Nutritional Evaluation of High Ash Meat and Bone Meal for Poultry

Thesis

By

Manli Liu

Submitted to the Faculty of Graduate Studies
In partial fulfillment of the requirements for
The Degree of Master of Science
Department of Animal Science
University of Manitoba

May. 2000

0-612-56136-4

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES
*****
COPYRIGHT PERMISSION PAGE

Nutritional Evaluation of High Ash Meat and Bone Meal for Poultry

BY

Manli Liu

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirements of the degree

of

Master of Science

Nutritional evaluation of high ash meat and bone meal for poultry

Manli Liu MSc
Department of Animal Science
University of Manitoba, 2000
Dr. L. D. Campbell, Advisor

Abstract

An initial study investigated the nutritional value of a high ash meat and bone meal (MBM) in terms of proximate composition, true amino acid (AA) digestibility and in vitro protein digestibility as well as available energy for poultry. Chemical analyses showed consistent contents of most nutrients in high ash MBM from batch to batch except for a small variation in contents of fat and ash. On a DM basis, it contained GE, 4250±200 kcal/kg; protein, 53±2.7%; ash, 29±2% with relatively balanced Ca (9%) and P (5%); fat 11.5% with 50% of unsaturated fatty acids. *In vitro* protein digestibility was 66% and true digestibilities of total essential AA, Lys, and total sulfur AA were 83, 77.5 and 71.3%, respectively. There was no statistical differences in AA digestibility in vivo and in vitro protein digestibility between high and low ash MBM despite of large difference in the content of ash. True metabolizable energy corrected to zero nitrogen retention was 2800 kcal/kg on a DM basis.

Based on the above nutrient values of high ash MBM, two experiments were conducted to evaluate the effect of various inclusion levels of high ash MBM in leghorn chick diets with 100 or 90% of NRC (1994) Lys requirement on bird performance, and to study the effect on broiler chicken performance of supplementation with Lys, Met, Thr and Try individually or in combination in 10% high ash MBM diets. The results indicated that greater than 5% inclusion level of high ash MBM into leghorn chick diets with 90% NRC Lys specification yielded a linear adverse effect on chick growth in comparison to those

chicks fed the same level of high ash MBM diets, but with 100% NRC Lys requirement. Amino acid deficiency was the primary cause of growth depression. Supplementation of Lys and Met in 10% high ash MBM diets significantly improved chick performance which reached the level of chicks fed a soybean meal control diet. Further addition of Thr and Try to the diet already containing Lys and Met did not yield significant improvement on chick performance. Both Lys and Met were the first limiting AA in corn-based practical broiler diets with 10% high ash MBM.

A final experiment evaluated formulation of broiler diets containing 10 or 15% high ash MBM on a total AA versus digestible AA basis at 100 or 90% NRC AA specification and the effect of high dietary level of Ca and P provided by high ash MBM and inorganic source on chick performance. Results showed that no difference in performance was observed when formulating diets with 10% MBM based on total vs. digestible AA values at 100% NRC specifications. Dietary formulation with high ash MBM on a total AA basis at 90% NRC significantly reduced chick performance dietary, however, for corn-soybean meal control diets, all AA being 90% of NRC did not result in performance decline. Dietary formulation with 10 or 15% high ash MBM based on digestible AA values significantly improved chick performance when AA requirements were at 90% NRC. It brought broiler performance up to the level of corn-soybean meal control diet, indicating that dietary formulation based on digestible AA values was superior to that based on total AA contents. Excessive Ca and P from high ash MBM were a secondary factor responsible for the reduced chick performance.

From the current study it can be concluded that the commercial high ash MBM is an effective source of protein and energy. The high ash MBM can be incorporated at a level of 10% to broiler diets.

Dedication

The thesis is dedicated to my husband Yonggang Liu, son Jake Xiaoyun Liu, and my parents.

Acknowledgements

This thesis is the result of three year's hard work with kind help from many people. Firstly, I am most grateful to my supervisor, Dr. L.D. Campbell, not only for the opportunity he offered to me as a MSc. student, but also for his constant concern and insightful supervision of this research project. Without it, the completion of this thesis would never have been possible. I thank my committee members Dr. C. M. Nyachoti and Dr. G. Blank for their supervision and suggestions regarding my thesis preparation.

I am highly appreciative of people in our poultry group for their help and their contributions regarding different aspects of this thesis. I would like to specifically thank Dr. W. Guenter, Dr. B. Slominski, Mr. H. Muc, Mr. J. Neufeld, and Ms. P. Robinson for their guidance and assistance with the in vitro experiments and the chick trials. I also appreciated the assistance of Dr. G. H. Crow and Dr. L. Onischuk in data analyses and Mr. P. Mills in amino acid analyses. My thanks also go to all secretaries in the Department of Animal Science.

I wish to acknowledge Natural Sciences and Engineering Research Council (NSERC) and Maple Leaf Foods Inc., Shur-Gain Div. for providing me with financial support.

My special thanks also go to my Chinese colleagues. Enjoyable talks, great help and friendship from these friends made my living pleasurable in Canada while my husband was away

Last, but not least, my sincere thanks go to my dear husband Yonggang and son Xiaoyun for all their love. I especially thank my husband for his encouragement and assistance in my study. I am indebted to my husband so much for the long time separation.

Contents

Chapter I Introduction .... 1 - 4

Chapter II Literature review .... 5 - 22

Chapter III. Manuscript I
Chemical composition and nutritional value of
high and low ash meat and bone meal for poultry .... 23 - 38

Chapter IV. Manuscript II
Effect of supplementing lysine, methionine, threonine
and Tryptophan to corn-based diets with high ash meat and
bone meal on broiler performance .... 39 - 54

Chapter V. Manuscript III
Formulation of broiler diets with high ash meat and bone meal
Based on total versus digestible amino acid content .... 55 –70

Chapter VI General discussion .... 71 - 77

Chapter VII General summary .... 78 - 79

Reference .... 80 - 86

Table and Figure List

Chapter II

Table 1. Chemical composition of meat and bone meal compared to soybean meal and canola meal (p: 7)

Table 2. Average percent fatty acid content of meat and bone meal in comparison to soybean meal and fish meal (p: 8)

Table 3. Mineral content in meat and bone meal in comparison with soybean meal and canola meal (p: 11)

Table 4. Amino acid profile of meat and bone meal and soybean meal (p: 14)

Table 5. True digestibility of essential amino acids in meat and bone meal for poultry

Chapter III, Manuscript 1

Table 1. Composition of high ash meat and bone meal from different batches (p: 29)

Table 2. Fatty acids of high ash meat and bone meal from different batches (p: 30)

Table 3. In vitro protein digestibility of high and low ash meat and bone meal (p: 31)

Table 4. True metabolizable energy of high and low ash meat and bone meal

Table 5. Amino acid content of high and low ash meat and bone meal from different batches and in comparison to that of soybean meal (p: 34)

Table 6. True amino acid digestibility of high and low ash meat and bone meal from caecectomized and intact roosters (p: 35)

Chapter IV, Manuscript 2

Table 1. Chemical composition and amino acid content of ingredients used in experimental diets (p: 45)

Table 2. Percent composition of experimental diets with various levels of high ash meat and bone meal used in Trial 1

Table 3. Basal composition of control experimental diets used in Trial 2

Table 4. The effect of different levels of protein and high ash meat and bone meal on chick weight gain during 10-day experiment (p: 48)

Figure 1. Effect of inclusion levels of high ash meat and bone meal with different levels of protein and lysine specifications on bird weight gain (p: 49)

Table 5. Effect on chick performance of supplementing diets containing 10% high ash meat and bone meal with amino acids (p: 50)

Figure 2. Effect on feed conversion ratio by supplementation of lysine or methionine alone or in combination with threonine and tryptophan to high ash meat and bone meal diets (p: 51)

Figure 3. Effect on feed intake by supplementation of lysine or methionine alone or in combination with threonine and tryptophan to high ash meat and bone meal diets (p: 52)

Chapter V, Manuscript 3

Table 1. Chemical composition and amino acid digestibility of high ash meat and bone meal, soybean meal, corn and barley used in experimental diets (p: 60)

Table 2. Composition of soybean meal and high ash meat and bone meal diets based on total amino acid requirements for broiler chickens

Table 3. Composition of soybean meal and high ash meat and bone meal diets based on digestible amino acid values for broiler chickens (p: 62)

Table 4. Performance of chicks fed diets containing various levels of high ash meat and bone meal with formulation based on total amino acids or digestible amino acids (p: 64)

Figure 1. Effect on chick performance of dietary formulation with high ash meat and bone meal on a total amino acid or digestible amino acid basis of NRC (1994) (p: 66)

Figure 2. Effect on chick performance of dietary formulation with 10 or 15% high ash meat and bone meal on 90% NRC (1994) total amino acid or digestible amino acid values (p: 67)

Figure 3. Effect of high dietary levels of Ca and P provided by high ash meat and bone meal or inorganic source on chick performance (p: 69)

Abbreviations

AA, Amino acids
Arg, Arginine
Ca, Calcium
CEC, Cecectomized
CM, Canola meal
Cys, Cystine
DAA, Digestible amino acids
DM, Dry matter
EAA, Essential amino acids
FCR, Feed conversion ratio
FM, Fish meal
GE, Gross energy
His, Histidine
Ile, Isoleucine
Leu, Leucine
Lys, Lysine
MBM, Meat and bone meal
ME, Metabolizable energy
Met, Methionine
N, Nitrogen
NND, Non-Nitrogen diet
P, Phosphorus
Phe, Phenylalanine
SBM, Soybean meal
TAA, Total amino acids
TEAA, Total essential amino acids
Thr, Threonine
TME, True metabolizable energy
TME/DM, True metabolizable energy calculated on a dry matter basis
TMEn, True metabolizable energy corrected to zero nitrogen retention
TNEAA, Total non-essential amino acids
Try, Tryptophan
TSAA, Total sulfur amino acids
Val, Valine

Chapter I

Introduction

Although the primary propose of farm animal production is to produce food/meat for human consumption, there is a considerable proportion of animal products that are undesirable for food use. Foxcroft (1984) investigated ratio of consumable products and by-products in UK meat processing plant and reported that the preparation of every metric tonne of meat for human food left at least 32% unusable animal materials such as feather, hair, gut, connective tissues and bone. Considering a total world meat production of 207 million tonnes in 1997 (FAO, 1998), the estimated total inedible animal residues would be about 66 million tonnes, which represents about 74% of total world production of soybean meal in 1997 (66 vs. 89 million tonnes). Should these materials be of no intrinsic value, disposal would not only cause an increased burden to the environment but also represent an additional cost to the food industry (Brooks, 1991). Therefore, adequate rendering of these residues into animal protein meal will contribute substantially to the human food industry and the environment as well as providing an alternative protein source to meet the demand of a fast growing animal industry.

Generally, meat and bone meal (MBM) is composed of meat residues, organ meat, bone, associated fat and to some extent, hair and blood. There is no fixed ratio among these components used by manufacturers, hence the resulting products are very variable in chemical composition and nutritive quality. Sibbald (1986) indicated that Lys and Cys digestibility of 21 MBM samples ranged from 45 to 86% and 30 to 64%, respectively. Batterham et al. (1986) reported that Lys availability varied from 0.31 to 0.86 % when MBM was processed at different temperatures. Wang and Parsons (1998a) reported that the protein content of 32 MBM samples from different commercial manufacturers varied from

40 to 60% and ash from 20 to 47%. Correspondingly, digestibilities of Lys and Cys ranged from 68 to 92% and 20 to 71%, respectively. Notwithstanding the high variability, MBM has been considered for a long time as a rich source of supplemental protein, calcium, phosphorus and B-vitamins as well as a good source of available energy.

Meat and bone meal has been used in poultry diets since 1950 (Patrick, 1953). However, today, it still remains as an underutilized protein ingredient, at usual dietary inclusion levels of about 5% in broiler and young-pig diets. The main constraints include variation in protein quality, as indicated by an unbalanced amino acid profile, and a high ash content, particularly high calcium and phosphorus that affect palatability and the environment. Salmonella contamination may be another limiting factor. However, with a high-standard quality-control program in place, the potential risk of salmonellae contamination has been minimized or eliminated by heating followed by the addition of suitable organic acids such as formic or propionic. According to Brooks (1991) and Veldman et al. (1995), the risk of animal contamination by salmonellae is not greater due to the feeding of MBM than it is for other ingredients such as fish meal, corn or soybean meal. Variation in protein quality and excessive Ca and P seem to be the major factors that limit the use of MBM in animal rations.

Numerous studies have been conducted to assess protein quality and feeding value of MBM for poultry and pigs, with a wide range of nutritive values being reported (Summers et al., 1964; Sathe and McClymont, 1965; Skurray and Herbert, 1974a; Sibbald, 1986, Knabe et al., 1989; Parsons et al., 1997; Wang and Parsons, 1998a). Limiting amino acids that may cause animal growth depression have been indicated in some reports (Summers et al., 1964; Atkinson and Carpenter, 1970b; Wang et al., 1997). However, studies on the order of limiting amino acids in MBM have not yielded a firm conclusion, which may be largely due to the variability in MBM quality, or as a consequence of test methodology including type of cereal grain used and the dietary inclusion rates of MBM (Skurray and Cumming, 1974b; Johri et al., 1980). Furthermore, there has been no conclusion on the effect of high ash content, especially high calcium, that may or may not depress protein digestibility and animal performance (Summers et al., 1964; Partanen, 1994; Johnson and Parsons, 1997). Given the variability in research results, it is understandable that the recommendation of MBM inclusion rate in animal diets is variable (Wilder et al., 1957; Runnels, 1968; Batterham et al., 1970). However, an inclusion constraint below 5% may not be justified at the present time because of improved processing systems and the development of quality-control procedures in MBM production. Recent studies have shown that 10% MBM included in young turkey diets supported satisfactory growth and feed efficiency (Sell, 1996). With synthetic amino acid supplementation, relatively high levels of MBM in broiler and growing pig diets have given good growth results (Anderson and Warnick 1971; Evans and Leibholz, 1979b; Cromwell et al., 1991). Moreover, in recent experiments, formulation of broiler diets with high levels of MBM based on digestible amino acids (DAA) showed performance results equivalent to that for chicks fed a corn-soybean diet (Wang and Parsons, 1998c).

For a protein ingredient, digestibility of amino acids is an important index. Meat and bone meal has protein level similar or higher than that of soybean meal, but amino acid digestibility, due to heat damage is generally poorer, especially digestibilities of Lys and total sulfur amino acids (TSAA). Additionally, today's feed industry tends to use DAA instead of total amino acids (TAA) to formulate animal diets in order to accurately meet animal requirements and to decrease nitrogen pollution. However, only a limited number of studies have demonstrated advantages of formulating poultry diets based on DAA values

(Fernandez et al., 1995; Rostagno et al., 1995; Wang and Parsons, 1998c) probably due to lack of a standard for DAA requirements.

A new version of high ash MBM processed from residues of a low ash MBM has been launched by a Canadian manufacturer. Because variation in chemical composition and protein quality among MBM is substantial due to its natural sources, tabulated data have limited practical value for the extrapolation of its nutritive value and effect on animal performance. A successful introduction of a new product would require not only for consistent production, but also for a detailed description of its chemical and nutritional properties. It is also essential to investigate its inclusion levels and effect on animal performance. The purpose of current study was to generate a database on nutritional profile of the new MBM to facilitate manufacture of a consistent quality product for both domestic and export markets. Moreover, it will also provide basic information for nutritionists to apply the new MBM adequately to diets for poultry and other livestock. Therefore, the overall objectives of the current study are:

- To analyze the chemical composition of a series samples of the new high ash MBM and conventional low ash MBM
- To assess protein quality and true digestibility of amino acids by *in vivo* and *in vitro* methods
- To determine feeding value of the high ash MBM by studying available energy, appropriate dietary inclusion level and supplementation of amino acids in practical broiler diets with the high ash MBM on chick performance
- To compare the effect of formulating broiler diets based on TAA vs. DAA values on chick performance at two inclusion levels of the high ash MBM.

Chapter II

Literature Review

1. Meat and bone meal

Soybean meal, canola meal and fishmeal which are major sources of supplemental protein, are extensively used in poultry and pig production. From time to time there are various constraints in using these ingredients such as price, fluctuation in supply and anti-nutritional factors. Meat and bone meal is an important alternative source which may become more important in the future as increases in MBM become available with annual increases in meat production in many parts of the world.

Meat and bone meal is a type of animal protein feed obtained by rendering animal offal, bones, heads and hooves, and soft tissues such as meat residues, organs, connective tissues as well as whole condemned carcasses. In general, animal hair and blood are not present in MBM. Typically, MBM consists of residues from pork, beef and sheep, either as individual or a mixture of all different animal species depending on the raw material available. Dependent on the level of protein in the final product MBM can be divided into meat meal (protein content >55%) and MBM (protein content around 40-55%). The proportion of bone to soft tissues used in the manufacturing process results in the finished products being named as low ash (ash<20%) or high ash (ash >20%) meal.

