AMINO ACID SUPPLEMENTATION OF HYDROLYZED FEATHER MEAL DIETS

FOR FINISHER PIGS

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Kalyan Chakravorty Divakala, son of Venkata Rao Divakala and Lakshmi Divakala, was born August 20, 1977 in Visakhapatnam, India. In 1994, he graduated from Dr. L. B. College (High School) in Vizag, India. He attended Rajiv Gandhi College of Veterinary and Animal Sciences in Pondicherry, India and graduated with a Bachelor of Veterinary Science and Animal Husbandry Degree in September, 2001. After working as a veterinarian in Andhra Pradesh, India for four years, he entered Graduate School, Auburn University under the guidance of Dr. Lee I. Chiba.

THESIS ABSTRACT

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The objective of this study was to determine the possibility of replacing soybean meal (SBM) in pig diets completely with hydrolyzed feather meal (FM). Corn-SBM, finisher 1 and 2 positive control (PC) diets were formulated to contain 6.1 and 4.7 g apparent ileal digestible (AID) Lys/kg, respectively, and corn-FM, negative control (NC) diets were formulated to be iso-N to the PC diet. The NC diet were supplemented with AA to satisfy all the AID indispensable AA requirements based on the 1998 NRC AID AA (NRC; NC + Lys and Trp) and the assumption that the apparent ileal digestibility of all indispensable AA in FM is 40% (40-2AA = NC + Lys, Trp, and Thr, but no His and Ile, and 40All = NC + Lys, Trp, Thr, His, and Ile). Forty-five gilts and 45 castrated males (57.8 ± 0.8 kg; 3 gilts or 3 castrated males/pen) were randomly assigned to 5 finisher 1 diets. At 81.0 ± 1.4 kg, pigs were offered finisher 2 diets. Pigs had ad libitum access to feed and water, and blood samples were collected before slaughter. Pigs were slaughtered

at the end of the study (112.1 ± 1.8 kg). As expected, overall ADFI, AID Lys intake (LysI), ADG, and G:F were greater and G:LysI was lower in pigs fed the PC diet than those fed the NC diet. ($P < 0.001$). Overall G:LysI tended to be lower in pigs fed the NRC diet than those fed the PC diet ($P = 0.083$) or the 40-2AA and 40All diets ($P = 0.094$), and pigs fed the 40All diet had numerically higher G:F ($P = 0.119$) and G:LysI ($P = 0.160$) than those fed the 40-2AA diet. Pigs fed the PC diet had more serum albumin and total protein ($P < 0.001$) but less glucose ($P = 0.031$) and cholesterol ($P < 0.001$) than those fed the NC diet, and total protein was higher ($P = 0.031$) in pigs fed the 40All diet than those fed the 40-2AA diet. Diets had no effect on urea N or triglycerides. Pigs fed the PC diet had less average backfat than those fed the NC diet ($P = 0.016$) or the NRC diet ($P = 0.020$). The LM was greater ($P < 0.001$) in pigs fed the PC diet or the 40All diet than those fed the NC or the 40-2AA diet, respectively. Pigs fed the PC diet had greater ($P < 0.01$) % fat-free lean, lean gain (LG), and LG:F than those fed the NC diet, but their LG:F or LG:LysI was similar to those fed the NRC diet. The LG:F ($P = 0.030$) and LG:LysI ($P = 0.028$) were lower in pigs fed the NRC diet than those fed the 40-2AA and 40All diets, and LG:LysI tended to be higher ($P = 0.068$) in pigs fed the 40All diet than those fed the 40-2AA diet. Pigs fed the 40All diet had greater ($P < 0.001$) meat color, firmness, and marbling scores than those fed the 40-2AA diet. Diets had no clear effect on organ weights. The results indicated that the FM diets supplemented with crystalline AA were not as good as the corn-SBM diets in terms of supporting weight gain. However, the results seemed to indicate that pigs fed the FM diets supplemented with the necessary AA can utilize AA and feed for weight gain and LG as efficiently as those fed the corn-SBM diet.

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I. INTRODUCTION

For a successful and sustainable swine production, it is not only important to increase nutrient utilization, but also explore fully the potential of all amino acid sources or finding alternative sources of amino acids as a feed ingredient (Chiba, 2001). The effort to find alternative amino acid sources is important because of increased demands for sources of the amino acids and continuous increase in feed costs. Nonruminant species, including humans, have similar nutrient requirements. For instance, they have requirements for amino acids and not crude protein per se. The primary sources of amino acids in nonruminant livestock diets have been conventionally byproducts of human food industry. With the ever-increasing human population, there is an increasing demand to extract quality amino acids from those byproducts for human consumption. Under such circumstances, the competition between humans and nonruminant species, such as swine and poultry, for quality sources of amino acids is likely to increase continuously.

It has been estimated that energy sources and protein supplements together account for more than 90% of the total feed costs in swine production (SCA, 1987), and, protein supplements are generally more expensive than energy sources in terms of the cost/unit. The ever-increasing feed costs in swine industry would further add to the necessity of exploring newer, viable, and inexpensive alternative feed ingredients that could replace conventional feed ingredients (Chiba, 2001). Furthermore, swine

producers are highly concerned about the optimum utilization of amino acid sources simply because any inefficient utilization of amino acids would lead to adverse environmental effects, which in turn could be detrimental for the success and sustainability of the swine industry. Thus, it is essential to find alternative sources of amino acids that can be utilized efficiently.

In modern swine industry, corn-soybean meal-based diets are the most popular as they provide a proper balance of amino acids (Aherne and Kennelly, 1985; Seerley, 1991; Cromwell, 1998). Soybean meal accounts for more than 85% of all the major protein supplements fed to swine (Cromwell, 1998). It is a byproduct of soy oil production, and it is available in either 44 or 48% crude protein for swine diets. In recent years, after oil extraction, soybeans have been also used to produce protein rich and medicinally beneficial human food products such as soy protein isolate and soy protein concentrate. Because of high protein availability (Erdman and Fordyce, 1989; Young, 1991) and specific potential health benefits, such as decreasing the risk of heart diseases (Lichtenstein, 1998), there is an increasing demand for those products for human nutrition. In future, there is a possibility of processing larger quantities of soybeans to produce those products than soybean meal. In such a scenario, the demand for soybean meal exceeds supply and thereby limiting its use as a major protein supplement in swine diets. Thus, it is essential to find alternative and viable protein or amino acid sources that can replace soybean meal as a major protein supplement in swine diets. Over the years, studies have been conducted to replace soybean meal, either partially or completely, with various alternative protein sources in typical swine diets (Aherne and Kennelly, 1985; Church, 1986; Thacker and Kirkwood, 1990). The

alternative protein sources are either plant or animal origin, and they differ in their feeding values due to variations in their nutrient contents, palatability, handling property, and other factors. Several factors such as economic feasibility, nutritive potential, and environmental implications should be considered while choosing an appropriate alternative protein feedstuff. Although several studies have been conducted to partially replace soybean meal in typical corn-soybean meal based swine diets with alternative protein sources, there are only limited number of studies that explored the possibility of completely replacing soybean meal in pig diets (Shelton et al., 2001).

One of the potential alternative protein sources is hydrolyzed feather meal (**FM**) because of its high protein content. In addition, it is highly available, and has no anti-nutritional factors and, apparently, no risks of disease transmission (unlike some of ruminant-based products, which are capable of transmitting diseases such as Bovine Spongiform Encephalopathy), thus, FM can be an attractive alternative protein source for nonruminant diets.

In this study, the possibility of replacing soybean meal in finisher pig diets completely with FM by amino acid supplementation was explored. The amino acids were added to the FM diets based on 1998 NRC apparent ileal digestibility values for indispensable amino acids and the assumption that the apparent ileal digestibility of all indispensable amino acids in FM is 40%. Specific objectives were to investigate the effect of crystalline amino acid supplementation of FM diets on growth performance, carcass traits, subjective meat quality scores, organ weights, and serum metabolites.

II. LITERATURE REVIEW

Poultry Feathers

***General Features*.** Poultry feathers represent about 5 to 7% of the body weight of the domestic fowl. They are high in N constituting 15% of their composition. The major protein in feathers is keratin, which constitutes 90% of feather weight (Harrap and Woods, 1964; Tiquia et al., 2005). Although feathers constitute the most abundant keratinous material in nature, their biological uses have not been fully exploited (Onifade et al., 1998). It is, therefore, essential to explore the potential of these N rich products for biological uses, including as a source of nutrients for livestock.

Keratin – Structure and Properties. The structure of keratin is tightly packed, as it exists in beta form with formation of a super coiled polypeptide chain (Parry and North, 1998). Keratin is characterized by the presence of a large quantity (about 8.8%) of Cys, a sulfur containing AA (Goddard and Michaelis, 1934; Harrap and Woods, 1964; Onifade et al., 1998). The Cys has a tendency to form disulphide bonds with another Cys, and, thereby, providing structural rigidity to keratin molecule. In addition, the rigid keratin structure is further enchanced by extensive hydrogen bonds and hydrophobic interactions among the polypeptides. Thus, the rigid structure of keratin also provides chemical resistance against many digestive enzymes.

Scale of Feather Production. Based on the estimates by the National Chicken Council (2000), more than 8.5 billion chickens are commercially grown in the United States annually, and the processing of those chickens generate more than 2.3 billion pounds of feathers (Mc Govern, 2000). It was also estimated that a typical broiler plants generate feathers at an average rate of about 4,000 pounds an hour and 65,000 pounds a day (McGovern, 2000). The enormous scale and rate of feather production would increase the cost and labor for their disposal, and thereby, decrease profit margin of the broiler production. In addition, if not disposed properly, feathers also affect environment adversely. It is, therefore, essential to find viable means or methods to dispose or recycle feathers in an efficient and environmentally friendly manner.

Disposal/Recycling Methods. Currently, the most popular method of recycling feathers is composting, a natural recycling method, which converts the N rich feathers into potential organic fertilizer (McGovern, 2000). Composting is considered to be economically feasible and environmentally friendly method of recycling feathers (Tiquia et al., 2005). However, this method alone cannot handle the overwhelmingly large volumes of feathers generated on a daily basis by the broiler industry. Therefore, alternative methods have been constantly explored to recycle feathers. Recycling feathers to generate commercially useful fiber is one such alternative method (Mc Govern, 2000). However, it seems that the most efficient method is to convert feathers into one of the components in animal feeds.

Recycling Feathers - Benefit both the Broiler and Swine Industries. Enforcement of stringent environmental policies, along with labor costs to handle feather waste, could actually impede competitiveness of the broiler industry. Increasing the

market value of the feathers by converting them into a value added product such as feather meal can enhance the competitiveness of the broiler industry (Mc Govern, 2000). On the other hand, evaluating a potential of feather meal as a source of AA would contribute to the effort to explore fully the potential of all AA sources or protein supplements for successful and sustainable pig production. Thus, any effort to increase utilization of feather meal as a major source of AA in swine feeds would be mutually beneficial for the poultry and swine industries.

Utilization of Keratin in Animals. Although feathers contain more than 80% protein, they are of very little nutritive value. This is simply because of the inability of most of the animal species to digest keratin protein in feathers (Naber and Morgan, 1956). Most animal species lack a hydrolyzing enzyme called keratinase that is capable of breaking disulphide bonds in keratin. Therefore, feathers are subjected to external hydrolysis to improve their nutritive value (Latshaw, 1990).

Hydrolyzed Feather Meal (FM) in General

Processing of Feathers

Introduction. The protein in feathers was found to be moderately susceptible to trypsin when it was subjected to ball-milling or fine grinding (Routh, 1938). Draper (1944) demonstrated first that the nutritive value of the feathers can be improved by subjecting them to heat. This indicated that a source of energy such as heat is essential to hydrolyze feather protein. A moderate growth was reported in young rats and chickens when they were fed feathers in powdered form (Routh, 1942; Newell and Elvehjem, 1947). In chickens fed feather meal, a positive correlation was reported between their growth rate and the degree of keratin breakdown during heat processing (Naber and

Morgan, 1956). These findings indicate that processing would improve the nutritional quality of feathers by hydrolyzing keratin. As the processing involves hydrolysis, the end product of processed feathers is often called hydrolyzed feather meal (FM).

***Processing Methods*.** Feathers can be processed by many different methods. But, it is important to select method that is most cost-effective (Dalev, 1994; Kherrati et al., 1998; Coward-Kelly et al., 2006) and environmentally friendly. The most efficient and modern method is hydrothermal treatment or autoclave (Papadopoulos, 1985). As the product obtained through this method yield much better results, it has become widely popular method in the rendering industry. It was also reported that subjecting feathers to enzymatic treatment prior to hydrolysis could improve the available protein and AA in FM (Barbour et al., 2002). Hydrothermal treatment involves three main steps to produce FM, i.e.: 1) hydrolysis, 2) drying, and 3) grinding. During the first step of hydrolysis, feathers are subjected to an optimum combination of temperature, pressure, and time to hydrolyze keratin protein (Mc Casland and Richardson, 1966). Hydrolysis is followed by drying, during which the moisture content of the hydrolyzed product is reduced to certain desired levels. Finally, the product is subjected to grinding, where it is ground to a size suitable for use in animal feeds. This is achieved by allowing the product to pass through a screen of specific size. However, certain AA are destroyed during hydrolysis, resulting in FM with low nutritional value. Some studies are done to produce the highest quality FM by optimizing temperature, time, and pressure during the hydrolysis. The hydrothermal treatment method demands more energy inputs and can destroy, again, certain AA (Wang and Parsons, 1997). It is, therefore, important to find alternative processing methods (Onifade et al., 1998).

