ABSTRACT

SUNGWARAPORN, YUWARES. Lipid and Protein Quality of Poultry By-Products Preserved by Phosphoric Acid Stabilization (Under the direction of Dr. Peter R. Ferket).

The increase in intensive poultry productions has raised the public concern about poultry waste disposal and its efficiency of nutrient utilization. When properly handled, nutrients from poultry mortality and poultry processing waste can be recovered and recycled into nutritionally valuable and biologically safe animal feed ingredients. There were two general objectives included in this dissertation. 1) to evaluate the effectiveness of different sources and levels of phosphoric acid, and lactic acid fermentation in preserving protein and lipid quality of poultry mortality and poultry processing waste (dissolved air floatation sludge, DAF), and 2) to evaluate the nutritional value of secondary protein nutrients (SPN), a product meal derived from processed DAF sludge, in broiler diets. Results showed that both feed-grade and food-grade phosphoric acid were more effective than lactic acid fermentation in preserving protein and lipid quality of silages for a short term storage (15 days), while food-grade phosphoric acid was found to be the most effective preservative for long term storage (45 days). Food-grade phosphoric acid was a more effective preservative of protein and lipid quality than feed-grade at 2.76% acidification. Regardless of acid sources, the inclusion of 5.52% phosphoric acid significantly improved protein quality of poultry mortality silages. Similar effects were observed when phosphoric acid was used to preserve nutrient quality of DAF sludge. However, phosphoric acid stabilization did not improve the lipid quality of DAF silages because extensive lipid oxidation occurred prior to acid preservation. Apparent nutrient digestibilities of SPN, including apparent metabolizable energy corrected for nitrogen retention (AMEn), apparent nitrogen retention (ANR, %) and

apparent fat digestibility (AFD, %), was evaluated in broilers using acid insoluble ash (Celite™) and titanium dioxide as digestibility markers. Results indicated that the acid insoluble ash method had higher accuracy and preciseness in measuring nutrient digestibility of the diets. The calculated AMEn, %ANR and %AFD of dietary SPN estimated by the use of Celite™ was 1650.37 kcal/kg, 23.66%, and 12.60%, respectively. These values were then used in the formulation of experimental diets containing increasing levels of SPN and growth performance and nutrient digestibility of broilers were observed. AMEn, ANR, AFD, and broiler performance decreased as the levels of SPN increased. At 14 days of age, birds fed the diet containing 20% SPN had significantly higher incidence of rickets due to vitamin D deficiency, and this led to a significantly higher mortality from 14 to 21 days of age. The reduction in bird performance demonstrated the adverse effects of including a high level of dietary SPN on nutrient availability and palatability of broiler diets.

In conclusion, phosphoric acid preservation, especially food-grade source, was proved to be an effective method of preserving the nutritional value of poultry mortality prior to further processing into valuable animal feed ingredient. However, only protein quality of material recovered from the dissolved air floatation (DAF) unit of a poultry processing plant can be successfully preserved by food-grade phosphoric stabilization. Product meal derived from DAF material should not be incorporated at the level higher than 7.5% in broiler diet due to the damaged of lipid and protein which could lead to the interference of nutrient availability.

LIPID AND PROTEIN QUALITY OF POULTRY BY-PRODUCTS PRESERVED BY PHOSPHORIC ACID STABILIZATION

by

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APPROVED BY:

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Chair of Advisory Committee

DEDICATION

To

My grand mother and my parents

BIOGRAPHY

Yuwares Sungwaraporn was born on December 14, 1970 in Trat, Thailand. Secondary education completed at Tratrakarnkune School in 1988. Graduated with B. S. in Animal Science from Kasetsart University, Bangkok, Thailand in 1992. Completed a M. S. in Animal Nutrition at Kasetsart University of Thailand in 1995. Served as a faculty member of Animal Science Department at Kasetsart University in October 1996. Won the full scholarship from the Royal Thai Government and joined the Department of Poultry Science at North Carolina State University for the degree of Doctoral of Philosophy in August, 1998.