In Britain, it was reported that the preparation of every tonne of meat for human consumption would leave about 1/3 of the raw material as residues. Except for approximately 6% of the by-products that were used as fresh parts in the pet-food industry, the rest was processed by commercial manufactures to yield a large quantity of protein meals such as feather meal, blood meal and MBM (Foxcroft, 1984). The world meat production in 1997 was approximately 207 million tonnes (FAO, 1998). Using the ratio of edible to unusable parts from meat processing in Britain, a total world output of animal protein meal was estimated at 54 million tonnes, of which MBM accounted for 64% or 33 million tonnes. Canada produces about 0.5 million tonnes per annum.

2. Processing of MBM

According to FDA (1996) the standard rendering process generally consists of three basic steps: 1) grinding of raw materials; 2) cooking or heating of raw materials to remove moisture by evaporation; and 3) separation of melted fat from the protein solids. Two systems, batch dry cooker and continuous dry rendering methods, are used in the industry to process MBM. Normally, in the old batch dry cooker system, raw materials are delivered to cookers in proportional amounts, and held in the cooker for 30-240 min with the temperature at final discharge varying from 121 to 132 C depending on the type of raw materials processed (Prokop, 1985). In a continuous rendering system, the raw material is fed continuously into the cooker and the cooked material is discharged at a constant rate with a residence time of only 6-15 min (Prokop, 1985). However, the temperature in the discharge phase may still vary from 90 to 110 C among systems. Moreover, high pressure is used in the continuous systems. For both systems, heating/cooking is a necessary step. Adequate heating helps to destroy microorganisms, alter physical form, decrease moisture and improve digestibility. On the other hand overheating will impair protein quality. The old batch drying system in comparison to the new continuous dry systems usually resulted in severe damage to Lys and Cys because of longer cooking time and higher temperature (Knabe et al., 1989; Wang and Parsons, 1998a).

3. Chemical composition

3.1 Proximate composition

There are numerous reports on the chemical composition of MBM (Eastoe and Long, 1960; Batterham et al., 1980; and Wang and Parsons, 1998a). Table 1 shows the general chemical composition and variability of MBM in comparison to common protein supplements, indicating that MBM is a rich source of protein and minerals, especially Ca and P. The percent composition of MBM is on average: gross energy (GE), 4000 kcal/kg; protein, 50.4%; fat, 10.0%; ash, 29%; Ca, 10.3%; P, 5.1%. However, chemical composition varies considerably in commercial MBM: (GE), 2800-4700 kcal/g; protein, 40-60%; fat, 8-15%; ash, 20-47%; Ca, 6-13%; P, 4-8%. The variation may be attributed to differences in raw material source, composition ratio and processing conditions (Atkinson and Carpenter, 1970a; Skuarry and Herbert, 1974a; Wang and Parsons, 1998a). Compared to soybean and canola meal, MBM has higher contents of protein, fat and non-phytate P.

Table 1. Chemical composition of meat and bone meal (MBM) compared to soybean meal (SBM) and canola meal (CM) (air-dry basis)

	MBM [1]	MBM [2]	Average [3]	SBM [4]	CM [4]
DM (%)	93.1	94.8	93	90	90
CP (%)	43.8-56.7	40-60	50.4	48.5	34.0
GE (kcal /kg)	3392-4359	2862-4698	4001	-	-
Fat (%)	7.8-13.9	8-15	10.0	3.0	3.8
Ash (%)	22.4-34.5	20-47	29.2	6.9	4.8
Ca (%)	6.7-12.9	6-12	10.30	0.34	0.63
Non-phytate P (%)	3.8-6.6	4-8	5.1	0.16	0.3

1. Batterham et al. (1980). Australia. 2. Wang and Parsons, (1998a). United States.
3. Based on data of NRC (1994), Waldroup and Adams (1994), Wang and Parsons (1998a).
4. Based on data of NRC (1994) and Canola Council of Canada (1997).

3.2 Fatty acids

Table 2 describes the fat level as well as the fatty acid profile of MBM in comparison with soybean meal and fishmeal. Depending on the fat removal processing procedure and commercial purpose, fat content in MBM ranges from 8 to 15%, with an average of 10% (Table 2). Fat content in MBM is much higher than that in soybean meal and similar to that of fish meal. When relatively high levels of MBM are used in animal diets, additional inclusion of fat to diets may not be required. This gives an important economical value to MBM since fat is an expensive ingredient. However, the high level of fat in MBM is prone to autoxidation potentially resulting in rancidity during storage.

The fat in MBM is composed mainly of fatty acids that are highly digestible with a high proportion of unsaturated fatty acids, accounting for 52% of total fat in MBM. There are few studies on fat digestibility in MBM. Lessire et al. (1985) reported digestibility of 74.0% for total fat, and 82.8% for total fatty acids for MBM containing 10.61% fat.

Table 2. Average percent fatty acid content [1] of meat and bone meal (MBM) in comparison to soybean meal (SBM) and fish meal (FM) (air-dry basis)

	MBM	SBM	FM
Fat	8.6	1.0	9.4
$C_{12:0}$	-	-	0.01
$C_{14:0}$	0.22	-	1.15
$C_{16:0}$	2.36	0.56	3.61
$C_{16:1}$	0.44	0.15	1.58
$C_{18:0}$	1.42	0.03	0.57
$C_{18:1}$	3.74	0.89	1.96
$C_{18:2}$	0.31	1.13	0.14
$C_{18:3}$	-	0.06	0.08

1. Based on data from NRC (1994).

3.3 Minerals

Average values of ash and minerals in MBM are presented in Table 3. In addition to its role as a protein supplement MBM is known as a good supplemental source of Ca and P. Inorganic P is usually an expensive component in animal diets, after energy and protein. When MBM is used in animal diets at levels greater than 5%, there is little need to add inorganic P and, in this regard, a benefit in reduced feed cost is realized

There are no reports on the digestibility of Ca from MBM, but it is assumed that digestibility of Ca in animal by-products is similar to that of inorganic Ca sources, being nearly 100 percent available (Partanen, 1994). Phosphorus in MBM is in an organic form and theoretically can be utilized by animals as well as inorganic P since there is no association with phytic acid. Results of early studies have indicated that the availability of P provided by animal protein is excellent (Waldroup et al., 1965). More recently, Orban and Roland (1992) reported that utilization of P from MBM was 90% for 0-3 week old broilers. Based on body weight, feed conversion ratio (FCR) and tibia growth data from studies with eleven MBM samples, Waldroup and Adams (1994) suggested that P from MBM was as equally available as the P from mono-dicalcium phosphate. The relative availability of P from MBM ranged from 97 to 105%. Waibel et al. (1984) found that young turkeys utilized P from MBM for weight gain and deposition of tibia ash as efficiently as P from dicalcium phosphate. However, contradictory results have been reported by Cromwell (1989) who indicated values for P availability in pigs of 64-72%. The inconsistency in P availability values may be attributable, at least in part, to particle size of processed bone in MBM (Waldroup and Adams, 1994).

It is considered that a high level of ash in MBM may be a disadvantage as it may interfere with digestion and absorption of amino acids and decrease protein quality

(Summers et al., 1964; Sathe and McClymont, 1964). Bone contains 14-25% protein, with 83% being collagen. Collagen contains almost no tryptophan and is deficient in several other essential amino acids (Eastoe and Long, 1960). However, research has yielded inconsistent results. Knabe et al. (1989) reported crude protein digestibility declining from 85 to 75% when shifting from low ash (21.0%) to high ash (34.5%) MBM. On the other hand, Partanen (1994) and Johnson and Parsons (1997) reported no effect on crude protein digestibility (75.5 vs. 74.8%) or protein efficiency ratio when ash content of MBM increased from 20.8 to 33.0% in growing-pig and chick diets. Johnson and Parsons (1997) suggested that a low protein efficiency ratio for chicks given high ash MBM was due to a deficiency of total sulfur amino acids (TSAA) in MBM and not as a consequence of the high ash content. High levels of ash in MBM may have negative effects on digestibility of other nutrients such as fat and energy digestibility/availability. Batterham et al. (1980), and Wang and Parsons (1998a) reported that ME was negatively correlated to bone content in MBM (r= -0.83), and fat digestibility was shown to decrease from 68 to 29% because of high Ca (Partanen, 1994).

In addition to Ca and P, MBM is also a good source of other essential minerals such as Na, Cl, Fe, Mg and Se in comparison to soybean meal and fish meal (Table 3).

Table 3. Mineral content in meat and bone meal (MBM) in comparison with soybean meal (SBM) and canola meal (CM) [1] (as fed-basis)

Items	MBM	SBM	CM
Ash (%)	25.6	6.9	4.32
Ca (%)	10.30	0.27	0.57
P (%)	5.10	0.62	0.91
Non-phytate P (%)	5.10	0.22	0.19
K (%)	1.45	1.98	1.10
Na (%)	0.7	0.02	0.63
Cl (%)	0.69	0.05	0.09
Mg (%)	1.12	0.30	0.46
S (%)	0.5	0.44	0.77
Fe (mg/kg)	490	170	128
Mn (mg/kg)	14	43	44.3
Cu (mg/kg)	2	15	5.13
Se (mg/kg)	0.25	0.1	0.99
Zn (mg/kg)	93	55	62.1

1. Data based on NRC (1994), Waldroup and Adams (1994), and Canola Council of Canada (1997).

4. Metabolizable energy

Earlier investigators reported that the metabolizable energy (ME) value of MBM ranged from 1,722 – 2,712 kcal/kg (Matterson et al., 1965). In later studies it was found that 2,300 – 2,500 kcal/kg seemed to be more adequate for practical poultry diets (Lessire and Leclercq, 1983; Martosiswoyo and Jensen, 1988; Dolz and DeBlas, 1992). NRC (1994) lists 2,150 kcal/kg MEn and 2495 kcal/kg TMEn for MBM containing 50.4% protein, 10.0% ether extract and 93% dry matter. More recently, Dale (1997) reported 2,450 and 2,850 kcal/kg ME, respectively for beef and pork meals (94% DM),. Wang and Parsons (1998a) obtained a range for TMEn of 1,940 – 3,400 kcal/kg, and an average of 2,580 kcal/kg for 32 MBM samples. Metabolizable energy level in MBM may be affected by raw material source and processing conditions. Wang and Parsons (1998a) reported TMEn values of 2,310 – 3,400 kcal/kg for pork meal, 2,250-2,850 kcal/kg for beef meal, and 1,940-3,240 kcal/kg for meal mixtures. The TMEn for MBM processed at low temperature was 2,180-3,400 kcal/g, and 1,940-2,850 kcal/kg at high processing temperature. High ash MBM (ash >35%) contained 1,826-2,270 TMEn kcal/kg for poultry. The data indicate a wide range in reported available energy values for MBM.

Difference in methodology may be a contributing factor in the inconsistency in MBM energy values (Schang and Hamilton, 1982). There are two *in vivo* methods currently used to measure ME. One is by a substitution method as modified by Farrell (1978) and another is the precision force-feeding rooster assay developed by Sibbald (1986). Metabolizable energy values from early reports were obtained by the substitution method (e.g. Matterson et al., 1965). However, reports have shown a reverse relationship between the level of substitution and ME value from the substitution assay. Using adult roosters, Lessire and Leclercq (1983) measured 10 MBM samples substituting MBM at levels of 10 or 20% for corn in the basal diet, and obtained lower ME values at 20% than at 10% replacement level. In another study (Lessire et al., 1985) two MBM samples were incorporated at 5, 10, 20, 40 and 60% in the diet, and the resulting ME value for both MBM samples decreased about 25% when the inclusion increased from 5 to 60%. Noticeable decline was observed as the level of MBM increased from 5-20% of the diets. However, they also noticed that up to 20% inclusion ME content of MBM was relatively constant. Recently, the precision force-feeding TME of Sibbald assay has been commonly accepted for determination of available energy in poultry because it avoids the problem associated with the substitution level of test material.

5. Protein and amino acids

5.1 Protein digestibility

Information on protein digestibility of MBM is limited in poultry since the tedious separation of urine N from fecal N is required due to the mixed excreta. Early research indicated protein digestibility of MBM to be 80% for growing chicks (Summers et al., 1964), and 69% for colostomized adult birds (Warring et al., 1969). More recently, Lessire et al. (1985) indicated that protein digestibility of low fat and high fat MBM in the adult rooster was 63.6 and 64.2%, respectively. Similarly, Rhone-Poulenc (1989) suggested a digestible protein value of 66.1 and 78.4%, respectively for low and high quality MBM.

In recent years, various *in vitro* methods have been developed to measure protein digestibility for poultry feedstuffs. By using pepsin digestion in a study of nine MBM samples, Johnston and Coon (1979) showed protein solubility to range from 60.0 to 82.0%, with an average value of 71.9%. Similarly, Parsons et al. (1997) tested 12 MBM samples for protein solubility and obtained values varying from 53.6 to 82.6%, with an average of 71.7%. For 20 New Zealand MBM samples, Moughan et al. (1989) used the multi-enzyme procedures proposed by Metz and Van der Meer (1985) and obtained average protein solubility of 70.1% with a range of 58.7-89.0%. A digestion coefficient of 70% as estimated by in vitro techniques may be suggested as a general reference for MBM.

5.2 Amino acids and their digestibility

Table 4 lists the range and average contents of essential amino acids (EAA) in MBM in comparison to those in soybean meal. Average values of most EAA in MBM are similar to those of soybean meal except for Try which is much lower in MBM. Phenylalanine, Val, Thr and His values are also slightly lower for MBM. The level of total EAA in MBM is

3.5% lower than that in SBM. However, the levels of Lys and Met, the most limiting AA for poultry are similar to those in soybean meal.

Table 4. Amino acid profile of meat and bone meal (MBM) and soybean meal (SBM) [1] (air-dry basis)

	MBM	SBM
Protein (%)	50.4 (43.8-56.7)	48.5
Arginine (%)	3.46 (3.04 - 4.57)	3.40
Cystine (%)	0.64 (0.32 – 1.16)	0.68
Histidine (%)	0.91(0.51 – 1.52)	1.22
Isoleucine (%)	1.38 (0.82- 1.63)	2.14
Leucine (%)	3.07(2.08 – 3.90)	3.50
Lysine (%)	2.59 (1.57 – 3.23)	2.77
Methionine (%)	0.63 (0.48 – 1.02)	0.68
Phenylalanine (%)	1.65 (1.12 – 2.12)	2.38
Tyrosine (%)	0.98 (0.53 – 1.25)	1.65
Threonine (%)	1.59 (0.99 – 1.84)	1.85
Tryptophan (%)	0.31 (0.23 - 0.35)	0.74
Valine (%)	1.97 (1.37 – 2.42)	2.38
TEAA [2] (%)	19.18	23.39

1. Based on data from NRC (1994), Partanen (1994), Waldroup and Adams (1994), Wang and Parsons(1998a).

2. TEAA, total essential amino acids.

Data of amino acid digestibility of MBM from several studies are summarized in Table 5. The average true digestibility of most AA in MBM for poultry is about 80-85%, Cys at below 60% being the lowest. Values from the four studies are variable, which may be related to methodology employed and to raw material source of MBM. Special caution is needed when using these coefficients.

Table 5. True digestibility of essential amino acids in meat and bone meal for poultry (%)

Amino acid	Study *				Average
	1	2	3	4	
Lys	80.5	78.4	80.9	82.2	80.5
Met	88.0	85.3	85.1	86.7	86.3
Cys	52.4	63.0	57.7	47.0	55.0
Thr	81.5	76.8	80.4	78.5	79.3
Ile	83.4	80.5	84.3	89.1	84.3
Leu	85.8	80.4	85.7	88.3	85.1
His	85.4	77.3	75.6	81.8	80.0
Val	83.2	77.6	84.5	87.8	83.3
Arg	85.1	79.3	87.1	86.0	84.4
Phe	84.0	80.6	86.6	83.7	83.7

* Based on data from 1, Sibbald (1986); 2, Rhone-Poulenc (1989); 3, Parsons et al., (1997); and 4, Wang and Parsons (1998a).

The database on availability of amino acids (AA) in MBM is limited since it is usually determined by slope-ratio growth assay that is more complicated and time consuming than measuring digestibility. Wang and Parsons (1998b) in studies with poultry reported that bioavailability for protein synthesis of digestible Lys or Met was above 90% for both high or low quality MBM, but for TSAA, the bioavailability was only 80% or less. Despite the criticism that digestibility data for low quality feedstuffs, especially heat damaged ingredients, may overestimate the amount of AA that are actually utilizable by poultry (Batterham, 1992), true digestibility of AA is often considered synonymous with AA availability in practice. This may be, in part, because measurement of digestibility is much simpler and quicker than that for availability.

5.3 Variation in protein quality

Meat and bone meal probably has the greatest variation in protein quality among protein ingredients. For example as shown in Table 4, Lys content ranged from 1.57 to 3.23%, Cys from 0.32 to 1.16%, and Met from 0.48 to 1.02%. True digestibility of Lys varied from 67.9 to 91.9%, Cys from 20.5 to 70.8%, and Met from 75.8 to 91.9% (Wang and Parsons 1998a). In studies with poultry, protein efficiency ratio of 14 MBM samples ranged from 0.61 to 2.89, and net protein ratio ranged from 1.76-3.55 (Parsons et al., 1997). Knabe et al. (1989) reported the ileal digestibility of EAA in growing pigs as varying from 56 to 79%, 73 to 86%, 35 to 65% for Lys, Arg and Try, respectively. Raw material source and processing conditions are two of the major factors that affect protein quality of MBM.