Microbial keratinolysis is one of the most innovative alternative strategies to hydrolyze keratin in feathers by using certain keratinolytic microbes. Microbes, such as *Bacillus licheniformis* (Williams et al., 1990; Lin et al., 1992), *Streptomyces fradiae* (Elmayergi and Smith, 1971; Young and Smith, 1975), *Kochuria rosea* (Vidal, 2000; Bertsch and Coello, 2005), and strain kr2 of *Vibrio* species (Sangali and Brandelli, 2000) have been found to possess keratinolytic activity to hydrolyze feathers. Further research is needed to evaluate the quality of FM produced by microbial keratinolysis. However, this seems to be an economically feasible and environmentally friendly. Any processing methods should be cost-effective and environmentally friendly with maximum improvement of the nutritional quality of the product.

Quality of FM

***Factors Influencing the Quality*.** Several factors influence the final composition of FM. For instance, FM composition is primarily affected by composition of raw feathers, which in turn is influenced by factors such as species, breed, and age of the bird. In addition, it was also reported that processing conditions can affect some of the proximate principles such as CP, EE, and ash (Sullivan and Stephenson, 1957; Combs et al., 1958; Han and Parsons, 1991), and AA contents (Gregory et al., 1956; Johnston and Coon, 1979a; Papadopoulos et al., 1985; Han and Parsons, 1991; Moritz and Latshaw, 2001), and AA digestibility (Sullivan and Stephenson, 1957; Naber et al., 1961; Papadopoulos, 1985; Latshaw et al., 1994) of FM. Among AA, Lys (Carpenter, 1973) and Cys (Papadopoulos et al. 1985; Latshaw, 1990; Wang and Parsons, 1997) were found to be more sensitive to processing conditions. The Cys losses during processing primarily due to the formation of a nonproteinogenic AA called lanthionine have been reported

(Baker et al., 1981; Wang and Parsons, 1997). It was reported that high concentration of lanthionine in FM reduces AA availability (Baker et al., 1981; Han and Parsons, 1991). The Lys can also form nonproteinogenic AA such as lysinoalanine during processing (Wang and Parsons, 1997). Most of the nonproteinogenic AA decrease the nutritive value of FM by causing considerable losses of available AA.

***Methods to Evaluate the Quality*.** Selecting appropriate procedure to evaluate nutritional quality can influence the reported nutritive values of FM (Moritz and Latshaw, 2001). In vitro pepsin digestibility assay is widely used to evaluate the protein quality of FM simply because it saves time, labor, and cost (Han and Parsons, 1991). However, the concentration of pepsin used in the assay influences the results (Han and Parsons, 1991). Some studies indicated that a concentration of 0.002% yielded much better results than 0.2% pepsin (Johnston and Coon, 1979b; Bielorai et al., 1982; Han and Parsons, 1991). Therefore, it is essential to standardize the concentration of pepsin used for the assay. Results obtained by in vitro pepsin digestibility of FM did not correlate well with those obtained by growth assay in chicks (Bielorai et al., 1982). In addition, most studies were conducted with experimentally or laboratory processed feathers, and not with commercial processed products (Wang and Parsons, 1997). It is, therefore, necessary to evaluate the nutritional quality of commercially prepared FM samples with a standard procedure. Despite some limitations, the pepsin digestibility assay is still widely used as a standard to define the quality of FM. The American Association of Feed Control Officials (1995) defines FM as “the product resulting from the treatment under pressure of clean, undecomposed feathers from slaughtered poultry, free of additives and or accelerators

and having a CP digestibility of not less than 75% in the in vitro pepsin digestibility assay".

Amino Acid Content of Hydrolyzed Feather Meal. Hydrolyzed feather meal was found to contain large amounts of Gly, Phe, Thr, and sulfur AA (Cys, and Met), and Arg (Eggum, 1970; Liu et al., 1989; Han and Parsons, 1991). However, it is low in Lys and certain other indispensable AA that limit its extensive use in swine diets, where Lys is considered to be the first limiting AA in typical pig diets. As in many other animal protein sources, the AA composition of FM is highly variable (Wang and Parsons, 1997).

Enhancing Nutritive Value of Hydrolyzed Feather Meal. Nutritional quality of FM was improved when small amounts of other protein sources such as blood meal and poultry by-product meal were added either during (Burgos et al.1974; Pate et al., 1995) or after (Goedeken et al., 1990b) processing feathers. Hydrolyzed feather meal complements blood meal simply because the former is high in sulfur-containing AA but low in Lys, whereas the latter is high in Lys (Blasi et al., 1991; Gibb et al., 1992).

Hydrolyzed Feather Meal vs. Soybean Meal

Hydrolyzed feather meal was found to be superior to SBM in some AA content such as total Cys, Val, and Thr (Bielorai et al., 1983). Except Lys, the bioavailability of other indispensable AA for nonruminants seemed to be similar between FM and SBM (Bielorai et al., 1983; Knabe et al., 1989; Han and Parsons, 1991). Thus, FM has a potential to replace SBM in nonruminant diets especially after supplementation with certain AA such as Lys.

Limitations of Hydrolyzed Feather Meal

As in many other feed ingredients, FM also has certain limitations for its use as a component of livestock feeds, especially in nonruminant diets. For instance, FM has low content of some indispensable AA such as Lys, Met, His, and Trp (Routh, 1942; McCasland and Richardson, 1966; Moran, et al., 1966; Wessels, 1972; Luong and Payne, 1977; Liu et al., 1989), and low digestibility of N and AA (Bielorai et al., 1982; Knabe et al., 1989). Also, although there is no data, FM diets might be low in palatability.

Hydrolyzed Feather Meal for Ruminants, Poultry, and Fish

Ruminants

General. As a source of high CP, FM can be fed to ruminants at a higher rate without adversely affecting performance. The nutritive value of FM in ruminants is primarily attributed to its high content of ruminally undegradable protein (**RUP**; Thomas and Beeson, 1977; Daugherty and Church, 1982; Goedeken et al., 1990b). It has been reported that it contains almost twice the RUP compared with SBM (Goedeken et al., 1990b), and feeding diets high in RUP would improve performance and productivity of ruminants by increasing N and AA flows into the small intestine where most absorption of nutrients take place (Cecava and Parker, 1993; Coomer et al., 1993; Sindt et al., 1993; Zinn and Owens, 1993). However, the important factor that limits the utilization of RUP is its digestibility. Some studies have been conducted to evaluate digestibility of RUP in FM compared with RUP sources such as SBM and cottonseed meal. It was found that digestibility of RUP in FM was similar to cottonseed meal (Aderibigbe and Church, 1983), but lower than SBM (Thomas and Beeson, 1977; Church et al., 1982).

Nevertheless, as a potential source of RUP, FM can be used to improve performance and production of ruminants.

***Lactating Cows*.** Only limited data are available with regard to utilization of FM in lactation diets (Harris et al., 1992). It has been suggested that FM could be used as a supplemental protein source in lactating dairy cows (Kellems et al., 1989). As a potential source of RUP, it can improve the flow of certain indispensable AA to intestine that might limit the quantity and quality of milk in lactating cows. By including FM in lactation diets, milk production was improved (Cunningham et al., 1994). However, when dairy cattle was fed both FM and blood meal (another RUP source) together, much better results were obtained than feeding FM or the SBM alone (Waltz et al., 1989; Johnson et al., 1994). This indicates that complementary effects exist between FM and blood meal with regard to supply of AA to the small intestine. Thus, the availability of AA in FM diets is low compared to those in diets containing a combination of FM and blood meal or only SBM. Contrary to these findings, Bas et al. (1989) indicated that feeding a combination of FM and blood meal in dairy cattle could affect the production adversely by reducing the flow of microbial protein to the small intestine. According to those authors, feeding FM and blood meal to dairy cattle can result in reduction of the ruminal ammonia N and the supply of peptides, AA, and branched chain volatile fatty acids (which are obtained by breakdown of true protein), and thereby inhibit microbial growth. Thus, FM diets could influence the flow of microbial protein, along with/without postruminal AA to the small intestine affecting the milk production in lactating animals. On the other hand, feeding FM to dairy cattle could have a protein sparing effect in lactating diets when included at 3% in 14% CP diets by providing a perfect balance of the

RUP, thereby, improving milk production similar to that obtained with 18% CP diets (Harris et al., 1992). With increasing rates of inclusion of FM in lactation diets, protein percentage can be decreased without affecting fat percentage (Harris et al., 1992). An interaction between the CP content and the inclusion rate of FM in lactating cow diets has been also found (Harris et al., 1992). The inclusion of FM in lactation cow diets could affect both the quantity and quality of milk in dairy cattle.

Growing Cattle. Hydrolyzed feather meal has been found to contain ruminal escape sulfur AA that may limit growth in ruminants (Goedeken et al., 1990b; Klemesrud et al., 2000). Hydrolyzed feather meal could be an excellent and economical source of RUP in molasses-based liquid supplements fed to yearling cattle consuming moderate-quality forage (Pate et al., 1995). In another study, Brown and Pate (1997) indicated that feeding FM to steers improved their performance more than when fed cottonseed meal at a lower rate of protein supplementation. Feeding FM to growing steers has been shown to be an excellent source of metabolizable protein, although no complementary effects to improve protein efficiency were noticed when fed along with poultry by-product meal (Klemesrud et al., 1998).

Sheep. Hydrolyzed feather meal may replace SBM in protein supplements fed to sheep grazing on low quality forages, and improve their performance (Thomas et al., 1994). Replacing SBM with FM improved weight gain in fattening lambs (Jordon and Croom, 1957; Thomas et al., 1994). However, FM decreased growth when it supplied all the supplemental protein in growing-fattening diets for lambs (Huston and Shelton, 1971). In another study, Ely et al. (1991) indicated that FM can replace SBM up to 50% in finisher diets. Feeding FM to sheep by substituting for SBM did not affect the rate or

extent of ruminal neutral detergent fiber fermentation and the wool characteristics such as wool fiber length, wool fiber diameter, or wool sulfur concentration (Thomas et al., 1994).

Poultry

The use of FM in poultry diets was well documented in the literature, and FM replaced a portion of protein sources in practical poultry diets (Wisman et al., 1958; Balloun and Khajarern, 1974; Cupo and Cartwright, 1991). Earliest studies indicated that the potential of FM to replace some portion of other protein sources, especially in high protein diets, owes primarily due to its nonspecific N content (Wilder et al., 1955; Naber and Morgan, 1956; Sullivan and Stephenson, 1957; McKerns and Rittersporn, 1958; Sibbald et al., 1962). It has been also postulated that FM might contain an unidentified factor that was responsible for growth in chicken (Naber and Morgan, 1956; Lillie et al., 1956). However, complete replacement of SBM with FM in a corn-SBM diet for broiler chicks decreased growth and feed efficiency (Naber et al., 1961). Abdella et al. (1996) concluded that FM can replace up to 75% of SBM in broiler diets. Hydrolyzed feather meal showed a positive complementary effect with broiler offal in broiler diets, and therefore FM and broiler offal could substitute major feed ingredients in broiler diets (Isika et al., 2006). In turkeys, it has been reported that FM can be included up to 6% in grower diets without negatively affecting performance (Eissler and Firman, 1996). On the whole, these findings indicate that FM cannot be used to replace SBM completely or at higher levels due to nonavailability of certain AA that limit growth in chickens. Moran et al. (1966) indicated that FM is limiting in four indispensable AA, Met, Lys, His, and Trp in 20% CP corn-FM diets for chicks. In another study, Smith (1968) indicated that Lys

and His in FM diets were poorly available. However, FM can be utilized as a potential source of extra dietary N to improve leanness of the carcass. The relationship between leanness and extra dietary N was discussed under the swine section. Feeding FM diets to the broilers provided extra dietary N and improved carcass leanness by decreasing abdominal fat (Cabel et al., 1987, 1988). In addition, some studies indicated that feeding FM to poultry can spare the use of synthetic Met (Wessels, 1972; MacAlpine and Payne, 1977). Thus, the feeding FM to poultry diets has wider applications in enhancing performance and leanness.

