year. Death loss estimates of 9% for turkeys and 4.9% for broilers have been reported (Blake, 1998). For broiler breeders, mortality rates of 0.35% and 1.5% per week can be expected for females and males, respectively (Wineland et al.,1997). Table egg production results in 0.5% per month animal loss to mortality. Disposal of dead fowl clearly presents a challenge to modern producers. Burial and composting have been the traditional methods employed, but these methods are under increased scrutiny. A quest for alternative disposal methods is underway and recycling the nutrients in the mortality into feedstuffs is a possibility.

To make the production of feedstuffs from mortality plausible, a means of on-farm storage will be necessary so that adequate volumes of material can be accumulated so as to make transportation economical. Whatever method of storage is utilized, it must preserve nutrients. The most obvious method of preservation is freezing. Producers could gather mortalities daily, place them in on-site freezers, and call for pick-up when volume warrants. Once at the processing facility, thawed mortalities could be ground and rendered or otherwise further processed.

Frozen and rendered whole layer mortality was successfully fed to laying hens (Damron, et al., 2001). Rendered meal replaced SBM and corn in isonitrogenous diets for 84 d. Inclusion of up to 10% of this product supported similar feed intake to a control diet. Egg production per day and feed conversion (kg feed per dozen eggs) improved with additions of rendered mortality; however, egg weight declined slightly, possibly due to lower amino acid availability in rendered hen meal as compared to SBM. Declines were not

deemed biologically significant and the rendered hen meal seemed to offer a plausible means of recycling the nutrients in mortalities.

Christmas et al. (1996) successfully fed rendered, frozen mortality meal to broiler chicks. No differences in body weight were observed between experimental and control chicks even at inclusion rates up to 12% mortality meal; however, chicks receiving the mortality meal ate more feed than their control counterparts and therefore also exhibited reductions in feed conversion ratio.

Tadtiyanant et al. (1993) successfully utilized mortalities in extruded feed ingredients for broiler chickens. Freezing was used as the preservation technique. Thawed carcasses were ground, mixed with 75 parts SBM to 25 parts ground mortality, and then extruded. The end products produced satisfactory gains when included in diets for broiler chick and turkeys. Similarly, the extruded products had no negative effects on egg production in laying hens.

Acidification of the carcasses would be a second possible means of preserving mortalities. Livestock producers have successfully utilized the process of ensiling for decades to preserve forage crops via anaerobic fermentation. Acids produced by the fermentation process are sufficient to prevent spoilage and nutrient loss in properly fermented forages. Attempts at ensiling poultry waste products have met with mixed results. Low levels of fermentable carbohydrate in animal tissue present a large hurdle.

Urlings et al. (1993) used waste from a broiler processing plant and attempted to reduce material pH to less than 4.0 within 48 h. Their secondary goal was to hold the pH below 5.0 for a year. Sugar beet pulp (10% by weight) was added to the poultry waste to

supply fermentable carbohydrate and silage inoculum was added to give 10^6 colony forming units per gram material. Additionally, a portion of the waste had 2% dextrose added to supply additional carbohydrate. The results from this initial attempt were that pH did not decline rapidly enough to prevent spoilage and nutrient degradation. Many of the samples contained unsuitable levels of enterobacteria and biogenic amines.

Other researchers have, however, overcame the challenges poultry mortality presents to the ensiling process and have fed the ensiled material to swine with success (Tibbetts et al., 1987; Tibbetts and Seerley, 1988). Mortality silage containing 30 parts corn, 60 parts poultry offal, 5 parts dried molasses, and 5 parts *Lactobacillus* culture could be incorporated at rates of up to 20% (air dry basis) of diets with no effect on digestibility, carcass characteristics, or feed conversion by gilts and barrows. Similar attempts to feed raw, ensiled poultry mortality to cattle encountered severe palatability problems (Matthew Poore, PhD., North Carolina State University, Raleigh, personal communication).

Direct acidification of the poultry mortality is an alternative to ensiling. Cai et al. (1995) compared the abilities of formic, acetic, propionic, lactic, sulfuric, phosphoric, and peracetic acids at a variety of strengths to preserve poultry offal in a laboratory scale over 144 h. Stable products were considered to have a final pH less than 4.4 and were obtained with 1.5% formic, 10% acetic, 3.8% propionic, and 4.8% lactic acids. Among these, formic acid in strengths of 2% or greater produced stable products which lasted 60 d with pH below 4.2. Formic acid at 1.5% was determined to be the most cost effective acidification means.

When applied in a field-scale situation, 2% formic acid applied to ground carcasses produced a stable product in which protein, fat, and pH levels were nearly constant over 8 d

of storage at 30°C (Cai et al., 1995). Fecal coliform bacteria and *Streptococci* were reduced in the stored product from 10^8 colony forming units to 10^4 to 10^5 colony forming units over the storage period. *Salmonella spp.* were not detected in the final product.

Middleton and Ferket (2001) acidified ground poultry mortality with phosphoric acid. Since phosphoric acid is commonly used in the feed industry, it is readily available and it provides a source of dietary phosphorus. Its use could therefore prove to be more economical than other acids. A minimum of 6.9% phosphoric acid was required to produce a product with stable pH over a 45-d storage period; however, a minimum of 8.3% acid was required to maintain constant volatile N levels (a measure of bacterial degradation of proteins in the mortality). This trial supported that of Cai et al. (1995) in demonstrating that direct acidification could successfully preserve poultry mortality on farms. Additional work is required, however, to determine the best ways to use preserved mortality in feeding regimes for ruminants and non-ruminants alike and further research is needed on alternative means for handling poultry mortalities.

Spent laying hens

According to the 2002 Census of Agriculture (NASS, 2002) there were 334.4 million hens in production in the U.S. Between one third and one half cease to produce profitably in any year meaning that 111.4 to167.2 million hens are labeled as "spent" and deemed waste. If each hen weighs 1.7 kg, the combined annual weight of the spent hens is 189,000 to 284,000 t annually.

Laying hens have been bred for egg production; therefore, the majority of the nutrients they consume are directed to this end. By the time they complete their productive

lives, their bones have become brittle as a result of demineralization for eggshell production and only about 250 g of usable meat remains on their bodies (Kersey et al., 1997; Aho, 1999). They are therefore of little use to the human food industry. Their on-farm value prior to disposal often approaches $0.00 and their removal from the farm may represent a net expense to the producer (Middleton, 2000).

Since no viable market is available for these birds, an increasing number are being euthanized and composted or buried, resulting in the loss of nutrients that remain in their tissues (Lyons and Vandepopuliere, 1996). Existing technologies can be combined in new combinations to change this situation, including deboning, flash dehydration, extrusion, and expulsion, and resulting in numerous potential feed ingredients.

Traditional rendering of the whole birds would be the most direct method of recycling nutrients; however, the feathers on the hens, which typically constitute 4% of body weight, make them difficult to handle for traditional rendering, since they tend to absorb fat and reduce digestibility (Webster *et al.*, 1996). Hamm (1976) successfully rendered spent hens on an experimental scale. The result was a product containing 20.6% moisture with about 50% of the initial weight of the hens. His feedstuff contained 48% CP, 38% fat, and 9% ash (dry basis). When the majority of the fat and remaining moisture was removed, the product contained 8% moisture, 73% CP, 5% fat, and 14% ash.

Kersey et al. (1997) examined spent hen meal (**SHM**) produced by commercial rendering companies in Texas, Oklahoma, and Nebraska. They found the products quite variable in quality with CP ranging from 65 to 71%, fat from 9 to 11%, and ash from 13 to 18%. Although amino acid content expressed as a percentage of CP was fairly constant

across products, the digestibility of the amino acids as determined by cecaectomized cockerels varied (Table 3). These researchers expressed concern about the inconsistency of nutrient content in SHM because variation in dietary ingredients can result in nutrient imbalances which in turn can reduce animal productivity or cause excessive nutrient excretion. Despite the deficiencies of spent hen meal, it was clear from this work that commercial-scale rendering of these birds is a possibility for nutrient recycling.

Kersey and Waldroup (1998) fed the commercially produced SHM to broiler chickens. Diets were formulated to provide similar amounts of digestible amino acids. Body weight gain, feed conversion, bone ash, and carcass yield were similar in control birds and birds receiving SHM when it supplied 10% or less of the digestible amino acids. At inclusion rates beyond 10%, reductions were seen in these performance parameters. This trial demonstrated, however, that the nutrients in spent laying hens can successfully be recycled and fed in limited quantities to broiler chicks. The usefulness of SHM in diets for other species remains to be determined.

Dissolved air floatation skimmings

It is impossible to process poultry into market-ready products without the use of considerable quantities of water. According to the U.S. Census of Agriculture (NASS, 2002), roughly 8.5 billion meat-type chickens and 283 million turkeys are processed annually. Current daily through-put in typical processing plants creates 19 to 38 L of waste water per bird processed or 3.8 to 7.6 million L/day wastewater that must be treated before it can be released into the environment (Kiepper, 2001). This water contains more than 4,000

mg/L total suspended solids and upward of 3,000 mg/L fats, oil, and grease (Kiepper, 2001). Both physical and chemical treatment methods are available to assist in the clean-up effort.

In a survey of 23 poultry processing plants across 11 states, Kiepper (2001) found that 83% employed the process of dissolved air floatation (**DAF**). Dissolved air floatation had its origins in the mining industry in which it was used to separate particles of mineral ore from water (Edzwald, 1995). The process involves supersaturating water with air under pressure and then injecting it through a specially designed nozzle into a floatation tank containing the contaminated water. Small bubbles (average diameter = 40 μm, Edzwald, 1995) are formed as a result of the injection process. The bubbles rise individually, grow when they merge together, or form clumps as they journey upward in the tank. As they rise, they form aggregates with the contaminants in the water (called floc) and carry them to the surface of the liquid in the tank. The floc forms a layer which can then be removed by skimming resulting in the product DAF skimmings (or sludge) as well as waste water with a significantly lower content of contaminants.

Successful floc formation depends on the creation of hydrophobic particles by the neutralization of charge on the suspended contaminants. To create hydrophobicity, chemical flocculating agents are often employed. Inorganic iron salts or aluminum sulfate are commonly used to pre-treat waste water as are polyacrylimides (Anonymous, 2000). While these agents increase the efficiency of the DAF process, they create several hurdles that must be considered. Flocculating agents can hasten the wear and tear on machinery and may be difficult to remove from equipment surfaces. They cause the sludge to present challenges to animal nutritionists because of their high mineral content and as a result of

their potential impact on other dietary components. Further challenges are created by variability in nutrient content which results from differences in species being processed or differences in processing methods in waste water treatment (Fransen et al., 1995).

Dissolved air floatation skimmings from processing facilities is typically 90% moisture, 6% fat, and 4% non-fat solids at harvest (Anonymous, 2000). It must therefore be dried before it can be handled effectively. If partially dewatered, it contains 75% moisture, 15% fat, and 10% non-fat solids. At this point, land application near point of origin is possible; however, this method of disposal raises environmental concern. It can be packaged and transported for further processing at other locations at expense to the generator or it can be further processed at its point of origin to create a meal called secondary protein nutrients (**SPN**).

Secondary protein nutrients from poultry plants commonly contain 7 to 9% moisture, 41 to 53% CP, 9 to 15% ash, and 12 to 25% fat (Fransen et al., 1995). According to a survey of multiple sources of SPN, ferric chloride was most commonly used as flocculating agent and resulted in high iron concentrations in the material (21,850 to 41,000 mg/kg DM, Fransen et al., 1995). Copper (38 to 167 mg/kg DM), zinc (165 to 550 mg/kg DM), lead (2.9 to 84.3 mg/kf DM) and cadmium (0.2 to 0.4 mg/kg DM) were present in lower concentrations and peroxide values of 148 to 568 meqO_2/kg fat were observed.

Peroxide values in feedstuffs below 20 meq O_2/kg fat are considered acceptable by nutritionists (Hamilton and Kirstein, 2003). Values above 20 meq O_2/kg fat indicate rancidity; however, feeding rancid fat does not necessarily have a negative impact on animal performance (Lea et al., 1966, broilers; Carpenter et al., 1966, pigs; Kirkland and Fuller,

1971, broilers; Zinn, 1995, cattle); however, it may be a concern in some cases. In the case of SPN, the high peroxide values were likely indicative of oxidative damage to the fat in the product facilitated by the Fe in the flocculating agent. Addition of antioxidants, such as ethoxiquin, can reduce progressive oxidative damage in the finished product, but can not prevent damage to fat during DAF.

The Fe can also act as a prooxidant to fat soluble vitamins in finished feed. Sungwaporn et al. (2004) reported linear declines in plasma 25-hydoxy vitamin D when SPN levels in chick diets exceeded 7.5%. Rickets was observed in chicks consuming diets containing 20% SPN. Antioxidants were present in the feed, albeit at levels reported as marginal. The rickets were attributed to oxidative damage to vitamin D in the feed that resulted in poor Ca and P absorption and utilization. Impaired micelle formation caused by high levels of free fatty acids in the feed, a common symptom of oxidative damage to fat, was also implicated as a potential cause of the observed rickets.

In addition to damaging fat and fat soluble nutrients in feed, high levels of Fe can also cause Cu deficiency and thereby limit animal performance. While the exact mechanism for this interference is not completely understood, dietary Fe levels of 250 mg/kg can result in Cu depletion in growing cattle (Humphries et al., 1985**)**. Dietary Fe at 600 mg/kg resulted in depletion of Cu (plasma Cu less than 0.5μg/mL) in pregnant heifers in 56 d (Gengelbach et al., 1994). Maximum Fe concentrations of 500 mg/kg (sheep) and 1,000 mg/kg (beef cattle) have been recommended by NRC (1985 and 1996, respectively) and may be worthy of consideration when adding SPN to animal diets.

A final challenge presented by DAF skimmings is bacterial content, which is high by nature. Clostridia were present at levels of 3.1 to 5.8 $\log_{10}$ N/g DM in sludge collected from 8 swine and 5 poultry processing plants (Fransen et al., 1996). Salmonella were also present in all the samples. *Campylobacter jejuni/coli* were more prevalent in poultry skimmings than in swine skimmings. These bacteria not only pose a pathogenic risk to humans and livestock that might consume them, they also contribute to protein degradation and production of toxic compounds such as biogenic amines. Steps to reduce bacterial load are therefore necessary if the skimmings are to be utilized effectively as a feedstuff.

CONCLUDING REMARKS

Based on the abundance of literature available describing the use of poultry byproducts as feedstuffs in a wide variety of species, it is clear that these products offer viable alternatives to traditional, plant-derived protein sources. Fed alone or in combination, research indicates that they supply nutrients required by producing ruminants in forms that allow their utilization. Their acceptance by and incorporation into feeds in the ruminant industry will depend on several factors including: 1) availability, 2) profitability, 3) convenience, and 4) consistent and predictable animal performance. Perception of their wholesomeness by the consuming public may also influence the level to which byproducts can be utilized. Some of the byproducts described (feather meal and poultry byproduct meal, for example) have successfully overcome common hurdles and are readily utilized. Others, such as poultry litter, are fairly well accepted by producers but continue to struggle to some extent with consumer acceptance. Meals produced from hatchery waste, mortality, spent hens, and DAF skimmings still remain in developmental stages. How these products

are produced and marketed, including the names they are given, will influence whether or not the valuable nutrients they contain can be utilized to their full potential.

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Table 1. Amino acid composition of dried poultry excreta (DPE), poultry litter (PL), hydrolyzed feather meal (FM), poultry byproduct meal (PBM), poultry hatchery byproduct (PHB), rendered hen meal (RHM), and soybean meal (SBM) as a percentage of crude protein[1]

	Casein[2]	Muscle[3]	DPE[2]	PL[2]	FM[2]	PBM[2]	PHB[4]	RHM[5]	SBM[2]
Crude protein (% of dry matter)	80.0	NL	32.2	29.8	91.4	56.4	34.0	70.8	54.3
Methionine	3.75	3.20	0.42	0.51	0.65	1.71	2.38	1.72	1.46
Lysine	9.72	10.00	1.36	1.93	1.24	4.24	5.66	5.45	6.32
Histidine	3.47	3.30	0.80	0.79	0.33	2.68	1.76	2.16	2.72
Tryptophan	1.39	1.40	1.85	NL	0.47	0.94	NL	0.70	1.46
Cystine	0.42	NL	1.85	0.55	3.53	1.70	NG	1.83	1.48
Threonine	5.28	5.00	1.22	2.05	3.29	3.55	3.93	3.82	4.18
Arginine	4.72	7.70	1.32	1.70	4.61	6.60	7.28	6.33	7.53
Phenylalanine	6.39	5.00	1.22	1.93	3.13	3.02	4.58	4.04	5.65
Isoleucine	7.92	6.00	1.26	2.29	3.13	3.96	5.26	3.57	5.44
Leucine	12.08	8.00	2.79	2.76	9.18	7.45	9.29	7.22	7.95
Valine	9.44	5.50	1.60	2.92	5.35	4.38	6.41	4.98	5.65

[1] Values for each feedstuff are from a single source. If one or more values are missing from that source, it is indicated as "NL" (not listed).
[2] Feedstuffs Reference Issue and Buyers Guide. 2001. Miller Publishing Co., Minnetonka, MN.
[3] VanSoest, P.J. 1994. Nutritional Ecology of the Ruminant. Cornell University Press, Ithaca, NY, p. 307.
[4] Calculated from data of Vandepopuliere, J.M., H.K. Kanungo, H.V. Walton, and O.J. Cotterill. 1977. Broiler and egg type chick hatchery by-product meal evaluated as laying hen feedstuffs. Poult. Sci. 56:1140-1144., assumed 95% dry matter and using egg-type chick meal.
[5] Calculated means from Kersey, J.H., C.M. Parsons, N.M. Dale, J.E. Marr, and P.W. Waldroup. 1997. Nutrient composition of spent hen meals produced by rendering. J. Appl. Poult. Res. 6:319-324.