Due to the variable protein quality of MBM, various in vitro and in vivo methods have been developed to predict protein quality of MBM for poultry. In general, the precision-feeding cockerel assay proposed by Sibbald (1979) and the pepsin or two-stage pepsin/pancreatin in vitro technique are the most widely used procedures to evaluate digestibility of AA and solubility of dry matter and protein for poultry feedstuffs. Data from the former has practical value as it can be used to make dietary formulations more accurate and economical. In this regard, the maximum effectiveness can be extracted from feedstuffs. The latter is relatively quick and inexpensive, but direct application in diet formulation is less useful than for amino acid digestible values.

In the precision-feeding bioassay, tested material is placed directly into the crop of fasted adult cockerels followed by 48 h excreta collection. Amino acid content in both tested ingredient and individual excreta samples are analyzed. Correction of endogenous loss of amino acids is obtained by collecting excreta from a group of fasted cockerels or those fed an N-free diet. Amino acid digestibility is calculated based on the difference between intake and output of AA. However, a criticism of the accuracy of this method is that dietary factors, especially protein concentration and amount of feed given to the roosters may influence the secretion and hence excretion of endogenous AA. Another claim is that microflora in the lower gut might affect the interpretation of the data by resulting in deaminating of undigested AA residues. To overcome this latter criticism, cecectomized roosters have been commonly used for assessment of AA digestibility in poultry feedstuffs.

5.4 Order of limiting amino acids in MBM

Although MBM has a high level of Lys, heat damage during processing may decrease content and digestibilities of Lys and also TSAA and consequently result in a reduction in the overall AA balance. Additionally, MBM protein is deficient in Try and a number of other essential AA due to the presence of large quantities of bone or connective tissues (Easton and Long, 1960). Amino acid imbalance is one of the constrains in the usage rate of MBM in animal diets. However, supplementation with synthetic AA to compensate the deficiencies may increase the inclusion level of MBM in animal diets.

Attention has been devoted to the definition of the order of limiting AA in MBM. Patrick (1953) suggested that Lys was the principle AA deficient in MBM. Kratzer and Davis (1959) used 10 MBM samples to determine AA deficiency by individual or combined addition of AA to a 20% CP diet for chicks, and the results showed Try and TSAA to be the first two limiting AA in MBM. Summers et al. (1964) used MBM as the sole protein source in poultry diets and found MBM to be deficient in six AA, namely Met the first limiting, Try and Ile the second and about equally limiting, and Cys, Thr, and Arg the 4^{th}, 5^{th} and 6^{th} limiting. However, In a study with nine MBM samples, Johnston and Coon (1979) reported that the first limiting AA for chick weight gain were TSAA. By using deletion and addition methods, Wang et al. (1997) investigated a number of commercial MBM varying in AA digestibility for chicks, and concluded that the order of limiting AA to be : 1st, Try and TSAA; 2nd, Thr; 3rd, Ile and Phe+Try; 4th, Met; 5th, Lys.

The variable results in order of AA deficiency may be related to incorporation levels of MBM (Summers et al., 1964; Atkinson and Carpenter, 1970b) and related to the type of cereal in the diet (Skuarry and Cumming, 1974b; Johri et al., 1980). Usually, Lys or Met is the first limiting AA in cereal diets such as barley, wheat and corn. Atkinson and Carpenter (1970b) reported that in barley plus MBM diets, Lys became the first limiting with the second being Met and Thr when MBM contributed 40% of total protein to the diet either for rats or pigs. In a wheat plus MBM diet, Lys was the first limiting AA for poultry (March et al., 1950). Sathe and McClymont (1968) showed that wheat plus MBM diets were equally limiting in Lys and Met for chicks. In contrast, Stockland et al. (1971) found Try to be the first limiting in a corn-based diet for pigs. When studying MBM as the sole protein source in the chick diet, Skuarry and Cumming (1974b) indicated the order of limitation of EAA was Met followed by Val, Lys, Try, Arg, Ile and His. When MBM contributed approximately 50% of the crude protein in barley, wheat, sorghum based diets, the major limiting AA were Met, Cys, His and Lys. Wilson (1967) found that when MBM and cereals each contributed 50% of the protein to the chick diet, Lys and Met were equally limiting, however, if cereal contributed two thirds of the protein, the order of limiting AA in the diet was determined by the cereal. It is apparent that when MBM is used at a low level in diets, the order of limiting AA in MBM may be masked by the type of cereal used. If MBM is used at a high level or as the sole protein source in diets, the order of limiting AA inherent in MBM will be realized. In this regard, variation of type and amount of cereal used in diets and the inclusion level and quality of MBM must be considered in evaluating the order of limiting AA for MBM.

6. Inclusion of meat and bone meal in poultry diets

Meat and bone meal is a good source of protein, Ca and P. In addition, its energy is at a relatively low cost in comparison to other protein sources such as fishmeal. These factors make MBM attractive for extensive use in monogastric diets as a supplemental protein to replace the soybean meal and fishmeal. However, inclusion levels vary widely. An early report from Wilder et al. (1957) evaluated 13 MBM samples from various sources with an average protein of 50% and found that MBM could be used in broiler diets at levels as high as 17% without any adverse effects on broiler weight gain or FCR. In Australia, Sathe and McClymont (1965) reported that 25 to 30% protein provided by low quality MBM in chick diets containing 20% protein was a maximum level. However, the results also showed that high quality MBM could be used to provide 35% of total protein in chick diets with growth rate equivalent to that for a soybean meal diet. Runnels (1968) showed that a MBM inclusion rate of 7.5% was the best level for optimum chick weight gain although 10% MBM could be used if energy and EAA in the diets were maintained at normal levels. Salmon (1977) stated that an increase of MBM from 7.5 to 15% in turkey diets reduced weight gain. Evans and Leibholz (1979a) reported that pig performance was linearly depressed as MBM replaced soybean meal at increasing levels from 2.5 to 10% in corn-based diets. These early studies showed little agreement for the inclusion level of MBM in poultry and pig diets. Usually, MBM inclusion level is restricted to 5%. The problems associated with the use of high inclusion of MBM in broiler or young-pig diets are AA imbalance, high dietary Ca and P level and salmonellae contamination.

The adverse effect from AA imbalance may be compensated by supplementation with synthetic AA. Early studies found that MBM could be used at 15% in chick diets with supplementation of Lys and Met (Sathe and McClymont, 1968). Anderson and Warnick

(1971) reported that chicks receiving diets containing 12% MBM supplemented with Lys and Met had growth rate and FCR as good as birds fed either herring meal or soybean meal. Waibel et al. (1987) reported that diets containing 7% MBM, with balanced AA, provided good weight gain and FCR for growing turkeys. More recently, with improved protein quality resulting from improved processing conditions, Sell (1996) reported that turkey toms fed on 10% MBM from 1 to 119 days had the same body weight gain and FCR as those fed on soybean meal diets. Wang and Parsons (1998 c) reported that chicks receiving 20% MBM diets formulated on digestible AA values grew as well as those birds fed corn-soybean meal diets. In other species, Stockland et al. (1971) found that pigs receiving a corn diet with 19.5% MBM but supplemented with Try, Lys and Met had the same growth rate and FCR as those fed soybean meal diets. Evans and Leibholz (1979b) also showed that supplementing Lys and Met in the diets containing 22% MBM increased the feed intake and weight gain of the pigs by 15 to 18%. According to Cromwell et al. (1991) in the studies with growing-finishing pigs, up to at least 10% of MBM could be used in corn-based diets fortified with Lys plus 0.03% L-Try. It may be concluded that satisfactory animal performance can be achieved when relatively high levels of MBM are used in monogastric diets as long as the diets are adequately supplemented with synthetic AA.

When MBM, particularly high ash MBM, is used as the major supplemental protein in animal diets, high dietary levels of Ca and P are usually obtained. Firm conclusions have not been made regarding the possible negative effect of excessive dietary Ca and P level on chick performance or protein digestibility. Summers et al. (1964) indicated that high dietary levels of Ca and P depressed chick performance in studies with MBM. However, Sathe and McClymont (1965) considered that the negative effect was due to the fact that dietary AA did not meet requirements. This conclusion was supported by Runnels (1968) and Evans and Leibholz (1979a) who reported that high levels of dietary Ca (1.3 and 3.2%) and P (1.0%) in commercial broiler and young pig diets containing MBM had no adverse effects on animal performance. Partanen (1994) and Johnson and Parsons (1997) also reported that high Ca and P provided by high ash MBM did not affect protein digestibility or protein efficiency ratio for pigs and chicks. The lack of effect of high dietary levels of Ca and P may be explained by the findings of Fernandez (1992) and Partanen (1994) who reported that an increase in Ca and P intake decreased their digestibility and/or absorption and as well increased urinary P excretion. Notwithstanding the apparent lack of the effect of high levels of Ca and P on animal performance, attention to the control of P pollution of the environment is important and consequently, excessive dietary P levels are not recommended in animal diets. Therefore, level of P in MBM may represent a real constraint on its dietary inclusion level.

As the animal by-products such as MBM may be a good material source for bacterial growth, one of the concerns in using MBM is bacterial contamination, especially salmonellae. In small-scale processes, inadequate heat treatment and ineffective separation of incoming and outgoing material can often result in salmonellae contamination and recontamination. In large, efficient MBM processing operations with a high hygiene standard, the potential for salmonellae contamination may be minimized or eliminated with the use of heating followed by the addition of suitable organic acids such as formic or propionic. Brooks (1991) reported that risk of salmonellae contamination was less when MBM was compared to other commonly used materials in animal diets such as fishmeal or soybean meal. Veldman et al. (1995) investigated the rate of contamination of poultry feeds with salmonellae from feed components used by the Dutch feed industry during 1990-1991, and showed that fish meal and corn presented higher rates of salmonellae contamination than did MBM (31 and 27% vs. 4%, respectively). These results indicate that the risk of salmonellae contamination from MBM is low and controllable and hence should not restrict usage rate of MBM in animal diets.

In commercial practice, the inclusion level of MBM depends on several factors. The advancement in synthetic AA production has made more industrial AA available commercially at a reasonable cost. Protein quality as reflected in AA balance can be overcome by supplementation with appropriate synthetic AA. Consequently, the restriction rate of MBM below 5% in poultry and young animal diets is now questionable due to the availability of inexpensive synthetic AA and because of improved processing conditions that result in the production of high quality MBM. Furthermore, the risk of salmonellae contamination may be controlled by improved quality control programs during MBM processing. While Ca + P at high dietary levels have not been shown conclusively to impair animal performance or protein digestibility, the risk of P pollution from a high ash MBM is probably the major limiting factor constraining the usage rate of MBM at 5% in broiler and turkey diet notwithstanding the improved quality of MBM and the corporation of inexpensive AA in diet adjustments.

Chapter III, Manuscript 1

Chemical Composition and Nutritional Value of High and Low Ash Meat and Bone Meals for Poultry

Abstract A newly developed high ash meat and bone meal (MBM) was subjected to a series of nutritional evaluations in comparison to a low ash MBM. Samples were collected as five batches over two years for high ash MBM. Proximate analyses showed that the contents of most nutrients in high ash MBM were consistent from batch to batch except for small variations in fat and ash content. On a DM basis, it contained: GE, 4249 ± 200 kcal/kg; protein, 53 ± 2.7% with a relatively favorable AA profile; ash, 29 ± 2% with well balanced Ca (9%) and P (5%); fat, 11.5% with a ratio of 1 between saturated and unsaturated fatty acids. In vitro protein digestibility was 66% and true digestibilities of total essential amino acids (TEAA), Lys and total sulfur amino acids (TSAA) determined with CEC roosters were 83, 77.5, and 71.3%, respectively. There were no statistical differences ($P>0.05$) in AA digestibility or in *in vitro* protein digestibility between high and low ash MBM despite of a difference in ash content. True metabolizable energy corrected to zero nitrogen retention was determined as 2800 kcal/kg on a DM basis. Chemical component analysis and AA digestibility and available energy indicate that the high ash MBM can be a good and effective source of supplemental protein.

Introduction

Meat and bone meal has long been considered as a rich source of supplemental protein, an effective source of Ca, P, and B-vitamins as well as a potential source of energy for monogastric animals. However, in comparison to many other protein ingredients such as soybean meal or fish meal, MBM is extremely variable in nutritive value. Wang and Parsons (1998a) showed that the nutrient content of 32 MBM samples obtained from different commercial manufacturers varied widely; protein from 40 to 60%, ash from 20 to 47%, true digestibility of Lys and Cys from 68 to 92% and 20 to 71%, respectively. Sibbald (1986) reported that Lys and Cys digestibilities of 21 MBM samples ranged from 45 to 86% and 30 to 64%, respectively. Batterham et al. (1986) found that Lys availability varied from 48 to 88%. This variation is a consequence of different systems being used in processing MBM and because no fixed ratio among the components which include meat residues, organ, bone, associate fat and to some extent, hair and blood is used by manufacturers in the production of MBM. Different production of MBM leads to differences in chemical composition and nutrient quality, specifically available energy and protein. In this regard, tabulated data of nutritive values for MBM are of somewhat limited value.

A new version of high ash MBM was produced as the residual product in the production of a high quality low ash MBM. To facilitate the use of this new MBM product in animal diets, a database on the nutrient characteristics is required, especially for protein quality. The objectives of this study were to determine the chemical composition, available energy value, AA profile, true AA digestibility and in vitro protein digestibility of high ash MBM and to compare these values with those of low ash MBM.

Materials and Methods

Chemical analyses

Five batches of high ash MBM and two batches of low ash MBM samples were obtained from the same rendering factory in Ontario. The MBM samples were composed of

90% pig offal and 10% beef residue with a small amount of hair and blood. The high ash MBM consisted of more bones and connective tissues than the low ash MBM. The samples were analyzed for dry matter (DM), gross energy (GE), fatty acids, protein, ash, Ca and P based on AOAC (1990) procedures. Amino acids were determined by ion-exchange chromatography following hydrolysis of samples in 6N HCl at 110 C for 24 h (Mills et al., 1989). Methionine and Cys were analyzed after performic acid oxidation (Andrews and Baldar, 1985) and Try was determined following alkaline hydrolysis (Hugli and Moore, 1972).

2.2 True metabolizable energy (TME)

True metabolizable energy was assayed by a method described by Sibbald (1986) with some modifications (Zhang et al., 1994) using conventional Leghorn (Dekalb-Delta) roosters kept in individual cages with raised wire floors in an environmentally controlled room with 14 h light and 10 h dark. Water and a wheat-based diet were supplied *ad libitum* when birds were not on experiment. The wheat-based maintenance diet was fortified to meet NRC (1994) specification for AA and macronutrients of roaster chickens. Prior to initiation of a balance trail, birds were moved into metabolism cages and deprived of feed for 28 h, but allowed water ad libitum. During starvation the birds were administered 32 ml glucose solution (25 g glucose) by gavage in order to limit AA catabolism, then each MBM sample (30 g) was randomly fed to individual roosters (n = 10 - 20) by crop intubation. A plastic tray was placed under each cage to collect excreta during the following 48 h. Excreta from individual roosters was freeze-dried, weighed and ground to pass through a 1 mm screen. Samples of MBM and individual excreta were assayed for N and GE. A correction coefficient of endogenous loss of energy from urine and feces was applied according to data from previous experiments in which unfed birds were treated similarly to the fed birds. True metabolizable energy and nitrogen-corrected true metabolizable energy (TMEn) were calculated for each sample according to Sibbald (1986).

Amino acid digestibility

The precision force-feeding rooster bioassay described for the TME determinations was used in the evaluation of true AA digestibility of MBM. Procedures for force-feeding test materials to roosters were the same as outlined above for TME, except that both cecectomized (CEC) and intact roosters were used. Correction applied for endogenous AA loss was determined following precision feeding 25g non-nitrogen diet (NND, glucose and canola oil 90:10). Twenty birds of each type were used. Amino acids in MBM, excreta and endogenous excreta were individually analyzed using ion-exchange chromatography as described above. True digestibility of AA was calculated by the following formula:

True digestibility of AA (%)=[Ingested AA (g)- (Excreted AA (g)- Endogenous AA (g))/Ingested AA]x100

In vitro protein digestibility

Digestible protein content was determined in vitro using a two-stage digestion/dialysis unit according to the procedure described by Slominski et al. (1999) for canola meal. A series of MBM samples varying in weight (3, 4 and 5 g) were used to select the best sample weight for measuring protein digestibility of MBM because MBM has a higher protein content than canola meal. A brief description of the procedure is as follows:

1) Grind test samples to pass through 1 mm screen; 2) Mix 3, 4 or 5 g MBM with 500 mg commercial pepsin (P 1700, Sigma, St. Louis, MO, USA) with 50 ml 0.1 M HCl/54 mM NaCl solution and incubate for 1 h at 40 C in an environmentally controlled incubator shaker; 3) Add 2.5 ml 2.0 M NaOH and adjust pH to approximately 7.0, then add 250 ul pancreatin solution (2g commercial pancreatin, P 1750, 4xUSP; Sigma, St. Louis, MO, USA, with 2 ml 0.1 M phosphate buffer, pH 7.0); 4) Transfer the contents into

dialysis tubes (Spectrum, Houston, TX, USA) with a molecular weight cut-off value of 12,000 - 14,000 and place into a thermal-controlled water bath filled with 0.05 M phosphate buffer at pH 7.0 to allow digestion for 6 h at 40 C in rotating dialysis tubes; 5) After digestion, replace the buffer with ice water and continue rotation of dialysis tubes for 72 h with regular changes of water; 6) Transfer all contents in a dialysis tube to individual containers with known weight and freeze-dried, then weigh and measure protein content (Kjeldahl N x 6.25); 7) Calculate protein digestibility as:

Protein digestibility (%)=(N in sample (g)-N in undigested sample (g))/N in sample (g) x100

Statistical analyses

Data for in vitro protein digestibility, TME were subjected to ANOVA (Steel et al., 1997) for a completely randomized design, followed by Duncan's multiply range test (Duncan, 1955) to detect differences among treatment means. Data for AA digestibility were analyzed by ANOVA (Steel et al., 1997) for a completely randomized design with a factorial arrangement of treatments (2 MBM x 2 bird types).