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CHAPTER 1: LITERATURE REVIEW

INTRODUCTION

In conjunction with the advances in modern technology, an adequate food supply and balanced diet are a very important factor in improving the living standards of the world population. The traditional ways to increase food supply have focused on increasing agricultural products, such as high-yielding crop varieties and livestock productions (El Boushy and van der Poel, 2000). During the 1990's, world poultry production has increased tremendously. World chicken meat production has grown from 29 million metric tons in 1990 to an estimated 50 metric tons in 2000, and the world egg production has grown from 32 to 50 metric tons. Total world chicken meat and egg production has increased at the rate of 4 metric ton per year during the last decade of the 20th century, and it will still continue to increase for the first decade of 21th century (Aho, 2002).

During the past 50 years, the chicken industry is among the fastest growing agribusinesses in the United States of America. Retail weight-based consumption of chicken meat increased from 9 pounds to 80 pounds per capita (Aho, 2000). However, the increase in intensive poultry production in many regions has lead to greater public concerns about poultry waste disposal and its efficiency of nutrient utilization versus nutrient emissions into the environment (Najafpour et al., 1994). Poultry production wastes originating from intensive farm production (manure, litter, and mortalities) and from poultry processing plants (blood offal, feather, protein and fat skimming) have raised public concern about pollution and the environmental safety. Currently, proper disposal of poultry wastes has been identified by the poultry industry as a high priority endeavor. If improperly handled, these wastes can cause environmental problems, as well as increase the risks of health hazards to humans and animals (El Boushy and van der Poel, 2000).

Detoxification mechanisms for toxic substances from protein degradation

Normally, there are two groups of amine oxidases, monoamine oxidases (MAO) and diamine oxidases (DAO), which play an important role in oxidative deamination and detoxification of naturally occurring amines and other foreign compounds. Most amine oxidases lack strict substrate specificity. For instance, diamine oxidase also uses histamine as a substrate and the enzyme has also been designated as a histaminase (Bachrach, 1985).

Monoamine oxidases are flavoprotein enzymes located in the mitochondria of the liver, kidney, and brain. The enzymes have been found also in blood platelets and intestinal mucosa. The MAO exists as a large group of similar enzymes with overlapping substrate specification and inhibition patterns. MOA will deaminate primary, secondary and tertiary aliphatic amines to oxidation products. The reaction rate will decrease as the complication of structures increase. Diamine oxidases oxidize diamines to the corresponding aldehydes in the presence of oxygen. The DAO are pyridoxal phosphate proteins containing a copper unit that are present in the soluble fraction of the liver, intestine and kidney. The rate of deamination is determined by chain length. The maximum activity of DAO occurs when the substrate is PUT followed by CAD. Although MAO can deaminate both substituted and primary amines, DAO can only deaminate primary amines (Hodgson and Glodstein, 2001). In mammalian cells, there are two possible means of polyamine catabolism. One is the recycling or interconversion pathway by which SPM and SPD can be converted to PUT and 3-acetamidopropanal. This PUT can be either recycled to SPD and possibly SPM or further metabolized. Another pathway is catabolic oxidation of the primary amino group by DAO to form an aminoaldehydes or a dialdehyde, ammonia and hydrogen peroxide (Morgan, 1999).

Malondialdehyde is a major secondary product of lipid oxidation, which has been shown to be the principle factor that involves protein cross-link reactions. The Shiff base formation between amino groups of lysine and other free amino groups in the protein could lead to the reduction in the availability of these amino acids (Crawford, et al., 1967, Nielsen et al., 1985). Moreover, this cross-link inactivates ribonuclease and bind covalently to nucleic acids (Crawford, et al., 1967). In general, malondialdehyde is considered as a complete carcinogen and a mutagen. Malondialdehyde has been reported to be toxic to living cells because it can be absorbed through the digestive system (Piche et al., 1988).

Wills (1961) suggested that the adverse effect of oxidized lipids on enzymes could result directly from the reactions with protein. The peroxides of linoleic acid or linolenic acid posses an inhibitory effect to certain enzymes, especially those containing –SH groups. Many of the inhibitions observed are similar to the effects produced by organic peroxides.