Table 2. Proximate analysis of poultry litter obtained from 106 sources in Alabama (percent of dry matter)[1]

Component	Minimum	Maximum	Least square mean	Standard error
Dry matter (%,)	61.0	95.3	80.5	0.58
Nitrogen	2.3	6.0	4.0	0.72
ADF[2]	18.0	69.1	41.1	1.08
ADIN[2]	0.1	3.4	0.6	0.02
Crude fiber	10.8	51.6	23.6	0.81
Ash	8.9	59.0	24.7	0.89
Ca	0.8	6.1	2.3	0.08
P	0.6	3.9	1.6	0.05
Cu (ppm)	25	1003	473	22.7
Fe (ppm)	529	12,604	2,377	262.7

[1] Stephenson, A.H., T.A. McCaskey, and B.G. Ruffin. 1990. A survey of broiler litter composition and potential value as a nutrient resource. Biol. Wastes 34:1-9.
[2] ADF = acid detergent fiber, ADIN = acid detergent insoluble N

Table 3. Content and digestibility of some amino acids essential to livestock in spent hen meal produced commercially at 3 locations[1]

Amino acid	Production location	% of crude protein	True digestibility (%)
Lysine	Tulsa, OK	5.40	77.8
	Omaha, NE	5.39	74.8
	Bastrop, TX	5.57	82.9
Methionine	Tulsa, OK	1.75	82.8
	Omaha, NE	1.80	83.0
	Bastrop, TX	1.61	88.4
Histidine	Tulsa, OK	1.93	73.0
	Omaha, NE	2.04	74.5
	Bastrop, TX	2.52	80.5
Arginine	Tulsa, OK	6.56	85.4
	Omaha, NE	6.49	79.8
	Bastrop, TX	5.95	85.1
Threonine	Tulsa, OK	3.81	76.9
	Omaha, NE	3.73	73.5
	Bastrop, TX	3.92	83.0

[1] Kersey, J.H., C.M. Parsons, N.M. Dale, J.E. Marr, and P.W. Waldroup. 1997. Nutrient composition of spent hen meals produced by rendering. J. Appl. Poultry Res. 6:319-324.

Alternative methods for disposal of spent laying hens: Evaluation of the efficacy of grinding, mechanical deboning, and of keratinase[1] in the rendering process

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ABSTRACT

Besides the challenges of mortality and litter disposal, the poultry industry must find economical means of disposing of laying hens that have outlived their productive lives. Because spent hens have low market value and disposing of them by composting and burial is often infeasible, finding alternative disposal methods that are environmentally secure is prudent. The feasibility of grinding or mechanically deboning spent hens with and without prior mechanical picking was evaluated for the production of various proteinaceous by-product meals. The end products were analyzed for nutrient content and found to be high in protein (35.3 to 91.9% CP) and, with the exception of the feathers, high in fat (24.1 to 58.3%), making them potentially valuable protein and energy sources. After considering physical and economic feasibility, mechanical deboning was determined to be a logical first step for the conversion of spent hens into value-added by-product meals. Because the hard tissue fraction (primarily feathers, bones, and connective tissue) generated by mechanically deboning the hens presents the greatest challenge to their utilization as feedstuffs, attention was focused on technologies that could potentially improve the nutritional value of the hard tissue for use as a ruminant protein source. Traditional hydrolysis of this hard tissue fraction improved its pepsin digestibility from 74% to 85%; however, subsequent keratinase enzyme treatment for 1, 2, 4, or 20h after steam hydrolysis failed to improve the pepsin or amino acid digestibility any further ($P > 0.10$). Enzyme hydrolysis did, however, increase the

quantities of the more soluble protein fractions (A: 45.5, 46.6, 52.8, 51.6, and 55.8% of CP; B_1: 3.2, 9.8, 6.0, 4.6, and 4.1% of CP; B_2: 11.7, 18.1, 22.8, 29.6, and 22.0% of CP for 0, 1, 2, 4, and 20h, respectively) and reduced quantities of the less soluble fractions (B_3: 30.2, 18.1, 10.8, 5.5, and 10.2% of CP; C: 9.4, 7.5, 7.6, 8.8, and 7.9% of CP for 0, 1, 2, 4, and 20h, respectively). The protein digestibility of the steam hydrolyzed hard tissue fraction from the mechanical deboning of spent hens was found to be comparable to the digestibility to feather meal, but post-hydrolysis keratinase treatment did not improve feeding value for ruminants.

Keywords: hard tissue, keratinase, mechanical deboning, spent hens, steam hydrolysis

INTRODUCTION

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

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

MATERIALS AND METHODS

Evaluation of Available Technologies

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

the feather yield was determined by subtraction of group picked weight from group initial weight.

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

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

In preparation for freeze drying for laboratory analysis, all samples were thawed and similar samples were pooled. The particle size of the hard tissue samples was reduced to facilitate freeze drying and analysis by alternately processing in a food cutter (Model #FC19, Blakeslee and Co., Cicero, IL) and grinding with a hand-crank meat grinder (Back to Basics Products, Inc., Draper, UT) using initially a 10 mm die screen and subsequently a 4 mm die screen. Because their initial particle size was smaller, the soft tissues were prepared for freeze drying by grinding with the hand grinder through

the 4 mm screen. All sample types were subdivided into tared, zipper-locking plastic bags, refrozen, weighed, freeze dried, and then re-weighed for calculation of DM percentage. Following freeze-drying, the samples were ground in a food processor (Little Oskar, Sunbeam Appliance Co., Boca Raton, FL) for subsequent laboratory analysis.

Evaluation of the Addition of Enzymatic Hydrolysis to the Rendering Process

An additional 1,000 spent hens from the same flock were euthanized by cervical dislocation and fractionated through the deboning device. The hard tissues were frozen at -23^{o}C prior to further processing. The soft tissue fraction was directed to another project for further study.

To create material with sufficient moisture for traditional hydrolysis, 29.2 kg of thawed hard tissues were mixed with 16.3 kg water to create a 30% DM product. A subsample of this material was frozen for later analysis (designated **E -1**). The remaining water and hard tissue mixture was hydrolyzed as the first replication of the hydrolysis process (**rep 1**) using a pilot-sized (133.1 L capacity) hydrolyser (Anco-Eaglin, Inc., Greensboro, NC) by holding the pressure in the unit at 10.8 torr for 45 min to approximate the cooking conditions in commercial rendering facilities. The product was agitated constantly during cooking. A maximum cooking temperature of 124^{o} C was achieved and maintained for the last 10 min of cooking time.

After the hydrolyzation process was completed and atmospheric pressure was reestablished inside the hydrolyzer, the product was placed into an improvised thermal

container (plastic drum wrapped in six layers of 2.54 cm diam. bubble-wrap) to reduce the rate of cooling. Sub-samples of the hydrolyzed material were frozen at -23°C for analysis (0 h enzyme hydrolysis, **E 0**). Keratinase enzyme (300,000 U/g activity) produced from *Bacillus licheniformis* PWD-1 (12.5% wt/vol stock solution) was added to the hydrolyzed material to give a final enzyme concentration of 1%. Enzyme addition occurred after the temperature of the hydrolyzed material had cooled to 63° C. The material was sampled at multiple times during the enzymatic digestion process (1 h, 2 h, 4 h, and 20 h, designated **E 1**, **E 2**, **E 4**, and **E 20**) and samples frozen and stored at -23°C.

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

Laboratory Analysis of Raw Hen Fractions

Dry matter, Kjeldahl nitrogen, NDF, ADF, and ash were determined according to AOAC procedures (1995). Protein fractions were determined according to the procedure of Licitra et al. (1996). Trichloroacetic acid was used to determine non-protein N in this

process. The samples were subjected to ether extraction for fat determination (Labconco extraction apparatus, Labconco Corp., Kansas City, MO). The amino acid content of the fractions was determined by AOAC procedures (AOAC, 2000) at the laboratory of Novus International (St. Charles, MO).

Laboratory Analysis of Hydrolyzed Hard Tissues

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

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

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

RESULTS

Evaluation of Available Technologies

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

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

Table 3 reports the amino acid content of the various fractions as a percent of CP. Lanthionine is included as an indicator of possible damage to the sulfur-containing amino acids as a result of processing.

Evaluation of the Addition of Enzymatic Hydrolysis to the Rendering Process as Applied to Hard Tissues

Hydrolysis replicate had an impact on DM content but not on CP content of the hydrolyzed hen tissues (Table 4). The LS mean DM for reps 1 and 2 were 34.0% and

39.9%, respectively (SE = 0.008, $P < 0.01$). The CP content of the samples averaged 53.8% and did not differ between reps 1 and 2 ($P = 0.17$). Hydrolysis time had linear, quadratic, and cubic effects on both DM and CP contents of the material ($P < 0.01$).

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

When enzymatic hydrolysis results from both sessions were combined, the linear and cubic relationships between time and pepsin digestibility were significant ($P < 0.01$ for linear and cubic relationships; $P = 0.20$ for quadratic relationship).

The results of partitioning the enzymatically hydrolyzed hen hard tissue from the second replicate into protein fractions are given in Table 5. Equivalent results for the unhydrolyzed material (t = -1 h) are included, but these data were not included in our statistical model. No statistical comparison between reps was possible because inadequate material from rep 1 remained after PEPD analysis to complete protein fraction analysis so protein fraction determinations were only made on material from rep 2. Enzymatic hydrolysis seemed to increase the solubility of the protein in the material in Rep 2. Linear increases ($P = 0.01$) in the A (non-protein N) fraction were observed. The highly soluble, B_1 fraction was increased by a cubic function ($P < 0.01$), peaking at 1h of hydrolysis. The moderately soluble B_2 fraction increased by a quadratic function

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

Enzymatic hydrolysis time had no impact on the TAAD of individual amino acids ($P > 0.10$) so only data from amino acids critical to ruminant diets are presented (Table 6). Average true amino acid digestibility before keratinase addition was 79.3% (SE = 1.9). The relationship between hydrolysis time and average TAAD is illustrated in Figure 2.

DISCUSSION

Evaluation of Available Technologies

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

Approximately two thirds of bird mass (Table 2) was partitioned into the MDM fraction with the remaining material being separated out as the hard tissue fraction. Based on observation of the hard tissue fraction, it contained bones, connective tissue,

feet, the exterior of the eyes, combs, and feathers (if they were present on the carcasses). It also contained a small amount of residual soft tissue. The soft tissues were pulverized during the deboning process and could not be distinguished, but the MDM fraction would logically contain muscles, fat, nervous tissue, and the viscera with its contents. Either fraction could be further processed to yield useful feed ingredients. The more digestible soft tissues would likely be directed to monogastric diets and the less digestible hard tissues to ruminant diets.

The nutrient composition of the WG hen (Table 2) was similar to that seen in other trials (Haque et al., 1991; Lyons and Vandepopuliere, 1996; and Kim and Patterson, 2000). The WH and WS materials from our trial are similar to the mechanically deboned hen fractions described by Lyons and Vandepopuliere (1997). Nutrient analysis of all the fractions (Table 2) confirms that each of these fractions has potential value of as a source of both protein and energy.

The protein fractions in the hen products (described as A, B_1, B_2, B_3, and C by VanSoest, 1994), are determined by chemical solubility and indicate how the protein might be utilized if incorporated into a ruminant ration. The A and all B fractions are largely available for use by ruminants and only the C fraction is indigestible. Results from the unhydrolyzed samples (Table 2) indicate that the products that do not contain feathers would be readily digested by ruminant animals, even in the absence of further processing, as evidenced by the low content of C protein. The elevated C fraction values for the feathers and for the bird products containing feathers indicate that, as expected,

the feathers cause a large reduction in feeding value and would require more aggressive further processing to enhance digestibility.

These tests were run on the raw material and for any of these bird fractions to be used as feed ingredients, further processing would be a necessity to insure feed safety. The processing involved would likely improve the availability of the protein in all the fractions, but particularly in those containing feathers.

The amino acid content of WG hens presented (Table 3) is similar to those presented by Douglas et al. (1997) and Kersey et al. (1997) for rendered spent hen meals. The amino acid composition we found in feathers was similar to that reported by Han and Parsons (1991) for seven batches of feather meal and those for WH and WS fractions are in agreement with data in Lyons and Vandepopuliere (1997). No data regarding the other hen fractions was available for comparison. Amino acid compositions should likely be considered in conjunction with the 0.02% pepsin digestibility and the true amino acid digestibility data illustrated in Fig. 1 and 2 since it is ultimately the availability of the amino acids that determines the feeding value of a protein source.

Lysine and methionine are often the first and second limiting amino acids in traditional corn and soybean meal-based animal diets (NRC, 1994; NRC, 1998; and NRC 2001, respectively). The hen soft tissue (Table 3) would potentially be a better source of lysine than soybean meal, which is listed as having a lysine content of 6.6% of CP (NRC, 1998). Lysine in the hard tissue had a true amino acid digestibility of 72.6%

(Table 6) after traditional hydrolysis and it would therefore likely be at least as available in the soft tissue fraction. With the absence of feathers, any of the hen products would supply more methionine than soybean meal (1.2% of CP as methionine). Feathers are high in cysteine, however, and so their inclusion raises the cysteine proportion (Table 3). Since cysteine can spare methionine, the inclusion of the feathers as a cysteine source might balance their diluting effect on methionine content. The true amino acid digestibility of methionine in the hydrolyzed hard tissue was 81.3% (Table 6) and that of cysteine was 72.0%, suggesting that these amino acids would also be fairly well utilized from any rendered hen products.

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

Evaluation of the Addition of Enzymatic Hydrolysis to the Rendering Process as Applied to Hard Tissues

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

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

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

The changes seen in the protein fractions of the cooked hard tissue as a result of enzymatic hydrolysis (Table 5) suggested that the enzyme was indeed acting on the

proteins. The enzyme appeared to be hydrolyzing less soluble protein (B_3) into more soluble amino acids, peptides, and proteins (A, B_1, and B_2). The cubic effect of hydrolysis time on the B_1 fraction suggested that it is initially increased by the attack of keratinase on the less soluble B_2 and B_3 protein fractions but is then reduced by its degradation to the even more soluble A fraction as the keratinase continued to work. No solid explanation for the cubic effect of time on the C protein fraction was apparent. The acid detergent insoluble residue was minute for the hard tissues and it was possible that analytical errors contributed to the observed effect.

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

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

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Table 1. Proportions of fractions produced by deboning spent hens with (whole) and without feathers (picked)

Fraction	Weight (kg)	% of recovered material
Whole, Hard[1]	13.56	33.2
Whole, Soft[1]	27.30	66.8
Picked, Hard	13.29	31.8
Picked, Soft	28.45	68.2

[1] Hard = hard tissue: bones, feathers, connective tissue, etc.; Soft = soft tissue: mechanically deboned meat

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

Component	WG[1]	WH[1]	WS[1]	PG[1]	PH[1]	PS[1]	Feathers
DM (%)	41.8	46.8	40.9	39.3	42.2	40.7	94.7
OM (% of DM)	89.2	83.2	96.1	90.3	80.1	96.8	98.0
CP (% of DM)	42.8	58.0	37.1	42.6	52.3	35.3	91.9
EE (% of DM)	45.8	24.1	57.0	48.5	26.1	58.3	5.2
NDF (% of DM)	45.6	53.3	47.4	53.4	50.2	48.8	82.6
ADF (% of DM)	23.8	3.4	4.3	18.8	15.0	4.5	9.9
Ash (% of DM)	10.8	16.8	3.9	9.7	19.9	3.2	2.0
Protein fractions[2]	% of CP						
A	14.5	7.2	11.9	17.4	8.0	11.3	1.5
B_1	12.4	2.1	8.4	3.3	2.9	9.6	1.3
B_2	42.3	56.7	53.1	40.8	56.6	51.3	10.4
B_3	5.4	17.8	20.2	36.4	24.7	26.3	17.4
C	25.5	16.2	6.5	2.1	7.8	1.4	69.3

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

[2] A: non-protein N; B_1: true protein soluble in borate-phosphate buffer; B_2: protein soluble in neutral detergent; B_3: protein soluble in acid detergent, but insoluble in neutral detergent; C: protein insoluble in acid detergent

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

Content as % of CP	WG[1]	WH[1]	WS[1]	PG[1]	PH[1]	PS[1]	Feathers
Arginine[2]	6.38	6.53	6.07	6.99	6.61	6.44	6.92
Histidine[2]	2.18	1.35	2.98	3.14	1.61	3.32	0.51
Isoleucine[2]	3.95	3.48	4.69	4.88	3.26	5.02	4.53
Leucine[2]	6.78	6.38	7.89	8.46	6.03	8.46	9.01
Lycine[2]	5.41	3.94	7.33	8.29	4.83	8.24	1.20
Methionine[2]	1.86	1.37	2.44	2.74	1.59	2.80	0.63
Phenylalanine[2]	4.71	3.53	3.99	4.31	3.35	4.25	4.94
Threonine[2]	3.42	3.15	3.71	3.96	3.12	4.06	4.52
Tryptophan[2]	0.81	0.51	0.93	0.95	0.56	1.16	0.29
Valine[2]	5.14	5.13	5.45	5.61	4.36	5.86	7.48
Alanine[3]	5.73	5.86	5.53	6.51	6.23	5.86	4.71
Aspartate[3]	7.75	6.80	8.51	9.73	6.84	8.51	6.27
Cystine[3]	1.56	2.64	1.49	1.41	1.45	1.54	6.95
Glutamate[3]	12.46	11.47	12.87	16.02	10.93	13.84	10.43
Glycine[3]	8.02	10.42	5.25	6.80	10.62	4.96	8.53
Proline[3]	6.27	7.71	4.38	5.26	6.69	4.22	9.92
Serine[3]	3.69	4.42	2.89	3.06	3.57	3.12	11.78
Taurine[3]	0.46	0.27	0.59	0.62	0.37	0.58	0.01
Tyrosine[3]	2.26	2.07	3.09	2.95	2.15	3.12	2.49
Lanthionine[4]	0	0.03	0.79	0	0.17	0.80	0.03