Results and discussion

Proximate composition

Table 1 shows that the contents of DM, GE, protein, Ca and P from five batches of high ash MBM were of low variability (CV<5%) although fat and ash appeared to be somewhat variable (CV, 7 and 13%, respectively). Generally, all nutrients in high ash MBM were very close to the NRC (1994) value for a MBM with 50.4% protein content, indicating a uniformity of product. All nutrients in low ash MBM were more consistent than those in high ash MBM. Compared to low ash MBM, the protein content of high ash MBM was 13% lower, whereas contents of ash, Ca and P were approximately two times higher. In the rendering plant, low ash MBM was the main product and consequently a resulting large quantity of bone and other residues were left for the production of high ash MBM. Therefore, it is not surprising that the content of ash in the new MBM was relatively high and variable. It is generally recognized that a higher ash content will result in a poorer protein quality of MBM since ash level reflects amount of bone added in MBM. Bone protein is composed of a large proportion of collagen, which is deficient in many essential amino acids (EAA), especially Try (Eastoe and Long, 1960). However, the ash content of the high ash MBM samples was within the range reported values 20 - 47% (Wang and Parsons, 1998a). Fat content in the MBM samples was high in comparison to most plant protein ingredients. In this regard, the high fat level in MBM may represent a risk of autooxidation in storage.

Table 1. Composition of high and low ash meat and bone meal from different batches [1] (DM basis)

Samples	Batch #	DM (%)	GE (kcal/kg)	Protein (%)	Fat (%)	Ash (%)	Ca (%)	P (%)
HAMBM [2]	1 (04/98)	94.83	4354.1	51.73	11.97	30.64	9.46	5.48
HAMBM	2 (08/98)	95.43	4157.9	55.34	10.64	29.40	9.24	5.51
HAMBM	3 (04/99)	94.40	4548.1	56.14	13.42	25.84	8.99	5.13
HAMBM	4 (09/99)	96.70	4064.4	51.20	9.49	30.54	9.00	5.16
HAMBM	5 (11/99)	95.13	4120.2	49.43	12.11	30.68	9.65	5.09
Mean ± Std		95.30±0.87	4248.9±199.7	52.87±2.72	11.53±1.50	29.42±2.07	9.26±0.28	5.27±0.21
CV (%)		0.91	4.70	5.14	13.05	7.05	3.00	3.90
LAMBM [2]	1 (04/98)	95.59	5250.2	60.74	14.16	15.73	3.81	2.60
LAMBM	2 (04/99)	96.42	5281.0	60.99	14.93	15.16	4.07	2.51
Mean ± Std		96.01 ±0.59	5265.6±21.8	60.87±0.18	14.55±0.54	15.45±0.40	3.94±0.18	2.55±0.08
CV (%)		0.61	0.41	0.29	3.74	2.61	4.63	3.15

1. All values were means of duplicate or triplicate analyses for each batch.

2. HAMBM, high ash meat and bone meal; LAMBM, low ash meat and bone meal.

Fatty acid composition

Fatty acid composition of high and low ash MBM is listed in Table 2. The difference in fat level between low and high ash MBM was 2.6 percentage units. Fatty acid profile remained constant between the two MBM and agreed well with NRC (1994). Approximately 45% of fat in MBM was composed of C 16:0 and C 18:0 fatty acids, while 42% was C 18:1 and C 18:2 unsaturated fatty acids, indicating a ratio of saturated to unsaturated fatty acids of about 1. There was a little variation among different sample batches.

Table 2. Fatty acids of high and low ash meat and bone meal from different batches [1]

	HAMBM* 1	HAMBM 2	HAMBM 3	Mean	LAMBM* 1	LAMBM 2	Mean	MBM[2]
Fat (%)	11.35	10.15	12.67	11.39	13.54	14.40	13.97	8.6
C 14:0	2.23	2.27	1.86	2.12	1.97	1.92	1.94	2.56
C 16:0	28.84	30.03	27.13	28.67	28.75	27.65	28.2	27.44
C 16:1 (n=7)	2.65	2.77	2.84	2.75	2.87	3.00	2.94	5.12
C 18:0	19.01	18.54	15.77	17.77	16.96	16.04	16.5	16.51
C 18:1 (n=9)	37.40	38.22	41.20	38.94	40.05	41.86	40.96	43.49
C 18:1 (n=7)	3.60	4.32	4.04	3.99	3.51	3.97	3.74	-
C 18:2 (n=6)	1.07	1.09	4.76	2.31	2.03	4.06	3.04	3.60
C 20:1 (n=9)	2.02	1.02	0.74	1.26	1.79	0.74	1.26	-
C 22:6 (n=3)	1.12	0.91	0.44	0.82	0.57	0.20	0.38	-

[1] Value of fatty acids for each batch sample was obtained on four replicates. All fatty acid composition was calculated on fat basis (%); fat content is based on as fed basis.

[2] Data was adapted from NRC (1994).

* HAMBM, high ash meat and bone meal; LAMBM, low ash meat and bone meal.

In vitro protein digestibility

As shown in Table 3, the coefficients of in vitro protein digestibility were not influenced by the amount (3 - 5 g) of sample used in the assay. The consistent value for protein digestibility indicates that a sufficient amount of enzyme was added to digest all of the protein in MBM. However, to obtain enough residue for measuring N or AA contents, a sample size of 4 g MBM was chosen.

The in vitro protein digestibility values ranged from 66% to 69% for high and low ash MBM with no significant difference among samples (Table 3). Protein digestibility coefficients from this study agreed well with other published data. Johnston and Coon (1979) reported protein solubility in 0.002% pepsin ranging from 60.0 to 82.0% with an average of 71.9% for nine MBM samples. Similarly, Parsons et al. (1997) assessed 12 MBM samples and obtained protein solubility values varying from 53.6 to 82.6% with an average of 71.7%. Using pepsin/pancreatin enzymes in an in vitro method, Moughan et al. (1989) tested 20 MBM samples and obtained an average protein solubility value of 70.1% with a range of 58.7-89.0%.

Table 3. In vitro protein digestibility of high and low ash meat and bone meal [1] (air-dry basis)

	Sample Wt. (g) (Mean ± Std)	Protein digestibility (%) (Mean ± Std)
HAMBM [2]	3.0017 ± 0.0007	66.46 ± 0.16[a]
	4.0081 ± 0.0062	66.21 ± 0.89[a]
	5.0033 ± 0.0036	65.82 ± 0.96[a]
LAMBM [2]	4.0124 ± 0.0028	69.29 ± 1.63[a]
	5.0052 ± 0.0013	66.02 ± 0.11[a]

1. Each value of high ash MBM is from four batches of three replicates; each mean of low ash MBM is from two batches of three replicates. Values with no common superscript within the same column differ significantly (P<0.05).

True metabolizable energy (TME)

True metabolizable energy values are shown in Table 4. There was no difference in TME between two batches of high ash MBM. The high ash MBM contained 2930-3010 kcal/kg TME, whereas the low ash MBM had 3540 kcal/kg, with a difference of approximately 550 kcal/kg (P<0.05). Similarly, TMEn, TME/DM and TMEn/DM were all lower in the high ash MBM. The difference in available energy content between high and low ash MBM may be mainly caused by the difference in ash content although relative differences in fat and protein content could also have an effect. It has been reported that high ash content was negatively correlated to fat and energy digestibility in MBM (r = -0.83) (Partanen, 1994; Wang and Parsons, 1998a). The value for TMEn (2750 kcal/kg) in high ash MBM was higher than that (2,495 kcal/kg) reported by NRC (1994) which is assigned to a sample with 50.4% protein, 10.0% ether extract and 93% DM. The value in the current experiment, however, is in agreement with several authors who indicated that ME for MBM in NRC is underestimated (Lessire et al., 1985; Martosiswoyo and Jensen, 1988; Dolz and Blas, 1992). More recently, Dale (1997) reported values of 2450 and 2850 kcal ME/kg for beef and pork meal, respectively. Wang and Parsons (1998a) indicated 2850 kcal/kg as an average TMEn for 32 MBM samples on as fed basis. Our data support the findings of others, showing that available energy of MBM is underestimated in NRC (1994) and indicating that a value of 2670 kcal/kg TMEn for the high ash MBM may be used for poultry diets.

Table 4. True metabolizable energy (TME) of high and low ash meat and bone meal [1]

Samples	Replicate No.	TME (kcal/kg)	TMEn* (kcal/kg)	TME/DM (kcal/kg)	TMEn/DM* (kcal/kg)
HAMBM* (4/98)	8	3010 ± 92^{a}	2730 ± 83^{a}	3174 ± 97^{a}	2879 ± 87^{a}
HAMBM (8/98)	16	2930 ± 131^{a}	2606 ± 109^{a}	3070 ± 137^{a}	2731 ± 114^{a}
LAMBM*	7	3538 ± 103^{b}	3174 ± 65^{b}	3702 ± 108^{b}	3321 ± 68^{b}

1. Values are Mean± Std. Values within a column not followed by the different character differed significantly ($P<0.05$).
*HAMBM, high ash MBM; LAMBM, low ash MBM; TMEn, true metabolizable energy correct to zero nitrogen retention. TME/DM, true metabolizable energy calculated on dry matter basis.

Amino acid profile and true amino acid digestibility

Amino acid profile

The AA profile of high and low ash MBM is reported in Table 5. Amino acid content of high ash MBM from three batches was consistent. There were no differences in AA levels, expressed as mg/g protein, between low ash MBM and high ash MBM. This implies that the protein quality of the new product, high ash MBM, may be as good as that for low ash MBM, in spite of a difference in ash content. Amino acid content in MBM is similar to that of NRC (1994) for MBM, and is in agreement with values reported by

Partanen (1994) and Johnson et al. (1998) for high and low ash MBM. The essential AA content of MBM is not as good as that in soybean meal (Table 5), particularly for Try, Ile, and Phe with values almost two times lower in MBM than in soybean meal. Generally, the total essential AA content is approximately 30% higher in soybean meal than in MBM.

Table 5. Amino acid content of high and low ash meat and bone meal (MBM) from different batches and in comparison to that of soybean meal [1] (as fed-basis)

	High ash MBM 1	High ash MBM 2	High ash MBM 3	Mean	Low ash MBM 1	Low ash MBM 2	Mean	Soybean meal
Protein (%)	49.1	53.0	49.5	50.5	58.1	58.8	52.7	48.5
Ash (%)	29.1	24.4	29.5	27.7	14.6	15.0	23.9	6.9
EAA* (mg/g protein)								
ARG	66.0	61.7	72.3	66.5	64.2	56.6	60.4	70.1
HIS	21.4	20.0	23.0	21.4	23.6	20.2	21.9	25.2
ILE	22.0	20.2	24.8	22.4	24.6	23.6	24.1	44.1
LEU	52.0	48.3	58.0	52.7	64.4	54.1	59.2	72.2
LYS	48.1	44.7	51.5	48.1	52.5	47.3	50.0	57.1
MET	8.8	12.6	16.0	12.5	9.5	13.4	11.5	14.0
PHE	28.5	21.5	29.9	26.5	33.4	23.3	28.4	49.1
THR	31.0	26.4	33.3	30.1	37.4	28.2	32.9	38.1
VAL	34.2	36.0	40.0	36.8	37.4	34.7	36.1	49.1
TRY	10.2	7.7	8.3	8.7	10.3	7.3	8.9	15.3
TEAA*	322.3	299.2	357.2	325.6	357.4	308.8	333.4	434.2
NEAA* (mg/g protein)								
ALA	84.0	65.3	76.6	75.0	82.7	56.6	69.7	
ASP	88.1	72.5	87.3	82.3	92.7	71.8	82.1	
CYS	7.9	9.4	9.1	8.9	10.7	8.7	9.8	
GLU	119.0	114.9	131.3	121.5	134.9	110.0	122.4	
GLY	158.2	130.0	157.0	147.9	116.8	103.9	110.4	
PRO	84.8	89.2	101.4	91.8	76.6	71.1	73.9	
SER	38.5	34.9	42.6	38.6	43.7	35.0	39.4	
TYR	17.5	12.1	16.4	126.3	23.9	17.0	20.5	
TNEAA*	598.0	528.3	621.6	581.4	582.0	474.1	528.1	

1. Each amino acid value in MBM is the mean of duplicate samples for each batch. Content of AA of soybean meal is based on NRC (1994).

*. EAA, essential amino acids; NEAA, non-essential amino acids; TEAA, total essential amino acids; TNEAA, total non-essential amino acids.

Table 6. True AA digestibility (%) of high and low ash MBM from caecectomized and intact roosters [1] (air-dry basis)

	High ash MBM	Low ash MBM	High ash MBM	Low ash MBM
Protein (%)	49.1	58.1	49.1	58.1
Ash (%)	29.1	14.6	29.1	14.6
Bird type	CEC[2]	CEC	Intact[2]	Intact
EAA[2]				
ARG	83.8 ± 4.6	86.4 ± 3.7	86.5 ± 3.3	83.7 ± 4.2
HIS	81.0 ± 5.0	79.6 ± 4.4	87.5 ± 3.5	80.9 ± 2.1
ILE	87.9 ± 3.2	87.8 ± 3.4	92.6 ± 1.4	89.3 ± 1.3
LEU	89.3 ± 2.1	90.6 ± 1.7	91.3 ± 1.4	90.4 ± 1.3
LYS	77.5 ± 13.6	84.5 ± 7.6	91.9 ± 3.0	81.9 ± 7.5
MET	77.4 ± 4.3	75.9 ± 3.3	92.6 ± 1.2	91.0 ± 2.6
PHE	89.1 ± 1.8	90.3 ± 1.9	90.7 ± 1.6	89.8 ± 1.3
THR	86.2 ± 3.2	87.6 ± 2.6	91.6 ± 2.4	87.6 ± 2.1
VAL	85.6 ± 2.8	85.4 ± 2.7	91.7 ± 1.5	88.1 ± 2.0
TRY[3]	73.2 ± 5.2	76.5 ± 2.4	-	-
TEAA[2]	83.1 ± 4.6	84.5 ± 3.4	90.7 ± 2.1	87.0 ± 2.7
NEAA[2]				
ALA	88.0 ± 2.4	90.0 ± 1.7	88.9 ± 2.7	90.7 ± 1.5
ASP	82.6 ± 4.3	82.1 ± 2.7	87.3 ± 2.4	86.0 ± 2.4
CYS	65.2 ± 10.4	63.2 ± 9.6	76.6 ± 4.6	66.6 ± 11.6
GLU	86.7 ± 2.6	88.4 ± 1.8	89.2 ± 1.8	89.2 ± 1.4
GLY	80.8 ± 7.9	81.5 ± 3.6	85.1 ± 4.4	85.2 ± 2.3
PRO	78.0 ± 7.5	79.9 ± 4.0	85.9 ± 3.6	85.5 ± 2.3
SER	83.6 ± 3.7	83.7 ± 3.3	87.7 ± 2.3	84.3 ± 2.4
TYR	88.7 ± 2.6	89.6 ± 2.4	92.8 ± 1.9	88.4 ± 1.5
TNEAA[2]	81.7 ± 5.2	82.3 ± 3.6	86.7 ± 3.0	84.5 ± 3.2
TAA[2]	82.4 ± 4.9	83.4 ± 3.5	88.7 ± 2.6	85.7 ± 3.0

1. Values are Mean± Std; All values were means of nine individual roosters. Main effect of bird type was significant for all AA ($P<0.05$). There was no significant effect of MBM type and no interaction between MBM type and bird type for all AA ($P>0.05$) except for Met, Cys.

2. CEC, cecectomized roosters; Intact, intact roosters. EAA, essential amino acids; TEAA, total essential amino acids; TNEAA, total non-essential amino acids; TAA, total amino acids.