Fish

In fish diets, the primary source of protein/AA is fish meal. About 12% of fish meal produced in the word is being used in fish feeds, making it a product of high demand in the aquaculture industry. To reduce excess demands on fish meal, it is essential to find other sources of AA/protein that can replace fish meal (Rumsey, 1993). Replacing a portion of fish meal by FM has been shown to have positive effects on performance and production of several species such as chinok salmon (Fowler, 1990), rainbow trout (Hughes, 1991; Bureau et al., 2000), Japanese flounder (Kikuchi et al., 1994), tilapia (Viola and Zohar, 1984; Bishop et al., 1995), and Indian major carp (Hasan et al., 1997). As in other species, the digestibility value of FM in shrimp and fish is also low primarily because of unbalanced AA profile (Lee, 2002). To overcome the low digestibility problems, FM has been supplemented with certain indispensable AA, and, that has resulted in improved growth performance of fish (Tacon and Akiyama, 1997) and shrimp (Cheng, 2002).

Hydrolyzed Feather Meal for Swine

Very few studies were conducted to evaluate the inclusion rates of FM in swine diets (van Heugten and van Kempen, 2002). This is simply because of the low Lys availability (Lys being the first limiting AA in most practical swine diets) from FM. In spite of this limitation, Papadopoulos (1985) reported that the FM was used traditionally as one of the components in practical swine diets.

Finisher Pigs

General. As the feed costs during the finisher phase are very high, any effort to reduce feed costs will pay dividends. Thus, the utilization of inexpensive protein sources such as FM would decrease the cost of pork production. Also, it is possible that the finisher pigs might be better adapted to utilize unconventional feeds such as FM. In poultry, it has been reported that older (mature birds) seemed to utilize FM more efficiently when compared to younger birds (Morris and Balloun, 1973). It is possible that a well developed digestive system in older/finisher phase might help them to utilize unconventional feedstuffs such as FM. Thus, FM could be a potential choice as an alternative protein feedstuff in finisher pig diets.

Inclusion Rates in Corn-SBM Diets. Some of the earliest feeding studies failed to evaluate the potential value of FM as a protein source in grower-finisher diets (Hall, 1957). In those studies, protein sources were replaced by FM on weight by weight basis. Thus, those studies do not contribute towards understanding the effects of including FM in modern day pig diets. Following those earliest studies, not many studies on FM inclusion in swine diets have been conducted until the mid 90's. Chiba et al. (1995) indicated that the finisher pigs can utilize FM at higher rates of inclusion than those

traditionally recommended as long as they were formulated isolysinic to the corresponding SBM diets. Based on NRC requirements, FM diets were found to be limiting not only in Lys, but also possibly other AA such as Trp, His, and others (Chiba et al., 1996). Based on this contention, Chiba et al. (1996) substituted SBM completely with FM by crystalline Lys supplementation in the finisher diets. They found that feeding FM with Lys supplementation did not affect growth performance, but carcass quality was not reduced. They concluded that FM diets are not only limiting in Lys but also in other AA. They reported that FM can be included up to 9% of the diet without adverse effects on growth performance.

In another study, van Heugten and van Kempen (2002) evaluated the inclusion of FM up to 10% in grower and finisher diets. Their study indicated that feeding FM up to 10% has no effects on the performance during grower phase, while it affected performance during the finisher phase and entire grower-finisher phase. Also, FM up to 10% affected carcass characteristics adversely by increasing backfat thickness. They concluded that FM can be included up to 8% in corn-SBM finisher diets. This inclusion rate exceeds the maximum rate of 5% recommended by Seerley (1991), and is consistent with the findings by Chiba et al. (1996).

Apple et al. (2003) evaluated the effects of including FM up to 6% in both corn-SBM diets and in corn-SBM-wheat middling diets in two separate studies. The study indicated that feeding FM up to 6% in either diet did not affect growth performance. Their study also indicated that feeding 6% FM in corn-SBM-wheat middlings diets did not have any adverse effects on the pork color or water-holding capacity, but influenced muscle pH. The study concluded that the dietary wheat middlings affected pork quality

more than dietary FM, and FM could be included up to 6% without any adverse effects on pork quality. However, further research is necessary to evaluate the effect of feeding FM on pork quality.

In general, castrated males have fatter carcasses than gilts of similar lean growth potential because of their higher energy intake and faster growth during the finisher phase (Bruner and Swiger, 1968; Nold et al., 1997). Energy intake can be reduced by decreasing their feed intake. In the previous study, van Heugten and van Kempen (2000) indicated a decrease in feed intake with the 10% FM diet. Thus, feeding FM at higher rates could possibly improve carcass leanness of castrated males (Ssu et al., 2004). Based on this idea, Ssu et al. (2004) conducted a study, and the results indicated interactions between the period at which FM diets were fed and the average daily gains (ADG). They also indicated that the pigs weighing 36 kg had shown a linear decrease in their average daily gain with increasing inclusion rates of FM in their diets as compared to those weighing 60 or 86 kg. They also indicated that 10 and 20% FM diets decreased ADG and average daily feed intake without affecting the carcass composition of castrated males as compared to those of gilts. They concluded that FM, even at higher inclusion rates in finisher diets, failed to modify the carcass composition of castrated males as compared to those of gilts.

Hydrolyzed Feather Meal - A Source of Extra Dietary N & Carcass Quality. Finisher pigs have a tendency to deposit more fat in their body simply because they consume more energy than their requirements (Whittemore, 1985). As the demand for leaner pork increases, it is essential to explore the most optimum ways to increase leanness in finisher pigs. In addition to genetics, nutritional management can be

important in enhancing carcass leanness (Chiba et al. 1995). Some findings indicate that excess N or AA content (irrespective of their quality) of diets can affect carcass composition or improve leanness (Griffiths et al., 1977; Asche et al., 1985; Chiba et al., 1991b). Several factors were responsible for the positive relationship between feeding extra dietary N and carcass leanness. Diets high in AA content lead to increased urea formation which increase energy expenditure, thus, reducing the metabolizable energy in pig's body. It has been indicated that the deaminated AA can be utilized by animals less efficiently compared with carbohydrates or lipids (Schultz, 1975; Whittemore, 1985). Diets rich in AA content also contribute towards increasing mass of internal organs (Chiba, 1992, 1994) and(or) whole-body protein turnover, thus, increasing the energy expenditure (Reeds et al., 1981). In addition, it has been suggested that feeding diets high in AA content decreases feed intake (Chiba et al., 1991a). Furthermore, it is possible that feeding diets high in AA content would suppress the rate of lipogenesis (Allee et al., 1971; Yeh and Leveille, 1969). Thus, some or all of these factors decrease the energy status in the body, and thereby affecting the necessary energy to deposit body fat,thus, improving carcass leanness. However, there are certain disadvantages when high-protein diets are fed to animals. One of which is an increase in the production costs of the pigs, especially during the finisher phase (Whittemore, 1985). A possible decrease in the body weight (Whittemore, 1985) and negative implications on environment because of increased N excretion (Lenis, 1989) are the other limitations. As providing extra dietary N can be more important than the quality of protein, FM can be a viable source of extra dietary N to improve carcass leanness (Chiba et al., 1995). Based on this contention, Chiba et al. (1995) evaluated the use of FM as a source of extra dietary N in finisher pig

diets, and, the results indicated that low quality protein sources such as FM has the potential to prove extra dietary nitrogen, thus, improving leanness of finisher pigs.

Lactating Sows

As the productivity of sows is constantly increasing because of genetics and increased use of white line crossbred females, the nutrient requirements of the animals also increase. It is, thus, important to explore various AA sources to maximize litter and sow performance. Although the role of Val and its requirements are still not clear, it has been reported that Val could play an important role in lactating sows (Richert et al., 1996, 1997a,b). As FM contains 5.88% of Val, with a Val to Lys ratio of 2.83 (NRC, 1998), it can be a potential substitute for synthetic Val for lactating sows. However, Southern et al. (2000) reported that the inclusion of FM as a source of Val in lactating diets failed to show any positive response on sow productivity or litter performance. Further studies are required to establish the exact role and requirements of Val in lactating sows, and explore the possibility of FM as a substitute for synthetic Val in lactating sow diets.

Amino Acid Availability in Hydrolyzed Feather Meal

Amino Acid Availability

Availability/bioavailability of dietary AA was defined as “the proportion of ingested dietary AA that is absorbed in a chemical form that renders them potentially suitable for metabolism or protein synthesis” (Batterham, 1992; Lewis and Bayley, 1995). Availability involves the processes of digestion, absorption, and utilization of the nutrients by the tissue after absorption. The terms bioavailability and availability are used interchangeably in the literature.

Importance of Amino Acid Availability

When the swine diets are formulated on the basis of the total dietary AA content, it increases not only the cost of production but also affect environment adversely by increasing N excretion. This is simply because pigs can utilize only those AA that are available to them rather than the total dietary AA. Thus, to effectively utilize FM in swine diets, it is necessary to formulate diets by expressing the composition of FM, as well as AA requirements of pigs, in terms of the bioavailable AA (Tanksley and Knabe, 1984). Unfortunately, there is limited data in the literature on the availability of AA of FM. Thus, it is obvious that there is a growing need for evaluating the availability of AA in FM, which in turn would help the optimum utilization of FM.

Methods for Measuring Availability of Amino Acid

Although several methods have been used to evaluate the bioavailability of indispensable AA in a feedstuff, accurate estimation of AA bioavailability in feedstuffs is a challenging task (Stein et al., 2007). Some of the methods commonly used to evaluate AA bioavailability of feedstuffs include growth trials (slope-ratio assays), in vivo digestive studies, in vitro assays, and chemical techniques such as dye-binding.

Slope-ratio Assays. Slope-ratio assays can be used for estimating bioavailability of AA (Batterham, 1992). However, these assays are time consuming and also expensive to conduct as they require a large number of pigs for conducting the assay. In addition, the estimated availability value for AA depends on the response criterion (Adeola et al., 1994). From the previous studies, it has been indicated that slope-ratio method underestimates bioavailability (Stein et al., 2007). Gabert et al. (2001) indicated that the estimated availabilities are only relative values with high standard error of determination

and are related to the experimental conditions. These assays were used in chicks to evaluate AA availabilities in FM. But, they failed to produce accurate results in measuring availability of certain AA such as Lys and Met in FM (Han and Parsons, 1991).

Digestibility Studies. The most limiting factor that affects the availability is digestibility (Fuller, 2003). It has been indicated that AA availability could be accurately estimated by measuring their digestibility (Bragg et al., 1969; Leibholz, 1985). The digestible assays save time and labour (Batterham et al., 1990a). The digestibility assays can measure both the ileal and fecal digestibility of AA. However, ileal assay is more accurate simply because they disregard any post-ileal microbial metabolism that alters the undigested dietary AA profile (Zebrowska, 1973). It has been indicated that the ileal assays showed a higher positive correlation between ileal digestible protein and(or) AA and the protein synthesis/deposition in the body than those obtained by fecal assays (Moughan and Smith, 1985; Just et al., 1985).

However, ileal AA digestibility assay has certain limitations. It was reported that considerable variations of the ileal AA digestiblities were found among different samples of the same feedstuff such as SBM (Sauer and Ozimek, 1986). In addition, it has been indicated that there might not be any positive correlation between the digestibility and bioavailability of AA from feed stuffs that are subjected to heat processing (Beech et al., 1991). For instance, Lys, a dibasic AA, when subjected to heat, involves in Maillard reaction and forms products that might be absorbed but cannot be utilized for protein synthesis (Carpenter, 1960; Batterham et al. 1990a; Moughan and Rutherford, 1996). Reactions other than Maillard reaction were also cited for the poor retention of ileal

digestible AA such as Thr (Beech and Batterham, 1990). Thus, it is obvious that certain ileally absorbed indispensable AA from heat processed feedstuffs such as FM might not be available for protein synthesis, and the calculated digestibility value usually overestimates bioavailability. In addition, there is also a possibility of microbial fermentation taking place even in the upper gut, resulting in net loss or gain of AA (Stein et al., 2007). In spite of some of these limitations, it can be concluded that apparent ileal digestible assays are more practical to be used as an index of bioavailability (Sauer and Ozimek, 1986).

As mentioned earlier, not many studies were conducted to evaluate available AA in FM. Knabe et al. (1989) made a comparative digestibility study of different protein sources, which included FM, in growing pigs. In their study, pigs were initially reluctant to eat when FM was the sole source of protein in the diets, indicating the poor palatability of FM diets. The diets were, therefore, formulated with 6% protein from SBM and 6% protein from FM, and the digestibility values were calculated by difference method that disregarded any possible associate effects between the SBM and FM. The results indicated that FM had lowest ileal and fecal N digestibility than other protein feedstuffs, except cottonseed meal. Apparent ileal digestibility of AA were 40, 35, 60, and 66% for Lys, His, Trp, and Thr respectively. These findings were consistent with those obtained in poultry (Bielorai et al., 1983; Nordheim and Coon, 1984). Based on the study by Knabe et. al (1989), it may be possible that AID value of indispensable AA in FM is 40% , which is the AID value for Lys. On the other hand, NRC (1998) reported an AID value of 54% for Lys. When FM diets are formulated based on AID values of either 54% or

40%, some AA are found to be deficient. Thus, supplementation of those AA to FM diets might be required to replace SBM completely in pig diets.