[1] WG = whole hen, ground; WH = whole hen, hard tissues; WS = whole hen, soft tissues; PG = mechanically picked hen, ground; PH = mechanically picked hen, hard tissues; PS = mechanically picked hen, soft tissues
[2] Amino acid considered essential to ruminants
[3] Amino acid considered non-essential to ruminants
[4] Indicator of damage to protein during processing

Table 4. DM and CP contents of raw hen samples and with traditional and enzymatic hydrolysis (means, *LS means)*

Enzymatic hydrolysis time (h)	-1	0	1	2	4	20	SE	L[1]	Q[1]	C[1]
Replicate 1 % DM[2]	30.1	42.2	32.4	33.0	32.4	33.7				
Replicate 2 % DM[2]	ND[3]	39.0	39.0	40.0	40.5	40.7				
Overall % DM[4]	*NE*[5]	*40.6*	*35.7*	*36.5*	*36.5*	*37.2*	*0.001*	*<0.01*	*<0.01*	*<0.01*
Replicate 1 % CP[2]	54.7	48.6	54.7	55.2	56.6	55.0				
Replicate 2 % CP[2]	ND[3]	54.4	54.6	53.4	52.5	53.5				
Overall % CP[4]	*NE*[5]	*51.5*	*54.6*	*54.3*	*54.5*	*54.3*	*0.24*	*<0.01*	*<0.01*	*<0.01*

[1] L, Q, C: *P* values for orthogonal contrasts of hydrolysis time and % DM or CP for linear, quadratic, and cubic relations
[2] Arithmatic mean
[3] Not determined: no sample taken
[4] Least square mean
[5] Not estimable due to missing data point

Table 5. Protein fractions of hen hard tissue hydrolyzed with and without keratinase

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

[1] L, Q, C: P-values for linear, quadratic, and cubic relationships between hydrolysis time and protein fractions, respectively
[2] A: non-protein N; B_1: true protein soluble in borate-phosphate buffer; B_2: protein soluble in neutral detergent; B_3: protein soluble in acid detergent, but insoluble in neutral detergent; C: protein insoluble in acid detergent

Table 6. True digestibility (%) of key amino acids for ruminant diets in hydrolyzed hard tissue from spent laying hens after addition of keratinase enzyme

Enzyme hydrolysis time	0h	1h	2h	4h	20h	SEM	*P* for time effect
Lysine	72.6	73.7	71.6	74.2	75.0	2.2	0.82
Methionine	81.3	82.9	79.9	82.6	83.6	1.4	0.44
Histidine	73.5	73.4	71.6	72.4	75.8	2.6	0.71
Tryptophan	99.3	94.6	96.1	96.6	96.8	2.3	0.69
Threonine	79.5	77.1	71.8	74.7	79.3	2.1	0.14
Cysteine	72.0	70.0	63.8	67.5	74.1	3.3	0.29

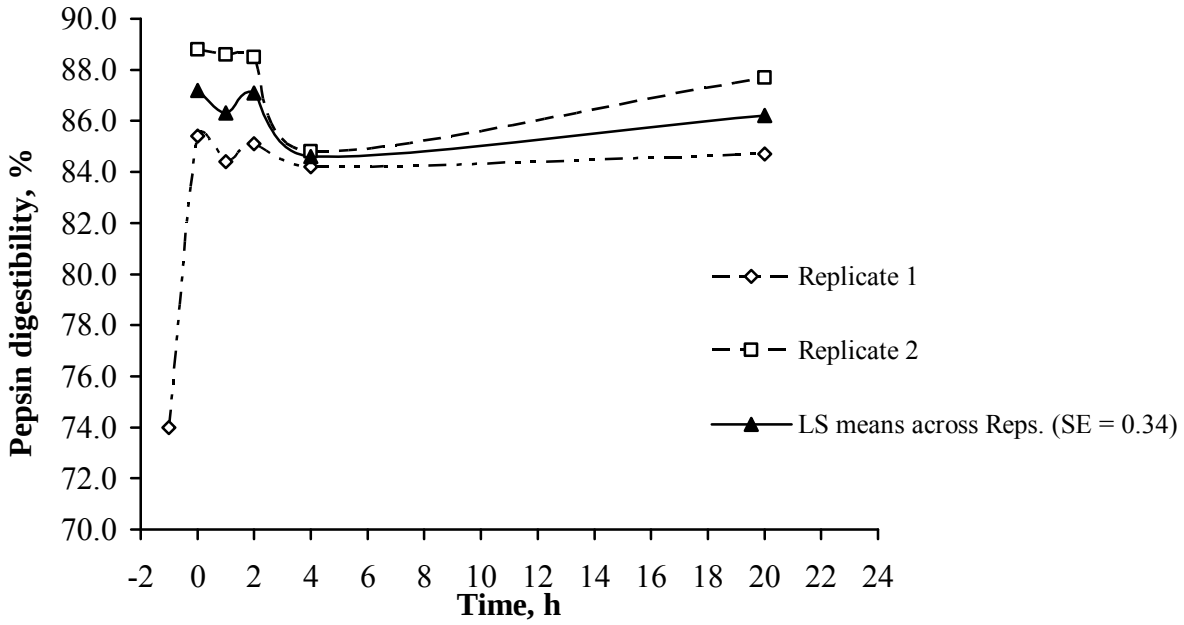

Figure 1. Relationship between hydrolysis time and 0.02% pepsin digestibility of hen hard tissues

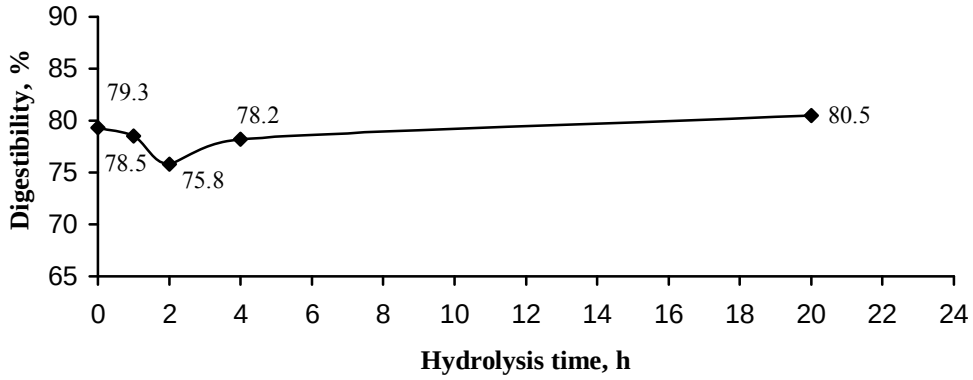

Figure 2. True amino acid digestibility of hen hard tissue as a function of enzymatic hydrolysis time (SE=1.9)

Alternative methods for disposal of spent laying hens: Determination of nitrogen balance in goats fed a meal produced from hydrolyzed spent hen hard tissue

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ABSTRACT

In order to provide an economically viable and environmentally sound method for disposing of spent laying hens, we manufactured a proteinaceous meal from the hard tissue fraction of mechanically deboned laying hens (primarily feathers, bones, and connective tissue). We hydrolyzed the hard tissue, co-extruded it with soybean hulls, and created a novel feather-bone meal (**FBM**) containing 94.2% DM, 23.1% CP, 54.5% NDF, and 7.3% fat. We evaluated the FBM in diets for meat goats in which it provided 0, 20, 40, or 60% of the N added to the diets *versus* a negative control concentrate with no added N source. The remainder of the N was contributed by soybean meal (**SBM**). Supplementation of N resulted in greater DMI than the negative control ($P = 0.005$) and DMI increased by a quadratic function ($P = 0.11$) as FBM increased in the concentrate. Digestibility of DM was similar in all diets, including the negative control ($P > 0.10$). Fiber digestibility increased linearly as dietary inclusion of FBM increased ($P = 0.04$ for NDF and 0.05 for ADF), probably as a result of the soybean hulls in the FBM. Nitrogen digestibility declined linearly as FBM supplementation increased ($P = 0.07$), but N retention increased by a quadratic function as FBM replaced SBM ($P = 0.06$). Negative control goats had lower N digestibility ($P < 0.001$) and N retention ($P = 0.008$) than the N-supplemented goats. Feather-bone meal had a greater proportion of ruminally undegradable B_3 protein than SBM (23.1 vs 0.3% of CP, respectively). Ruminal VFA and pH were unaffected by replacing SBM with FBM, but supplying no source of N in the concentrate resulted in reduced total

VFA in ruminal fluid (P = 0.04). Ruminal ammonia level increased quadratically (P = 0.07) as FBM increased, reflecting increased intake, and it was much lower in unsupplemented goats (P < 0.001). Blood urea N had less variation between 0 and 4h after feeding in goats receiving 40 or 60% of added N as FBM in comparison to those receiving only SBM or 20% FBM. Evidently, FBM promotes a more stable rumen environment, possibly due to reduced rumen degradability. A palatable by-product meal for ruminants can be made from spent laying hen hard tissue, one that supports similar N metabolism to traditional protein sources.

Key Words: Meat goats, nitrogen balance, protein byproduct, spent laying hens,

INTRODUCTION

As in any industry, table egg producers must balance input with output to maintain optimum profitability. When the economic value of egg yield in egg laying operations no longer exceeds the input costs, producers terminate the flock of hens and the "spent" hens are disposed of. Over 144 million live, spent layers are removed annually from production (C.A.S.T., 1995; Lyons and Vandepopuliere, 1996), but the economic value of the low white meat yield (166 g per bird valued at $0.69), barely justifies the cost of transport and processing ($0.68 per bird; Middleton, 2000). Because the cost of removing spent laying hens from the farm often exceeds their value for meat (Aho, 1999), finding methods of disposal that minimizes nutrient and biohazard emissions into the environment while yielding residual value to the poultry producer is a great challenge.

Conversion of food production waste materials into value-added feedstuffs is often an economically and environmentally feasible means of solving disposal problems. Novel application of existing processing methods to spent hens may help improve the sustainability and competitiveness of laying hen operations. The processing technologies of mechanical deboning, hydrolysis, and extrusion are potential options for processing whole hens. Mechanical deboning would result in the production of two fractions: mechanically deboned meat and hard tissue residues composed mainly of bones, feathers, and connective tissue. The mechanically deboned meat can be further processed into a low-ash protein by-product meal for monogastric diets through co-extrusion or traditional rendering, while the hard tissue fraction could be processed by steam hydrolysis and extrusion into a proteinaceous meal for ruminants. The objectives of our research were to: 1) evaluate the efficacy of processing hen hard tissue by hydrolysis and co-extrusion with soybean hulls; and, 2) determine the acceptability and feeding value of this meal in diets for growing meat goats.

MATERIALS AND METHODS

The experimental procedures used on both hens and goats were approved by the Institutional Animal Care and Use Committee at North Carolina State University (**NCSU**, Raleigh, NC).

Production of Feather-Bone Meal

A group of 1000 Leghorn-type hens were transported to the Animal and Poultry Waste Management Center at NCSU (Raleigh, NC) and euthanized by cervical dislocation. The

hen carcasses were then fractionated using a commercial deboning device (Beehive Model RSTD06, Sandy, UT). The mechanically deboned meat was directed to a separate project. The hard tissue was frozen and stored at -23°C for further processing at a later date.

Prior to hydrolysis, 29.2 kg of thawed hard tissue were mixed with 16.3 kg water to yield a product with approximately 70% moisture. This product was heat-processed using a pilot-sized hydrolyzer (133.1 L capacity, Anco-Eaglin, Inc., Greensboro, NC) at 121°C and 10.8 torr for 45 min., followed by drying in the hydrolyzer for 25 min. to yield 30 kg of material at 46% DM. The hydrolyzed material was blended to uniformity with 57.7 kg soybean hulls using a variable speed, double ribbon mixer (NCSU Dept. of Agricultural Engineering, Raleigh, NC) resulting in an interim product blend containing 28.2% DM.

The interim product blend containing soybean hulls and hydrolyzed hard tissue was further processed through an Insta-Pro dry extruder (Model 2000R, Des Moines, IA) with single flight augers, a #2000-6 steam lock, and a 0.95 cm nose cone. Upon exiting the extruder, the material, now termed feather-bone meal (**FBM**), was 113°C and contained 80.8% DM. The FBM was air dried and allowed to cool over night under ambient conditions. Subsequent drying in a forced-air oven at 55°C for 48h yielded 56.0 kg of FBM containing 92.6% DM. It was stored in plastic containers at -15°C until its evaluation as a feed ingredient for goats.

Goat N-balance Trial

Experimental diets. Corn and SBM-based experimental concentrates were formulated according to NRC (1981) to achieve 100g/d gain when fed as 50% of the total diet. The

remainder of the diet was provided as Eastern Gamagrass hay (*Tripsacum dactyloides*) harvested in a single batch from a field at NCSU (90.3% DM, 96.6% OM, 9.4% CP, 77.8% NDF, and 39.9% ADF). A negative control diet was formulated with no added source of N (**0AN,** Table 1) along with a positive control (**0FBM**) containing its entire quantity of added N in the form of soybean meal (**SBM**). Three additional diets contained FBM as a replacement for a 20, 40 or 60% of added N (**20FBM, 40FBM,** and **60FBM,** respectively). When hay and concentrates were fed to the goats, the total diets contained 9.2, 13.0, 12.5, 12.7, and 12.7% CP (0AN, 0FBM, 20FBM, 40FBM, and 60FBM, respectively). As formulated, the 0AN, negative control diet contained less CP than the N-supplemented diets.

Treatment of animals. Thirty weanling Boer (≥ 75%) x Spanish cross wether goats were selected from the NCSU herd and moved to the NCSU Metabolism Educational Unit (Raleigh, NC) for adaptation to the experimental diets. They were initially group-penned and offered 1.5% group BW as commercial pellets and 2% group BW as Gamagrass hay. Free-choice access to water and limited pasture was available.

Once the goats had acclimated, dietary adaptation to liquid molasses began; the content increasing 1%wt/wt every other day until it reached 3% wt/wt with the pellets and this level continued throughout the trial. Dietary adaptation continued by blending 60FBM pellets with the commercial pellets (25% with 75% by weight, respectively). The proportion of 60FBM pellets increased by 25% every fourth day until the pellets offered were 100% 60 FBM (22d of adaptation). The pellets were well accepted by the goats, with the exception of 1goat that refused to eat pellets having a molasses coating. Once adapted to the 60FBM

pellets, 25 goats were selected for a N-balance trial (BW = 22.8 ± 2.0 kg). The goats were stratified by weight, blocked in groups of five, and placed into metabolism crates(0.5 m x 1.1m) with *ad libitum* access to water bowls. Subsequent treatment for internal parasites (2 mL/10 kg BW Cydectin, 5 mg moxidectin/mL, Fort Dodge Animal Health, Fort Dodge, IA and 1.25mL Corid, 9.6% amprolium, per L drinking water, Merial, Ltd., Duluth, GA) reduced fecal egg counts to < 450 eggs/g feces. Application of Co-Ral dust (1% coumaphos, Agri-labs, St. Joseph, MO) eliminated lice.

Feeding, N-balance sampling. Upon placement into metabolism crates, the goats were initially offered an amount equal to 3.0% of their individual BW of a diet consisting of 50% Gamagrass hay and 50% 60FBM concentrate. They were switched to their randomly assigned treatments over a 4-d period. One wether within each weight block received each dietary treatment. Once the animals were receiving 100% of their assigned treatments, total feed was offered at a rate to obtain 15% total feed refusals. Concentrate was offered 30 min. before hay to allow its consumption before hay addition to reduce wastage. By the end of a 12-day *ad libitum* feeding period, intake levels had reached a plateau for the majority of the wethers and from this point on, the goats were fed a constant amount of feed equal to 115% of the average intake for the last 5 d of the 12-day *ad libitum* period.

Fecal collection bags were placed on the goats at the initiation of the constant feeding period. After a 3-day adaptation to the collection bags, total feces and urine were collected for 5 d. Fecal collection bags were emptied twice daily and the contents weighed once daily. The total feces were sub-sampled at a percentage set for individual goats to obtain

approximately 100g of DM from each goat over the 5-day collection. The fecal samples were composited daily into containers in a forced air oven at set 55°C. Following the final addition, the feces were allowed to dry for an additional 48 h after which they were air equilibrated for 48 h and then weighed for air-equilibrated DM determination. Dry samples were placed in sealed plastic bags and stored for later laboratory analysis.

To maintain pH 3 to 5 (determined with pHydrion 1 to 12 paper, Micro Essential Lab, Brooklyn, NY), urine was acidified by the addition of 10 to 30 mL 6N HCl to the collection vessels at the start of each 24-h collection period. Deionized water (100 mL per vessel per d) was added in addition to minimize the crystallization of urinary compounds which are sensitive to low pH. Total urine was removed from the collection vessels daily, weight and volume determined, and a sub-sample obtained by weight. The percentage of the total urine saved varied by animal, was established on d 1 of the collection period, was set to yield 50 g of urine, and remained constant for the 5-d collection. Urine samples were composited, frozen, and stored at -10° C for later laboratory analysis.

In addition to fecal and urine samples, feed samples (100 g of each feed/d) were collected and feed refusals were quantitatively saved and composited. Sub-samples of the feed refusals were taken at the end of the 5-d period. All feed and feed refusal samples were placed into a forced air oven at 55°C, dried for 48 h, air equilibrated for 48 h, and weighed for air equilibrated DM determination.

Ruminal fluid and blood sampling and analysis. Following the total excreta collection, the goats were put onto a staggered feeding regime in which the individual weight blocks

were stagger-fed at 15 min intervals. On the second day of the staggered feeding regime, blood samples were collected into untreated 10 mL Vacutainer tubes (Becton Dickinson and Co., Franklin Lakes, NJ) with 20 ga. x 2.5 cm needles *via* jugular venipuncture before feeding (time = 0 h) and then 2, 4, and 8 h after feeding. All blood samples were placed on ice during the collection period and subsequently allowed to clot at room temperature for 30 min prior to centrifugation at 1470 x *g* for 30 min. The serum obtained was stored at -10^{o}C until analyzed.