3. Digestibility of Try for intact roosters was not analyzed.

True digestibility of amino acids

For both low and high ash MBM, true digestibilities of AA determined using both CEC and intact roosters are presented in Table 6. There were no significant differences in true digestibilities of all AA between low and high ash MBM ($P>0.05$). Although digestibility of Lys in low ash MBM was 7% higher than that in high ash MBM the difference did not reach significance ($P>0.05$), probably due to a large variation in Lys excretion among individual roosters. Digestibility values of most AA determined with CEC roosters were higher than 80% although Lys, Met and Try had relatively low digestibility values (77.5, 77.4 and 73.2%, respectively). Cystine showed the lowest digestibility with a value of 63%. Average of true digestibilities of TEAA and TAA was 83.1 and 82.4%, respectively for high ash MBM. All AA digestibility of low ash MBM showed the same tendency as high ash MBM. The data agree well with that of Johnson et al. (1998) for high and low ash MBM.

Amino acid digestibility determined by intact birds showed the same tendency as by CEC birds as there was no statistical difference ($P>0.05$) observed between MBM types. However, bird type had a significant influence on determination of AA digestibility within MBM although interaction between bird and MBM types was not significant for most AA except for some AA such as Met and Cys. Generally, effect of bird type on AA digestibility determination for high ash MBM is greater than for low ash MBM. Digestibility determined in intact birds was higher than the coefficients obtained in CEC birds for high ash MBM, particularly for Met, Lys, His and Cys (Table 6). Differences reached about 10 to 14 percentage points between the two type birds ($P<0.05$), indicating a function of microflora in the caeca. However, with regard to determination of AA digestibility for low ash MBM bird type showed a negligible influence due to microflora ($P>0.05$) except for

Met and Cys. In general, digestibilities of Cys and Met determined by intact birds were always high for both MBM. The results agreed with Parsons et al. (1997) who reported no consistent results on AA digestibility assessed by CEC and intact birds among 16 MBM samples, but the largest difference was observed for Cys digestibility between bird type. Reasons for the large difference in Cys digestibility between bird type are not clear, but may be due to microbial metabolism of Cys in the ceca (Parsons et al., 1997). In general, AA digestibility determined by CEC roosters is closer to literature reports (Rhone-Poulenc, 1989; Wang and Parsons, 1998a). To minimize the effect of ceaca microflora, the use of CEC birds is recommended for the determination of AA digestibility.

The overall AA digestibility results for both MBM showed a similar tendency with *in vitro* protein digestibility as shown in Table 3. These data indicate that protein quality of high ash MBM is similar to that of low ash MBM, consequently protein quality of the MBM is not impaired by its high ash content. This conclusion is in agreement with Partanen (1994) who reported no difference in protein digestibility (75 vs. 76%) for high and low ash (33 vs. 20.8%) MBM for pigs. Johnson et al. (1998) also reported no detrimental effect of higher ash content on AA digestibility and protein efficiency ratio in roosters. Based on AA digestibility and protein digestibility in vitro, it can be concluded that the high ash MBM is a good source of supplemental protein for poultry.

Conclusions

Chemical component analyses showed that most nutrients of the new product, high ash MBM, were consistent from batch to batch except for small variations in fat and ash. On average the MBM (DM basis) contained: GE, 4048 kcal/kg; protein, 53% with a relatively favorable amino acid profile; ash, 29% with balanced levels of Ca (9%) and P

(5%); fat, 11.5% with a ratio of 1 between saturated and unsaturated fatty acids.

There were no statistical differences in true AA digestibility and protein digestibility in vitro between the high and low ash MBM ($P>0.05$). Protein digestibility determined in vitro averaged 66%. True digestibility of most AA determined by CEC roosters was higher than 80%. Cystine had the lowest digestibility (65.2%), followed by Try (73.2%), Lys (77.5%) and Met (77.4%). High Ash content in the new product did not affect protein quality either measured by in vivo or in vitro methods. True metabolizable energy, corrected to zero nitrogen retention was 2800 kcal/kg (DM basis) for the high ash MBM. In general, the current data demonstrated that protein quality of the new high ash MBM is similar to that of low ash MBM.

Chapter IV, Manuscript 2

Effect of Supplementation with Lysine, Methionine, and Threonine andTryptophan to Corn-based Diets with High Ash meat and bone meal (MBM) on Broiler Performance

Abstract Two experiments were conducted to investigate the effect of inclusion levels of high ash MBM and supplementation of AA in practical broiler diets containing 10% high ash MBM on chick performance. The study involved 480 day-old male Leghorn chicks and 400 day-old male broiler chicks. In the first trial, a 2×5 factorial design contained two protein/Lys levels (90% NRC , 17/0.76 and 100% NRC, 18/0.85%) and five MBM levels (0, 2.5, 5.0, 7.5, and 10%). In the second trial, a corn-soybean meal diet was used as a positive control. High ash MBM was substituted at 10% to the positive control diet to serve as a negative control. Both diets contained 20% protein and 3100 kcal/kg TMEn. Eight treatments were derived from the MBM diet with supplementation of Lys, Met, Thr and Try individually or in combination. All treatments with MBM had similar nutrient content except for the four AA. Results from the first trial indicated that when using high ash MBM to replace soybean meal, chickens fed the diets with inclusion levels of MBM over 5% formulated on 90% of NRC protein, Lys specifications yielded significantly lower growth rate (8-12%) than those birds fed the same level of MBM but with protein, Lys requirements at 100% NRC ($P<0.05$). Results in the second trial showed that supplementation of Lys or Met or Lys and Met or Lys and Met combined with Thr and Try in broiler diets containing 10% high ash MBM significantly improved chick body weight gain (9-16%) and feed conversion ratio (FCR) (5-8%) ($P<0.05$). No response was observed for supplementing Thr or Try alone ($P>0.05$). Chicks receiving Lys and Met together had superior performance to those receiving supplemental Lys or Met alone

(P<0.05) and performance was equal to that of chicks receiving a corn-soybean meal control diet (P>0.05). Further supplementation of Thr and Try to the diets already containing Met and Lys did not yield significant improvement in chick performance (P>0.05). Overall the data indicated that Lys and Met were equally limiting when 10% MBM was included in a corn-soybean meal based diet and this inclusion had no adverse effect on chick performance as long as Lys and Met meet NRC (1994) specifications.

Introduction

Meat and bone meal (MBM) has always been considered as a good source of supplemental protein, and an excellent source of Ca, P and energy. As dietary inclusion rate for MBM increases, the need for dicalcium phosphate and supplemental fat in the diet is reduced and since both these ingredients are costly, this increases the value of MBM as a protein supplement. Furthermore, a consistent supply of MBM for most parts of the world is guaranteed because it is a by-product of the livestock industry, which is increasing worldwide.

Commercial MBM may contain large quantities of collagen protein which is derived from skin, connective tissues and bone resulting in a deficiency of Try and low levels of other essential amino acids (EAA) (Eastoe and Long, 1964). The digestibility of total sulfur amino acids (TSAA), Lys, and Try in MBM is low and variable (Knabe et al., 1989; Wang and Parsons, 1998a). Early studies indicated that animal performance was depressed when cereal-type diets with increasing levels (2.5-10%) of MBM were fed to chicks (Summers et al., 1964; Sathe and McClymont, 1965; Skurray and Cumming, 1974b) or growing pigs (Evans and Leibholz, 1979a). The authors concluded that reduction in animal performance was related to dietary AA imbalance and to high Ca and P provided by MBM.

In the studies by Summers et al. (1964) and Skurray and Cumming (1974b) it was demonstrated that with MBM as a sole protein source in chick diets, the first limiting AA was Met, followed by Try and Ile. In contrast, Wang et al. (1997) found that Try and TSAA were the first limiting AA, followed by Thr, Met and Lys. However, these conclusions were obtained with MBM as a sole source of dietary protein. In more practical poultry diets with wheat-plus MBM, March et al. (1950) indicated that Lys was the first limiting AA. Patrick (1953) also reported that Lys was the principal AA deficient in a corn-MBM diet for growing chicks. Similarly, Atkinson and Carpenter (1970b) stated that Lys was first limiting and Met and Thr were second and equal limiting in cereal-based diets for rats and pigs. In contrast, Kratzer and Davis (1959) found that Try in combination with either Met or Cys improved chick growth when added to corn-based diets containing MBM. Stockland et al. (1971) also found that Try was the first limiting AA in a corn-based diet for growing pigs. It may be concluded from the studies cited that the order of limitation of AA in MBM-containing diets for poultry is inconsistent. This inconsistency may be related to variation in MBM quality, the level of substitution and the cereal type used in the diet.

The objectives of the current study were: 1) to assess the ability of a high ash MBM to support chick growth; 2) to determine the effect of supplementation of Lys, Met, Thr and Try individually or in combination on chick performance when 10% of high ash MBM is used to replace soybean meal in corn-based diets.

Materials and methods

Ingredients

A sample of high ash MBM was obtained from a commercial rendering plant. Soybean meal, corn, wheat and hulled barley were purchased from local commercial suppliers. Protein and essential AA content of the five ingredients were determined and are given in Table 1. Protein was determined by the Kjeldahal method (AOAC, 1990) and AA were analyzed by ion-exchange chromatography following hydrolysis in 6N HCl at 110 C for 24 h (Mills et al., 1989). Methionine and Cys were analyzed after performic acid oxidation (Andrews and Baldar, 1985) and Try was determined following alkaline hydrolysis (Hugli and Moore, 1972). Calcium and phosphorus in the MBM were analyzed by standard AOAC procedures (AOAC, 1990). Digestibility of AA and TMEn of MBM were assessed by using the precision-fed rooster assay described in detail previously (Zhang et al., 1994). Cecectomized birds were used for AA digestibility assay and conventional intact roosters were used for the TMEn assay.

Dietary treatment design

A preliminary trial (Trial 1) was designed to evaluate the ability of high ash MBM to support chick growth, at inclusion levels of 0, 2.5, 5, 7.5 and 10% replacing soybean meal in a corn and wheat-based diet at two levels of dietary protein and Lys (17, 0.76% and 18, 0.85%, respectively). Diets with 18% protein met NRC (1994) total essential AA requirements for leghorn chicks. Diets with 17% protein level were designed to be 90% of NRC Lys specification (1994) in order to increase sensitivity for the detection of chick performance when fed different levels of the high ash MBM. Diets 2, 3, 4 and 5 in Table 2 were formulated to contain equivalent levels of protein and Lys to those in the control diet (Diet 1, 17% protein/ 0.76% Lys). Diets 7, 8, 9, and 10 contained protein, and AA equivalent to the control diet (Diet 6, 18% protein, 0.85% Lys) or NRC (1994). All diets were iso-coloric (ME 3100 kcal/kg) with slight differences in the levels of Ca and P. Composition of diets used in Trial 1 is given in Table 2.

Trial 2 was designed in an attempt to improve chick performance by supplementing Lys, Met, Thr and Try alone or in combination in diets containing 10% high ash MBM. As shown in Table 3, a corn-based soybean meal diet (Diet 1) was designed to contain protein at 20% with all AA meeting NRC (1994) specifications and a TMEn of 3100 kcal/kg. This diet served as a positive control in order to compare the effect of supplementation with the four AA. A negative control diet (Diet 2) contained 10% high ash MBM in place of soybean meal in the positive control diet and had concentrations of protein and energy equivalent to the soybean meal control diet but different Ca and P levels. Both control diets had similar levels of corn and barley. In order to eliminate the interference of AA other than the four being studied, all AA, except Lys, Met, Thr and Try, in the 10 dietary treatments exceeded the NRC requirements (1994) for 0-3 week broilers. The levels of Lys, Met, Thr and Try added in dietary treatments 3 to 9 were based on digestible AA values of high ash MBM to meet NRC (1994) specifications. The level of the four AA added in Treatment 10 was based on total AA values of high ash MBM. The inclusion of AA was at the expense of sucrose. Meat and bone meal provided 25% of total protein in the diets.

Chick assay

Trial 1. Day-old male White Leghorn chicks were housed in thermostatically controlled Jamesway brooder batteries with 24 h lighting. Feed containing 20% crude protein and water were provided *ad libitum* until 10 days of age. Prior to initiation of the experiment all birds were deprived of feed for 4 h, then weighed individually and placed randomly to 70 pens in an electrically-heated Petersime brooder batteries in an environmentally controlled room. Each treatment was completely randomized in 70 pens with seven replicates (pens) of six birds per pen. Initial body weight per bird was 102 g with a standard deviation of 2 g. Experimental diets were fed for 10 days. During the experimental period, the birds had free access to water and feed with 24 h artificial lighting. Feed intake and body weight were measured on a pen basis at the end of the experiment. Birds were weighed following 4 h feed withdrawal.

Trial 2. Day-old Arbor Acre male broiler chicks from a local commercial hatchery were placed in Jamesway brooder batteries with free access to water and feed. A commercial chick starter diet containing 21% crude protein was supplied to chicks for the first five days. On day 6, the birds were randomly assigned to each experimental treatment with eight replicates (pens) of five birds per pen. Each treatment was completely randomized in 80 pens. The procedure for weighing birds and allocation to dietary treatments and housing conditions were the same as described for Trial 1. Average initial body weight per bird was 94 g with a standard deviation of 1.2 g. From day 6 to day 20, the birds were fed the experimental diets. Feed and water were provided *ad libtium* with 24 h artificial lighting. Feed intake and bird weight were measured weekly on a pen basis. Birds were weighed following 4 h feed withdrawal. Feed conversion ratio (FCR) was calculated as feed intake/gain basis (g/g).

Statistical analysis

In Trial 1, a 2 x 5 factorial design and in Trial 2, a completely randomized design were applied. All data were subjected to analysis of variance (ANOVA, Steel et al., 1997) by SAS (SAS Institute, 1985), followed by Duncan's (1955) multiply range test to rank the different means.

Table 1. Chemical composition and amino acid content of ingredients used in experimental diets [1] (air-dry basis)

	High ash meat and bone meal [2]	Soybean meal	Corn	Barley	Wheat
DM (%)	96.2	93.5	92.5	91.7	90.0
CP (%)	50.9	46.5	8.13	11.9	16.0
ME (kcal/kg)	2760	2400	3350	2800	3150
TMEn (kcal/kg)	2670	2485	3470	2900	3260
Ca (%)	8.76	0.28	0.02	0.03	0.09
Non-phytate P (%)	5.10	0.23	0.08	0.16	0.13
Amino acids					
THR (%)	1.65 (86.2)	1.84	0.35	0.27	0.43
CYS (%)	0.45 (65.2)	0.70	0.28	0.19	0.33
MET(%)	0.80 (77.4)	0.69	0.22	0.21	0.29
VAL (%)	1.98 (85.6)	2.10	0.49	0.35	0.59
ILE (%)	1.23 (87.9)	2.05	0.34	0.24	0.47
LEU (%)	2.87 (89.3)	3.68	0.74	0.87	1.01
TYR (%)	0.81 (88.7)	1.35	0.23	0.19	0.34
PHE (%)	1.48 (89.1)	2.13	0.47	0.29	0.66
HIS (%)	1.14 (81.0)	1.25	0.23	0.22	0.34
LYS (%)	2.55 (77.5)	2.95	0.37	0.28	0.40
ARG (%)	3.58 (83.8)	3.37	0.48	0.33	0.62
TRY (%)	0.41 (73.2)	0.55	0.13	0.05	0.16

1. ME, TMEn, Ca and P of soybean meal, corn, barley and wheat were adapted from NRC (1994). Amino acid and protein contents of the four ingredients were determined in our laboratory.

2. All nutrient values for high ash meat and bone meal (MBM) were determined in our laboratory. Amino acid digestibility (value in parenthesis) and TMEn of MBM was determined using cececotomized and conventional roosters, respectively. ME was obtained from NRC (1994).