Environmental Implications

High protein diets with low digestibility could affect environment adversely by increasing urinary N (Lenis, 1989). Thus, FM with high protein and low digestible N and AA could affect environment adversely. However, not many studies have been conducted to study the effects of feeding FM diets on the environment. van Heugten and van Kempen (2002) evaluated dry matter, N, and phosphorus digestibilites to study their excretion rates. Although their results were not conclusive, they indicated that the inclusion of FM in corn-SBM diets influence the excretion rates of N, phosphorus, and various odorous compounds that include volatile fatty acids, phenols, and indoles.

Crystalline Amino Acids in Nutrition

In nonruminant nutrition, crystalline AA have been used to replace a portion of CP to supply deficient AA, and thereby improving performance and alleviating environmental problems associated with high CP diets. It has been indicated that a 1% drop in CP by supplementing with crystalline Lys resulted in 8% drop in N excretion (Kerr and Easter, 1995). Currently, feed grade form of Lys, Thr, Trp, and Met are commercially available. Most of these synthetic AA are available in L-form as the L-form has more bioactivity than that of the D-form. For instance, feed grade Lys is most commonly available as L-Lys monohydrochoride. However, Met is available both in D- and L- form as both their forms have bioactivity.

As pigs are fed ad libitum most of the time, problem of inefficient utilization of crystalline AA is not of practical importance. When compared to protein-bound AA,

crystalline AA have been shown to have rapid absorbtion in small intestine (Yen et al., 2004). Digestibility of crystalline Lys and Met has been assumed to be 100% in pigs (Batterham, 1984; Izquierdo et al., 1988).

SUMMARY

In the United States, more than 8.5 billion chickens are commercially grown annually, and the processing of those chickens generate more than 2.3 billion pounds of feathers. Feathers are N rich products that could be a potential source of nutrients for livestock. The rigid structure of keratin in feathers provides chemical resistance against many digestive enzymes, thus, necessitating external processing. The most efficient method to process feathers is hydrothermal treatment, which converts feathers to FM.

Hydrolyzed feather meal is rich in many AA, and it can be an attractive source of AA for livestock diets. Hydrolyzed feather meal can be used in different species such as a potential source of RUP for ruminant species, partial replacement of protein sources for poultry diets, and a source of extra dietary N to improve carcass leanness of broilers and finisher pigs. Hydrolyzed feather meal is, however, deficient in lysine and certain other AA for nonruminant diets. Very few studies were conducted to evaluate the inclusion rates of FM in swine diets, probably, because of its low lysine content, which is crucial for typical swine diets. Those limited number of studies indicate that FM can be included up to 9 to 10% in pig diets.

Supplementation with deficient indispensable AA might be the most effective way to utilize FM in swine diets, and also it is necessary to formulate diets based on bioavailable AA. Unfortunately, there are limited data in the literature on the availability of AA in FM. In one study, AID of indispensable AA in FM was investigated, and the

AID value of Lys has been estimated to be only 40%, which is lower than that reported for Lys (54%) by the 1998 NRC.

Crystalline AA have been used in nonruminant diets to replace a portion of protein supplementation to supply deficient AA, thus, improving performance and alleviating environmental problems associated with, especially, high CP diets. When compared with protein-bound AA, crystalline AA have been shown to be absorbed more rapidly, which may affect the efficiency of AA utilization. But, it may not be important because most growing pigs are fed ad libitum. And, digestibility of Lys and other AA has been assumed to be 100% in pigs.

III. AMINO ACID SUPPLEMENTATION OF HYDROLYZED FEATHER MEAL FOR FINISHER PIG DIETS

Amino Acid Supplementation of Hydrolyzed Feather Meal Diets for Finisher Pigs[1,2]

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ABSTRACT: The objective of this study was to determine the possibility of replacing soybean meal (SBM) in pig diets completely with hydrolyzed feather meal (FM). Corn-SBM, finisher 1 and 2 positive control (PC) diets were formulated to contain 6.1 and 4.7 g apparent ileal digestible (AID) Lys/kg, respectively, and corn-FM, negative control (NC) diets were formulated to be iso-N to the PC diet. The NC diet was supplemented with AA to satisfy all the AID indispensable AA requirements based on the 1998 NRC AID AA (NRC; NC + Lys and Trp) and the assumption that the apparent ileal digestibility of all indispensable AA in FM is 40% (40-2AA = NC + Lys, Trp, and Thr, but no His and Ile, and 40All = NC + Lys, Trp, Thr, His, and Ile). Forty-five gilts and 45 castrated males (57.8 ± 0.8 kg; 3 gilts or 3 castrated males/pen) were randomly assigned to 5 finisher 1 diets. At 81.0 ± 1.4 kg, pigs were offered finisher 2 diets. Pigs had ad libitum access to feed and water throughout the study. At the end of the study (112.1 ± 1.8 kg), blood samples were collected by vena cava puncture using a sterile syringe and needle to assess serum metabolites. Pigs were slaughtered using conventional procedures, and standard carcass data were collected. To assess gross metabolic alterations, internal organs were collected and weighed separately. As expected, overall ADFI, AID Lys (representing indispensable AA) intake (LysI), ADG, and G:F were greater and G:LysI was lower in pigs fed the PC diet than those fed the NC diet ($P < 0.001$). Overall G:LysI tended to be lower in pigs fed the NRC diet than those fed the PC ($P = 0.083$) or 40-2AA and 40All diets ($P = 0.094$), and pigs fed the 40All diet had numerically higher G:F ($P = 0.119$) and G:LysI ($P = 0160$) than those fed the 40-2AA diet. Pigs fed the PC diet had more serum albumin and total protein ($P < 0.001$) but less glucose ($P = 0.031$) and cholesterol ($P < 0.001$) than those fed the NC

diet, and total protein was higher (P = 0.031) in pigs fed the 40All diet than those fed the 40-2AA diet. Diets had no effect on urea N or triglycerides. Pigs fed the PC diet had less average backfat than those fed the NC (P = 0.016) or the NRC diet (P = 0.020). The LM was greater (P < 0.001) in pigs fed the PC or the 40All diet than those fed the NC or the 40-2AA diet, respectively. Pigs fed the PC diet had greater (P < 0.01) % lean, lean gain (LG), and LG:F than those fed the NC diet, but their LG:F or LG:LysI was similar to those fed the NRC diet. The LG:F (P = 0.030) and LG:LysI (P = 0.028) were lower in pigs fed the NRC diet than those fed the 40-2AA and the 40All diets, and LG:LysI tended to be higher (P = 0.068) in pigs fed the 40All diet than those fed the 40-2AA diet. Pigs fed the 40All diet had greater (P < 0.001) meat color, firmness, and marbling scores than those fed the 40-2AA diet. Diets had no clear effect on organ weights. The results indicated that FM diets were not as efficient as corn-SBM diets in terms of supporting weight gain in pigs. However, the results seemed to indicate that pigs fed the FM diets supplemented with the necessary AA can utilize AA and feed for weight gain and lean gain as efficiently as those fed the corn-SBM diet. Further research is needed to investigate the optimum AA supplementation strategies to alleviate the weight gain depression associated with AA-supplemented FM diets.

INTRODUCTION

More than 8.5 billion chickens are commercially grown and processed in the United States, and those chickens can generate more than 2.3 billion pounds of feathers each year. Obviously, turning its major waste product into valuable commercial products would be extremely beneficial for the competitive poultry industry. Although the production of hydrolyzed feather meal (**FM**), along with composting, is the

recycling method capable of keeping up with the volume of feathers generated on a daily basis, the sheer volume of feathers can be overwhelming. Thus, increasing the market demand for FM would be obviously, important for successful and sustainable poultry production. The competition between humans and animals, especially nonruminant species, for quality sources of AA is likely to increase continuously because of the ever-increasing world population. Clearly, it would be essential to find alternative sources of AA for successful and sustainable pig production. The effort to increase the market demand for FM by increasing its use in pig production is, therefore, mutually beneficial for the poultry and pig industries.

Hydrolyzed feather meal can be an excellent source of dietary AA for pigs, but the available data in the literature are quite limited. Because FM is low in Lys and certain other AA, it must be incorporated into diets based on the AA content. Unfortunately, this can increase the dietary protein content, which may lead to contamination of water and odorous emissions because of excess excretion of unused dietary N. Clearly, alleviating public concerns on the environmental issues is imperative for sustainable animal production. Supplementation of FM diets with appropriate AA, therefore, would be the most plausible and effective way to utilize FM in pig diets.

In this study, the possibility of replacing soybean meal in finisher pig diets completely with FM by amino acid supplementation was explored. The AA were added to the FM diets based on 1998 NRC reported apparent ileal digestible **(AID)** values for indispensable AA and the assumption that the apparent ileal digestibility of all indispensable AA in FM is 40%. Specific objectives were to investigate the effect of

supplementing crystalline AA to FM in finisher pig diets on growth performance, carcass traits, subjective meat quality scores, organ weights, and serum metabolites.

EXPERIMENTAL PROCEDURES

General

A total of 90 (45 gilts and 45 castrated males) crossbred (Yorkshire X Duroc) finisher pigs were used in the study. The protocol for animal care was approved by the Institutional Animal Care and Use Committee of Auburn University. The FM without added blood meal (American Proteins, Inc., Hanceville, AL) was used, and it was analyzed for CP and AA (Ajinomoto Heartland LLC, Eddyville, IL). The composition of FM, along with corn and SBM, is presented in Table 1.

Animals and Facilities

Pigs approaching 50.0 kg were selected based on their BW, sex, and ancestry, and moved into the open-sided grower finisher unit. Pigs were allocated to 30 pens (1.35 m^2/pig) with 3 gilts or 3 castrated males per pen, and pens were assigned randomly to 5 dietary treatments with 3 gilt and 3 castrated male pens per treatment. Because of availability of pigs, the study was conducted in 2 trials. The first trial used 15 gilts and 15 castrated males, whereas the second trial used 30 gilts and 30 castrated males. Two trials were approximately 4 wk apart, and the average minimum and maximum daily temperatures during the study were 14.4 and 27.5ºC, respectively.

When the average pen weight reached 57.8 ± 0.8 kg BW, pigs were offered one of the 5 finisher 1 diets. At an average pen weight of 81.0 ± 1.4 kg, they were switched to the finisher 2 diets. Pigs were allowed ad libitum access to feed and water during the study. Pig weights and feed consumption data were collected once a week.

Experimental Diets

Fundamental Assumptions on Amino Acid Availability. To utilize FM effectively, it is necessary to formulate diets based on AA availability. The most limiting factor in the complete utilization of protein is digestibility, i.e., once digested, AA are absorbed and utilized efficiently unless AA disproportions and other factors impede those processes. Therefore, the terms, AA availability and ileal digestibility are used interchangeably in this study. Also, the determination of true ileal AA digestible values would be affected more by the status of animals and other extraneous factors rather than inherent features of a feed ingredient itself. Therefore, it would be preferable to use AID values, which can be determined directly and much more easily and precisely, instead of the true digestible values.

Unfortunately, the AA availability data for FM in pigs are rather limited (Chiba, 2001). And, even with a plethora of data, those estimates are likely to vary widely simply because of inherent difficulties and variations associated with the determination of AA availability. The adequacy of Lys is the primary concern for most practical pig diets, and its AID in FM for pigs has been estimated to be as low as 40% by Knabe et al. (1989) who may have published the first report on the ileal digestibility of FM in pigs. Although the NRC (1998) publication includes both apparent and true ileal digestible values for FM, those estimates may not be universally applicable. Therefore, the use of an assumed range or ranges of availability values, such as the ileal digestibility of 40% for all the indispensable AA, in evaluating the nutritional value of FM may have immediate practical applicability, or it may have more practical significance in utilizing FM for pig production.

Diet Formulation. To maximize economic efficiency and minimize environmental impacts, supplying nutrients as close as possible to meeting but not exceeding the requirements of pigs would be advantageous (Chiba, 2000), thus, 2 diets, finisher 1 and 2, were used to satisfy the AA needs during the finisher phase. The experimental diets were formulated based on the results of the AA analysis of FM samples (Table 1). However, for corn and SBM, the energy and nutrient contents reported by the NRC (1998) were used for diet formulation because more than 1 batch of corn and SBM were used during the study. A corn-SBM positive control (**PC**) diet was formulated to contain 6.1 g AID Lys/kg to satisfy the requirement during the finisher 1 phase (Table 2). A corn-FM negative control (**NC**) diet was formulated to be iso-nitrogenous to the PC diet. The NC diet was supplemented with crystalline Lys and Trp (**NRC**; NC + Lys and Trp) to alleviate apparent deficiencies based on AID values reported by NRC (1998). In addition, the NC diet was supplemented further with the third limiting AA, Thr (**40-2AA** = NC + Lys, Trp, and Thr, but no His and Ile), and fourth and fifth limiting AA, His and Ile (**40All** = NC + Lys, Trp, Thr, His, and Ile) based on the assumption that the apparent ileal digestibility of all AA in FM is only 40% as reported for Lys by Knabe et al. (1989). The 40-2AA diet was included to assess whether it is necessary to supplement very minute amounts of His and Ile.