Ruminal fluid was obtained by rumenocentesis 2 h after feeding as described by Nordlund and Garret (1994) and modified for goats using 14 ga. x 5 cm needles with no local anesthesia. A minimum of 5 mL of fluid was withdrawn from each goat. Ruminal fluid pH was determined immediately upon sample removal using a Fisher Scientific Accumet AP63 portable pH meter (Cole-Parmer Instruments, Vernon Hills, IL), after which samples were placed on ice. Ruminal fluid was stored at -10^{o} C until analyzed.

Laboratory analysis. Feed, feed refusal, and dry fecal samples were ground in a Model 4 Wiley Mill (Arthur A Thomas Co, Philadelphia, PA) to pass through a 1 mm screen. Dry matter (105^{o} C), ash, and Kjeldahl N were determined according to AOAC procedures (1995). Concentration of NDF and ADF were determined with α-amylase as described by Van Soest et al. (1991) modified for use with an Ankom apparatus (Ankom Technology, Macedon, NY). Protein fractions were determined on the concentrate pellets by procedures recommended by Licitra et al. (1996). Non-protein N was determined by the subtraction of N in the precipitate resulting from treatment of samples with tricarboxylic acid from total N.

Soluble N was calculated by subtracting the N in the residue remaining after treatment with a borate-phosphate buffer from total N. Protein insoluble in neutral and acid detergents was determined by subjecting samples to neutral or acid detergent digestion prior to N analysis. Lipid levels were determined by ether extraction with anhydrous diethyl ether (Soxtec HT6, Foss Tecator AB, Höganäs, Sweden) at Dairy One Laboratories (Ithaca, NY).

Urea N in blood (**BUN**) and urine (**UUN**) was determined colorimetrically by an automated, diacetyl-monoxime method (Marsh et al., 1965). Total urinary Kjeldahl N was determined as per AOAC (1995).

VFA concentrations were determined in ruminal fluid using a Varion 3800 gas chromatograph (Varian Chromatography Systems, Walnut Creek, CA) using a Nikol fused silica capillary column (15 m; 0.53 mm i.d.; 0.5 μm film thickness; Supelco, Bellefonte, PA). Ruminal ammonia was determined by the colorimetric procedure used for Kjeldahl N (AOAC, 1995).

Statistical analysis. Statistical analysis of goat data was by PROC GLM of SAS (SAS Institute, Cary, NC). The model for all parameters except BUN included class variables of treatment, weight block, and crate type (wood or metal). No significant differences ($P > 0.10$) were detected for crate type, so it was removed from the model. Weight block had an impact only on DMI, but this was expected since the animals were blocked by weight. When intake was expressed as a percentage of body weight, the block effect was eliminated. Weight block remained in our model; however, only treatment differences are presented. Orthogonal polynomial contrasts between 0AN and N-supplemented diets and among N-

supplemented diets were conducted. There were no significant cubic effects so these *P*-values are not shown. For BUN data, the independent variables were goat within treatment, treatment, time, and time by treatment interaction. The goat within treatment error term was used to test for treatment differences across sampling times.

RESULTS

Production of Feather-Bone Meal

The FBM utilized in the goat trial was golden colored, with the slight odor of cooked poultry. Texture was similar to soybean hulls and suggested no palatability problems would be encountered when incorporating FBM into livestock feed. Selected proximate analysis parameters of the FBM, the SBM, and the 5 experimental concentrates are listed in Table 2. The FBM was higher in NPN (A fraction, rapidly degraded in the rumen) than SBM and lower in buffer soluble, true protein (B_1 fraction, rapidly degraded in the rumen). Soybean meal was much higher in neutral detergent soluble protein (B_2 fraction, moderately degraded in the rumen) than FBM, but FBM had more neutral detergent insoluble protein (B_3 fraction, slowly degraded in the rumen) than SBM. The FBM was also higher in the acid detergent insoluble and largely indigestible C fraction than the SBM.

Goat N-balance Trial

Dry matter intake was lower in the unsupplemented than in the supplemented goats ($P = 0.005$, Table 3). Dry matter intake tended to increase by a quadratic function ($P = 0.11$) as dietary FBM inclusion increased (Table 3); however, DM digestibility did not vary among treatment groups. The digestibilities of NDF and ADF were similar among the

supplemented and unsupplemented goats (Table 3). Both NDF and ADF digestibilities increased linearly ($P = 0.04$ and 0.05, respectively) as dietary FBM inclusion level increased.

As anticipated, N intake was lower among the negative control treatment group than the other groups ($P < 0.001$, Table 4). Within the supplemented treatment groups, N intake increased quadratically ($P < 0.01$). Fecal N (Table 4) followed a similar pattern, with fecal N excretion being lower in unsupplemented goats than in supplemented ones ($P < 0.001$) and fecal N increased linearly as dietary FBM inclusion increased ($P < 0.05$).

Intake and fecal excretion of N both contributed to the observed pattern of N absorption from the digestive tract. Absorption of N (g/d) increased by a quadratic function ($P < 0.01$) among supplemented goats. Nitrogen absorption in the unsupplemented goats was only about half that of supplemented goats ($P < 0.001$). Digestibility of N (Table 4) declined linearly as FBM increased in the feed ($P = 0.07$) and was lowest in goats that did not receive supplemental N in their feed ($P < 0.001$).

Absorbed dietary N which was not retained within tissues was likely excreted in the urine. Urinary N excretion rates (g/d, Table 4) were similar among supplemented goats ($P > 0.10$) and were lower in goats that did not receive supplemental N in their diets ($P < 0.001$). This led to quadratic increases in the quantities of N retention in the animals receiving supplemental N (Table 4, $P = 0.06$) and reduced N retention in unsupplemented goats as compared to those receiving added N in their feed ($P = 0.008$). Retained N as a percentage of absorbed N was similar for all treatment groups (Table 4, $P > 0.10$).

Urinary N present as urea (Table 4) was lower in the negative control goats than in supplemented goats ($P < 0.001$). It was similar in all the supplemented treatment groups ($P > 0.10$). A similar pattern emerged when urinary N present as urea was expressed as a percentage of total urinary N (**%UUN**), with unsupplemented goats having a lower %UUN than supplemented goats ($P < 0.001$) and supplemented goats having similar %UUN.

Few ruminal parameters were influenced significantly by treatment (Table 5). Ruminal NH_3 was lower in unsupplemented goats than in supplemented ($P < 0.001$). It responded with a quadratic increase ($P = 0.07$) to level of FBM in the diet with minimal level occurring in the range of 20% inclusion rate for FBM. Likewise, total VFA concentration was lower in goats receiving no supplemental N source than in goats receiving supplemental N ($P = 0.04$). It was similar among supplemented goats ($P > 0.10$). Molar proportions of VFA did not vary with treatment ($P > 0.10$) with the exception of valerate, which increased linearly as FBM increased in the feed ($P = 0.01$).

Treatment played a significant role in determining BUN even when variation within animal across time was taken into account ($P = 0.001$, Table 6). Blood urea N was lower in the goats that received no supplemental N across all sampling times than in goats on the N-supplemented diets. Time of sampling also affected BUN ($P = 0.001$, Table 6) and there was a time by treatment interaction ($P = 0.02$).

DISCUSSION

Production of Feather-Bone Meal

Both hydrolysis and extrusion are processes that involve heating ingredients with the goals of improving digestibility and reducing pathogen load (Tadtiyanant et al., 1993). Our hydrolysis conditions for the raw feather-bone material were similar to those used in commercial facilities for producing feather meal (Kevin Custer, American Proteins, Inc., Cumming, GA, personal communication) and our preliminary data suggested an improvement in 0.02% pepsin digestibility from 74% to 85% as a result of hydrolysis (S. Freeman, unpublished data). Extrusion equipment and conditions were similar to those employed by Tadtiyanant et al. (1993) and the FBM exited the extruder at 113^{o} C, exceeding the FDA guideline of 110^{o} C for safe production of rendered products (9CFR166.1).

Our final FBM product blended well within the concentrate mixtures, having similar flow qualities to soybean hulls. It contributed a lower percentage of CP than more traditional sources of protein, such as SBM (Table 2); however, it also contributed larger amounts of fat and fiber, potentially providing a better balance of protein and energy. Additionally, the protein within the FBM had a greater proportion of B_3 fraction than SBM. This protein would largely escape degradation within the rumen and be available for digestion in the lower gastrointestinal tract of ruminants, making FBM superior to SMB as a source of ruminal escape protein.

The negative values obtained for the B_1 fraction in the 0 AN pellets (Table 2) were not biologically possible. They were likely the result of small systematic and random errors in the analytical approach for determining protein fractions.

Goat N-balance Trial

All diets were well accepted by the goats as evidenced by similar DMI among goats receiving supplemental N. Evidently, FBM did not present palatability issues even when incorporated to provide 60% of added N. Intake as a percentage of BW in our trial was approximately 3.1%, slightly lower than that reported by Moore et al. (2002) for goats of similar size and genetic background fed a variety of plant byproduct feeds and housed in pens during fecal collections. Differences in hay quality and housing style likely influenced these differences. Dairy does fed feather meal (**FM**) to replace SBM in control diets had similar DMI to control does (Lu et al., 1990) and a mixture of FM with blood meal (**BM**) did not impact DMI of lactating does (Andrighetto and Bailoni, 1994). These results reinforce our conclusions that rendered poultry products are feasible feedstuffs for goats.

Dry matter digestibility was similar among treatments and slightly less than that reported by Andrighetto and Bailoni (1994) in does receiving a diet containing a mixture of FM and BM. Moore et al. (2002) reported similar DM digestibilities to ours in meat goats being offered hay and plant byproducts as supplement. Dietary differences (corn silage vs. grass hay) and the use of lactating dairy goats in place of meat goats could have contributed to the differences in DM digestibility.

An inadequate supply of ruminally degradable protein (**RDP**) is the most likely explanation for the reduced DMI observed among the negative control goats. Goats receiving 0 AN consumed 7.6 g N daily, above the NRC (1981) maintenance recommendation of 6.1 g/d; however, ruminal NH_3 in this same group of animals was 2.5 mg/dL (Table 5), below the levels of 5.0 to 23.5 mg/dL found in literature and suggested as minimal for optimum ruminal fermentation (Satter and Slyter, 1974; Mehrez et al., 1977; and Van Soest, 1994). Since apparent total tract fiber digestibility was not reduced in this treatment group (Table 4), sub-optimal NH_3 may have caused reduced fiber degradation rates in these goats, which in turn could have led to a reduced rumen passage rate and correspondingly reduced intake levels.

The trend for a quadratic increase in DMI (P = 0.11, Table 3) among the N-supplemented goats may have been the result of improvements in fiber digestion with these dietary treatments. Both NDF and ADF apparent digestibilities increased linearly as FBM increased in the diets. This was likely due to the increased quantities of soybean hulls contributed by the FBM. Soybean hulls have been reported to be highly digestible with *in vitro* DM disappearance of 94.6% (Moore et al., 2002) and they supported a DM digestibility similar to those obtained in our trial.

Nitrogen intake was dictated by DMI and CP content of the various concentrates so that the negative control goats ate less N than supplemented goats (Table 4). Quadratic increases were seen in N intake as FBM increased in the concentrates. The quadratic effect likely

resulted from the variation in CP content of the concentrates, which followed a similar pattern to the N intakes, with 20FBM being lower than the other diets. The 60FBM goats had the highest N intake as a result of their elevated DMI.

Fecal N excretion was lowest in the 0AN goats since they had lower N intakes than the goats receiving supplemented diets. Among goats receiving supplemental N, the linear increase in fecal N was probably the result of increasing C protein fraction in the feed combining with increasing DMI. The indigestible C protein was 4.5% of CP in the FBM as compared to 0.4% in SBM and therefore would increase in the diet as FBM levels were increased. The apparent amount of N absorbed from the gastrointestinal tracts of the supplemented goats increased with the same pattern as N intake, despite the increase in fecal losses as FBM increased in the diet. None the less, N digestibility declined as FBM increased in the concentrates along with the C protein fraction it contained. Fecal N was also reported to be increased in does receiving a FM and BM mix in their concentrate as compared to meat meal (Andrighetto and Bailoni, 1994). The difference was explained on the basis of protein degradability and supported our results.

The similarity in N excretion among goats receiving supplemental N indicated that dietary proteins that escaped ruminal degradation were digested and absorbed in the small intestine, making them bioavailable. Feather meal (Lu et al., 1989) and a mixture of FM and BM (Andrighetto and Bailoni, 1994), which also supply ruminally undegradable protein, successfully supported lactation in dairy goat does. These findings confirmed the theory that

the proteins which are not degraded in the rumen can be digested in the small intestine to support production functions.

The lack of difference in urinary N excretion was accompanied by a quadratic increase in N retention, which mirrored the intake patterns. The lower N quantity found in the urine of the unsupplemented goats was expected and reflected their lower intake levels. The value of 1.0 g/d N retention supported the notion that even the negative control 0 AN diet exceeded the goats' minimal requirement for N.

The percentage of absorbed N retained was similar across treatments, suggesting that the amino acids in the diets were being utilized with similar efficiencies and indicated that no one diet presented any advantage or disadvantage for N utilization. The retention percentages obtained in this trial were lower than those we obtained in a previous trial with a different N source (Freeman et al., 2008) but are within the range reported by Lindberg (1989) and Merkel et al. (2001).

The %UUN can be an indicator of the overall N status of an animal. Archibeque et al. (2001) and Huntington et al. (2001) stated that increasing dietary N above the amount required by an animal led to increases in %UUN. The lower N intakes of the unsupplemented goats were accompanied by lower %UUN (Table 4), which was in agreement with these statements. Likewise, similar N intakes among supplemented animals resulted in there being no differences in %UUN (Table 4). Since the unsupplemented goats also had lower ruminal NH_3 than those receiving supplemental N (Table 5), the reduced

%UUN found in the 0AN goats' urine suggested that they were recycling a greater proportion of N to the rumen instead of excreting it into the urine.

There were few treatment effects on the ruminal parameters we examined (Table 5). With reduced DMI and N intakes, the lower ruminal NH_3 in the negative control goats was anticipated. The lack of decrease in ruminal NH_3 among the FBM-supplemented goats suggested that the increasing DMI offset the reduced degradability of the protein in the concentrates containing FBM. Increased recycling of NH_3 to the rumen could have also been a contributing factor. Ammonia levels detected in our supplemented goats were similar in magnitude to those reported in other studies in which goats received sources of RDP (Andrighetto and Bailoni, 1994; Moore et al., 2002; Freeman et al., 2007). Researchers who fed diets low in RDP reported ruminal NH_3 concentrations that were intermediate to those we reported for supplemented and unsupplemented goats (Lu et al., 1990; Soto-Navarro et al., 2006).

Total VFA concentrations in our goats (Table 5) likely reflected the differences in DMI and fiber degradation supported by the diets, since the 0 AN goats had reduced levels of total VFA as compared to the goats receiving supplemental N, which in turn had similar VFA concentrations. Total VFA concentrations were somewhat higher in our goats as compared to those reported in other studies (Lu et al., 1990; Andrighetto and Bailoni, 1994; Soto-Navarro et al., 2006). Since all groups reported *ad libitum* intake, dietary ingredient

differences may also contribute to the differences in total VFA concentrations between experiments.

Among the individual VFA, only valerate responded to dietary inclusion level of FBM (Table 5). The linear increase we detected could have been the result of increased DMI and greater availability of precursors within the rumen.

Both dietary treatment and time of sampling affected BUN (Table 6). Ikuta et al. (2005) demonstrated that BUN levels reflect ruminal NH_3 levels in dairy cattle. These findings supported our data since the 0AN goats had the lowest ruminal NH_3 and the lowest BUN. The results of Freeman et al. (2007) in goats of similar size and breeding also demonstrated that BUN declined when ruminal NH_3 decreased.

All the goats, including those on the 0 AN diet, had non-significant increases in BUN at 2 h after feeding. Between 2 and 4 h after feeding, BUN dropped in all treatment groups, significantly so in the 0AN, 0FBM, and 20FBM goats. By 8 h after feeding, BUN in all the treatment groups had decreased significantly below pre-feeding (0 h) levels. The pattern of increase followed by a decrease like we observed was not unusual for nutrients in blood following a meal and it is likely the result of the rate of ruminal organic matter degradation exceeding the supply of ruminal N causing urea to be pulled from the blood to meet ruminal needs. The lower 0 and 2-h BUN levels seen in the 40- and 60FBM treatment groups as compared to 0FBM and 20FBM suggested that the proteins in FBM were either not being degraded or were being degraded more slowly than those in the SBM. This was in

agreement with Cozzi et al. (1995) who found reduced degradation percentages and rates when SBM was replaced with FM in diets for sheep. It was also interesting to note that the animals receiving the 40 and 60 FBM had more stable BUN levels through 4h after feeding than did the other treatment groups suggesting again a more gradual and sustained release of NH_3 into the ruminal environment.

No differences in BUN were found among the goats receiving diets containing supplemental N. Blood urea N in goats receiving the 0 AN diet remained lower than BUN in the supplemented groups. This finding supported our urinary N excretion and %UUN data and may have been the result of increased recycling of N to the rumen.

Our study demonstrated that whole, spent laying hens can successfully be processed into mechanically deboned meat and hard tissue. Soft tissues would likely be directed to monogastric feeds and the hard tissue can be further processed by conventional hydrolysis and co-extrusion with soybean hulls into a meal for use in ruminant diets. The meal successfully replaced soybean meal in concentrates for meat goat wethers, supplying up to 60% of added N. Because the protein in the meal was less degradable in the rumen than the protein in SBM, diets containing the FBM product maintained a more stable ruminal environment and may have provided more protein for digestion in the small intestine. The opportunity to utilize whole, spent hens to produce both monogastric and ruminant feedstuffs would aid the poultry industry by providing an avenue for hen disposal that would

potentially achieve both economic and environmental goals and which would provide other animal industries with useful alternative feedstuffs.