Table 2. Percent composition of experimental diets with various levels of high ash meat and bone meal (MBM) used in Trial 1

	Diets									
	17% protein, 0.76% Lys					18% protein, 0.85% Lys				
Ingredients	1	2	3	4	5	6	7	8	9	10
High ash MBM	0	2.50	5.00	7.50	10.00	0	2.50	5.00	7.50	10.00
Soybean meal	17.50	13.40	9.30	5.20	1.10	20.90	16.80	12.71	8.62	4.52
Corn	46.53	42.28	38.04	33.80	29.55	46.53	42.28	38.04	33.80	29.55
Wheat	21.00	25.65	30.30	34.95	39.60	21.00	25.66	30.32	34.97	39.63
Barley	8.28	10.17	12.06	13.95	15.84	4.71	6.57	8.42	10.28	12.14
Limestone	1.54	1.48	1.41	1.34	1.28	1.52	1.46	1.40	1.34	1.28
Mono-dicalcium phosphate	1.35	1.01	0.68	0.34	0	1.34	1.00	0.67	0.34	0
Vitamin premix	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Mineral premix	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vegetable oil	2.30	1.94	1.58	1.21	0.85	2.50	2.16	1.81	1.46	1.12
DL-MET	0	0.01	0.02	0.03	0.04	0.00	0.01	0.02	0.02	0.03
LYS.HCl	0	0.06	0.12	0.18	0.24	0.00	0.06	0.12	0.18	0.23
Calculated analyses[1]										
ME (kcal/kg)	3100	3100	3100	3100	3100	3100	3100	3100	3100	3100
Ca (%)	0.91	1.03	1.16	1.29	1.41	0.90	1.03	1.16	1.29	1.42
Non-Phytate P (%)	0.40	0.46	0.52	0.57	0.63	0.40	0.46	0.52	0.57	0.63
Lys (%)	0.76	0.76	0.76	0.76	0.76	0.85	0.85	0.85	0.85	0.85
Met (%)	0.32	0.32	0.32	0.32	0.32	0.34	0.33	0.33	0.33	0.33
Met+Cys (%)	0.64	0.63	0.62	0.61	0.60	0.67	0.66	0.65	0.63	0.62
Thr (%)	0.63	0.61	0.58	0.56	0.53	0.69	0.67	0.64	0.62	0.59
Try (%)	0.21	0.20	0.19	0.18	0.18	0.23	0.22	0.21	0.20	0.20

1. All nutrient values are calculated based on Table 1 data.

Table 3. **Basal composition of control experimental diets used in Trial 2**

Items	Positive control (Diet 1)	Negative control (Diet 2)
Soybean meal	31.5	20.5
High ash meat and bone meal	-	10
Corn	50.2	50.2
Barley	10	10
Limestone	1.63	1.00
Mono-dicalcium phosphate	1.54	-
Vegetable Oil	2.9	0.6
Sucrose	0.54	5.83
Vitamin premix	1	1
Mineral premix	0.5	0.5
DL-Met	0.164	-
Thr	0.053	-
Val	0.016	0.077
Arg	-	0.047
Ile	0.001	0.119
Phe	-	0.11
*Calculated analyses**		
TMEn (kcal/kg)	3100	3100
Fiber (%)	2.87	2.77
Ca (%)	1.00	1.35
Non-phytate P (%)	0.45	0.61
Protein (%)	20.0	20.0
Arg (%)	1.27	1.31
His (%)	0.52	0.50
Phe+Tyr (%)	1.41	1.37
Ile (%)	0.80	0.81
Leu (%)	1.67	1.56
Val (%)	0.90	0.93
Lys (%)	1.10	1.04
Met (%)	0.56	0.40
Met+Cys (%)	0.90	0.71
Thr (%)	0.80	0.71
Try (%)	0.21	0.19

*. All nutrient values are calculated based on Table 1 values.

Results and discussion

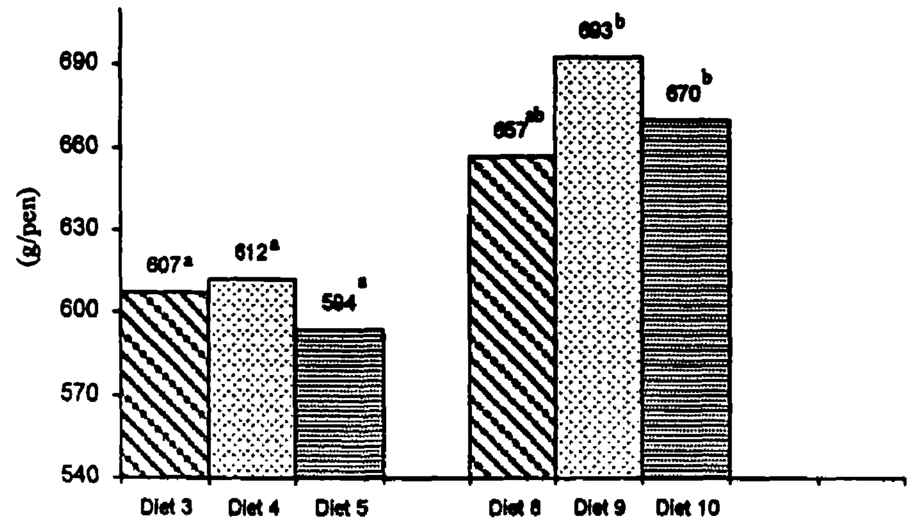
Trial 1. Average bird weight gain during the 10-day experimental period is listed in Table 4. Main effect analysis showed that chick weight gain was not affected by inclusion of various levels of MBM in the diet ($P>0.05$). Dietary protein/Lys levels significantly affected weight gain as chicks fed the 18%, 0.85% level grew 7% faster than those fed 17%, 0.76% level ($P<0.05$). No interaction ($P>0.05$) between protein level and MBM inclusion rate was observed. However, further comparison among means by Duncan's test showed that when MBM was added at a level greater than 7% in 17%, 0.76% protein and Lys diets, chick growth rate was significantly depressed in comparison to birds receiving the diets with same level MBM at 18%, 0.85% protein/Lys diets (Figure 1) ($P<0.05$). Additionally, at the same protein and Lys level (17%, 0.76%), the inclusions of MBM at 5% or greater tended to depress chick growth (Table 4). Lysine deficiency may be the major factor limiting chick weight gain.

Table 4. The effect of different levels of protein and high ash meat and bone meal (MBM) on chick weight gain during 10-day experiment (Trial 1)

Diets.	High ash MBM (%)	Protein (%)	Lys (%)	Gain, g/pen*
1	0	17	0.76	638±17 [abc]
2	2.5	17	0.76	640±17 [abc]
3	5.0	17	0.76	607±18 [bc]
4	7.5	17	0.76	612±17 [bc]
5	10.0	17	0.76	594±17 [bc]
6	0	18	0.85	657±16 [ab]
7	2.5	18	0.85	660±18 [ab]
8	5.0	18	0.85	657±16 [ab]
9	7.5	18	0.85	693±17 [a]
10	10.0	18	0.85	670±18 [a]

[a,b] Means within the same column with no common superscript differ significantly ($P<0.05$). * Values are LSM±SEM.

Figure 1. Effect of inclusion levels of high ash meat and bone meal (MBM) with different levels of protein and Lys specifications on bird weight gain



Diets 3, 4 and 5 with same levels of protein and Lys (17%, 0.76%), but different levels of MBM (5, 7.5 and 10%, respectively).

Diets 8, 9 and 10 with same levels of protein and Lys (18%, 0.86%), but different levels of MBM (5, 7.5 and 10%, respectively).

Trial 2. The average feed intake, weight gain, and FCR in the 14 d experimental period are presented in Table 5. Data showed that 10% inclusion of MBM to replace soybean meal yielded an adverse effect on chick weight gain and FCR ($P<0.05$), but with no significant effect on feed intake (Diets 2 vs. 1) ($P>0.05$). This is in agreement with Trial 1 and previous reported findings for growing chicks or pigs (Sathe and McClymont, 1965; Evans and Leibholz, 1979a).

Table 5. Effect on chick performance of supplementing diets containing 10% high ash meat and bone meal (MBM) with amino acids [1]

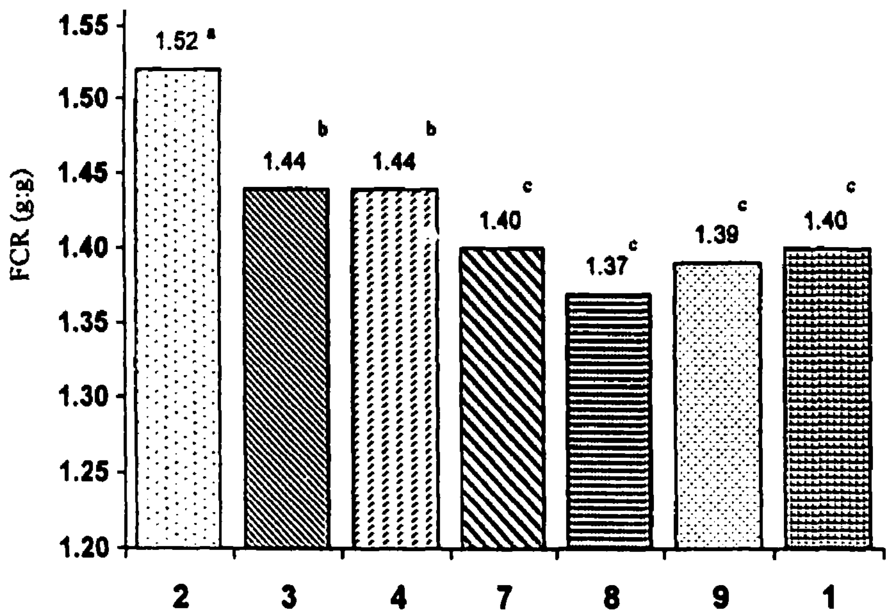
Treatments	Formulation method	Feed intake g/bird	Weight gain g/bird	FCR [2] g:g
1	Soybean meal (Positive control)	723.8^{a}	514.4^{ab}	1.40^{c}
2	10% MBM (Negative control)	712.7^{a}	466.5^{c}	1.52^{a}
3	2 + Lys 0.152%	739.2^{a}	509.0^{ab}	1.44^{b}
4	2 + Met 0.226%	736.3^{a}	508.5^{ab}	1.44^{b}
5	2 + Thr 0.112%	709.8^{a}	471.3^{c}	1.49^{a}
6	2 + Try 0.029%	731.0^{a}	482.8^{bc}	1.50^{a}
7	2 + Lys 0.152, Met 0.226%	741.5^{a}	526.3^{a}	1.40^{c}
8	2 + Lys 0.152, Met 0.226, Thr 0.112%	750.2^{a}	543.2^{a}	1.37^{c}
9	2 + Lys 0.152, Met 0.226, Thr 0.112, Try 0.029%	751.3^{a}	535.4^{a}	1.39^{c}
10	2 + Lys 0.079, Met 0.191, Thr 0.089, Try 0.018%	711.0^{a}	510.7^{ab}	1.38^{c}
Pooled SEM		12.2	9.0	0.01

1. [a,b,c] Means within the same column with no common superscript differ significantly ($P<0.05$). Each value was LSM of eight replicates of five male chicks from 6-20 d of age.
2. FCR, feed conversion ratio.

Body weight gain of birds was significantly improved with an increase of 9 to 16% when supplementing Lys or Met alone or Lys and Met combined with Thr and Try (Diets 3, 4, 7, 8 and 9) to the MBM negative control diet (Diet 2) ($P<0.05$). Growth rate of these birds was brought up to the level of soybean meal control diet ($P>0.05$). Threonine or Try added individually had no positive effect on chick growth ($P>0.05$). Further addition of Lys and Met, or Lys and Met combined with Thr or Lys, Met combined with Try and Thr (Treatments 7, 8 and 9) showed a slight improvement in weight gain compared to the diets with Lys or Met alone (Treatments 3 and 4), but the effect did not reach statistical significance ($P>0.05$).

Feed conversion ratio showed the same tendency as body weight gain, a significant improvement (5-8%) was obtained when supplementing Lys or Met alone or in combination with Thr and Try ($P<0.05$) compared to birds fed the negative control MBM diet (Diet 2). Supplementation of either Thr or Try in the MBM diet gave no response in FCR ($P>0.05$). The improvement on FCR was more pronounced when Met and Lys in combination were added to the MBM diet as compared to supplementation the two AA alone ($P<0.05$), and the resulting FCR value was equivalent to that for chicks fed the positive control soybean meal diet (Diet 1). Further addition of Thr and Try to a diet already containing Lys and Met tended to give a small additional response, but the effect was not statistically significant ($P>0.05$) (Figure 2).

Figure 2. Effect on feed conversion ratio (FCR) by supplementation of Lys or Met alone or in combination with Thr and Try to high ash meat and bone meal (MBM) diets



2, MBM diet (negative control); 3, MBM diet plus 0.152% Lys;

4, MBM diet plus 0.226% Met; 7, MBM diet plus 0.152% Lys and 0.226% Met;

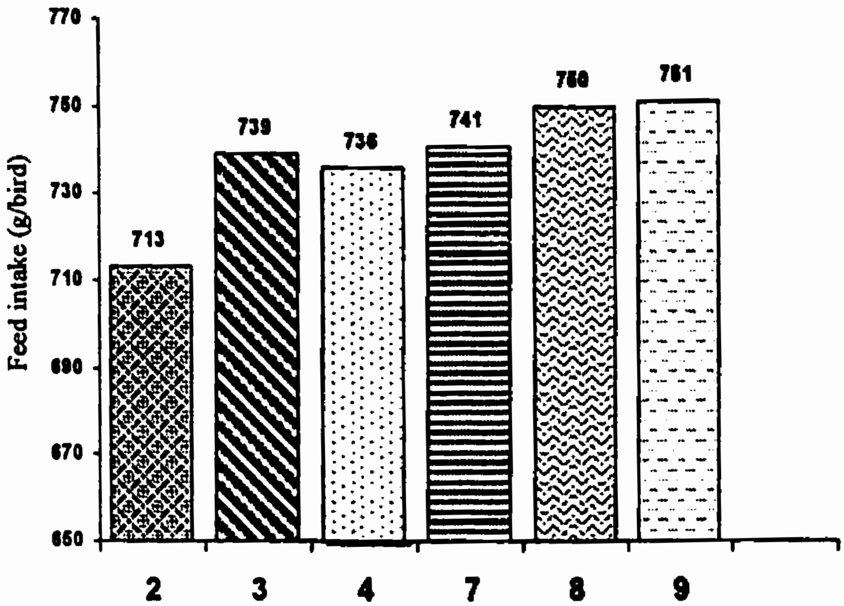
8, MBM diet plus 0.152% Lys, 0.226% Met and 0.112% Thr;

9, MBM diet plus 0.152% Lys, 0.226% Met, 0.112% Thr and 0.029% Try;

1, Soybean meal positive control diet.

Feed intake tented to be improved when supplementing MBM diets with Lys or Met individually or in combination with Thr and Try although the differences were not statistically significant (P>0.05) (Figure 3).

Figure 3. Effect on feed intake of supplementation of Lys or Met or Met and Lys combined with Thr and Try in high ash meat and bone meal (MBM) diets



2- MBM diet (negative control); 3- MBM diet plus 0.152% Lys;
4- MBM diet plus 0.226% Met;
7- MBM diet plus 0.152% Lys and 0.226% Met;
8- MBM diet plus 0.152% Lys, 0.226% Met and 0.112% Thr.
9- MBM diet plus 0.152% Lys, 0.226% Met, 0.112% Thr and 0.029% Try.

The MBM diet with supplementation of a combination of Lys, Met, Thr and Try based on digestible AA (Diet 9) did not yield superior improvement in chick performance to a diet (Diet 10) with the supplementation of these four AA based on total AA values (P>0.05). However, adding these four AA based on digestible values of the high ash MBM tended to improve feed intake and weight gain by approximately 7% ($P<0.20$) (Table 4). Further study is needed to study the effect of diet formulation with high ash MBM based on digestible AA vs. total AA values on chick performance.

Consideration of the overall responses in chick performance, indicating that birds fed MBM diets with supplementation of Lys and Met in combination showed superior performance to birds fed diets with Lys or Met supplemented individually, and had similar performance to those birds receiving diets with all four AA supplemented (Lys, Met, Thr and Try). Supplementation of Lys and Met to the corn-based MBM diet effectively improved chick growth and FCR. It can be suggested that Lys and Met were equally limiting in the corn-soybean meal based diets with 10% high ash MBM. These results agreed well with the findings of Partric (1953), Summers et al. (1964), Sathe and McClymont (1968), Skurray and Cumming (1974), and Evans and Leibholz (1979b) who for growing chicks and pigs reported that Lys and Met, single or in combination, were the first limiting AA in corn or wheat-plus MBM diets as well as in diets containing MBM as the sole protein source. The order of limitation of AA found in the current study differed from that of Meade and Teter (1957), Stockland et al. (1971) and Wang et al. (1997) who found that Try was the first limiting AA when MBM contributed 60% of protein in corn-based diets for growing pigs or when MBM was used as a sole protein source for chicks. The reason for the disagreement may relate to the inclusion rates for MBM or the cereal type used in the diets. In our experiments, the MBM only contributed 25% of protein in a 20% protein diet, in which cereals (corn and barley) contributed 26% of the protein and soybean meal supplied almost 50% of the protein to the diet. Therefore, the deficiency order for AA would be determined by the combination of the three protein sources (MBM, soybean meal and cereals). Fernandez et al. (1994) reported that in soybean meal, corn or corn-soybean meal diets the first limiting AA were TSAA, Lys and Met, respectively. Consequently, it was not surprising that Lys and Met together were the first limiting AA in the current experiment. Deficiency of Try in the high ash MBM may have been masked by the relatively high level of soybean meal in the diets.

Conclusion

Growth of Leghorn chick was significantly depressed when using high ash MBM over 7% to replace soybean meal in the diets containing 17% protein and a 90% of NRC (1994) Lys specification in comparison to those diets with same levels of the MBM, but in which NRC Lys requirement was met.

Body weight gain and FCR were significantly improved with supplementation of Lys and Met or in combination with Thr, and Try in broiler chick diets containing 10% high ash MBM. Birds receiving Lys and Met together had superior performance to those chicks fed diets with supplementation of Lys or Met individually. Further addition of Thr and Try to the diet already containing 0.152% Lys and 0.226% Met did not yield statistical improvement. No response in chick performance from adding Thr or Try individually to the MBM diet was observed. It can be concluded from the results of the current study that Lys and Met are the first limiting AA in the corn-based soybean meal diets with 10% high ash MBM. In addition, the inclusion of 10% high ash MBM in chick diets had no adverse effect on performance as long as Lys and Met in the diet met NRC (1994) requirements.