Adverse effects of lipid degradation of by-products on poultry feeding

Middleton et al. (2001) reported that poorly preserved poultry mortality silage contained low lipid quality as indicated by lower oxidative stability index (OSI) and higher in free fatty acid content. Oxidative stability index represents the capability of oils to resist oxidation over time in the presence of oxygen and heat. The longer the induction period of lipid oxidation, the greater the oil stability. Free fatty acid content has been widely used to indicate lypolysis in animal tissues during storage (Sklan et al., 1983). Normally, fat in animal tissues contains nearly no free fatty acid. However, it can be produced either by the activity of endogenous lypolytic enzyme or the action of microbial enzyme degradation of lipid material (Matti et al., 1964; Ostby et al., 1986; Hierro et al., 1997).

Primary, type I, or chain breaking antioxidants are free radical acceptors that delay or inhibit the initiation step or interrupt the propagation step of autoxidation. Primary antioxidants react with lipids and peroxy radicals and convert them to more stable, nonradical products (Buck, 1991). Primary antioxidants donate hydrogen atoms to the lipid radical and produce lipid derivatives and antioxidant radicals. The antioxidant radical produced by hydrogen donation has a very low reactivity with lipid, which slow down the rate of oxidation (Rajalakshmi and Narasimhan, 1996).

The most commonly used antioxidants in foods are synthetic compounds. The primary synthetic antioxidants approved for use in food include butylate hydroxyanisole (BHA), butylated hyroxytoluene (BHT), propyl gallate (PG), ethoxyquin, ascorbyl palmitate and tertiary butylhydroquinone (TBHQ). However, a few natural primary antioxidants, such as tocopherols and carotenoids are also commonly used as primary antioxidants in foods (Reische et al., 2002).

Secondary antioxidants, preventive, or type II antioxidants act through numerous possible mechanisms. These antioxidants slow the rate of oxidation by several different actions, but they do not convert free radicals to more stable products (Labuza, 1971). Secondary antioxidants can chelate prooxidant metals and deactivate them, replenish hydrogen to primary antioxidants, decompose hydrogen peroxides to nonradical species, deactivate singlet oxygen, absorb ultraviolet radiation, or act as oxygen scavengers. These antioxidants often referred as synergists because they promote the antioxidant activity of type I antioxidant. Citric acid, ascorbic acid, ascorbyl palmitate, lecithin and tartaric acid are good example of these synergist compounds (Reische et al., 2002).

Chitosan, a biodegradable polymer, has been used as a coagulating agent and flocculant aid to recover coagulated by-products from poultry processing wastewater. Chitosan is particularly effective in removing protein in processing wastewater (Bough, 1976). It has been demonstrated that the addition of chitosan at 5-10mg/l of wastewater provided the greatest coagulation of suspended material and resulted in the lowest turbidity values of poultry processing wastes (Bough et al., 1975). Coagulated solid, also called secondary protein nutrients (SPN), recovered by DAF in conjunction with chitosan contains 34.4% crude protein and 45.9% crude fat. This material can be dried, extruded or rendered along with other by-products from poultry processing to produce value-added feed ingredient (Bough et al., 1975; Lyons and Vandepopuliere, 1997).

DAF sludge in animal feeding

The protein rich-product recovered from herring fillet wastewater has been shown to contain comparable nutritive value to herring meal. The inclusion of this material at 8.5% and 17% showed no significance effect on broiler performance when compared with birds fed diets containing herring meal at equal levels (Herstad and Hvidsten, 1973). Similar flocculated sludge obtained from slaughterhouses and fish processing plants were sterilized and used as a substitute for soybean meal in growing swine diets. No significant effect on swine performance was observed when 50% of soybean meal was substituted with sterilized DAF material. When DAF material was included in swine diet at 200% of the soybean meal used in control group, the reduction in acceptability and palatability of the diet were observed, especially during the first week of the experiment. Significantly lower body weight gain was found when DAF material was substituted for 100% and 200% soybean meal, however, there was no significant difference in feed efficiency among these treatments.

The Use of Markers Digestibility Studies for Domestic Animals

Introduction

Good estimates of the feeding value (digestible protein and energy) of feed ingredients is essential in the formulation of animal diets. Although, the nutritional value of feed ingredients can be readily measured by chemical analysis, these evaluations do not present the actual availability of nutrients that animal can utilize (Short et al., 1998). The commonly used method to determine metabolizable energy and nutrient digestibility of animal feeds is total collection. This method relies on the quantitative measurement of excreta voided from a known amount of feed consumed (Sibblad, 1982). However, this method is laborious, requires special equipments, and is subjected to criticism on animal welfare because of the restriction of housing on the animal freedom of movement (Bakker and Jongbloed, 1994).