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Table 1. Formulation of concentrates containing feather-bone meal (FBM) for goat N-balance trial (%, DM basis)

Ingredient	0 AN[1]	0 FBM[1]	20 FBM[1]	40FBM[1]	60 FBM[1]
Corn	88.4	70.6	65.8	60.4	54.8
Soybean meal	0	17.7	14.8	11.6	8.1
FBM	0	0	8.7	18.3	28.6
Wheat flour	5.0	5.0	5.0	5.0	5.0
Dry molasses	2.0	2.0	2.0	2.0	2.0
Limestone	1.7	1.9	1.4	1.0	0.6
TM salt[2]	1.0	1.0	1.0	1.0	1.0
Dicalcium phosphate	0.9	0.5	0.4	0.2	0
Poultry oil	1.0	1.4	1.0	0.5	0

[1] 0 AN, 0 FBM, 20 FBM, 40 FBM, and 60 FBM = no added N source; 100% of added N from soybean meal; 20, 40, and 60% of added N from FBM, respectively

[2] Southern States, Inc., Richmond, VA. Contains 16.5% Ca; 7.0% P; 28.0% salt; 3.5% Mg; 2.0% S; 1.0% K; 70ppm I; 1050ppm Cu; 32ppm Co; 52ppm Se; 3200ppm Zn; 3000ppm Mn; 572,000IU vitamin A/kg; 44,000IU vitamin D/kg; and 440IU vitamin E/kg.

Table 2. Nutrient content of feather-bone meal (FBM), soybean meal (SBM), and concentrates offered to wether goats to determine N-balance (% of DM)

Component	FBM	SBM[1]	0AN[2]	0FBM[2]	20FBM[2]	40FBM[2]	60FBM[2]	Hay
DM (%)	94.2	89.5	85.1	84.7	84.7	85.7	86.6	90.3
OM	93.0	93.6	96.2	95.5	95.6	95.5	95.6	96.6
CP	23.1	53.8	8.9	16.5	15.5	16.0	15.9	7.8
NDF	54.5	9.8	8.8	9.8	12.7	16.6	21.4	77.8
ADF	34.7	6.2	6.3	6.6	7.5	8.5	10.2	39.9
Fat	7.3	1.1	4.0	3.4	2.4	3.0	3.4	
Calcium	2.0	0.4	1.0	0.9	0.9	0.9	1.0	0.2
Phosphorus	0.7	0.7	0.6	0.5	0.6	0.5	0.5	0.2
Magnesium	0.2	0.3	0.2	0.2	0.2	0.2	0.2	0.02
Protein Fractions	(% of CP)							
A	25.2	15.0	7.1	.2	4.8	5.2	9.1	13.3
B_1	4.7	4.7	-0.1	5.3	6.8	5.9	6.8	0.9
B_2	42.6	70.8	83.4	81.3	78.7	75.6	66.9	21.1
B_3	23.1	0.3	9.4	6.6	8.5	12.1	15.0	58.8
C	4.5	0.4	0.2	0.6	1.3	1.3	2.2	5.8

[1] From NRC (2001). Nutrient Requirements of Dairy Cattle,7th Ed.,National Academy Press, Washington, DC.
[2] 0AN, 0FBM, 20FBM, 40FBM, and 60FBM: no added N source, 100% of added N from SBM, 20, 40, and 60% of added N from FBM, respectively

Table 3 Dry matter intake and digestibility of DM, NDF, and ADF by goats fed diets containing feather-bone meal (FBM)

Item	0 AN[1]	0 FBM[1]	20 FBM[1]	40 FBM[1]	60 FBM[1]	SEM	- vs + [2]	L[2]	Q[2]
DMI (g/d)	608.0	702.7	709.8	672.6	779.3	29.7	0.005	0.17	0.11
DM digestibility (%)	69.2	69.3	68.0	68.0	68.9	0.75	0.40	0.77	0.14
NDF digestibility (%)	53.0	53.0	51.2	53.9	56.9	1.51	0.66	0.04	0.13
ADF digestibility (%)	51.4	53.4	51.8	54.1	57.6	1.63	0.14	0.05	0.14

[1] 0 AN, 0 FBM, 20 FBM, 40 FBM, and 60 FBM = no added N source; 100% of added N from soybean meal; 20, 40, and 60% of added N from FBM, respectively

[2] *P* values for contrasts: - vs + = 0 AN vs N-supplemented, L = linear among N-supplemented, Q = quadratic among N-supplemented

Table 4 Nitrogen balance in goats fed diets containing graded levels of feather-bone meal (FBM)

Item	0 AN[1]	0 FBM[1]	20 FBM[1]	40 FBM[1]	60 FBM[1]	SEM	-/+[2]	L[2]	Q[2]
N intake (g/d)	7.6	12.4	12.0	12.0	14.3	0.48	<0.001	0.02	0.01
Fecal N (g/d)	3.6	4.5	4.6	4.5	5.4	0.27	0.002	0.05	0.14
N absorbed (g/d)	4.0	7.9	7.5	7.5	8.9	0.30	<0.001	0.04	0.01
N digestibility (%)	47.0	60.5	57.0	57.5	55.6	1.63	<0.001	0.07	0.61
Urinary N (g/d)	3.0	5.7	6.1	5.4	6.4	0.26	<0.001	0.27	0.31
N retention (g/d)	1.0	2.2	1.4	2.1	2.5	0.31	0.008	0.21	0.06
N retained/N absorbed (%)	22.9	27.2	18.0	27.4	27.8	4.68	0.68	0.60	0.32
Urinary N as urea N (g/d)	2.0	4.7	5.1	4.5	5.5	0.31	<0.001	0.17	0.30
Urinary urea N/urinary N (%)	66.7	81.8	83.0	82.6	86.8	3.77	0.001	0.40	0.70

[1] 0 AN, 0 FBM, 20 FBM, 40 FBM, and 60 FBM = no added N source; 100% of added N from soybean meal; 20, 40, and 60% of added N from FBM, respectively

[2] *P* values for contrasts: - vs + = 0 AN vs N-supplemented, L = linear among N-supplemented, Q = quadratic among N-supplemented

Table 5. Ruminal parameters in goats receiving graded levels of feather-bone meal (FBM) in their diets

Item	0 AN[1]	0 FBM[1]	20 FBM[1]	40 FBM[1]	60 FBM[1]	SEM	-/+[2]	L[2]	Q[2]
pH	5.8	5.8	5.6	5.7	5.7	0.08	0.36	0.56	0.30
Ammonia (mg/dL)	5.1	27.8	24.5	24.6	31.6	2.67	<0.001	0.34	0.07
Total VFA (mM)	88.1	98.9	106.2	94.8	100.8	4.81	0.04	0.79	0.89
VFA proportions				%					
Acetate	63.0	62.2	61.9	63.0	63.2	1.46	0.81	0.53	0.87
Propionate	20.4	21.7	19.3	21.5	20.1	1.79	0.89	0.74	0.79
Isobutyrate	0.5	0.5	0.4	0.5	0.4	0.03	0.51	0.17	0.65
Butyrate	14.1	13.6	15.7	13.9	14.5	0.84	0.75	0.86	0.40
Isovalerate	1.0	1.0	0.9	0.8	0.7	0.17	0.34	0.18	0.99
Valerate	1.0	1.0	1.1	1.1	1.3	0.07	0.41	0.01	0.92
Acetate: propionate ratio	3.25	2.88	3.46	2.96	3.25	0.36	0.80	0.70	0.69

[1] 0 AN, 0 FBM, 20 FBM, 40 FBM, and 60 FBM = no added N source; 100% of added N from soybean meal; 20, 40, and 60% of added N from FBM, respectively

[2] *P* values for contrasts: - vs + = 0 AN vs N-supplemented, L = linear among N-supplemented, Q = quadratic among N-supplemented

Table 6. Changes in blood urea N (mg/dL) over time after feeding goats diets containing graded levels of feather-bone meal (FBM, SEM = 0.46)

Time after feeding	0 AN[1]	0 FBM[1]	20 FBM[1]	40 FBM[1]	60 FBM[1]
0 h	13.6[a, e]	20.5[b, e,f]	21.5[c, e]	19.1[d, e]	19.7[b,d, e]
2 h	14.2[a, e]	21.3[b, e]	21.7[b, e]	19.7[c, e]	19.8[c, e]
4 h	8.9[a, f]	19.6[b, f]	19.4[b, f]	18.8[b, e]	18.9[b, e]
8 h	7.4[a, g]	16.0[b, g]	15.8[b, g]	15.4[b, f]	16.3[b, f]
Across time[2]	11.5[a]	19.4[b]	19.6[b]	18.3[b]	18.7[b]

[1] 0 AN, 0 FBM, 20 FBM, 40 FBM, and 60 FBM = no added N source; 100% of added N from soybean meal; 20, 40, and 60% of added N from FBM, respectively
[2] LS means calculated using the animal within treatment error term (SEM = 1.30)
a, b, c, d: values within a row with no common superscripts differ ($P < 0.10$)
e, f, g: values within a column with no common superscript differ ($P < 0.10$)

Evaluation of secondary protein nutrients as a substitute for soybean meal in diets for beef steers and meat goats[1]

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ABSTRACT

Finding appropriate disposal techniques for waste products is one of many challenges facing the poultry processing industry. One waste generated in significant quantities is dissolved air floatation sludge, a product of wastewater treatment. Converting dissolved air floatation sludge into a dry feed product (meal) for incorporation into livestock feed appears to be a viable solution. This meal, called secondary protein nutrients (**SPN**), is high in protein (45% CP), fat (28% crude fat), and minerals. The protein consists of 85% B_2 and B_3 fractions, which are moderately to slowly degradable in the rumen and therefore may potentially escape ruminal degradation and be available for digestion in the lower gastrointestinal tract. The goal of this research was to evaluate SPN as an alternative to traditional protein sources for ruminants by substituting it on equal N basis for soybean meal in cattle and meat goat diets (0, 25, 50, 75, and 100% for cattle; 0, 20, and 40% for goats). When included in corn silage-based steer diets, increasing SPN resulted in linear and quadratic declines in both DMI and ADG ($P < 0.001$). Dry matter intake diminished with inclusion rates above 50% and ADG were reduced after inclusion of SPN reached 25% of added N. Feed efficiency declined linearly ($P < 0.001$) with each incremental increase in SPN. Addition of up to 40% of added N as SPN in goat diets caused no change in DMI, digestibility of DM or fiber, or N retention. Ruminal VFA concentrations showed little variation in either species. Increasing the proportion of SPN in the feed caused linear declines in ruminal NH_3 in steers ($P < 0.001$). Increasing SPN in goat diets, however, resulted in only a trend towards reductions of this parameter ($P = 0.14$). The decreases

observed may have resulted from decreasing ruminal protein degradability or increasing fat caused by increasing the proportion of SPN in the feed. Urinary urea N as a percentage of urinary N showed significant declines in urinary urea N as a percentage of urinary N in cattle, but not in goats, over the ranges of SPN offered. These results indicate that SPN can be included in diets for ruminants to supply up to 40% of supplemental N with little negative impact on animal performance.

Keywords: meat goats, N-balance, ruminally undegradable protein, secondary protein nutrients, steers

INTRODUCTION

The production of market-ready poultry products results in the generation of wastes which cannot enter the human food chain. Public concern over environmental quality has demanded that poultry processing plants creatively dispose of these materials. Converting them into livestock feedstuffs has proven to be an economically and environmentally sound means of removing them from their site of production.

Current daily through-put in many processing plants creates 3.8 to 7.6 million L of wastewater. Approximately 83% of surveyed plants employed the treatment technology of dissolved air floatation (**DAF**) to aid in wastewater purification (Kiepper, 2001). The DAF process, as described by Edzwald (1995), yields approximately 106,340 t DAF sludge yearly (K. Custer, American Proteins, Inc., Cummings, GA, personal communication).

The nature of DAF sludge (75% water, 15% fat, 10% non-fat solids) makes its utilization a challenge. It must be land-applied on sites near its point of origin or dried to facilitate handling, packaging, and transport. Nutrient content varies as a result of changes in the species being processed or in the specific procedures used by the processor (Fransen et al., 1995). Addition of flocculants (frequently trivalent salts of Fe and Al) is common and may result in high residual levels of Fe and Al in the sludge, raising toxicity concerns. Iron can cause auto-oxidation and rancidity in the fat (Black et al., 1992).

Removal of a portion of the protein and fat and subsequent drying yields a proteinaceous meal called secondary protein nutrients (**SPN**). The nutritive value of SPN as compared to traditional protein sources must be determined. Our research objectives were**:** 1) to evaluate the acceptability of SPN to cattle and meat goats, and 2) to determine the impact of substituting SPN for soybean meal **(SBM)** on an equal N basis on animal performance, N-balance, and metabolic parameters.

MATERIALS AND METHODS

The experimental procedures used were approved by the Institutional Animal Care and Use Committee at North Carolina State University (**NCSU**). Two separate trials were conducted to evaluate the performance of SPN in livestock diets.

Steer Growth Trial

Treatment of Animals. Seventy-two Angus and Angus-cross steers that graded USDA M-1 were purchased from North Carolina graded feeder cattle sales and transported to the NCSU Butner Beef Cattle Field Laboratory. Upon arrival, they were ear tagged and

vaccinated to prevent bovine diseases of concern in central North Carolina (Bovi-Shield 4, Pfizer, Inc., Exton, PA and Vision 7 with SPUR, Intervet, Inc., Millsboro, DE). They were also treated to eliminate internal and external parasites (Cydectin, Fort Dodge Animal Health, Fort Dodge, IA). After a 28-d quarantine period, 60 steers were selected, stratified by BW, and placed in groups of 12 in 2.74 x 9.14 m pens equipped with Calan gate electronic feeders (American Calan, Northwood, NH), 1 automatic water bowl per pen, and slotted floors. They were fed a corn silage-based diet while they acclimated to the feeding system.

The steers were implanted with Synovex S (Fort Dodge Animal Health, Fort Dodge, IA) on d 0. Two steers per pen (mean initial BW ± SD = 255 ± 15 kg) were randomly assigned to 1 of 6 experimental diets. Initial (d 1) and final (d 85) shrunk weights were determined on all the steers by removing uneaten feed at 1600 and weighing them the following morning at 0700, before feeding. These weights were used to calculate ADG.

Feeding, Feed Sampling, and Analysis. Diets based on corn silage and a supplement mix comprised of varying amounts of ground corn, SBM, SPN, vitamins, and minerals were formulated according to NRC (1996) guidelines to allow for 1.0 kg ADG. With the exception of a protein-deficient, negative control diet, diets were formulated to be similar in energy and CP. The negative control diet (**0 AN**) contained no supplemental N source. For the purpose of formulating diets, an ME value of 3.4 Mcal/kg was assumed for SPN based on values listed in NRC tables (NRC, 1996) for ingredients with similar protein and fat contents. Diet formulation is listed in Table 1. A positive control diet (**0 SPN**) diet

contained all added N in the form of SBM. The remaining 4 diets, **25 SPN, 50 SPN, 75 SPN,** and **100 SPN**, contained graded amounts of SPN which replaced SBM on an equal-N basis to give 25, 50, 75, or 100% of the added N from SPN, respectively.

Diets were offered to the steers as total mixed rations (**TMR**) and were fed once daily to allow approximately 5% orts. Orts were removed from the feeders as often as necessary to prevent spoilage from limiting intake. One steer receiving the 0 AN diet and 1 steer receiving the 100 SPN diet were removed from the trial when they became overly aggressive towards pen mates, causing feed waste and inaccurate intake records. The TMR and feed ingredients were sampled weekly and composited monthly for laboratory analysis. All feed and orts samples were kept frozen (-10°C) prior to analysis.

Days 63 through 70 of the feeding period were designated as a collection period for individual intake samples with the intent of determining if the steers were sorting their feed. The TMR were sampled daily during this collection period and composited for the week. Individual orts were quantitatively saved for the week and subsampled for laboratory analysis. The ratio of CP consumed to CP offered was used to detect sorting.

Feed and orts samples were dried in a forced air oven at 55°C to constant weight and then air equilibrated for 48 h to determine air-equilibrated DM. Samples were then ground in a Model 4 Wiley Mill (Arthur A Thomas Co, Philadelphia, PA) to pass through a 1-mm screen. Dry matter (105°C), ash, and Kjeldahl N were determined according to AOAC (1999) procedures. Concentration of NDF and ADF were determined with α-amylase as described by Van Soest et al. (1991) modified for use with an Ankom apparatus (Ankom

Technology, Macedon, NY). Protein fractions were determined on feed ingredients by procedures recommended by Licitra et al. (1996). Non-protein N (NPN) was determined by the subtraction of N in the precipitate resulting from treatment of samples with tricarboxylic acid from total N. Soluble N was calculated by subtracting the N in the residue remaining after treatment with a borate-phosphate buffer from total N. Protein insoluble in neutral and acid detergents was determined by subjecting samples to neutral or acid detergent digestion prior to N analysis. Lipid levels were determined by ether extraction of SPN and TMR samples using the Goldfisch ether extraction technique (AOAC, 1999) on a Labconco extraction apparatus (Model 35001, Labconco Corp., Kansas City, MO). Analysis of SPN and the 6 TMR samples for Ca, P, Na, Mg, S, K, Cu, Fe, Mn, and Zn was accomplished by optical emission spectrometry (Perkin-Elmer Optima 5300, Shelton, CT) by the North Carolina Department of Agriculture and Consumer Services forage analysis laboratory (Raleigh, NC).