A similar approach was used to formulate finisher 2 diets, and the PC and AA-supplemented NC diets contained 4.7 g AID Lys/kg (Table 3). Because of reduced requirements, the NC diet was not supplemented with crystalline His and Ile during the finisher 2 phase. To supplement the NC diets with AA, the amount of corn was adjusted accordingly based on the product specifications for each crystalline AA source. To

avoid possible confounding effects of the energy density, poultry fat was added to maintain a constant DE content for all diets. No effort was made to maintain a constant AA balance, but the proportions of indispensable AA relative to Lys for the PC and AA-supplemented FM diets were above the balanced protein (NRC, 1998). Minerals and vitamins for all diets were provided in amounts calculated to meet or exceed the NRC (1998) requirements. Feed samples (approximately 500 g each) were collected from each batch of feed mixed, and were stored frozen until they were pooled, subsampled, and analyzed for CP (AOAC, 1995).

Blood Samples

When the average pen weight reached the final target weight, approximately 10 mL of blood sample was collected from each pig via vena cava puncture using a sterile syringe and needle after an overnight fast. Serum were separated by centrifugation for 15 min at 1,500 x g to obtain cleaner samples, and an aliquot was stored frozen at -20°C until analyzed for urea N, total protein, albumin, triglycerides, cholesterol, and glucose using the automatic analyzer (Clinical Pathology Laboratory, Auburn University).

Slaughter Procedures

At an average pen weight of 112.1 ± 1.8 kg, pigs were slaughtered using conventional procedures after 24-h fast. The eviscerated carcass was split longitudinally through the vertebral midline, and warm carcass weight was recorded. To make gross assessment of metabolic or physiological alterations, heart, liver, and kidneys were collected and weighed separately. After chilling for 48 h at 2°C, the right side of the carcass was weighed. Backfat thickness at the first rib, last rib, and last lumbar vertebra was measured. Longissimus muscle of the right side was exposed by a perpendicular cut

between the 10th and 11th ribs, and LM area was traced. Backfat thickness at the 10th rib (about ¾ distances along the LM toward the belly) was also measured. The exposed LM area was used to determine subjective meat quality scores for color, firmness, marbling, and muscling (NPPC, 1991). The rate of carcass lean accretion and the percentage carcass lean were estimated by using the equation reported by NPPC (2000).

Statistical Analysis

Data were analyzed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Initially, the data for the 2 trials were tested for the homogeneity of variance (Pearson and Hartley, 1956). The results indicated that the variances for 2 trials were homogeneous, thus, the data were combined and analyzed accordingly. The treatment, sex, trial, and interactions, and appropriate BW as a covariate(s) were included in the statistical models initially, and those interactions and covariates that did not reach a statistically significant trend (i. e., $P > 0.10$) were deleted from the final models. Preplanned, nonorthogonal contrasts were used to assess the treatment effects: a) PC vs. NC diet, b) PC vs. NRC diet, c) NRC vs. 40-2AA and 40All diets, and d) 40-2AA vs. 40All diet. The pen was considered as the experimental unit. The results were considered significant if $P \leq 0.05$.

RESULTS

Growth Performance during the Finisher 1 Phase

Average daily feed intake was lower in pigs fed the NC diet than those fed the PC diet ($P = 0.041$; Table 4). The ADFI of pigs fed the PC diet was not different from those fed the NRC diet. The ADFI of pigs fed the 40All diet tended to be lower than those fed the 40-2AA diet ($P = 0.088$). Pigs fed the NC diet had lower AID Lys intake

(**LysI**) than those fed the PC diet ($P < 0.001$). Average daily gain was lower in pigs fed the NC diet than those fed the PC diet ($P < 0.001$). Pigs fed the NRC diet had lower ADG than those fed the PC diet ($P = 0.002$). Gain to feed intake was also lower in pigs fed the NC diet than those fed the PC diet ($P < 0.001$). However, pigs fed the NRC diet had lower G:F than those fed the PC diet ($P = 0.032$). Gain to AID Lys intake (**G:LysI**) was greater in the pigs fed the NC diet than those fed the PC diet ($P < 0.001$). The G:LysI tended to be higher in pigs fed the PC diet than those fed the NRC diet ($P = 0.059$).

Growth Performance during the Finisher 2 Phase

Pigs fed the NC diet had lower ADFI ($P = 0.005$), ADG ($P < 0.001$), G:F ($P < 0.001$), and LysI ($P < 0.001$) during the finisher 2 phase than those fed the PC diet. The G:F ($P < 0.001$) and G:LysI ($P = 0.015$) were lower in pigs fed the NC diet than those fed the PC diet. Pigs fed the NRC diet tended to have lower ADG than those fed the PC diet ($P = 0.079$). The ADFI tended to be higher ($P = 0.057$) and LysI was higher ($P = 0.039$) in pigs fed the NRC diet than those fed the 40-2AA and the 40All diets.

Overall Growth Performance during the Finisher Phase

The overall LysI ($P < 0.001$), ADG ($P = 0.004$), and G:F ($P < 0.001$) were lower in pigs fed the NC diet than those fed the PC diet. Pigs fed the NC diet had better G:LysI than those fed the PC diet ($P < 0.001$), and, pigs fed the NRC diet tended to have lower G:LysI than those fed the PC diet ($P = 0.083$) or the 40-2AA and 40All diets ($P = 0.094$). Pigs fed the 40All diet had numerically higher G:F ($P = 0.119$) and G:LysI ($P = 0.160$) than those fed the 40-2AA diet.

Carcass Characteristics

Diets did not affect dressing percentage (Table 5). A decrease in HCW was observed in pigs fed the NC diet than those fed the PC diet ($P < 0.001$). Average backfat thickness was higher in pigs fed the NC diet than those fed the PC diet ($P = 0.016$), and, also, in pigs fed the NRC diet than those fed the PC diet ($P = 0.020$). Diets had no clear effect on the 10th rib backfat. Longissimus muscle was lower in the pigs fed the NC diet than those fed the PC diet ($P < 0.001$). The LM was greater in pigs fed the 40All diet than those fed the 40-2AA diet ($P < 0.001$). Pigs fed the 40All diet had greater color ($P < 0.001$), firmness ($P = 0.004$), and marbling ($P < 0.001$) scores than those fed the 40-2AA diet. A tendency for a greater marbling score ($P = 0.082$) and a lower muscling score ($P = 0.002$) were observed in pigs fed the NC diet than those fed the PC diet.

Pigs fed the NC diet had lower fat-free lean percentage **(% lean**; $P = 0.005$), fat-free lean gain (**LG**; $P < 0.001$), and LG to feed intake (**LG:F**; $P < 0.001$) than those fed the PC diet. However, LG to ADI Lys intake (**LG:LysI**) tended to be higher in pigs fed the NC diet than those fed the PC diet ($P = 0.084$). Pigs fed the NRC diet tended to have lower LG than those fed the PC diet ($P = 0.054$), and lower LG:F ($P = 0.030$) and LG:LysI ($P = 0.028$) than those fed the 40-2AA and the 40All diets. Pigs fed the 40All diet tended to have higher LG:LysI than those fed the 40-2AA diet ($P = 0.068$).

Organ Weights and Serum Metabolites

Dietary treatments had no clear effects on the weight of livers in the pigs. Pigs fed the NRC diet had heavier kidneys than those fed the 40-2AA and the 40All diets (*P*

= 0.048). Pigs fed the 40-2AA diet had heavier hearts than those fed the 40All diet (P = 0.009).

Pigs fed the NC diet had lower concentrations of serum total protein ($P < 0.001$) and albumin ($P < 0.001$), and higher concentrations of glucose ($P = 0.031$) and cholesterol ($P < 0.001$) than those fed the PC diet. Serum total protein ($P = 0.031$) concentration was higher, and cholesterol concentrations tended to be higher (P = 0.060) in pigs fed the 40All diet than those fed the 40-2AA diet. Diets had no effect on BUN or triglycerides.

DISCUSSION

Hydrolyzed feather meal is rich in many AA, and it can be an attractive source of AA for pig diets. However, FM is deficient in Lys and certain other AA, and must be incorporated into diets based on the Lys content. Unfortunately, this method can increase the dietary protein content, which may lead to environmental problems. Considering AA profile and availability, it might be possible to completely replace SBM with FM by supplementing only with crystalline Lys. Chiba et al. (1996) evaluated this possibility, and the results indicated that corn-FM diets may have to be supplemented with not only Lys but also certain other AA.

When a corn-FM finisher pig diet is formulated based on the AID AA values reported by NRC (1998), it can be deficient in Lys and Trp. Besides Lys and Trp, a corn-FM diet can be also deficient in Thr, His, and Ile if the diet is formulated based on the assumption that AID values of all indispensable AA in FM is only 40%, which is the AID value reported for Lys by Knabe et al. (1989). It is, therefore, possible that the

FM diet must be supplemented with these additional AA to completely replace SBM in finisher pig diets.

In the present study, FM was included slightly above 9% during the finisher 1 phase and slightly above 6% during the finisher 2 phase. Previous studies indicated that the optimum inclusion rate in corn-SBM finisher diets range from 6 to 9% (Chiba et al., 1996; van Heugten and van Kempen, 2002; Apple et al, 2003). However, most of those studies replaced FM only partially, and, therefore, it is difficult to compare the results of the present study and those reports on the optimum inclusion rates of FM in pig diets.

In the present study, the NC diets were supplemented with AA to satisfy all the AID indispensable AA requirements based on the NRC (1998) AID AA, and the assumption that the AID of all indispensable AA in FM is 40%. As expected, pigs fed the NC diet had lower ADFI, LysI, ADG, G:F than those fed the PC diet. These findings were consistent with the earlier findings of Chiba et al. (1996), and can be attributed to the deficiency of bioavailable Lys and other AA in the diet. All the required AA in adequate amounts must be available simultaneously for protein synthesis (Everson et al., 1989), and thus, it may be possible that protein synthesis mechanism in the pigs fed the NC diet was adversely affected due to the deficiency of AA such as Lys. Earlier studies indicated that AA deficient diets can decrease growth performance of animals (Leung et al., 1968; Harper et al., 1970). However, pigs fed the NC diet had better G:LysI than those fed the PC diet, which can be attributed to the sparing effect of AA associated with pigs fed the diet deficient in Lys and other AA as mentioned by Chiba et al. (1991).

In consistent with the results of the growth performance, pigs fed the NC diet had adverse effects on carcass traits such as lower HCW, higher average backfat, and lower LM than those fed the PC diet. Lower HCW for the pigs fed the NC diet could be attributed to lower final BW simply because HCW and final BW at slaughter are positively related. Pigs fed the NC diet had poor growth and some of them were slaughtered before reaching the target weight. In one report, where FM was included at 10% in corn-SBM finisher diet, a reduction of 5% final BW was observed (van Huegten and van Kempen, 2002). Pigs fed the NC diet also had lower % lean, LG, and LG:F than those fed the PC diet, perhaps, because their diet did not have adequate amounts of bioavailable indispensable AA. These findings are, again, consistent with the earlier findings by Chiba et al. (1996). On the other hand, pigs fed the NC diet had higher LG:LysI compared to pigs fed the PC diet possibly because of, again, a sparing effect of AA in pigs fed the AA deficient diet.

Although comparisons between the NC diet and the NRC diet were not made statistically, the pigs fed the NRC diet seemed to have increased growth performance compared to those fed the NC diet. In one study, supplementation of corn-FM diet with only Lys did not improve growth performance (Chiba et al., 1996). Thus, addition of both Lys and Trp to the NRC diet might have played a crucial role in improved growth performance of pigs compared to those fed the NC diet. Gallo and Pond (1968) indicated that growth and carcass traits was improved only when Trp was also added to the corn diet supplemented with Lys. It has been reported that appetite, and, thereby, growth performance in pigs, can be reduced by feeding Trp deficient diets (Han et al.,

1993). Addition of Trp to corn-meat meal diets also improved growth performance of pigs (Cromwell et al., 1991).

Pigs fed the NRC diet should have satisfied NRC (1998) requirements for AID Lys and Trp, as well as other indispensable AA. But, those pigs, had lower ADG and G:LysI than those fed the PC diet and lower G:LysI than those fed the 40-2AA and 40All diets, thus, indicating that bioavailability of other indispensable AA in FM are lower than those reported by NRC (1998). Similarly, pigs fed the NRC diet had higher average backfat and lower LG than those fed the PC diet. This indicates that addition of AA to corn-FM diets based on the AID AA reported by NRC (1998) would be still inadequate to supply necessary available indispensable AA to support lean accretion similar to that observed in the pigs fed the PC diet.

Pigs fed the 40-2AA and 40All diets seemed to have better growth performance than those fed the NRC diet as indicated by numerically greater G:F, trends for higher G:LysI and higher LG:F and LG:LysI. Although the NRC diet was not compared to the 40-2All or 40All diets separately, it seems clear that the supplementation of Thr, His, and Ile, in addition to Lys and Trp, resulted in better growth performance of pigs compared with those fed the NRC diet. Threonine, in particular, is one of the most important limiting AA in typical swine diets, and its dietary supplementation has been reported to improve feed efficiency in growing pigs (Li et al., 1998).