Properties of an effective indigestible marker

Dietary inclusion of indigestible substances (markers) has been frequently used as alternative to total collection method in the study of nutritional availabilities (Kotb and Luckey, 1972). These methods assume that the marker is not absorbed during its passage through the gastrointestinal tract. Therefore, the amount of food absorbed by the bird can be determined from the ratio of marker in feed and excreta samples (Jagger et al., 1992). The addition of a marker helps reduce the errors associated with the inaccurate measurement of feed intake, excreta output and contamination of excreta (Sibblad, 1987) and it is particularly suitable for the study where total collection cannot be undertaken (Barton and Houston, 1991).

Shrivastara and Talapatra (1962a) used acid-insoluble ash of feed and feces as an internal marker for ruminant feed. They observed an average recovery rate of the marker to be 99.8% and the estimated digestibility coefficients were not different from those values obtained from the total collection method. Similar effects have also been reported in using this marker to determine the pasture consumption and level of nutrition digestibility of grazing sheep (Shrivastara and Talapatra, 1962b).

Van Keulen and Young (1977) successfully used acid-insoluble ash to determine the dry matter digestibility of sheep fed diets containing pelleted hay plus grain. Similarly, Block et al. (1981) reported that acid-insoluble ash was a suitable natural marker for the determination of dry matter digestibility when hay and grain diets were fed *ad-libitum* to sheep and dairy cows. The recovery rates of acid-insoluble ash have been shown to be close to 100% and were not significantly different from those obtained from total collection method.

However, the acid-insoluble ash marker method is not suitable for the determination digestibility of feedstuffs containing low acid-insoluble ash content because of large sampling variation (Van Keulen and Young, 1977; Thonney et al., 1985). The contamination of ingesta and excreta with soil, dust or the consumption of bedding material has been observed to reduce accuracy when acid-insoluble ash was used as marker as compared with other markers (Van Dyne and Lofgreen, 1964; Van Keulen and Young, 1977). The absorption and excretion of soluble siliceous substance in urine and the temporary accumulation of inert silica in digestive tract of grazing ruminants (Van Dyne and Lofgreen, 1964) could lead to the inaccuracy of digestibility determination.

The high recovery rate of acid-insoluble ash could also be the result of the ashing method used to determine acid-insoluble ash content in a sample. It has been indicated in most studies that metabolizability and digestibility values obtained from Vogtmann et al. (1975)'s method were significant higher than those obtained from the method of Van Keulen and Young (1977).

Sale and Janssens (2003) observed lower apparent metabolizable energy and nitrogen retention when acid-insoluble ash was used as internal marker in pigeons. The lower apparent digestibility values is believed to be the result of a low internal marker content in the diet that could partially be attributed to analytical error. Thonney et al. (1985) recommended that the acid-insoluble ash content should exceed 0.75% on dry matter-basis in order to get accurate measurement. However, the acid insoluble ash values in pigeon diets demonstrated by Sale and Janssens (2003) were only 0.06 and 0.04% for corn and peas, respectively.

Conclusion

Analysis of acid-insoluble ash is less expensive, less hazardous, and requires less analysis time than chromic oxide method. In addition, acid-insoluble ash eliminates electrostatic separation problems associated with other digestibility marker, such as chromic oxide (Schang et al., 1983). For the sake of accuracy, however, at least 3 g of sample with the analysis of acid-insoluble ash is needed (Scott and Boldaji, 1997). The inaccuracy of measurement using acid-insoluble ash in feed containing too low acid-insoluble ash content by gravimetric method could be overcome by the addition of exogenous ash component to the feed. With all these advantages, acid-insoluble ash is increasing in popularity as maker in digestibility studies.

Titanium dioxide has also demonstrated to be a very useful digestibility marker because it can be legally added to feed (AAFCO, 1996). In addition, this method requires only a small amount of sample (0.1 g) for titanium dioxide content determination (Short et al., 1996). Since the ideal marker has not been found yet, more research should be deliberate on comparison of the most suitable markers for accuracy, safety, and standardization of analyses, repeatability between laboratories and species, as well as the recovery rate in excreta (Sales and Jansens, 2003).

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