Chapter V, Manuscript 3

Formulation of broiler diets with high ash meat and bone meal based on total versus digestible amino acid contents

Abstract Nine diets in three sets were designed to investigate effects of formulating high ash MBM diets based on total amino acid (TAA) vs. digestible amino acid (DAA) values and the influence of high dietary Ca and P diets with MBM or inorganic source on broiler performance. The study involved 405 broilers, in which NRC (1994) requirement and corn-soybean meal diets were used as references. Results showed no significant difference in chick performance when formulating diets with 10% MBM based on either TAA or DAA values with all AA meeting NRC requirements. For corn-soybean meal diets, all AA being 90% of NRC did not result in performance decline ($P>0.05$). However, with all AA at 90% of NRC and MBM substituted at 10 or 15% level, dietary formulation based on DAA was superior to TAA values, and resulted in broiler performance equivalent to that of a corn-soybean meal diet. The study also indicated that high dietary Ca and P level provided by high ash MBM was not the major detrimental factor causing a depression in broiler growth. Rather, amino acid deficiency was the main factor responsible for a depression of chick performance. High ash MBM can be used at 10% in broiler diets with adequate AA supplementation and formulated on a DAA basis.

Introduction

Feed accounts for 60-70% of poultry production costs of which a large portion relates to protein ingredients that are usually expensive. Improved precision in amino acid (AA) formulation to meet bird requirements can reduce feed cost and also bring about a reduction in nitrogen excretion through animal effluent. In recent years attention has been paid to the reduction of dietary protein content by formulating diets based on digestible AA (DAA) instead of total AA (TAA) values to improve animal performance and profitability. This is particularly meaningful for those ingredients of poor AA digestibility, such as sunflower, rapeseed, cottonseed meals or meat and bone meal (MBM). The advantage, however, is only demonstrated by a limited number of studies using diets which contained relatively high levels of these ingredients (Fernandez et al. 1995; Rostagon et al. 1995; Wang and Parsons 1998c). The use of relatively high dietary levels of poor digestibility ingredients, however, is uncommon in practice, particularly for high ash MBM.

The transformation of TAA to DAA formulation involves the establishment of a database and listings of requirements on DAA basis. Today, a large number of data on digestibility of AA for poultry feedstuffs have been generated. However, a requirement for DAA in broiler diets has not been adequately established. The NRC requirement for TAA for broiler chickens was established from data based on corn-soybean meal diets, which are ingredients with relatively high digestibility. In this regard, the recommended requirements for AA may actually be high enough to meet broiler demand when formulating on TAA values. Consequently, the use of DAA values to formulate animal diets has not been widely applied in practice.

Previous studies conducted in our laboratory showed that weight gain and feed efficiency of the birds tended to be improved when supplementation of MBM was based on DAA rather than on TAA values. In the current study, further investigation of growth performance of broiler fed diets containing 10 or 15% high ash MBM in place of soybean meal using TAA and DAA values was carried out with diets containing a marginal amount of protein. The effect of high Ca and P levels in diets with a relatively high substitution level of MBM on chick performance was also studied.

Materials and Methods

1. Feed ingredients and analysis

The chemical composition, AA digestibility and true metabolizable energy corrected to zero nitrogen retention (TMEn) of corn, barley, soybean meal and high ash MBM used in the trial are given in Table 1. Dry matter (DM), protein, Ca and P were analyzed according to procedures of AOAC (1990). Amino acid analyses were performed by ion-exchange chromatography following hydrolysis of samples in 6N HCl at 110 C for 24 h (Mills et al., 1989) except for Met and Cys which were analyzed after performic acid oxidation (Andrews and Baldar, 1985). Tryptophan was determined following alkaline hydrolysis (Hugli and Moore, 1972). True AA digestibility and TMEn of the MBM were determined using the Sibbald rooster assay (1986) with some modifications as described previously (Zhang et al., 1994).

2. Diet formulation

In order to increase sensitivity of detecting performance differences between dietary formulation on a TAA vs. DAA basis, nine diets in three sets were used in the experiment. All diets contained similar nutrient content except for levels of Ca and available P. The first set included Diets 1, 2 and 6. Diet 1 was a corn-soybean meal control diet in which all AA levels met or exceeded NRC (1994) requirements for 0-3 week broilers. Diets 2 and 6, containing 10% MBM, were formulated to contain levels of TAA or DAA equivalent to those in the corn-soybean diet (Diet 1) or to NRC (1994). The second set involved Diets 3, 4, 5, 7 and 8. Diet 3 was a second corn-soybean meal control diet with all AA levels at 90%

of NRC (1994) requirements for 0-3 week broilers. Diets 4 and 5 and diets 7 and 8 containing 10 or 15% high ash MBM, respectively, were formulated to have all TAA or DAA levels equivalent to those in the corn-soybean meal control (Diet 3) or to 90% of NRC (1994). A third set (Diets 3, 5, 8 and 9) was designed to enable an estimation of whether the chick growth depression was caused by excessive Ca and P in the diets with high levels of MBM. Extra Ca and P from limestone and mono dicalcium phosphate were added to Diet 9, a corn soybean meal diet, to make energy, protein, Ca and P levels equivalent to those in Diet 5. All AA in Diet 9 was equivalent to those in Diet 5 or NRC (1994). Diet 8 had all nutrients equivalent to Diet 5 except for AA which were on a DAA basis. Diet 3 contained normal levels of Ca and P at NRC (1994) specifications (1.0 and 0.45%, respectively). The levels of Ca and P in Diets 5, 8 and 9 were 1.89 and 0.85%, respectively, or approximately 89% higher than the requirements of NRC (1994). Composition of all diets is given in Tables 2 and 3.

3. Chick assay

One-day old Arbor Acre male broiler chicks purchased from a local commercial hatchery were placed in Jamesway brooder batteries with free access to water and feed. A commercial chick starter diet containing 21% crude protein was supplied to chicks for the first five days. On day 6, all birds were deprived of feed for 4 h, and weighed individually. Nine replicates (pens) of five birds were randomly allotted to dietary treatments. Each treatment was completely randomized in 81 pens. Average initial bird weight was 92 g with a standard deviation of 1g. From day 6 to day 20, all birds were housed in thermostatically-controlled Petersime brooder batteries in an environmentally regulated room and fed on the experiment diets which were prepared in a mash form. Feed and water were provided *ad libitum*, with 24 h artificial lighting. Birds were weighed weekly following 4 h feed withdrawal. Feed weight back was taken to calculate feed intake.

Statistical analysis

All data were subjected to analysis of ANOVA (Steel et al., 1997) for completely randomized design using SAS (SAS Institute, 1985) and differences among treatment means were assessed using Duncan's multiply range test (Duncan, 1955). In addition, some single degree of freedom contrasts among treatments were made to aid in interpretation of the results.

Table 1. **Chemical composition and amino acid digestibility of high ash meat and bone meal, soybean meal, corn and barley used in experimental diets[1]**

	High ash meat and bone meal[2]		Soybean meal[1]		Corn[1]		Barley[1]	
DM (%)	96.2		93.5		92.5		91.7	
Protein (%)	50.9		47.9		8.7		11.9	
TMEn (kcal/kg)	2670		2485		3470		2900	
Ca (%)	8.76		0.28		0.02		0.03	
Non-phytate P (%)	5.10		0.23		0.08		0.16	
Amino acid	Content	Digestibility	Content	Digestibility	Content	Digestibility	Content	Digestibility
ARG (%)	3.58	83.8	3.47	92.0	0.35	89.0	0.48	85.0
CYS (%)	0.45	65.2	0.72	82.0	0.20	85.0	0.28	81.0
HIS (%)	1.14	81.0	1.29	88.0	0.23	94.0	0.23	87.0
LYS (%)	2.55	77.5	3.04	91.0	0.30	81.0	0.37	78.0
MET(%)	0.80	77.4	0.71	92.0	0.22	91.0	0.22	79.0
VAL (%)	1.98	85.6	2.16	91.0	0.39	88.0	0.49	81.0
ILE (%)	1.23	87.9	2.11	93.0	0.26	88.0	0.34	82.0
LEU (%)	2.87	89.3	3.79	92.0	0.94	93.0	0.74	86.0
THR (%)	1.65	86.2	1.89	88.0	0.29	84.0	0.35	77.0
TYR (%)	0.81	88.7	1.39	91.1	0.21	91.6	0.23	87.6
PHE (%)	1.48	89.1	2.19	92.0	0.31	91.0	0.47	88.0
TRY (%)[3]	0.41	73.2	0.57	92.6	0.06	80.5	0.13	82.4

1. Analyzed values except TMEn, Ca and non-phytate P of soybean meal, corn and barley that were from NRC (1994). Amino acid digestibility coefficients of soybean meal, corn and barley were adapted from NRC (1994).

2. Digestibility of AA and TMEn in high ash meat and bone meal were determined in our laboratory using cecectomized and conventional roosters, respectively.

3. Digestibility of Try for soybean meal, corn and barley was calculated based on the digestibility ratio between Try and Thr in swine (NRC, 1998).

Table 2. Composition of soybean meal (SBM) and high ash meat and bone meal (MBM) diets based on total amino acid requirements for broilers

Ingredient (%)	SBM Diet 1	10% MBM [2] Diet 2	SBM Diet 3	10% MBM [3] Diet 4	15% MBM [3] Diet 5	SBM [4] Diet 9	NRC (1994)	90% of NRC(1994)
SBM	29.95	19.02	30.22	19.9	14.717	31.51		
MBM	0	10	0	10	15	0		
Corn	58.4	60.8	58.4	60.45	59	54.52		
Barley	2.5	2.4	2.4	2.4	4	0		
Limestone	1.63	1.04	1.63	1.04	1.35	3.04		
Mono-dicalcium phosphate	1.58	0	1.57	0	0	3.505		
DL-MET	0.165	0.187	0.071	0.085	0.095	0.081		
LYS. HCl	0.006	0.095	0	0	0	0		
THR	0.056	0.092	0	0	0.009	0		
VAL	0.013	0.043	0	0	0	0		
ARG	0	0.009	0	0	0	0		
ILE	0.008	0.111	0	0.012	0.059	0		
TRY	0	0.011	0	0	0	0		
PHE	0	0.097	0	0	0	0		
Vitamin premix	1	1	1	1	1	1		
Mineral premix	0.5	0.5	0.5	0.5	0.5	0.5		
Vegetable Oil	3.54	1.52	3.59	1.79	1.95	5.64		
Sucrose	0.652	3.075	0.619	2.823	2.32	0.204		
Calculated analyses[1]								
TMEn (kcal/kg)	3200	3200	3200	3200	3200	3200		
Ca (%)	1.00	1.34	1.00	1.34	1.89	1.89	1	1
Non-phytate P (%)	0.45	0.61	0.45	0.61	0.85	0.85	0.45	0.45
Protein (%)	20	20	20	20	20	20	23	
HIS (%)	0.527	0.505	0.530	0.516	0.506	0.532	0.350	0.315
PHE+TYR (%)	1.394	1.340	1.402	1.273	1.206	1.412	1.340	1.206
MET (%)	0.563	0.590	0.472	0.495	0.507	0.477	0.500	0.450
MET+CYS (%)	0.900	0.900	0.810	0.810	0.810	0.810	0.900	0.810
LYS (%)	1.100	1.100	1.103	1.050	1.022	1.121	1.100	0.990
TRY (%)	0.209	0.200	0.210	0.194	0.186	0.212	0.200	0.180
ILE (%)	0.800	0.800	0.798	0.720	0.720	0.807	0.800	0.720
LEU (%)	1.702	1.597	1.712	1.627	1.572	1.707	1.200	1.080
THR (%)	0.800	0.800	0.749	0.725	0.720	0.754	0.800	0.720
ARG (%)	1.256	1.250	1.265	1.271	1.272	1.284	1.250	1.125
VAL (%)	0.900	0.900	0.892	0.875	0.864	0.893	0.900	0.810

1. Calculated analyses are based on nutrient values of ingredients in Table 1.
2. Total AA content in Diet 2 is equivalent to that in Diet1 or that of NRC (1994).
3. Total AA in Diet 3 is 90% of NRC (1994). Total AA contents in Diets 4 and 5 are equal to those in Diet 3 or 90% of NRC (1994).
4. Levels of energy, protein, Ca and P in Diet 9 are equivalent to those in Diet 5; AA in Diet 9 is equivalent to that in Diet 5 or NRC (1994).

Table 3. Composition of soybean meal (SBM) and high ash meat and bone meal (MBM) diets based on digestible amino acid values for broilers

Ingredient	SBM Diet 1	10%MBM[2] Diet 6	SBM Diet 3	10%MBM[3] Diet 7	15%MBM[3] Diet 8
SBM	29.95	19.02	30.22	19.9	14.717
MBM	0	10	0	10	15
Corn	58.4	60.8	58.4	60.45	59
Barley	2.5	2.4	2.4	2.4	4
Limestone	1.63	1.04	1.63	1.04	1.35
Mono-dicalcium phosphate	1.58	0	1.57	0	0
DL-MET	0.165	0.201	0.071	0.101	0.119
LYS.HCl	0.006	0.133	0	0.106	0.162
THR	0.056	0.092	0	0.024	0.038
VAL	0.013	0.051	0	0.026	0.042
ARG	0	0.042	0	0.023	0.036
ILE	0.008	0.111	0	0.09	0.137
TRY	0	0.027	0	0.024	0.035
PHE	0	0.144	0	0	0.065
Vitamin premix	1	1	1	1	1
Mineral premix	0.5	0.5	0.5	0.5	0.5
Vegetable Oil	3.54	1.52	3.59	1.79	1.95
Sucrose	0.652	2.919	0.619	2.526	1.849
Calculated analyses[1]					
TMEn (kcal/kg)	3200	3200	3200	3200	3200
Ca (%)	1.00	1.34	1.00	1.34	1.89
Non-phytate P (%)	0.45	0.61	0.45	0.61	0.85
Protein (%)	20	20.16	20	20.3	20.4
HIS (%)	0.475	0.448	0.478	0.458	0.445
PHE+TYR (%)	1.272	1.271	1.281	1.156	1.156
MET (%)	0.529	0.559	0.438	0.465	0.48
MET+CYS(%)	0.811	0.81	0.721	0.721	0.721
LYS (%)	0.982	0.982	0.984	0.984	0.984
TRY (%)	0.19	0.19	0.192	0.192	0.192
ILE (%)	0.736	0.737	0.733	0.733	0.733
LEU (%)	1.569	1.465	1.578	1.493	1.438
THR (%)	0.7	0.701	0.648	0.648	0.647
ARG (%)	1.147	1.146	1.155	1.155	1.155
VAL (%)	0.811	0.811	0.803	0.803	0.803

1. Calculated analyses are based on Table 1 nutrient values of ingredients.
2. Digestible AA content in Diet 6 is equivalent to that in Diet 1.
3. Digestible AA contents in Diets 7 and 8 are equivalent to those in Diet 3.

Results and Discussion

1. Effect on chick performance of formulating diets with 10% high ash meat and bone meal on total amino acid or digestible amino acid values according to NRC (1994) specification

Chick body weight gain, feed intake and feed conversion ratio (FCR) during the 14 day experimental period are presented in Table 4 and Figure 1. The data indicate that inclusion of 10% high ash MBM with AA based on TAA (Diet 2) vs. DAA (Diet 6) values resulted in no differences in chick performance when all AA met NRC (1994) requirements. Weigh gain and FCR for both MBM diets were similar to that for the corn-soybean meal control (Diet 1) ($P>0.05$). However, feed intake in both MBM diets was inferior to that for the corn-soybean diet (Diet1) ($P<0.05$). The reason for this difference is not known. Consideration of overall performance parameters showed no statistical differences between Diets 2 and 6. These results imply that there was no significant advantage gained from formulating diets with 10% MBM based on DAA rather than TAA values. The results are not in agreement with those from Wang and Parsons (1998c), who reported that formulation of diet with 10% low quality MBM based on DAA values had superior performance over formulation on a TAA basis. The difference may be related to the level of TSAA used in experimental diets and digestibility of Cys in MBM. In our study, the level of TSAA (0.9%) reached NRC (1994) requirement for 0-3 week broilers as shown in Table 2. Therefore, even without supplementation of Met, the diet formulated on a TAA basis already contained 0.8% digestible TSAA, which may have met the growth requirement as some literature reports indicate requirement levels of 0.75% or 0.81% digestible TSAA for broiler body weight gain, and 0.77% for FCR for 0-3 week broilers (Leesons and Summers, 1997; Dalibard and Paillard, 1995). In the trial by Wang and Parsons (1998c) a marginal level of TSAA (0.78%) was used and in addition the MBM had a low Cys digestibility (31%). It has been reported that TSAA is the first limiting AA in corn-soybean meal diets (Fernandez et al., 1994) and also in MBM (Summers et al., 1964). This may explain the superior chick performance observed when formulating diets with 10% MBM based on DAA instead of TAA values in the experiment reported by Wang and Parsons (1998c).