Although no clear differences were observed between the performance of pigs fed the 40-2AA and 40All diets, pigs fed the 40All diet had numerically higher G:F and G:LysI than those fed the 40-2AA diet. It has been reported that supplementation of corn based diets with His (Eggert et al., 1955) and Ile (Kerr et al., 2004; Parr et al.,

2004) have positive effects on growth performance. In the present study, His and Ile were added in minute quantities and during only the finisher 1 phase, but those AA seemed to have positive effects on growth performance.

Although there were no marked changes on backfat in the pigs fed the 40-2AA and 40All diets, pigs fed the 40All diet had improved LM compared to those fed the 40-2AA diet. As mentioned before, the adequacy of His and Ile might have complemented the beneficial effect of additional Lys, Trp, and Thr on protein synthesis or accretion. Pigs fed the 40All diet tended to have a greater LG:LysI than those fed the 40-2AA diet. This indicates that supplementing FM with all the necessary AA may alleviate AA inadequacies, and the pigs seemed to be as efficient as those fed the PC diet in utilizing AA for lean growth.

The weight of metabolically active organs, such as liver, kidney, and heart, may indicate the rate of protein turnover in the body (Ferrell, 1988). It has been indicated that pigs with greater growth rate or lower backfat have heavy internal organs (Pond et al., 1988). In the present study, the kidney weight of pigs fed the NRC diet were heavier than those of pigs fed the 40-2AA and 40All diets. In previous study, it was found that the kidney weight tended to increase when 12% FM was included in the diet (Chiba et al., 1996). Overall, diets had no clear effects on the weight of metabolically active organs. As pigs were fed iso-N diets, perhaps, protein turnover may have been similar among the PC and AA supplemented FM diets, thus, metabolically active organ weights were not affected to a large extent.

Most of the serum metabolites reflect the changes in metabolic and physiological activities due to difference in diets. In the present study, diets seemed to

have no effect on BUN, an important serum metabolite that reflects the efficiency of N utilization or urea synthesis. Serum total protein and albumin concentrations in pigs fed the NC diet were lower than those fed the PC diet. Pigs fed the NC diet may had lower rates of protein synthesis because of insufficient available AA, and, thus, some proteins, including albumin, were not synthesized at a rate observed in pigs fed the PC diet. It has been indicated that protein or Lys restriction can reduce serum albumin concentration in pigs (Atinmo et al., 1976; Pond et al., 1980). Pigs fed the NC diet had higher concentrations of glucose and cholesterol compared to pigs fed the PC diet. As insulin is an important hormone that influences blood glucose concentration, it is possible that pigs fed the NC diet, which is deficient in Lys and other indispensable AA, were not able to secrete adequate insulin to lower serum glucose concentration. It has been reported that feeding Lys deficient diets in pigs resulted in decreased concentrations of serum insulin (Fernandez-Figares et al., 2006). In this study, however, as hormones were not measured, it is difficult to draw any conclusion on the exact effect of insulin on serum glucose concentration. Serum cholesterol was negatively correlated with Lys intake (Mule et al., 2006). Pond et al. (1986) reported a hypercholesteremic effect of dietary protein restriction in growing pigs. Although the exact mechanisms are not clear, it is possible that there may be some changes in lipoprotein composition and(or) transport that are associated with lipid metabolism during the protein restriction (Pond et al., 1986) or Lys restriction, thus, affecting serum cholesterol.

Pigs fed the 40All diet had higher serum total protein and tended to have higher albumin than those fed the 40-2AA diet. It is possible that total protein and albumin are the two metabolites that may reflect the AA content in the diet and the efficiency of AA

utilization. It is, therefore, possible that the efficiency of AA utilization in pigs fed the 40All diet may have been improved by the addition of His and Ile, which in turn resulted in higher serum concentrations of total protein, including albumin. It is possible that pigs fed the 40All diet did not have any protein/AA restriction in the body, and, therefore, they were not supposed to show hypercholesteremia compared with those fed the 40-2AA diet. However, in this study, serum cholesterol was higher in pigs fed the 40All diet than those fed the 40-2AA diet. There is no clear explanation for that effect.

Pork quality can be influenced by dietary manipulations. It has been reported that the adequacy of Trp in pig diets would reduce stress at slaughter, thus, improving meat quality (Adeola and Ball, 1992; Pethick et al., 1997). However, Guzik et al. (2006) reported no positive effect of dietary Trp inclusion in pig diets on meat quality. In terms of the inclusion of the FM in pig diets, it has been reported that up to 6% in the corn-soy-wheat middlings diet decreased color scores and increased lightness scores (Apple et al., 2003). Thus, it is possible that carcass quality can be influenced by the inclusion of the FM in the diets. Pigs fed the NC diet had higher marbling and lower muscling scores than those fed the PC diet in the present study. In earlier studies, it has been indicated that low Lys to DE ratio improve marbling greatly (Szabo et al., 2001), and the results of the present study agree with that report. Thus, in this study, low Lys to energy ratios in the NC diet improved marbling. The low muscling scores in pigs fed the NC diet is associated with their lower LM without affecting 10th rib backfat. Pigs fed the 40All diet, showed higher values of color, firmness, and marbling than those fed the 40-2AA diet. Thus, it may, once again, indicate that supplementation of the FM diet with all the necessary AA would improve subjective meat quality scores.

In summary, the results indicated that FM diets supplemented with crystalline AA were not as good as corn-SBM diets in terms of supporting weight gain. However, the results seemed to indicate that pigs fed FM diets supplemented with the necessary AA can utilize AA and feed for weight gain and lean gain as efficiently as those fed the corn-SBM diet. Further research is needed to investigate optimum AA supplementation strategies to alleviate the depression of weight gain associated with feeding AA-supplemented FM diets.

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Table1. Composition of hydrolyzed feather meal, soybean meal, and corn (%; as-fed basis)[1,2]

Item	Hydrolyzed feather meal	Soybean meal	Corn
Dry matter	93.66	88.30	87.85
Crude protein	83.82	47.83	8.12
Lysine	2.03	3.01	0.26
Threonine	3.79	1.85	0.30
Methionine	0.65	0.64	0.17
Cystine	3.97	0.72	0.18
Methionine + cystine	4.62	1.37	0.35
Arginine	5.71	3.51	0.39
Isoleucine	3.66	2.16	0.27
Leucine	7.76	3.62	0.93
Valine	5.74	2.27	0.38
Histidine	0.78	1.30	0.23
Alanine	3.85	-	-
Glutamic acid	8.76	-	-
Glycine	6.67	-	-
Aspartic acid	5.76	-	-
Phenylalanine	4.35	2.43	0.39
Proline	7.94	-	-
Serine	9.54	-	-
Tyrosine	2.28	-	-
Tryptophan	0.56	0.65	0.07

[1]Corn and soybean meal were analyzed by Evonik-Degussa, Keenesaw, GA; Hydrolyzed feather meal was analyzed by Ajinomoto Heartland LLC, Eddyville, IA.

[2]For corn and soybean meal, reported the average of several batches of samples, and some AA values, e.g., Ala, Glu, and Gly, were not reported.

Table 2. Composition of finisher 1 diets (as-fed basis)[1]

Item	PC[2]	NC[3]	NRC[4]	40-2AA[5]	40All[6]
Ingredients, g/kg					
Corn	791.9	858.8	851.5	850.3	850.1
Soybean meal (47.8% CP)	184.6	-	-	-	-
Hydrolyzed feather meal	-	97.96	97.96	97.96	97.96
Poultry fat	-	17.78	17.78	17.78	17.78
Dicalcium phosphate	10.86	14.09	14.09	14.09	14.09
Limestone	6.65	5.37	5.37	5.37	5.37
Salt	3.50	3.50	3.50	3.50	3.50
Vitamin-trace mineral premix[7]	2.50	2.50	2.50	2.50	2.50
L-Lysine (50.7%)[8]	-	-	7.00	7.55	7.55
L-Tryptophan	-	-	0.32	0.45	0.46
L-Threonine	-	-	-	0.47	0.51
L-Histidine	-	-	-	-	0.09
L-Isoleucine	-	-	-	-	0.04
Calculated composition[9]					
DE, Mcal/kg	3.47	3.47	3.47	3.47	3.47
Crude protein, g/kg	153.4	153.4	158.0	158.0	158.0
Ca, g/kg	6.00	6.00	6.00	6.00	6.00
P, g/kg	5.50	5.50	5.50	5.50	5.50
Ca:P	1.09	1.09	1.09	1.09	1.09
Lysine, g/kg	6.10	2.55	6.10	6.10	6.10
Lysine:DE, g/Mcal	1.758	0.735	1.758	1.758	1.758
Tryptophan, g/kg	1.274	0.672	1.000	1.000	1.000
Threonine, g/kg	4.247	4.465	3.700	3.700	3.700
Histidine, g/kg	3.529	2.051	2.051	1.929	2.000
Isoleucine, g/kg	5.098	4.802	4.802	3.332	3.400
Analyzed composition					
Crude protein, g/kg	15.0	15.1	15.8	15.0	15.4

[1]All corn-hydrolyzed feather meal (FM) diets were formulated to be iso-nitrogenous and iso-caloric to the corn-soybean meal, positive control diet. TM = trace mineral elements. The amount of crystalline AA supplemented in FM diets was adjusted accordingly based on the product specifications, and they were included by replacing a portion of corn. Finisher 1 diets were fed from 57.8 ± 0.8 kg to 81.0 ± 1.4 kg.

[2]PC = corn-soybean meal positive control diet.

[3]NC = corn-FM negative control diet.

[4]NRC = corn-FM diet supplemented with crystalline Lys and Trp to satisfy the NRC (1998) apparent ileal digestible AA requirements based on the apparent ileal digestibility of AA reported by NRC (1998).

[5]40-2AA = corn-FM diet supplemented with crystalline Lys, Thr, and Trp to satisfy the NRC (1998) apparent ileal digestible requirements based on the assumed apparent ileal digestible AA value of 40% for all the indispensable AA, but minute amounts of His and Ile were not included.

[6]40All = corn-FM diet supplemented with crystalline Lys, Thr, Trp, His, and Ile to satisfy the NRC (1998) apparent ileal digestible requirements based on the assumed apparent ileal digestible AA value of 40% for all the indispensable AA.

[7]Provided the following (unit/kg diet): Fe (ferrous sulphate), 150 mg; Zn (zinc oxide), 150 mg; Mn (manganous oxide), 37.5 mg; Cu (copper sulfate), 150 ppm; iodine (ethylenediamine dihydroiodide), 5 ppm; Se (sodium selenite), 3 ppm; vitamin A, 6,614 IU; vitamin $D_{3,}$ 1,102 IU; vitamin E, 26; vitamin B_{12}, 0.03 mg; menadione (menadione Na bisulfite complex), 1 mg; riboflavin, 6 mg; D-Pantothenic acid (D-Ca pantothenate), 45 mg; niacin, 28 mg; and choline, (choline chloride), 110 mg.

[8]Lysine was added in the form of Biolys (Evonik-Degussa Feed Additives, Kennesaw, GA). The composition of Biolys is L-Lys, 50.7%; water, 5.0%; Met, 0.2%; Cys, 0.1%; Met + Cys, 0.3%; Thr, 0.4%; Trp, 0.14%; Arg, 0.6%; Ile, 0.4%; Leu, 0.7%; and Val, 0.7%.

[9]For convenience, AA content expressed on the apparent ileal digestible AA reported by NRC, 1998.

Table 3. Composition of finisher 2 diets (as-fed basis)[1]

Item	PC[2]	NC[3]	NRC[4]	40-2AA[5]	40All[6]
Ingredients, g/kg					
Corn	849.35	895.13	890.11	889.45	889.45
Soybean meal (47.8% CP)	126.18	-	-	-	-
Hydrolyzed feather meal	-	66.93	66.93	66.93	66.93
Poultry fat	-	12.14	12.14	12.14	12.14
Dicalcium phosphate	12.17	14.38	14.38	14.38	14.38
Limestone	6.30	5.42	5.42	5.42	5.42
Salt	3.50	3.50	3.50	3.50	3.50
Vitamin-trace mineral premix[7]	2.50	2.50	2.50	2.50	2.50
L-Lysine (50.7%)[8]	-	-	4.79	5.16	5.16
L-Tryptophan	-	-	0.22	0.31	0.31
L-Threonine	-	-	-	0.18	0.18
L-Histidine	-	-	-	-	-
L-Isoleucine	-	-	-	-	-
Calculated composition[9]					
DE, Mcal/kg	3.47	3.47	3.47	3.47	3.47
Crude protein, g/kg	130.6	130.6	130.6	130.6	130.6
Ca, g/kg	6.00	6.00	6.00	6.00	6.00
P, g/kg	5.50	5.50	5.50	5.50	5.50
Ca:P	1.09	1.09	1.09	1.09	1.09
Lysine, g/kg	4.70	2.273	4.70	4.70	4.70
Lysine:DE, g/Mcal	1.354	0.655	1.354	1.354	1.354
Tryptophan, g/kg	0.988	0.576	0.800	0.800	0.800
Threonine, g/kg	3.519	3.668	3.668	3.000	3.000
Histidine, g/kg	2.995	1.984	1.984	1.890	1.890
Isoleucine, g/kg	4.166	3.963	3.963	2.946	2.946
Analyzed composition					
Crude protein, g/kg	12.9	12.4	13.0	12.3	12.3

[1]All corn-hydrolyzed feather meal (FM) diets were formulated to be iso-nitrogenous and iso-caloric to the corn-soy, positive control diet; The amount of crystalline AA supplemented in FM diets was adjusted accordingly based on the product specifications; Finisher 2 were fed from 81.0 ± 1.4 kg to 112.1 ± 1.8 kg.