Blood, Ruminal Fluid, and Urine Sampling and Analysis. Blood was drawn on d 28, 56, and 84 by jugular venipuncture for determination of plasma Cu levels using potassium EDTA-treated vacutainer tubes (Becton Dickinson and Co., Franklin Lakes, NJ) and 3.81 cm x 20 gauge needles and placed immediately on ice for transport to the laboratory. Plasma was obtained by centrifugation at a 2,316 x *g* for 30 min and frozen for later analysis. Plasma Cu concentrations were determined on the supernatant resulting from the centrifugation of a mixture of 1 mL thawed plasma and 3 mL of 5% nitric acid at 1,050 x *g* for 20 min followed by aspiration into the flame of an atomic absorption spectrophotometer

(Shimadzu Atomic Absorption Flame Emission Spectrophotometer, Model AA-6701F, Shimadzu, Japan).

Individual urine samples were collected from the steers on d 70 or 71 before the morning feeding by confining 1 group of 12 steers at a time in a narrow chute. To accomplish collections, plastic bags held open by rubber rings were secured with elastic straps over the steers' abdomens to cover the prepuce. Bags were removed as soon as each animal urinated or after 1 h, at which time all the steers were returned to their pen. Urine pH was measured immediately after sample collection (Fisher Scientific Accumet AP63 portable pH meter, Cole-Parmer Instruments, Vernon Hills, IL). Samples were acidified to pH < 3.0 with 6 *M* HCl and stored frozen for total N and urea N determination. Total N was determined as Kjeldahl N (AOAC, 1999) and urine urea was determined colorimetrically by an automated, diacetyl-monoxime method (Marsh et al., 1965).

On d 84 of the trial 2 h (± 20 min) after feeding, ruminal fluid was collected via stomach tube from steers restrained in a squeeze chute. Ruminal fluid pH was determined immediately (Fisher Scientific Accumet AP63 portable pH meter, Cole-Parmer Instruments, Vernon Hills, IL). Samples were strained through cheese cloth and frozen for later determination of concentrations of VFA and ammonia (**NH_3**). Additional blood samples were drawn by jugular venipuncture for determination of serum urea nitrogen (**BUN**) using untreated vacutainer tubes (Becton Dickinson and Co., Franklin Lakes, NJ). The blood was allowed to clot at room temperature for 30 min, was centrifuged at 1,470 x *g* for 30 min, and

serum was frozen and stored until BUN was determined as previously described for urine samples.

Statistical Analysis. All data were analyzed using PROC GLM of SAS (SAS Institute, Inc., Cary, NC) with class variables of pen and dietary treatment. Orthogonal contrasts were made of the 0AN diet against the added N diets and among the added N diets to determine if linear and quadratic effects were present. With the exception of DMI, pen effects were not significant so they were dropped from the model and only treatment effects are reported. Intake differences were expected among pens because the animals were penned by weight. Standard error for steer weights within a pen was 1.72 kg.

Goat N-balance Trial

Treatment of Animals. Twenty four weanling 7/8 Boer wether goats were selected from the NCSU herd. They were dewormed with an oral dose of 2 mL of Cydectin (5mg/mL moxidectin, Fort Dodge Animal Health, Fort Dodge, IA) and moved to a lot at the NCSU Metabolism Educational Unit (Raleigh, NC) for adaptation to experimental feed. The kids were subsequently dewormed with Valbazen (4mL/45.4kg BW, 11.36% albendazole, Pfizer, Exton, PA) 18 d after the initial deworming to insure minimal parasite load.

During adaptation, the goats were group-fed 2.0% of BW daily as concentrate pellets and also offered free choice hay and water. They were adapted gradually to the experimental diets by a regime that made dietary changes every third day. The entire group was switched over the course of 21 d from mixture of 50% commercial pellets and 50% 0 SPN pellets to experimental pellets containing 40% of added N as SPN (40 SPN) with a

liquid molasses coating. When the goats accepted the molasses coating, it was continued throughout the experiment in all treatment groups. The pellets were well accepted by the goats, with the exception of 1 goat that refused to eat any of the experimental concentrates.

Once the goats had been adapted to the 40 SPN pellets, 20 were selected for a N-balance trial (BW = 17.0 ± 1.8 kg). The wethers were blocked by weight (4 goats/block) and placed into metabolism crates. Fecal egg counts were performed using a Paracount-EPG fecal analysis kit (Chalex Corp., Issaquah, WA) on a composite of fresh feces collected from 1 wether randomly selected from each weight block on the second day the goats were housed in metabolism crates. High counts (> 1000 eggs/g feces) resulted in the re-treatment orally of all the wethers with 2 mL Cydectin (Fort Dodge Animal Health, Fort Dodge, IA). Egg counts were reduced to < 750 eggs/g by this treatment. All the goats were dusted with Co-Ral (1% coumaphos, Agri-labs, St. Joseph, MO) to eliminate lice. Two goats (1 receiving 20 SPN and 1 receiving 40 SPN) developed coccidial diarrhea on d 10 of the feeding period. Both were treated for 5 consecutive days with Corid (9.6% amprolium, Merial, Ltd., Duluth, GA) given at a rate of 20 mg/kg BW. The diarrhea was resolved and feces were normal by the third day of treatment.

Feeding, N-balance Sampling, and Analysis. Corn and SBM-based experimental concentrates were formulated according to NRC (1981) to achieve 100 g/d gain when fed as 50% of the total diet. As in the steer trial, a negative control was formulated with no supplemental N (0 AN). Concentrates containing supplemental N in the form of SBM (0 SPN) and in which SPN replaced SBM to provide 20 or 40% of supplemental N (20 SPN

and 40 SPN, respectively) were also formulated. These levels of SPN inclusion were selected based on steer performance data coupled with previous experiences with feeding goats novel feed ingredients. Poultry oil was added to the formulations in an attempt to balance fat across the rations and a pellet binder (Super-bind, Uniscope, Inc, Johnstown, CO) was included to improve pellet quality. All concentrates were pelleted through a 5-mm die (Sprout-Waldron Model D1173 pellet mill, Muncy, PA). Concentrate formulations are listed in Table 2.

Endophyte-free fescue hay (*Lolium arundinaceumn (Schreb.) Darbysh = Festuca arundinacea (Schreb.)*) from a single harvest contributed 50% of the diet. During adaptation to the diets, the hay was offered directly out of bales. To reduce particle size to prevent spillage during the experimental period, the hay was processed through an S600 VanDale Bale Processor (J-Star Industries, Fort Atkinson, WI**)** with the blades spaced 12.5 cm apart.

The day the goats were placed into metabolism crates, they were fed an amount equal to 3.5% of their individual BW. Half of the feed (by weight) was fescue hay and the other half was 40 SPN concentrate. They were then switched to their randomly assigned treatments over 2 d. One wether in each weight block received each treatment. Once the animals were receiving 100% of their assigned treatments, total feed was offered at a rate to obtain 10% total feed refusals. Concentrate was offered 30 min before hay to allow its consumption before hay addition to reduce the amount of hay the goats would spill. By the end of a 14-d ad libitum feed intake period, intake levels had reached a plateau. From this point on, the

goats were fed a constant amount of feed equal to 110% of the average intake for the last 3 d of the 14-d ad libitum period.

Fecal collection bags were placed on the goats at the initiation of the constant feeding period. After a 3-d adaptation to the collection bags, total feces and urine were collected for 5 d. Fecal collection bags were emptied twice daily and the contents weighed once daily. The total feces were sub-sampled at a percentage set for individual goats to obtain approximately 100 g of DM from each animal over the 5-day collection. The fecal samples were composited daily into containers in a forced air oven at 55°C. Following d 5 additions for the collection, the feces were allowed to dry for an additional 48 h after which they were air equilibrated for 48 h and then weighed for air-equilibrated DM determination. Dry samples were placed in sealed plastic bags and stored for later laboratory analysis.

Urine was acidified by the addition of 6 *M* HCl to the collection vessels to maintain pH 3 to 5 as determined with pH paper (pHydrion 1 to 12, Micro Essential Lab, Brooklyn, NY). Acid additions varied by animal but were with the range of 10 to 30 mL per day. Additionally, 100 mL deionized water was added to the collection vessels to minimize the crystallization of urinary compounds which are sensitive to low pH. Total urine was removed from the collection vessels daily. Total weight and volume of the acidified urine were determined and a sub-sample obtained by weight. The percentage of the total urine saved also varied by animal and was established on d 1 of the collection period. It was set to yield 50 g of urine and remained constant for the 5-d collection. Urine samples were composited, frozen, and stored at -10°C for later laboratory analysis.

In addition to fecal and urine samples, feed samples (100 g of each feed/d) were collected and feed refusals were quantitatively saved and composited. Sub-samples of the feed refusals were taken at the end of the 5-d period. All feed and feed refusal samples were placed into a forced air oven at 55°C and dried for 48 h. After a 48 h air equilibration period, the samples were weighed for barn DM determination. Procedures for laboratory analysis of these samples were those described for similar samples generated during the steer trial with the exception of fat determination. Feed samples from the goat trial were analyzed for fat by Dairy One Laboratory (Ithaca, NY) by ether extraction with anhydrous diethyl ether (Soxtec HT6, Foss Tecator AB, Höganäs, Sweden). No mineral determinations were done on feeds from the goat trial.

Ruminal Fluid and Blood Sampling and Analysis. Following the total excreta collection, the goats were put onto a staggered feeding regime in which the initial groups of 4 goats blocked by weight were fed at 15 min intervals. On the second day of the staggered feeding regime, blood samples were collected into untreated 10 mL Vacutainer tubes (Becton Dickinson and Co., Franklin Lakes, NJ) with 20 gauge x 2.5 cm needles via jugular venipuncture before feeding (time = 0 h) and then 2, 4, and 8 h after feeding. All blood samples were immediately placed on ice and later processed as previously described to obtain serum.

Ruminal fluid was also obtained by rumenocentesis 2 h after feeding as described by Nordlund and Garret (1994) and modified for goats using 14 gauge x 5 cm needles with no local anesthesia. A minimum of 5 mL of fluid was withdrawn from each goat. Ruminal

fluid pH was determined immediately upon sample removal using a Fisher Scientific Accumet AP63 portable pH meter (Cole-Parmer Instruments, Vernon Hills, IL), after which samples were placed on ice. Ruminal fluid and serum samples were then frozen and stored at -10°C. These were analyzed as described for the steer trial.

Statistical Analysis. Statistical analysis of goat data was by PROC GLM of SAS (SAS Institute, Cary, NC). The model included class variables of treatment, weight block, and crate type (wood or metal). No significant differences ($P > 0.10$) were detected for crate type or weight block, so they were removed from the model and only treatment differences are presented. Orthogonal contrasts between 0 AN and N-supplemented diets and among N-supplemented diets were conducted. For BUN data, the independent variables were goat within treatment, treatment, time, and time by treatment interaction. The latter played no significant role in the model and was dropped. The goat within treatment error term was used to test for treatment differences.

RESULTS

Feed Composition

Steer Growth Trial. The SPN used in this trial contained 46.5% CP (Table 3) with the CP being fractionated with lower solubility or as undegradable. It also contained 27.8% ether extract, 0.95% Ca, and 1.78% P. Because ferric chloride **($FeCl_3$)** had been used as a flocculating agent during water treatment, the SPN was exceptionally high in iron (28,200 ppm). The concentrations of Zn and Cu were also substantial (329 ppm and 178 ppm, respectively).

The corn silage had a greater DM content and lower CP content than estimated during diet formulation (Tables 1 and 3); therefore, the diets contained less than the 13% CP for which they were formulated (Table 4). Nonetheless, the 5 N-supplemented diets were similar in CP content (mean CP ± SD = 10.7 ± 0.33%) with the 0 AN (negative control) diet lower in CP (7.6%). Iron and copper contents of the diets increased with the increase in SPN, as did the ether extract content of the diets (Table 4).

Goat N-balance Trial. As was the case in the steer trial, the 0 AN (negative control) animals received diets that were lower in CP than supplemented animals (Table 5). The protein fractions of the feeds were determined by laboratory analysis rather than by calculation to account for changes that may have occurred in the fractions as a result of pelleting the feed.

Animal Performance

Steer Growth Trial. Dry matter intake showed both linear and quadratic decreases in response to dietary additions of SPN (Table 6). Intake peaked when SPN contributed 25% of added N and declined dramatically when SPN was increased to 75 and 100% of added N. Steers that received their entire supply of supplemental N as SPN consumed similar quantities of DM to the negative control (0 AN) steers.

Average daily gain showed both linear and quadratic declines with the substitution of SPN for SBM (Table 6) with similar gains for the 0 SPN and 25 SPN steers and with additional SPN in the diet causing ADG to decline. As was observed for DMI, steers receiving the 0 AN diet performed similarly to those receiving the 100 SPN diet.

Daily CP intake was determined for the steers for d 63 to 70 based on the feed and orts samples collected during the intake measurement week (Table 6). Intake of CP followed the same pattern as DMI with both linear and quadratic declines in CP intake as SPN increased. Crude protein ratio was calculated as % CP in feed consumed divided by % CP in feed offered to evaluate the extent to which the steers sorted the SPN out of their feed (Table 6). Linear and quadratic declines in this ratio suggest that the steers began sorting their feed as SPN level increased over 75% of added N.

Diets containing added N had higher G:F than the 0 AN diet ($P < 0.001$). The substitution of SPN for SBM caused greater reductions in gain than in DMI because G:F decreased linearly as SPN increased (Table 6).

Goat N-balance Trial. The addition of a supplemental N source to diets for the goats increased DMI ($P = 0.01$) and daily N intake ($P < 0.001$); however, it did not change the digestibility of DM, NDF, or ADF ($P > 0.10$, Table 7). Fecal N increased linearly ($P = 0.06$) as the proportion of SPN increased in the diet (Table 7); however, there was no change ($P > 0.10$) in N absorption as a result of increasing SPN in the feed. Goats that received the unsupplemented feed absorbed less N than supplemented goats ($P < 0.001$, Table 7).

The goats that received no added N in their diets excreted less urinary N than goats that received supplemental N (Table 7; $P < 0.001$), whereas urinary N was similar among supplemented goats ($P > 0.10$). Nitrogen retention was also similar among supplemented goats, but there was a trend ($P = 0.11$) for a quadratic relationship between SPN level and N

retention with peak retention being obtained with the 20 SPN diet. Goats that did not receive supplemental N retained less N than their supplemented peers ($P = 0.01$, Table 7).

Despite the differences in the quantities of N retained between the supplemented and unsupplemented goats, there were no differences between treatment groups in the proportion of feed N retained or in the proportion of absorbed N retained ($P > 0.10$, Table 7).

Blood, Urine, and Ruminal Parameters

Steer Growth Trial. All the steers consuming diets with added N had higher BUN than did the negative control steers ($P < 0.001$), and BUN exhibited a linear decline as the proportion of SPN in the diet increased ($P < 0.001$, Table 8). Plasma Cu was quadratically related to SPN proportion at d 28, but this relationship was no longer present by d 84 (Table 8). Because d 0 blood samples were not obtained, it can not be determined if this shift represented an overall change in Cu status for the steers which was the result of diet. Changes in plasma Cu level between d 28 and 84 (Table 8) were not impacted by treatment.

Urine pH (Table 8) did not differ when negative control animals were compared to N-supplemented animals ($P > 0.10$). Increasing the proportion of the supplemental N supplied by SPN, however, decreased urine pH linearly ($P < 0.001$). The percentage of urinary N as urea N (%UUN; Table 8), which can be an indicator of the overall N status of ruminants, declined linearly ($P < 0.001$) and quadratically ($P = 0.01$) as SPN increased in the diet. Additionally, steers receiving supplemental N in their feed excreted urine with higher %UUN than the negative control steers ($P < 0.001$).

Ruminal NH_3 (Table 9) declined linearly with each addition of SPN to the diets ($P < 0.001$) and was higher in fluid from N-supplemented steers than from 0 AN ($P < 0.001$). Ruminal pH was unaffected by increasing the proportion of SPN in the diets (Table 9). Similarly, total VFA concentration (Table 9) did not vary as a result of SPN substitution level in the diets, although there was a trend ($P = 0.11$) towards a linear decline with increasing levels of SPN. Unsupplemented steers had lower total VFA than steers supplemented with N ($P = 0.002$, Table 9).

Molar proportions of acetate, isovalerate, and valerate did not vary significantly with diet (Table 9). Butyrate and isobutyrate declined linearly as SPN increased, and propionate exhibited both linear and quadratic increases with SPN concentration (Table 9). Changes in propionate proportions resulted in a linear decrease in the acetate to propionate (A:P) ratio as the proportion of SPN increased.

Goat N-balance Trial. Goats which received supplemental N in their feed excreted larger quantities of urea than goats which received no supplemental N (Table 7). The %UUN was similar for goats on all treatments (Table 7).

The addition of SPN to the concentrates had several impacts on ruminal parameters (Table 11). Ruminal pH declined linearly ($P = 0.10$) as SPN increased in the diets but no difference was observed between supplemented and unsupplemented animals.

Ruminal NH_3 was lower ($P < 0.001$) in the 0 AN goats than in goats receiving supplemental N (Table 10). It was similar among the goats receiving varied levels of SPN

in their feed with only a slight trend ($P = 0.14$) for a linear decline in ruminal NH_3 concentration.

Total VFA were lower in ruminal fluid from the goats receiving the 0 AN diet than in the goats receiving supplemented diets. There were no differences among the supplemented goats in total VFA concentrations.

The molar proportion of acetate increased linearly as SPN increased in the diet and the proportion of propionate declined linearly (Table 10). This resulted in a linear increase in the A:P ratio with a trend towards a quadratic relationship ($P = 0.11$). There were few differences in the proportions of the other ruminal acids when treatments were compared (Table 10), although goats receiving no supplemental N had reduced proportions of butyrate ($P = 0.04$) and valerate ($P = 0.03$) as compared to supplemented animals.

There were no treatment differences in BUN as a result of increasing SPN in the feed (Table 10). Negative control goats had lower BUN than supplemented goats ($P = 0.03$).