[2]PC = corn-soybean meal positive control diet.

[3]NC = corn-FM negative control diet.

[4]NRC = corn-FM diet supplemented with crystalline Lys and Trp to satisfy the NRC (1998) requirements based on the apparent ileal digestible AA reported by NRC (1998).

[5]40-2AA = corn-FM diet supplemented with crystalline Lys, Thr, and Trp to satisfy the NRC (1998) requirements based on the assumed apparent ileal digestible AA value of 40% for all the indispensable AA.

[6]40All = corn-FM diet supplemented with crystalline Lys, Thr, and Trp to satisfy the NRC (1998) requirements based on the assumed apparent ileal digestible AA value of 40% for all

the indispensable AA; His and Ile were not added during the finisher 2 phase as their requirements were satisfied without their supplementation.

[7]Provided the following (unit/kg diet): Fe (ferrous sulphate), 150 mg; Zn (zinc oxide), 150 mg; Mn (manganous oxide), 37.5 mg; Cu (copper sulfate), 150 ppm; Iodine (ethylenediamine dihydroiodide), 5 ppm; Se (sodium selenite), 3 ppm; vitamin A, 6,614 IU; vitamin D_3, 1,102 IU; vitamin E, 26; vitamin B_{12}, 0.03 mg; menadione (menadione Na bisulfite complex), 1 mg; riboflavin, 6 mg; D-Pantothenic acid (D-Ca pantothenate), 45 mg; niacin, 28 mg; and choline, (choline chloride), 110 mg.

[8]Lysine was added in the form of Biolys; The composition of Biolys (Evonik-Degussa, Kennesaw, GA) is L-Lys, 50.7%; water, 5.0%; Met, 0.2%; Cys, 0.1%; Met + Cys, 0.3%; Thr, 0.4%; Trp, 0.14%; Arg, 0.6%; Ile, 0.4%; Leu, 0.7%; and Val, 0.7%.

[9]For convenience, AA content expressed on the apparent ileal digestible AA reported by NRC (1998).

Table 4. Least square means of growth performance during the finisher 1 and finisher 2 phases and overall[1]

Item	PC[2]	NC[3]	NRC[4]	40-2AA[5]	40All[6]	SEM[7]	*P*-values[8]			
							Contrasts[9]			
							PC vs. NC	PC vs. NRC	NRC vs. 40-2AA & 40All	40-2AA vs. 40All
Finisher 1										
Average daily feed intake, g/d	2,498	2,143	2,405	2,351	2,074	107	0.041	-	-	0.088
Average AID lysine intake, g/d	15.0	5.5	14.2	14.3	12.9	0.6	< 0.001	-	-	0.139
Average daily gain, g/d	990	509	835	837	834	32	< 0.001	0.002	-	-
Gain:feed, g/kg	413	235	347	367	405	20	< 0.001	0.032	0.136	-
Gain:AID lysine intake, g/g	68	90	59	60	67	4	< 0.001	0.133	-	-
Finisher 2										
Average daily feed intake, g/d	3,482	2,642	3,367	3,029	3,053	129	0.005	-	0.057	-
Average AID lysine intake, g/d	16.4	5.8	15.9	14.3	14.5	0.5	< 0.001	-	0.039	-
Average daily gain, g/d	1,093	458	967	883	957	48	< 0.001	0.079	-	-
Gain:feed, g/kg	307	191	279	287	312	14	< 0.001	0.172	-	-
Gain:AID lysine intake, g/g	64	82	57	60	65	3	0.015	-	-	-
Overall										
Average daily feed intake, g/d	3,055	2,375	2,843	2,747	2,651	99	< 0.001	0.144	-	-
Average AID lysine intake, g/d	16.1	4.9	15.3	14.5	14.0	0.5	< 0.001	-	0.139	-
Average daily gain, g/d	1,041	511	909	873	901	29	< 0.001	0.004	-	-
Gain:feed, g/kg	336	220	308	316	344	12	< 0.001	0.122	0.164	0.119
Gain:AID lysine intake, g/g	63	89	57	60	65	2.5	< 0.001	0.083	0.094	0.160

[1]Least squares means based on 6 pens containing 3 gilts or 3 castrated males/pen; Finisher 1: 57.8 ± 0.8 kg to 81.0 ± 1.4 kg; Finisher 2: 81.0 ± 1.4 kg to 112.1 ± 1.8 kg; All 5 diets were iso-nitrogeneous and iso-caloric and fed during both the finisher 1 and finisher 2 phases; AID = Apparent ileal digestible.

[2]PC = corn-soybean meal positive control diet.

[3]NC = corn-hydrolyzed feather meal negative control diet.

[4]NRC = corn-hydrolyzed feather meal diet supplemented with crystalline Lys & Trp to satisfy the NRC (1998) AID AA requirements based on the AID AA reported by NRC (1998).

[5]40-2AA = corn-hydrolyzed feather meal diet supplemented with crystalline Lys, Thr, and Trp to satisfy the NRC (1998) AID AA requirements based on the assumed AID AA value of 40% for all the indispensable AA, but minute amounts of His and Ile were not included.

[6]40All = corn-hydrolyzed feather meal diet supplemented with crystalline Lys, Thr, Trp, His, and Ile to satisfy the NRC (1998) AID AA requirements based on the assumed AID AA value of 40% for all the indispensable AA.

[7]pooled SEM.

[8]Reported *P*–values ≤ 0.20.

[9]Preplanned nonorthogonal contrasts.

Table 5. Least square means of carcass traits and subjective meat quality scores at the end of the finisher phase[1].

Item	PC[2]	NC[3]	NRC[4]	40-2AA[5]	40All[6]	SEM[7]	*P*–values[8] Contrasts[9]			
							PC vs. NC	PC vs. NRC	NRC vs. 40-2AA & 40All	40-2AA vs. 40All
Carcass traits										
Dressing, %	74.1	74.2	76.0	75.6	76.9	1.0	-	0.148	-	-
Hot carcass weight, kg	85.1	75.5	86.9	88.2	85.9	1.6	< 0.001	-	-	-
Average backfat, mm	24.8	28.5	28.3	27.7	29.2	1.0	0.016	0.020	-	-
10th rib backfat, mm	21.1	23.8	21.5	21.4	23.1	1.5	-	-	-	-
Longissimus muscle area, cm^2	49.9	41.8	51.6	48.4	55.7	1.4	< 0.001	-	-	< 0.001
Fat free lean, %	54.0	51.0	54.0	53.0	54.3	0.7	0.005	-	-	0.189
Fat free lean gain, g/d	411.7	162.8	372.1	387.6	403.2	15.9	< 0.001	0.054	-	-
Fat free lean gain:feed intake, g/kg	137.6	68.8	131.1	141.3	151.9	5.4	< 0.001	-	0.030	0.182
Fat free lean gain:AID lysine intake, g/g	26.3	29.9	24.3	25.9	29.1	1.1	0.084	-	0.028	0.068
Subjective meat quality scores										
Color	3.57	3.63	3.11	2.63	3.67	0.20	-	0.115	-	0.001
Firmness	3.51	3.45	3.11	2.48	3.62	0.25	-	-	-	0.004
Marbling	2.40	3.12	2.67	1.71	3.00	0.22	0.082	-	-	0.001
Muscling	2.51	2.21	2.40	2.41	2.47	0.03	0.002	0.135	-	-

[1]Least squares means based on 6 pens containing 3 gilts or 3 castrated males/pen; All five diets were iso-nitrogeneous and iso-caloric and fed during both the finisher 1 and finisher 2 phases; AID = Apparent ileal digestible.

[2]PC = corn-soybean meal positive control diet.

[3]NC = corn-hydrolyzed feather meal negative control diet.

[4]NRC = corn-hydrolyzed feather meal diet supplemented with crystalline Lys & Trp to satisfy the NRC (1998) AID AA requirements based on the AID AA reported by NRC (1998).

[5]40-2AA = corn-hydrolyzed feather meal diet supplemented with crystalline Lys, Thr, and Trp to satisfy the NRC (1998) AID AA requirements based on the assumed AID AA value of 40% for all the indispensable AA, but minute amounts of His and Ile were not included.

[6]40All = corn-hydrolyzed feather meal diet supplemented with crystalline Lys, Thr, Trp, His, and Ile to satisfy the NRC (1998) AID digestible AA requirements based on the assumed AID AA value of 40% for all the indispensable AA.

[7]pooled SEM.

[8]Reported *P*-values ≤ 0.20.

[9]Preplanned nonorthogonal contrasts.

Table 6. Least square means of organ weights and serum metabolites at the end of the finisher phase[1].

Item	PC[2]	NC[3]	NRC[4]	40-2AA[5]	40All[6]	SEM[7]	*P*–values[8] Contrasts[9]			
							PC vs. NC	PC vs. NRC	NRC vs. 40-2AA & 40All	40-2AA vs. 40All
Organ weights										
Liver, g	1,427.4	1,462.0	1,408.5	1,305.7	1,364.6	36.4	-	-	0.115	-
Kidney, g	322.5	259.4	369.6	312.0	290.7	26.7	0.108	-	0.048	-
Heart, g	375.7	367.7	381.6	416.9	331.6	21.1	-	-	-	0.009
Serum metabolites										
Total protein, g/dL	6.3	5.2	6.0	5.8	6.2	0.1	< 0.001	0.171	-	0.031
Albumin, g/dL	5.0	3.4	4.8	4.6	4.9	0.1	< 0.001	-	-	0.155
Blood urea nitrogen, mg/dL	11.2	12.8	11.2	8.9	10.5	1.0	-	-	-	-
Cholesterol, mg/dL	107.8	145.7	118.8	102.8	116.9	5.0	< 0.001	0.164	0.199	0.060
Triglycerides, mg/dL	38.2	37.5	51.0	40.7	50.4	6.9	-	-	-	-
Glucose, mg/dL	90.0	100.9	96.0	95.7	100.4	3.3	0.031	-	-	-

[1]Least squares means based on 6 pens containing 3 gilts or 3 castrated males/pen; All five diets were iso-nitrogeneous and iso-caloric and fed during both the finisher 1 and finisher 2 phases.

[2]PC = corn-soybean meal positive control diet.

[3]NC = corn-hydrolyzed feather meal negative control diet.

[4]NRC = corn-hydrolyzed feather meal diet supplemented with crystalline Lys & Trp to satisfy the NRC (1998) requirements based on the AID AA reported by NRC (1998).

[5]40-2AA = corn-hydrolyzed feather meal diet supplemented with crystalline Lys, Thr, and Trp to satisfy the NRC (1998) AID AA requirements based on the assumed AID AA value of 40% for all the indispensable AA, but minute amounts of His and Ile were not included.

[6]40All = corn-hydrolyzed feather meal diet supplemented with crystalline Lys, Thr, Trp, His, and Ile to satisfy the NRC (1998) AID AA requirements based on the assumed AID AA value of 40% for all the indispensable AA.

[7]pooled SEM.

[8]Reported *P*- values ≤ 0.20.

[9]Preplanned nonorthogonal contrasts.

IV. SUMMARY AND CONCLUSIONS

More than 8.5 billion chickens are commercially grown and processed in the United States, and those chickens can generate more than 2.3 billion pounds of feathers each year. Obviously, turning its major waste product into valuable commercial products would be extremely beneficial for the competitive poultry industry. Although the production of hydrolyzed feather meal, along with composting, is the recycling method capable of keeping up with the volume of feathers generated on a daily basis, the sheer volume of feathers can be overwhelming. Thus, increasing the market demand for feather meal would be obviously, important for successful and sustainable poultry production. The competition between humans and animals, especially nonruminant species, for quality sources of amino acids is likely to increase continuously because of the ever-increasing world population. Clearly, it would be essential to find alternative sources of amino acids for successful and sustainable pig production. The effort to increase the market demand for feather meal by increasing its use in pig production is, therefore, mutually beneficial for the poultry and pig industries. Hydrolyzed feather meal can be an excellent source of dietary amino acids for pigs, but the available data in the literature are quite limited. Because hydrolyzed feather meal is low in Lys and certain other amino acids, it must be incorporated into diets based on the amino acid content. Unfortunately, this can increase the dietary protein content, which may lead to environmental problems. Supplementation of hydrolyzed feather meal diets with

appropriate amino acids, therefore, would be the most plausible and effective way to utilize hyrdrolyzed feather meal in pig diets.