DISCUSSION

Feed Composition

The protein content of the SPN used in our studies (Table 3) was similar to the range of protein concentrations reported by Fransen et al. (1995), who surveyed similar material from 5 poultry processing plants and 9 swine processing plants. The products evaluated by Fransen et al. (1995), which were produced with similar chemical treatment, also contained high levels of Fe, Cu, and Zn (21,832 to 49,444 ppm Fe; 38 to 183 ppm Cu; 165 to 550 ppm

Zn). The product used in our studies fell into the reported ranges for these minerals. It appeared likely that for use in ruminant feeds, SPN would be characterized in a similar manner to other non-ruminant, non-specified risk material protein by-products such as blood meal or meat and bone meal.

The high Fe content raised concerns that animals eating this product might develop Cu deficiency. We did not measure mineral digestibility or balance in the goat trial nor is any data on these parameters available in the literature. Because we did not see any treatment impact on plasma Cu changes over the course of the steer trial, however, we believe that the available Cu concentration in the SPN is high enough to balance available Fe concentrations.

The protein fraction profile of SPN (Table 3) indicated that it may be a good source of ruminally undegradable protein (**RUP**). The B_2 and B_3 protein fractions of SPN comprised 41 and 44% of the CP, respectively, whereas soybean meal, which is a more common source of protein in ruminant diets, contained 70.2% as B_2 and 5.4% as B_3.

The negative values obtained for the B_1 fraction in the corn silage and SBM (Table 3) are not biologically possible. This anomaly in the data is the result of the analyzed amount of NPN present in these feeds being slightly higher than the calculated amount of soluble true protein present. Soluble true protein values were obtained by subtracting the amount of insoluble CP (which is assumed to be all true protein) and NPN from total protein. It may therefore contain error caused the presence of insoluble NPN compounds. An overestimation of insoluble true protein would result in an underestimation of soluble true

protein. When the NPN and soluble true CP fractions are similar in size, the calculation of soluble true protein (CP - insoluble CP - NPN) may result in a negative number if insoluble protein is overestimated.

The effects of SPN additions on the proportions of the protein fractions were apparent in the goat concentrates (Table 5). Although the total percentage of N as fractions B_2 plus B_3 remained fairly constant, the B_2 fraction declined as the B_3 fraction increased. Additionally, the unavailable C fraction increased, again reflecting the increasing SPN concentration in the goat concentrates.

Animal Performance and Metabolic Parameters

The changes observed in both steer and goat performance and metabolic status are those expected when a protein deficient diet is supplemented with protein and when a ruminally degradable protein source is replace by one of lower degradability. The DMI results presented from our steer trial (Table 6) were similar to those of Swartz et al. (1991), who replaced SBM with blood meal in diets for growing Holstein calves. Blood meal also contains high levels of B_2 and B_3 protein and is considered a good source of RUP.

Increased DMI when a source of supplemental N was added to the diets was a common response to both steers and wethers. This change may have been the result of improved fiber digestion due to more NH_3 being available for use by cellulolytic bacteria, which prefer NH_3 as their N source. The ruminal NH_3 concentrations we observed (Tables 9 and 10) are consistent with this explanation.

Reported values of optimal NH_3 for ruminal fermentation vary from 5mg/dL to 23.5mg/dL (Satter and Slyter, 1974; Mehrez et al., 1977; Van Soest, 1994) and likely vary with species and diet. Ammonia in ruminal fluid from our steers fell below or towards the lower end of this range (Table 9). Goat ruminal fluid, with the exception of that from the negative control group exceeded this range (Table 10). Reduced rate of fiber degradation as a result of lower ruminal NH_3 could therefore have limited the ability of the cattle and 0 AN goats to digest fiber as quickly as animals with higher NH_3. This could have resulted in reduced passage from the rumen which would have in turn caused reduced intake. Total tract fiber digestibility, however, was not impacted by treatment (Table 7).

Declines in ruminal NH_3 as SPN increased in the steer diets (Table 9) reflected both the lower ruminal degradability of SPN as compared to SBM and the reduced DMI of the steers. Similar reductions in ruminal NH_3 were seen by Cunningham et al. (1994) and Bohnert et al. (1998) when they substituted high-RUP protein sources for lower RUP protein sources in diets for dairy cattle and steers, respectively.

The lack of change in DMI and ruminal NH_3 in the goats receiving SPN (Tables 7 and 10) may have resulted from the lower inclusion rates used for the N-balance study. The SPN inclusion rates for the goat concentrates were intentionally chosen to be below the levels where negative responses were seen in cattle.

Another explanation for the difference in response between steers and goats could be the difference in fat content. The steer diets were not balanced for the fat in the SPN and so fat

content increased from 2.4 to 5.1% as SPN increased (Table 4). The goat diets were balanced for fat content and averaged 5.2% fat (Table 5). Onetti et al. (2001) found that adding fat to the diets of lactating dairy cows reduced DMI and ruminal NH_3. Lower ruminal NH_3 was attributed to a reduced protozoa population which was also observed with fat supplementation. Although we did not measure microbial populations, these findings agreed with what we observed in regards to other ruminal parameters and could explain why the species seemed to respond differently to SPN.

Data presented by Ikuta et al. (2005) demonstrated that changes in BUN levels occur about 2 h after changes in ruminal NH_3. The changes in BUN observed in our trials (Tables 8 and 10) therefore likely reflected the changes in ruminal NH_3 and may have been in response to declining protein degradability and increased fat as SPN increased in feed. They were also in agreement with the findings of other trials (Knaus et al., 1998; Huntington et al., 2001; Knaus et al., 2002).

Palatability may have been an issue at higher SPN substitution levels. The decline in CP ratio as SPN level increased in the steer diets (Table 6) did not become obvious until SPN supplied 100% of the supplemental protein, suggesting that the animals accepted the SPN until it exceeded 8.6% of DM. Sungwaporn et al. (2004) noted declines in intake when SPN from the same batch of product was added to broiler diets at the rate of 7.5% of DM (equivalent to a 64.7 SPN diet in our steer trial). They offered palatability as an explanation for the decreased DMI with SPN diets.

Reduced ADG among steers receiving SPN in their feed may have been caused by a combination of reduced feed intake and lower G:F when SPN increased in the diets (Table 6). Sungwaporn et al. (2004) attributed decreased gain in broilers to reduced feed intake when SPN was added to the feed in place of SBM. They also reported linear declines in feed efficiency as SPN increased from 7.5 to 20% of DM. El Boushy et al. (1984) described reduced gain and efficiency among broilers as dehydrated poultry slaughterhouse effluent (37.5% CP, 28.6% fat, 41,000 ppm Fe, air dry basis) increased from 2 to 7% in their diets. Nelson et al. (1985) observed a depression in ADG in steers fed wheat straw and corn silage diets supplemented with blood meal instead of SBM, although they did not observe reduced DMI. This depression was partially alleviated when the wheat straw was ammoniated, suggesting that limiting ruminal NH_3 may have played a role. The negative control steers in our study had lower G:F than the supplemented animals (Table 6) indicating that even the 100 SPN diet had benefits over offering no supplemental protein source. The low N level of the 0 AN diet and the reduced protein degradability of the higher SPN diets were apparently limiting animal performance.

Another possible explanation for the reduction in performance is the high Fe content (1004 to 3135 ppm) of the SPN-supplemented feed caused by the $FeCl_3$ flocculant. Harrison et al. (1992) found that additions of 100 to 1000 mg Fe per mL ruminal fluid resulted in depressed degradation rates for forage substrate DM in vitro. Total VFA concentrations were not altered by the Fe, but rates of production were reduced. They suggested that feed Fe concentrations in excess of 500 ppm (DM basis) could supply adequate Fe to have toxic

effects on ruminal microorganisms and result in reduced intakes and daily gains. The TMR containing SPN all contained more than 500 ppm Fe meaning toxic effects on ruminal microorganisms could have occurred, reducing intake and gain by depressing the rate of fiber degradation.

In addition to reduced intake and feed efficiency, Sungwaporn et al. (2004) reported increased incidence of rickets among broiler chicks receiving the higher levels of SPN in their diets. The rickets were attributed to vitamin D deficiency which was the result of dietary vitamin D destruction by Fe and other prooxidants contributed to the feed by the SPN.

As mentioned earlier, preventing fat oxidation and rancidity and the destruction of fat soluble nutrients is one of the challenges presented by using DAF sludge as a nutrient source. Rancidity does not have detrimental impacts on palatability or fat utilization in cattle (Zinn, 1995), but can reduce milk fat and protein production in lactating animals (Heinrichs et al., 2005). We did not monitor the feeds for presence of rancidity or nutrient destruction, and we did not observe any indications in the animals that these issues were presenting themselves.

According to NRC (1981), goats similar to those used in our trial require 10.6 g N/d to gain 100 g/d. With the exception of the 0 AN treatment, N intake by the goats was similar across treatments (Table 7), exceeded their requirement, and therefore should have supported 100 g/d gain or more if no other nutrients were lacking. Nitrogen intake for the 0 AN diet exceeded the maintenance recommendation of 6.1 g N/d suggesting that the animals

on the 0 AN treatment should have maintained their BW if the diet was not lacking in other nutrients. This expectation was also supported by a N-retention value greater than zero (Table 7).

The lower fecal excretion of N by the unsupplemented animals compared with the N-supplemented animals was expected. Linear increases in fecal N as SPN increased in the diet were anticipated because of increases in both the slowly degraded B_3 fractions and the largely indigestible C protein fraction contributed by the SPN. Despite the increase in fecal N caused by the SPN, all supplemented goats absorbed similar amounts of N, but N digestibility declined linearly with SPN additions to the diet. This result was expected because C protein fraction increased in the diets. Our results were similar to those reported in other trials when RUP was increased in feed (Cunningham, 1994, with dairy cows; Bohnert, 1998, with steers).

Similar urinary excretion of N among supplemented goats suggests that a large portion of the B_3 protein described earlier is indeed being digested and absorbed in the lower gastrointestinal tract. In agreement with our findings, Brun-Bellut et al. (1991) found that similar quantities of urinary N were excreted by non-lactating does receiving diets containing both high and low levels of ruminally degradable protein.

The lack of a decline in N retention in goats as SPN increased seemed to contradict the decline in ADG observed in steers; however, as stated earlier, the goat diets were designed to contain SPN levels lower than those that caused negative responses in the steers (50%

SPN inclusion for ADG). There was a trend (P = 0.11) for a quadratic relationship between SPN level and amount of N retained. If the SPN content of the goat diets had been increased beyond the 40% level, declines in N retention may have occurred. The trend suggested that the optimal inclusion rate for SPN in the diet would be approximately 20% of supplemental N. With only 3 levels of SPN, however, precise estimation of the optimal SPN level is difficult. The 20 SPN level in the goat diets and the 25 SPN level in the steer rations (which produced the highest ADG among the levels tested in cattle) were similar in dietary proportion (% of diet DM) and this supports the theory that the optimum level of SPN inclusion would be to supply 20 to 25% of supplemental N as SPN. Additional support for the 20 to 25% optimal inclusion rate range came from data on retained N as a percentage of N intake (Table 7), which also peaked with the 20 SPN goat diet and which was similar to rates reported by others (Lindberg, 1989; Woodard and Reed, 1997; Merkel et al., 2001). Retaining the highest proportion of dietary N is of paramount importance to maximizing profit and minimizing the proportion that is excreted into the environment.

The %UUN can give a glimpse at the N status of a ruminant animal. Increasing N intake above the required level resulted in higher % UUN (Archibeque et al., 2001; Huntington et al., 2001). Based on the reductions in CP intake with increasing SPN and assuming that the differences between supplemented and unsupplemented steers reported during d 63 to 70 (Table 6) are representative of protein intakes for the entire trial, we expected our observations that the 0 AN steers had lower %UUN than steers receiving the supplemented

diets and that %UUN declined among the supplemented steers (Table 8) with increasing SPN.

Declining %UUN among supplemented treatment groups with increasing SPN and the lower %UUN of the negative control steers (Table 8) might also have indicated increased urea recycling to the rumen associated with reduced ruminal NH_3. Rémond et al. (1993) demonstrated that increasing NH_3 concentrations within the rumen limited urea uptake by the rumen. Applied in reverse, the reduced NH_3 concentrations observed with the higher levels of SPN in the diets and when the 0 AN diet was fed would have led to enhanced urea uptake by the rumen, leaving less urea in the blood to be excreted in the urine. The reduced ruminal degradability of the dietary protein created by adding SPN to the feed caused lower ruminal NH_3 which compounded with reductions in intake to depress %UUN.

The absence of difference in %UUN between the negative control goats and the N-supplemented goats (Table 7) was unexpected and is difficult to explain. The concentrate fed to this group of animals was 90% corn (Table 2) and so provided a substantial supply of rapidly fermented carbohydrate along with protein which was 87% B_2 and B_3 fractions (Table 5). The hay, which was fed 30 min later than the pellets, contained 74.6% B_2 and B_3 fractions. The diets and feeding regime may have resulted in an asynchronous delivery of protein and energy, preventing the protein from being efficiently utilized by the animal and resulting in it being excreted as urea in the urine. Cronjé (1992) found that inadequate energy reduced % N retention in goats fed adequate levels of protein and that N recycling increased as the supply of energy increased. Other possible explanations were an imbalance

in the AA supplied to the goats which prevented them from being utilized efficiently or, that the reduced DMI among the negative control goats (Table 7) resulted in a greater proportion of the available AA being used as an energy source.

An explanation for the decline in steer urine pH as SPN level increased was not readily apparent. Huntington et al. (2001) also reported declines in urine pH when the proportion of ruminally degradable protein in the diet decreased. An explanation for the observation was not offered. It is possible that the $FeCl_3$ used in the production of the SPN was involved in the pH decline we observed. The diets were not analyzed for Cl content; however, if it was present in the same magnitude as Fe, it could have created an anionic physiological state within the steers which would have resulted in reductions in urine pH. Sodium remained fairly constant in the diets at 0.15% of DM (Table 4), but potassium declined as SPN increased. This could have compounded with high Fe concentrations to lower urine pH.

Lower total VFA concentrations in the negative control steers and wethers (Tables 9 and 10, respectively) likely reflected the lower DMI of these animals. The trend for a linear decline in total VFA concentration among SPN supplemented cattle can be explained by falling DMI as SPN proportion in the diet increased. Changes in ruminal VFA proportions as a result of SPN additions to the diets, however, varied with species and are more difficult to explain.

The increase in molar proportion of propionate in ruminal fluid from cattle receiving increasing levels of SPN (Table 9) could have been the result of decreased acetate

production which could in turn have been caused by low ruminal NH_3 and the inhibition of fiber fermentation (Van Soest, 1994). It could also have been explained by the declines in the C_4 acids (Table 9) which are produced by the fermentation of AA (isobutyrate) or fiber (butyrate). The increased proportion of propionate in ruminal fluid accompanied by no change in acetate across treatments explained the decrease in the A:P ratio (Table 9). The alterations in VFA proportions (Table 9) could have also resulted from high ruminal Fe concentrations. Harrison et al. (1992) noted that A:P ratio increased with 100 to 500 mg/L added Fe but then dropped with 1000 mg/L additions to in vitro fermentation flasks. Concentration of Fe was not determined in the ruminal fluid collected during our study.

The increase in fat in the steer diets (Table 4) also added a plausible explanation for the changes seen particularly because poultry fat is less saturated than tallow, which is a common source of fat in cattle diets. Pantoja et al. (1994) observed no change in total VFA or rumen pH when fat was added to dairy cow rations. Onetti et al. (2001) found that adding fat caused a trend for declines in total VFA and that the A:P ratio declined due to decreased acetate and increased propionate proportions as fat increased from 2 to 4% of dairy cow diets.

The changes seen in the VFA proportions in goats (Table 10) seemed to contradict those reported for the cattle. Increased acetate and decreased propionate and the concomitant increase in A:P ratio suggested improved fiber digestion with increasing SPN rather than impaired fermentation; however, this was not supported by NDF and ADF digestibility data (Table 7). The largest decline in the cattle A:P ratios was observed at SPN levels beyond

those fed to the goats. If the cattle results from 0, 25, and 50 SPN are compared to the goat results, the conflict diminished. Additionally, if the trend toward a quadratic relationship between A:P ratio and SPN in the goats was considered ($P = 0.11$; Table 10), the possibility existed that further increases in SPN in goat diets would have led to reduced A:P as was reported in the cattle. Balanced fat content in the goat diets while fat increased in the steer diets may have also contributed to the difference observed between goat and steer A:P responses.

Analyses of SPN suggested that it could be a useful source of protein in livestock diets. It would fit especially well into ruminant feeds, providing RUP based on its high B_2 and B_3 protein fractions. Steers and goats receiving SPN performed as well as control animals when it supplied up to 50% of supplemental N; however, including SPN to supply more than 50% of added N caused reductions in DMI and ADG. Inclusion of SPN in ruminant diets in place of traditional protein sources should therefore be limited to 50% of added N. This use of secondary protein nutrients would provide an environmentally and economically sound means of recycling the nutrients in dissolved air floatation sludge. Additional research to determine flocculant effects on protein quality and to evaluate AA supplied to the animal is needed.