In this study, the possibility of replacing soybean meal in finisher pig diets completely with FM by amino acid supplementation was explored. The amino acids were added to the FM diets based on 1998 NRC reported apparent ileal digestible values for indispensable amino acids and the assumption that the apparent ileal digestibility of all indispensable amino acids in hydrolyzed feather meal is 40%. Specific objectives were to investigate the effect of crystalline AA supplementation on growth performance, carcass traits, subjective meat quality scores, organ weights, and serum metabolites. Corn-soybean meal, finisher 1 and 2 positive control (PC) diets were formulated to contain 6.1 and 4.7 g apparent ileal digestible lysine/kg, respectively, and corn-hydrolyzed feather meal, negative control (NC) diets were formulated to be iso-nitrogeneous to the PC diet. The NC diet was supplemented with amino acids to satisfy all the apparent ileal digestible indispensable amino acid requirements based on the 1998 NRC apparent ileal digestible amino acids (NRC = NC + Lys and Trp) and the assumption that the apparent ileal digestibility of all indispensable amino acids in hydrolyzed feather meal is 40% (40-2AA = NC + Lys, Trp, and Thr, but no His and Ile, and 40All = NC + Lys, Trp, Thr, His, and Ile). Forty-five gilts and 45 castrated males (57.8 ± 0.8 kg; 3 gilts or 3 castrated males/pen) were randomly assigned to 5 finisher 1 diets. At 81.0 ± 1.4 kg, pigs were offered finisher 2 diets. Pigs had ad libitum access to feed and water throughout the study. Blood samples were collected by vena cava puncture using a disposable needle and syringe at the end of the study (112.1 ± 1.8 kg).

Pigs were slaughtered to collect standard carcass measurements. To assess gross metabolic alterations, internal organs were collected and weighed separately.

As expected, overall average daily feed intake, apparent ileal digestible lysine (representing indispensable amino acids) intake, average daily gain, and gain to feed were greater and gain to lysine intake was lower in pigs fed the PC diet than those fed the NC diet. Overall gain to lysine intake tended to be lower in pigs fed the NRC diet than those fed the PC diet or the 40-2AA and 40All diet, and pigs fed the 40All diet had numerically higher gain to feed and gain to lysine intake than those fed the 40-2AA diet. Pigs fed the PC diet had more serum albumin and total protein but less glucose and cholesterol than those fed the NC diet, and total protein was higher in pigs fed the 40All diet than those fed the 40-2AA diet. Diets had no effect on urea nitrogen or triglycerides. Pigs fed the PC diet had less average backfat than those fed the NC diet or the NRC diet. The longissimus muscle was greater in pigs fed the PC diet or 40All diet than those fed the NC or 40-2AA diets, respectively. Pigs fed the PC diet had greater % fat-free lean, lean gain, and lean gain to feed than those fed the NC diet, but their lean gain to feed or lean gain to lysine intake was similar to those fed the NRC diet. The lean gain to feed and lean gain to lysine intake were lower in pigs fed the NRC diet than those fed the 40-2AA and 40All diets, and lean gain to lysine intake tended to be higher in pigs fed the 40All diet than those fed 40-2AA diet. Pigs fed the 40All diet had greater meat color, firmness, and marbling scores than those fed the 40-2AA diet. Diets had no clear effect on internal organ weights. The results indicate that hydrolyzed feather meal diets were not as good as corn-soybean meal diets in terms of supporting weight gain. However, the results seem to indicate that pigs fed hydrolyzed feather meal diets

supplemented with the necessary amino acids can utilize amino acids and feed for weight gain and lean gain as efficiently as those fed the corn-soybean meal diet. Further research is needed to investigate the optimum amino acid supplementation strategies to alleviate the depression of weight gain associated with amino acid-supplemented hydrolyzed feather meal diets.

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APPENDICES

Appendix A: Principle of the Total protein Analysis (Roche Diagnostics, Indianapolis, IN)

Under alkaline conditions, divalent copper in the biuret reagent reacts with protein peptide bonds to form the characteristic purple-colored biuret complex:

$$\text{Protein} + Cu^{2+} \xrightarrow{\text{Alkaline solution}} \text{Cu-protein complex}$$

The color intensity of this complex is directly proportional to the protein concentration, which can be measured photometrically.

Appendix B: Principle of the Urea nitrogen Analysis (Roche Diagnostics, Indianapolis, IN)

Urea is hydrolyzed by urease to form CO_2 and ammonia:

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2\ NH_4^+ + CO_2$$

The ammonia formed then reacts with α-ketoglutarate and NADH in the presence of GLDH to yield glutamate and NAD^+:

$$\alpha\text{-ketoglutarate} + NH_4^+ + \text{NADH} \xrightarrow{\text{GLDH}} \text{L-glutamate} + NAD^+ + H_2O$$

The decrease in absorbance due to consumption of NADH is measured kinetically.

Appendix C: Principle of the Albumin Analysis (Roche Diagnostics, Indianapolis, IN)

It is a colorimetric assay with endpoint method. At a pH of 4.1, albumin displays a sufficiently cationic character to be able to bind with bromocresol green (BCG), an anionic dyestuff to form a blue-green complex:

$$\text{Albumin} + \text{BCG} \xrightarrow{\text{pH 4.1}} \text{albumin BCG complex}$$

The color intensity of the blue-green color is directly proportional to the albumin concentration and can be measured photometrically.

Appendix D: Principle of the Triglyceride Analysis (Diagnostic chemicals Ltd., Oxford, CT)

Serum triglycerides are hydrolyzed to glycerol and free fatty acids by lipase:

$$\text{Triglycerides} + H_2O \xrightarrow{\text{Lipase}} \text{glycerol} + \text{fatty acids}$$

In the presence of ATP and glycerol kinase (GK), the glycerol is phosphorylated to glycerol-1-phosphate:

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK, } Mg^{++}} \text{glycerol-1-phosphate} + \text{ADP}$$

Glycerol-1-phosphate is then oxidized by glycerol phosphate oxidase (GPO) to yield hydrogen peroxide (H_2O_2):

$$\text{Glycerol-1-phosphate} + O_2 \xrightarrow{\text{GPO}} H_2O_2 + \text{dihydroxyacetone phosphate}$$

The hydrogen peroxide causes oxidative coupling of p-chlorophenol and 4-aminoantipyrine, producing a red colored quinoneimine dye complex:

$$H_2O_2 + \text{p-chlorophenol} + \text{4-aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{quinoneimine dye} + H_2O$$

The increase in absorbance at 520 nm due to the formation of the quinoneimine dye is directly proportional to the concentration of triglycerides in the sample.

Appendix E: Principle of Cholesterol Analysis (Roche Diagnostics, Indianapolis, IN)

Cholesterol is determined enzymatically using cholesterol esterase and cholesterol oxidase as follows. Cholesterol esters are cleaved by the action of cholesterol esterase to yield free cholesterol and fatty acids:

$$\text{Cholesterol esters} + H_2O \xrightarrow{\text{Cholesterol esterase}} \text{cholesterol} + \text{RCOOH}$$

Cholesterol is converted by oxygen with the aid of cholesterol oxidase to cholest-4-en-3-one and hydrogen peroxide:

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{cholest-4-en-3-one} + H_2O_2$$

The hydrogen peroxide created forms a red dyestuff by reacting with 4-aminophenazone and phenol under the catalytic action of peroxidase:

$$2H_20_2 + \text{4-aminophenazone} + \text{phenol} \xrightarrow{\text{Peroxidase}} \text{4- (p-benzoquinone-monoimino)-phenazone} + 4\ H_2O$$

The color intensity is directly proportional to the concentration of cholesterol and can be determined photometrically.

Appendix F: Principle of Glucose Analysis (Diagnostic Chemicals Ltd)

Glucose is phoshorylated to hexokinase in the presence of adenosine triphosphate (ATP) and magnesium to form glucose-6-phosphate (G-6-P) and adenosine diphosphate (ADP):

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{Hexokinase, } Mg^{2+}} \text{Glucose-6-phosphate} + \text{ADP}$$

G-6-P is then oxidized by glucose-6-phosphate dehydrogenase (G-6-PDH) in the presence of nicotinamide adenine dinucleotide (NAD^+) producing 6-phosphogluconate and NADH:

$$\text{Glucose-6-phosphate} + NAD^+ \xrightarrow{\text{G-6-PDH}} \text{6-phosphogluconate} + \text{NADH} + H^+$$

The formation of NADH causes an increase in absorbance at 340 nm which is directly proportional to the concentration of glucose in the sample.

Appendix G. Minimum and maximum daily temperatures (°C) during the animal study[1]

Table 1. Daily minimum and maximum temperatures inside the building during the animal study

Date	Min. T	Max. T	Date	Min. T	Max. T	Date	Min. T	Max. T
24-Jul		25.6	11-Sep	18.9	37.8	30-Oct	6.7	20.6
25-Jul	21.1	31.1	12-Sep	16.7	33.9	31-Oct	11.1	23.9
26-Jul	19.4	31.1	14-Sep	21.1	26.7	1-Nov	10.6	25.6
27-Jul	20.0	31.1	15-Sep	18.3	30.6	2-Nov	10.6	27.8
28-Jul	20.0	32.2	16-Sep	17.8	35.6	3-Nov	7.8	26.7
1-Aug	21.1	30.6	17-Sep	18.3	31.1	4-Nov	2.8	28.9
2-Aug	20.0	31.7	18-Sep	15.6	32.2	5-Nov	3.3	26.7
3-Aug	18.9	32.2	19-Sep	15.0	31.1	6-Nov	8.9	27.2
4-Aug	18.9	33.3	20-Sep	17.8	30.0	7-Nov	-0.6	22.2
9-Aug	23.3	35.6	21-Sep	21.1	25.6	8-Nov	-2.2	20.0
10-Aug	22.2	36.7	22-Sep	20.0	31.1	9-Nov	-2.2	14.4
11-Aug	22.2	36.7	23-Sep	21.1	34.4	10-Nov	0.0	24.4
12-Aug	19.4	33.3	24-Sep	19.4	33.9	11-Nov	5.0	25.6
13-Aug	18.9	33.3	25-Sep	21.1	34.4	12-Nov	5.6	20.0
14-Aug	18.9	34.4	26-Sep	18.9	32.2	13-Nov	5.6	25.6
15-Aug	20.0	35.6	27-Sep	22.2	33.3	14-Nov	5.6	27.8
16-Aug	25.0	35.0	28-Sep	21.1	30.0	15-Nov	6.7	20.0
17-Aug	18.3	35.6	29-Sep	20.0	31.1	19-Nov	7.2	25.6
18-Aug	18.3	31.1	30-Sep	20.0	33.3	20-Nov	6.7	28.9
19-Aug	21.1	35.0	1-Oct	21.1	32.2	21-Nov	8.9	23.3
20-Aug	22.2	35.0	2-Oct	20.6	31.7	22-Nov	1.1	16.7
21-Aug	18.3	28.9	3-Oct	21.1	30.0	23-Nov	1.1	17.8
22-Aug	20.6	30.6	4-Oct	21.1	31.1	24-Nov	0.6	11.1
23-Aug	20.0	31.1	9-Oct	31.7	17.2	25-Nov	7.2	11.1
24-Aug	19.4	23.3	10-Oct	12.2	28.9	26-Nov	4.4	17.8
25-Aug	18.9	23.3	11-Oct	6.7	30.0	27-Nov	-2.2	20.0
26-Aug	18.9	26.7	12-Oct	8.9	31.1	28-Nov	7.8	22.2
27-Aug	16.1	31.1	13-Oct	8.9	30.0	29-Nov	4.4	27.2
28-Aug	20.0	36.7	14-Oct	14.4	30.0	30-Nov	5.6	23.3
29-Aug	21.1	37.8	15-Oct	14.4	30.0	1-Dec	8.9	21.1
30-Aug	21.1	35.6	18-Oct	17.8	21.1	2-Dec	8.9	21.1
31-Aug	21.1	27.8	19-Oct	8.9	26.7			
1-Sep	21.1	27.2	20-Oct	10.0	29.4			
2-Sep	20.0	26.1	21-Oct	19.4	26.7			
3-Sep	17.2	33.3	22-Oct	20.0	21.1			
4-Sep	21.1	36.7	23-Oct	8.9	25.6			
6-Sep	21.1	31.7	24-Oct	5.6	-			
7-Sep	20.0	32.2	26-Oct	8.9	16.7			
8-Sep	18.9	33.3	27-Oct	6.7	23.3			
9-Sep	16.7	35.6	28-Oct	7.8	25.6			
10-Sep	16.7	37.2	29-Oct	8.9	25.6			

[1]Missing data were not included. Min. T = minimum temperature. Max. T = maximum temperature.

Table 2. Mean minimum and maximum temperatures

Month	Minimum temperature	Maximum temperature
July	20.1	30.2
August	20.2	32.5
September	19.2	32.2
October	13.4	26.4
November	4.4	22.5
December	8.9	21.1
Mean	14.4	27.5