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Table 1. Diet formulation[1] for steers fed graded levels of secondary protein nutrients (SPN)

			% of diet DM					
Ingredient	%DM[2]	%CP[2]	0 AN[3]	0 SPN[3]	25 SPN[3]	50 SPN[3]	75 SPN[3]	100 SPN[3]
Corn silage	33.3	8.6	80.6	84.0	82.3	81.2	80.2	78.5
Ground corn	90.6	8.6	17.7	3.8	5.3	6.2	7.0	8.5
Soybean meal	88.3	51.6	0	10.8	8.1	5.4	2.7	0
SPN	95.1	48.6	0	0	2.9	5.8	8.6	11.5
Trace mineralized salt	--	--	0.5	0.5	0.5	0.5	0.5	0.5
Limestone	--	--	1.0	1.0	1.0	1.0	1.0	1.0
Dicalcium phosphate	--	--	0.2	0	0	0	0	0

[1] Mineral premixes provided 15 ppm Zn, 15 ppm Mn, 10 ppm Cu, and 0.2 ppm Se to the diet DM. A vitamin A, D, and E premix provided 2200 IU vit A, 489 IU vit D, and 0.98 IU vit E per kg diet DM. Rumensin was added at a rate of 22 mg per kg diet DM.
[2] Estimated composition used for formulation
[3] 0 AN = no added N source; 0, 25, 50, 75, or 100 SPN = % of added N supplied by SPN

Table 2. Composition of concentrates (DM basis) for goats fed graded levels of secondary protein nutrients (SPN) to determine N-balance

Ingredient (%)	0 AN[1]	0 SPN[1]	20 SPN[1]	40 SPN[1]
Corn	90.5	71.1	71.9	72.6
Soybean meal	0	18.7	14.9	11.1
SPN	0	0	4.4	8.8
Dry molasses	2.0	2.0	2.0	2.0
Liquid molasses[2]	3.0	3.0	3.0	3.0
Poultry oil	1.8	2.4	1.2	0
Limestone	1.3	1.3	1.2	1.1
Mineral mix[3]	1.0	1.0	1.0	1.0
Super-Bind[4]	0.5	0.5	0.5	0.5

[1] 0 AN = no added N source; 0, 20, or 40 SPN = % of added N supplied by SPN.
[2] Liquid molasses was added to pellets as a coating, immediately prior to feeding.
[3] Southern States, Inc., Richmond, VA. Contains 16.5% Ca; 7.0% P; 28.0% salt; 3.5% Mg; 2.0% S; 1.0% K; 70 ppm I; 1,050 ppm Cu; 32 ppm Co; 52 ppm Se; 3,200 ppm Zn; 3,000 ppm Mn; 572,000 IU vitamin A/kg; 44,000 IU vitamin D/kg; and 440 IU vitamin E/kg.
[4] Super-Bind: Condensed lignin sulfonaate mid-inclusion pellet binder, Uniscope, Inc., Johnstown, CO

Table 3. Chemical composition of feed ingredients used in steer performance diet

Item	Corn Silage	Ground Corn	SBM[1]	SPN[1]
DM, %	38.6	88.6	88.2	95.0
OM, % of DM	97.0	98.8	93.2	85.9
NDF, % of DM	42.3	11.6	12.8	40.8
ADF, % of DM	22.6	3.1	5.3	9.0
CP, % of DM	6.7	8.7	58.7	46.5
Protein fractions		% of CP		
A	54.5	7.3	23.4	7.1
B_1	-1.2	4.5	-0.5	1.3
B_2	30.8	72.4	70.2	41.3
B_3	8.4	7.5	5.4	44.4
C	7.5	8.3	1.5	5.9

[1] SBM = soybean meal; SPN = secondary protein nutrients.

Table 4. Chemical composition of experimental diets with graded levels of secondary protein nutrients (SPN) fed to steers for performance measurement (DM basis)

Component	0 AN[1]	0 SPN[1]	25 SPN[1]	50 SPN[1]	75 SPN[1]	100 SPN[1]
DM, %	42.6	42.0	42.4	42.9	42.7	43.7
OM, %	96.0	95.7	95.3	95.2	95.4	95.2
CP, %	7.6	10.4	10.3	11.0	10.5	11.1
NDF, %	37.8	37.2	39.1	37.4	38.5	38.3
ADF, %	19.8	19.5	20.4	19.4	19.7	19.5
Ether extract, %	2.6	2.4	2.9	3.7	4.3	5.1
Fe, ppm	332	253	1004	1857	2379	3135
Cu, ppm	15	15	18	20	20	23
Na, %	0.15	0.15	0.15	0.16	0.15	0.15
K, %	0.74	0.86	0.82	0.79	0.68	0.65

[1] 0 AN = no added N source; 0, 25, 50, 75, or 100 SPN = % of added N supplied by SPN.

Table 5. Nutrient composition of feeds used for the goat N-balance trial (% of DM)

Component	0AN[1] pellets	0SPN pellets	20SPN pellets	40SPN pellets	Hay
DM (%)	83.1	83.8	83.5	83.6	87.3
OM	95.9	94.6	94.4	94.4	94.4
NDF	10.0	10.3	11.3	10.6	73.3
ADF	2.3	3.4	3.5	3.2	40.2
CP	9.8	17.4	18.2	17.7	9.6
Fat	4.9	5.1	5.2	5.2	1.3
Overall diet % CP[2]	9.7	13.5	13.9	13.6	--
Protein fractions			% of CP		
A	5.8	1.5	6.4	4.8	1.4
B_1	5.9	8.9	8.2	5.2	4.2
B_2	75.5	80.0	68.4	67.7	29.7
B_3	11.4	8.0	14.9	19.3	44.9
C	1.4	1.7	2.1	3.0	5.5

[1] 0AN = no added N source; 0, 20, or 40SPN = % of added N from secondary protein nutrients (SPN).
[2] 50% concentrate pellets + 50% hay, as fed.

Table 6. Performance of steers fed increasing proportions of secondary protein nutrients (SPN)

	0 AN[1]	0 SPN[1]	25 SPN[1]	50 SPN[1]	75 SPN[1]	100 SPN[1]	SEM	-/+[2]	L[2]	Q[2]
DMI,[3] kg/d	5.3	6.8	7.3	6.9	6.0	5.2	0.20	<0.001	<0.001	<0.001
ADG,[3] kg/d	0.54	1.26	1.21	1.11	0.94	0.67	0.050	<0.001	<0.001	0.01
G:F, [3] kg/kg	0.11	0.20	0.18	0.18	0.17	0.14	0.008	<0.001	<0.001	0.61
DMI,[4] kg/d	5.9	7.4	7.5	7.4	6.7	5.5	0.27	<0.001	<0.001	<0.001
Diet,[5] % CP	7.9	12.8	11.6	12.7	12.5	12.6	--	--	--	--
CP intake,[4] g/d	468	950	909	935	832	677	36	<0.001	<0.001	0.01
CP ratio[6]	0.997	1.002	1.005	1.002	0.995	0.981	0.004	0.85	<0.001	0.01

[1] 0 AN = no added N source; 0, 25, 50, 75, or 100 SPN = % of added N from SPN.
[2] *P* values for preplanned contrasts: -/+ = 0 AN vs. other diets; L = linear among added-N diets; Q = quadratic among added-N diets.
[3] For d 1 to 84.
[4] For d 63 to 70.
[5] Lab analysis of samples from d 63 to 70.
[6] Ratio of % CP in feed consumed to % CP in feed offered during d 63 to 70.

Table 7. Nitrogen metabolism of goats fed graded levels of secondary protein nutrients (SPN)

	0 AN[1]	0 SPN[1]	20 SPN[1]	40 SPN[1]	SEM	-/+[2]	L[2]	Q[2]
DMI, g/d	489.2	631.5	628.1	643.4	44.4	0.01	0.84	0.85
DM digestibility, %	70.5	69.8	69.8	69.6	0.01	0.53	0.92	0.93
NDF digestibility, %	50.8	47.1	49.2	51.2	2.38	0.56	0.23	0.97
ADF digestibility, %	45.3	40.4	42.4	47.1	3.22	0.59	0.16	0.74
N intake, g/d	7.6	13.9	14.3	14.6	0.97	<0.001	0.62	0.93
Fecal N, g/d	2.8	3.8	4.0	4.9	0.40	0.01	0.06	0.40
N absorption, g/d	4.8	10.1	10.3	9.7	0.62	<0.001	0.63	0.53
N digestibility, %	63.5	72.8	72.6	66.4	1.48	<0.001	<0.001	0.12
Urinary N, g/d	3.0	6.7	5.9	6.4	0.73	<0.001	0.74	0.43
N retention, g/d	1.8	3.4	4.5	3.3	0.59	0.01	0.89	0.11
N retention, % of N intake	21.9	25.2	31.3	22.3	4.6	0.42	0.64	0.17
N retention, % of N absorbed	34.7	34.3	43.2	33.5	6.6	0.77	0.93	0.22
Urine urea-N, g/d	2.5	5.8	4.5	5.3	0.62	<0.001	0.57	0.15
Urine urea-N, % urinary N	82.6	85.9	79.1	83.5	5.48	0.96	0.75	0.37

[1] 0 AN = no added N source; 0, 20, or 40 SPN = % of added N supplied by SPN.
[2] *P* values for preplanned contrasts: -/+ = 0 AN vs. others; L = linear among added N diets; Q = quadratic among added N diets.

Table 8. Blood and urine parameters in steers as influenced by increasing dietary levels of secondary protein nutrients (SPN)

Item	0 AN[1]	0 SPN[1]	25 SPN[1]	50 SPN[1]	75 SPN[1]	100 SPN[1]	SEM	-/+[2]	L[2]	Q[2]
Serum urea-N, m*M*	1.1	5.7	5.1	4.2	3.5	3.4	0.32	<0.001	<0.001	0.30
Plasma Cu (d28), ppm	1.07	1.14	1.11	1.31	1.17	1.08	0.06	0.16	0.83	0.03
Plasma Cu (d84), ppm	0.99	0.98	0.96	1.15	1.00	1.01	0.05	0.60	0.56	0.15
Cu change (d84 – d28)	-0.08	-0.15	-0.15	-0.15	-0.17	-0.08	0.05	0.34	0.43	0.41
Urine pH	6.77	7.76	6.93	6.48	6.32	5.58	0.221	0.53	<0.001	0.57
Urinary urea-N, % of N	9.8	57.1	53.6	48.1	38.3	20.4	0.035	<0.001	<0.001	0.01

[1] 0 AN = no added N source; 0, 25, 50, 75, or 100 SPN = % of added N supplied by SPN.
[2] *P* values for preplanned contrasts: -/+ = 0 AN vs. other diets; L = linear among added N diets; Q = quadratic among added N diets.

Table 9. Effects of substitution of secondary protein nutrients (SPN) for soybean meal on ruminal parameters in steers

Item	0 AN[1]	0 SPN[1]	25 SPN[1]	50 SPN[1]	75 SPN[1]	100 SPN[1]	SEM	-/+[2]	L[2]	Q[2]
pH	7.06	6.89	6.97	6.94	6.90	7.02	0.057	0.07	0.32	0.76
NH_3,mg/dL	3.2	15.6	12.2	8.2	3.0	2.8	1.20	<0.001	<0.001	0.23
Total VFA, m*M*	74.1	90.4	86.6	89.4	87.5	81.1	3.39	0.002	0.11	0.45
				Molar %						
Acetate	60.5	61.6	61.8	60.9	62.4	60.9	0.75	0.22	0.73	0.74
Propionate	21.2	19.4	19.6	19.4	20.4	22.2	0.64	0.18	0.003	0.08
Isobutyrate	0.74	0.88	0.85	0.76	0.79	0.76	0.043	0.21	0.05	0.47
Butyrate	11.1	11.4	10.9	12.2	9.6	9.8	0.64	0.65	0.04	0.26
Isovalerate	1.2	1.6	1.6	1.7	1.8	1.3	0.16	0.04	0.29	0.16
Valerate	1.3	1.5	1.4	1.4	1.5	1.5	0.06	0.01	0.71	0.20
Acetate:propionate ratio	2.89	3.20	3.16	3.17	3.09	2.80	0.13	0.19	0.03	0.20

[1] 0 AN = no added N source; 0, 25, 50, 75, or 100 SPN = % of added N supplied as SPN.
[2] *P* values for preplanned contrasts: -/+ = 0 AN vs. other diets; L = linear among added N diets; Q = quadratic among added N diets.

Table 10. Ruminal and blood parameters of goats fed graded levels of secondary protein nutrients (SPN)

	0 AN[1]	0 SPN[1]	20 SPN[1]	40 SPN[1]	SEM	-/+ [2]	L[2]	Q[2]
pH	6.17	6.10	6.10	5.77	0.14	0.25	0.10	0.30
NH_3, mg/dL	16.5	40.4	31.9	32.6	3.7	0.001	0.14	0.29
Total VFA, m*M*	85.7	110.8	96.7	113.4	9.5	0.07	0.84	0.17
VFA			Molar %					
Acetate	60.1	58.0	59.3	63.1	1.14	0.99	0.005	0.34
Propionate	27.7	27.6	26.6	21.0	1.35	0.11	0.003	0.15
Butyrate	9.5	11.4	11.1	13.0	0.88	0.04	0.21	0.28
Isobutyrate	8.2	12.9	10.5	14.7	1.56	0.48	0.52	0.45
Valerate	0.92	1.57	1.32	1.48	0.14	0.03	0.52	0.73
Isovalerate	0.86	1.13	1.00	1.35	0.21	0.67	0.57	0.92
Acetate:propionate ratio	2.18	2.14	2.23	3.08	0.20	0.19	0.003	0.11
Serum urea-N, m*M*[3]	5.8	7.8	7.2	8.0	0.70	0.03	0.84	0.45

[1] 0 AN = no added N source; 0, 20, or 40 SPN = % of added N supplied by SPN
[2] *P* values for preplanned contrasts: -/+ = 0 AN vs. others; L = linear among added N diets; Q = quadratic among added N diets.
[3] Least square means and *P*-values calculated using type III mean square for animal within treatments as error term.

CONSIDERATIONS FOR THE FUTURE

Generating waste materials is an inevitable result of creating value-added products for human consumption. As our society becomes more proactive in reducing its negative impact on the environment, disposal of waste materials will become increasingly challenging. "Reduce, reuse, and recycle" will become our mantra as we seek creative ways to avoid filling landfills and polluting air, soil, or water.

As discussed earlier, many agricultural waste materials have already found their way into the recycling chain and are commonly used as sources of feed or fertilizer. The research previously described demonstrated that existing technology can be applied to waste materials to create useful products, elevating waste materials to waste product or byproduct status. Several challenges remain, however, and our ability to address them effectively may impact the success of the various disposal strategies we develop.

As we experiment with new feedstuffs, it is of paramount importance that we have the most accurate means of evaluating their potential impact on the livestock which ultimately will consume them. Because many of the products will be sources of supplemental protein, protein quality should be assessed; however, the protein quality evaluation techniques used in our research each had inherent inaccuracies. The pepsin digestibility assay, which is a chemical evaluation, gave results that were about 10 percentage units higher than the cecaectomized cockerel test, a biological evaluation. Cockerels may not be an accurate model to use for ingredients that will be fed mainly to

ruminants. Neither of these assays adequately estimated where in the ruminant gastrointestinal tract the protein would be digested.

The protein solubility fraction assays, which may give a better indication of where in the ruminant digestive system the protein might be degraded, were developed for proteins in fibrous feeds and therefore may not offer the most accurate results for proteins in products of animal origin, since animal tissues do not contain any true fiber. Development and use of a standardized procedure for predicting digestibility and location of digestion for proteins of animal origin in ruminant feeds would prove extremely valuable for the evaluation of novel feed ingredients, such as SPN and FBM, as well as for traditional animal-original feedstuffs.

Laboratory tests used in our research also did not evaluate the protein products for damage caused by bacterial activity. Since the feedstuffs were produced from wastes that were certain to contain high bacterial loads, presence of biogenic amines was possible. While every effort was made to produce pathogen-free feedstuffs, bacterial attack on free amino acids could still be a threat to the overall acceptance by and quality of the final products for livestock. Neither SPN nor FBM was evaluated for the presence of biogenic amines. Because elevated concentrations of amines can reduce intake and pose health risks for both livestock and humans (Shalaby, 1996; Phuntsok et al., 1998), a technique such as thin-layer chromatography, which can detect several different biogenic amines quickly and quantitatively (Shalaby, 1996), would fit well into the feedstuff evaluation scheme. Establishing guidelines for maximum livestock dietary concentrations could be useful.

Fat quality in feed ingredients was another issue that warranted consideration. This was particularly true for SPN, since the product used in our research was high in minerals which can hasten fat deterioration and which can also damage fat soluble vitamins, as discussed earlier. The anti-oxidant, ethoxiquin, was added to the SPN to prevent oxidative damage to the fat in the final product; however, it could not have prevented oxidative damage during the floatation process, which was carried out prior to its addition to the SPN. A huge contributor to the potential for oxidative damage was ferric chloride, which is a commonly used flocculant for the DAF process. Use of a non-metallic flocculant could possibly reduce fat oxidation during flotation. If the skimmings are to be utilized as a feed ingredient, however, consideration must be given to regulations regarding compounds permissible in livestock diets.

Carboxymethylcellulose (**CMC**) is currently utilized in filters to aid in removal of impurities from water and gases. It also has FDA approval for many common uses and is incorporated into many household food and drug items as a bulking agent or thickener. Carboxymethylcellulose is degraded to varying extents both anaerobically and aerobically by microbial cellulase. Rusten et al. (1993) replaced the standard ferric chloride and alum flocculant system for DAF with lactic acid and CMC. They found that the CMC system removed 4-6% less of the chemical oxygen demand than the ferric chloride system; however, both systems removed approximately 60% of chemical oxygen demand. The CMC system removed about 60% of suspended protein as compared to the 80% removal rate of the standard system. Even though the CMC was less effective than ferric chloride,

the benefits it might offer to final feed product quality and stability and to the environment, should the skimmings be land-applied, might offset its sub-standard performance as a flocculant.

With the current push for development of biologically renewable fuel alternatives growing stronger, the need for disposal of waste materials will only increase. At the same time, scrutiny of the environmental impact of disposal techniques will also rise, forcing industries to use caution when they develop their manufacturing processes. Our research has demonstrated that recycling waste materials into feedstuffs is possible and that the feedstuffs so generated can support animal production. A concerted effort between industry and science could allow the development of novel processes for manufacture that not only result in the production of the desired consumer product, but also generate waste materials which have a minimum of biohazards and so would be more readily utilized as feedstuffs. Industry and academia can work together to foster acceptance of recycled nutrient feedstuffs by demonstrating their benefits not only to the environment, but also to everyone's financial security. Scientists must also demonstrate that these recycled nutrient feedstuffs are safe not only to the livestock that consume them, but also to the consumers of animal products.

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