Table 4. Performance of chicks fed diets containing various levels of high ash meat and bone meal (MBM) with formulation based on total amino acids (TAA) or digestible amino acids (DAA) [1]

Diets [3]	MBM (%)	Formulation method	Feed intake (g)	Weight gain (g)	FCR[2] (g:g)
1	0	TAA = NRC (1994)	753^{ab}	531^{a}	1.42^{a}
2	10	TAA = Diet 1 or NRC (1994)	721^{c}	509^{a}	1.42^{a}
6	10	DAA = Diet 1	723^{c}	508^{a}	1.42^{a}
3	0	TAA = 90% of NRC	756^{a}	528^{a}	1.43^{a}
4	10	TAA = Diet 3 or 90% of NRC	731^{abc}	490^{b}	1.49^{b}
5	15	TAA = Diet 3 or 90% of NRC	721^{c}	468^{c}	1.54^{c}
7	10	DAA = Diet 3	738^{abc}	520^{a}	1.42^{a}
8	15	DAA = Diet 3	724^{bc}	511^{ab}	1.42^{a}
9	0	Ca and P = Diet 5, TAA	757^{a}	512^{ab}	1.48^{b}
Pooled SEM			9.6	7.8	0.01

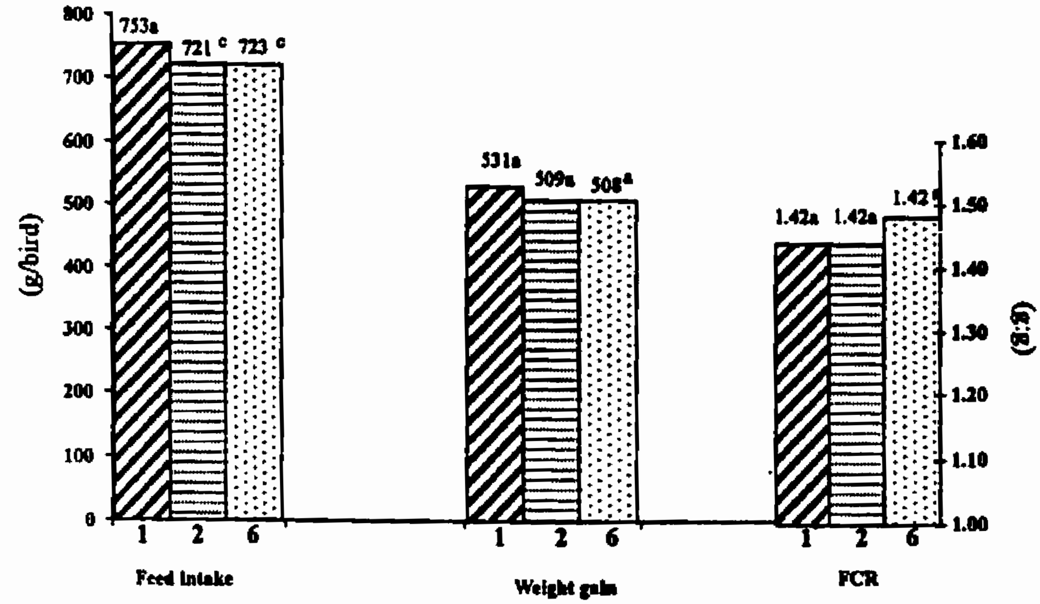
a-c Means within the same column with no common superscript differ significantly ($P<0.05$).

1. All values are LSM of the nine replicates (pens) of five male chicks from 6-20 day of age. Initial body weight for each treatments was 92 ± 1 g.

2. FCR, feed conversion ratio.

3. Single degree of freedom contrasts were not significant ($P>0.05$) for all parameters of Treatments 1 vs 3; Treatments 7 and 8 vs 3 and 1, but differed in feed intake for Treatment 2 and 6 vs 1 ($P<0.05$); weight gain and feed efficiency for Treatments 4 and 5 vs 7 and 8. All parameters for Treatments 5 vs 9 differed significantly ($P<0.05$).

Figure 1. Effect on chick performance of dietary formulation with high ash meat and bone meal (MBM) on a total amino acid (TAA) or digestible amino acid (DAA) basis of NRC (1994)



1, Soybean meal diet (Diet 1); 2, MBM diet (Diet 2), TAA basis; 6, MBM diet (Diet 6), DAA basis.

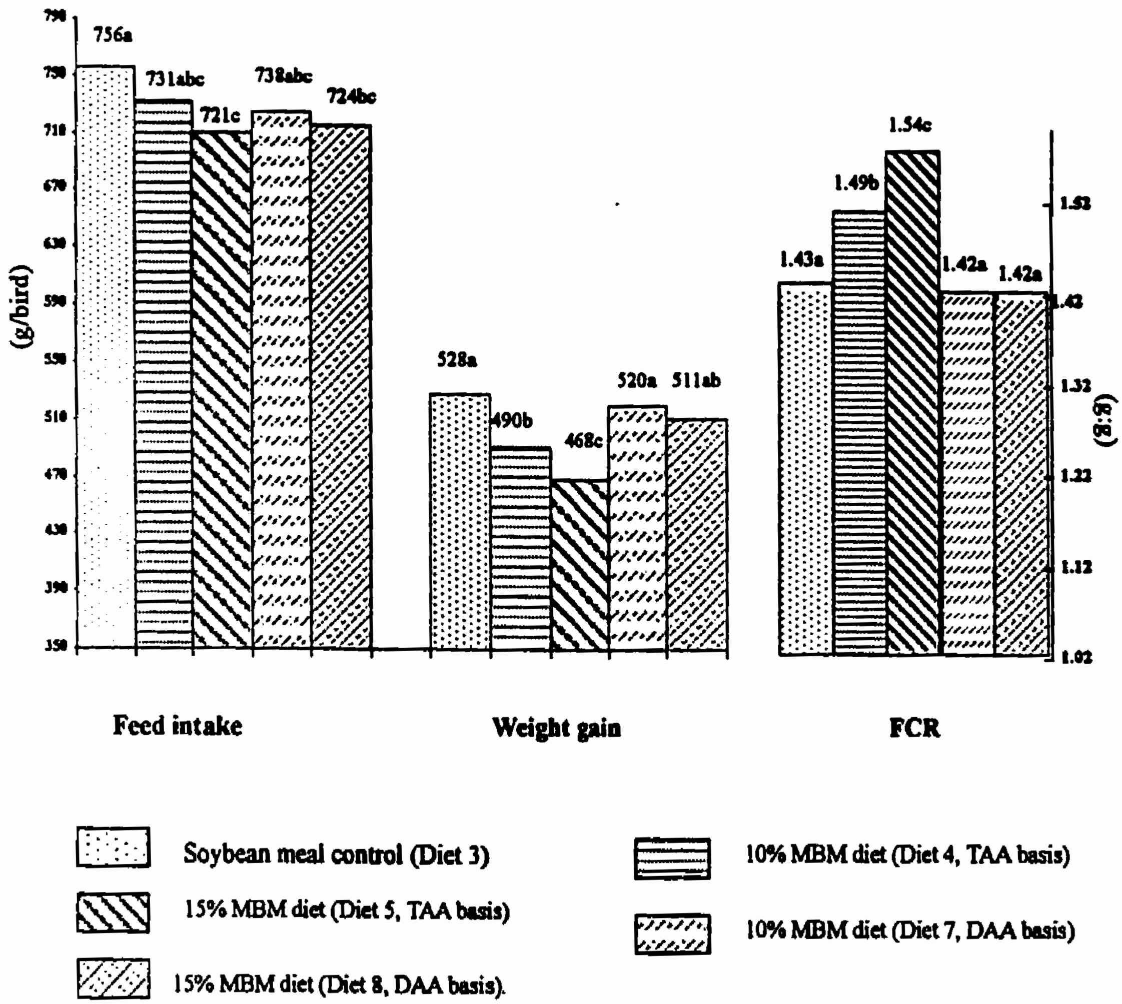
2. *Effect on chick performance of formulating diets with two levels of high ash MBM and based on total amino acid or digestible amino acid values according to 90% of NRC (1994)*

Chick performance when fed corn-soybean meal control diet or MBM diets formulated to 90% NRC specification is shown in Table 4 and Figure 2. The data indicated that for corn-soybean meal diets (Diets 1 and 3), a reduction of 10% for all AA values did not result in significant differences in chick performance for the 14 d experimental period of observation ($P>0.05$). However, chicks receiving diets with 10 or 15% high ash MBM formulated at 90% NRC AA basis (Diets 4 and 5) resulted in significantly lower weight gain and FCR compared to the corn-soybean meal control (Diet 3) ($P<0.05$). Chicks fed the diet containing 15% MBM showed the lowest gain and FCR ($P<0.05$). However, when using the same dietary composition as Diets 4 and 5, but formulated on a DAA basis, performance of all chicks fed diets containing 10 or 15% MBM (Diets 7 and 8) was improved to the level of chicks fed on the corn-soybean meal control diet (Diet 3). No statistical differences in any parameters were observed among Diets 7 and 8 vs. Diet 3 ($P>0.05$). The results clearly demonstrated that diet formulation on a DAA value was superior to that on a TAA value for MBM-based diets. The results agree well with Wang and Parsons (1998c), Dalibard and Paillard (1995), Fernandez et al. (1995), and Rostagno et al. (1995).

The different conclusions regarding the advantage of formulating diets with DAA instead of TAA arrived at from the two sets of experimental diets indicate that dietary AA level, per se, has an important influence. Lack of a standard for DAA requirements can lead to such contradictory results. At present, although research has been carried out to determine digestible Lys and TSAA requirements for broiler chickens (Parsons, 1990; Baker and Han, 1994; Dalibard and Paillard, 1995), available information is limited.

In this study chick performance was at a high level with 14 day weight gain of 520 g and FCR of 1.42, consequently the results indicate that a reduction of 10% in the AA requirements for 0-3 week-old broilers is practical when formulating on a DAA basis. This is in agreement with Parsons (1990) who indicated that digestible Lys and TSAA requirements should be 8 to 10% lower than that listed in NRC for AA specifications. The results may have considerable economic implications. Protein ingredients are costly, and a 10% reduction in the level of AA in diets would definitely reduce feed cost in addition to producing a benefit in reduced nitrogen excretion. The data also showed that commercial high ash MBM can be used at 10% in the diet of growing broiler chickens when formulating on a DAA basis.

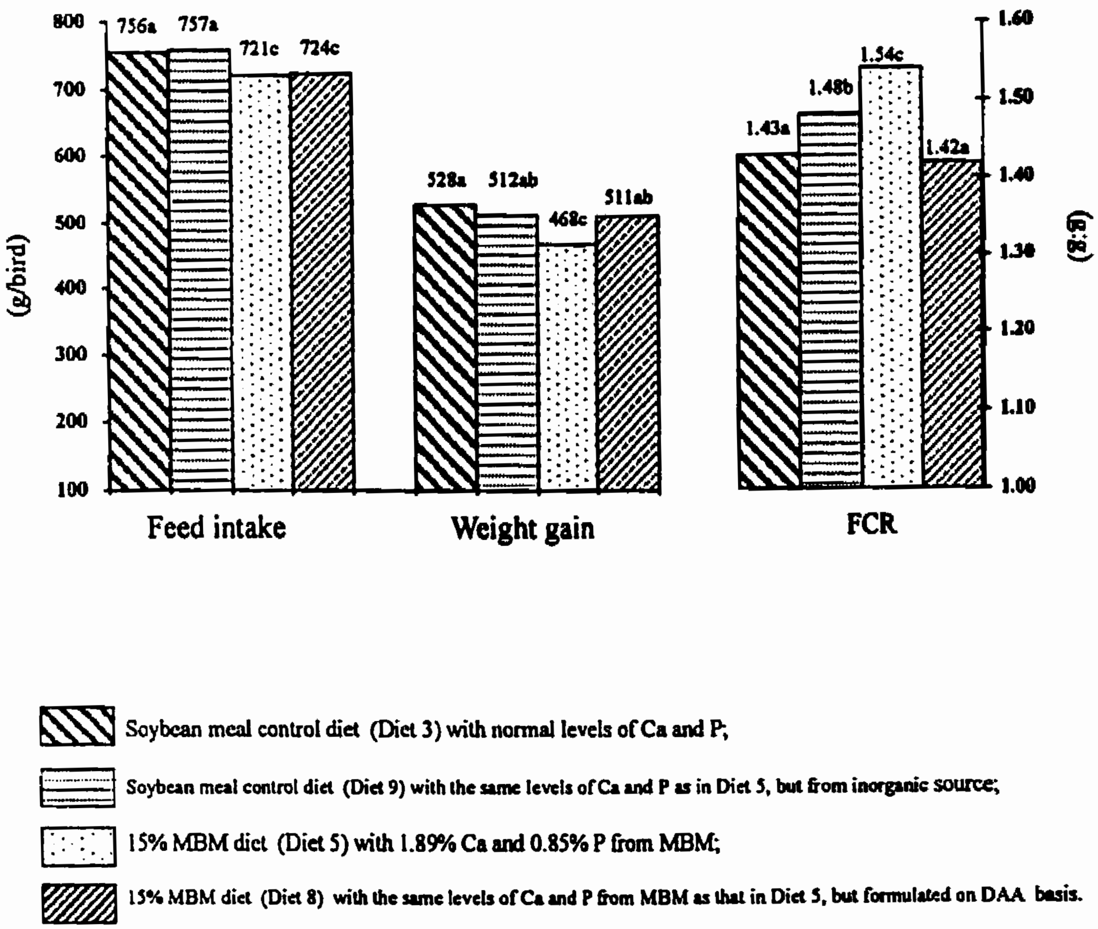
Figure 2. Effect on chick performance of dietary formulation with 10 or 15% high ash meat and bone meal (MBM) on 90% NRC (1994) total amino acid (TAA) or digestible amino acid (DAA) values



3. *Effect of dietary level and source of Ca and P on chick performance*

Chick performance showing the effect of adding Ca and P provided by limestone and mono dicalcium phosphate to a soybean meal diet (Diet 9) to equal the levels of Ca and P found in MBM diets (Diets 5 and 8) is presented in Table 4 and Figure 3. No significant adverse effects of high dietary Ca and P from inorganic sources on feed intake and chick growth were observed when compared to the corn-soybean meal control (Diet 3) which had a normal level of Ca and P ($P>0.05$). However, feed conversion ratio in Diet 9 was lower than that in the corn-soybean meal control (Diet 3) ($P<0.05$), probably due to a slight tendency for a reduction in weight gain (512 vs. 528 g, $P>0.05$). In contrast, high dietary Ca and P in Diet 5 supplied by high ash MBM significantly reduced feed intake, growth rate and FCR in comparison to chicks fed Diet 9 ($P<0.05$). However, the depression in chick performance may be due only partially to high Ca and P levels provided by 15% MBM. When dietary AA were supplemented on a DAA basis (Diet 8), performance of chicks receiving the same levels of Ca and P from MBM as that in Diet 9 was improved ($P<0.05$) and equivalent to that of chicks fed the corn-soybean meal control (Diet 3) ($P>0.05$). This implies that AA deficiency, probably Lys and TSAA due to their low digestibility (Table 1), is the primary reason for the depression in growth rate and feed intake. In our previous study, the data indicated that 10% MBM diet was deficient in Lys and Met. However, excessive Ca and P (89% higher than the requirement, Table 2) in the diet may be partly responsible for the reduction in FCR due to a tendency in reduction of growth rate as shown in the soybean meal diet comparison (Diets 3 vs 9). These results are in agreement with literature reports. Sathe and McClymont (1965) reported that protein quality was the main cause of growth depression of chicks fed MBM diet while high levels of Ca and P provided by MBM was a secondary factor influencing chick performance. Runnels (1968) indicated that dietary Ca as high as 2% would not affect chick growth provided energy and essential AA levels met bird requirements. Evans and Leibholz (1979a) reported that additional dietary Ca content (3.2%) from MBM did not reduce pig performance. Johnson and Parsons (1997) also reported that high levels of Ca (2.31%) and P (0.87%) which were added to corn-soybean meal diets as limestone and dicalcium phosphate to be equal to that supplied by high ash MBM had no significant effect on growth or protein efficiency ratio (PER) in growing chicks. In their study, high ash MBM resulted in a low PER which was explained by a deficiency of TSAA. In contrast, Summers et al. (1964) considered that excessive Ca and P were responsible for the poor chick performance when using MBM. The current study shows that AA deficiency in high level of MBM diet is the major factor restricting chick performance with excessive dietary Ca and P being a secondary factor causing low feed efficiency.

Figure 3. Effect of high dietary levels of Ca and P provided by high ash meat and bone meal (MBM) or inorganic source on chick performance



Conclusions

The results of current study demonstrate that formulating diets at various levels of high ash MBM based on DAA is superior to formulation based on TAA values. Performance of growing broiler chickens fed MBM was equivalent to those fed corn-soybean meal diets even though all AA were specified at 90% of NRC (1994) requirements. A 10% reduction of NRC (1994) AA requirement for 0-3 week broiler is reasonable when formulating on DAA basis.

Amino acid deficiency is the main factor to be considered in the low chick performance when using 15% MBM in the diet. High levels of dietary Ca and P from MBM represent a secondary factor causing a depression in chick performance.

Notwithstanding the equivalent performance of MBM diets based on DAA formulation, consideration of environmental pollution due to excess P excreting makes it necessary to recommend that the usage rate of high ash MBM in broiler diets should, generally not be greater than 10%.

Chapter VI

General Discussion

A new high ash meat and bone meal (MBM) produced in Ontario, Canada, was made available for nutritional evaluation. The product was processed from a secondary residue after producing a low ash MBM. Raw materials consisted of 90% pig offal and 10% beef residues, with possibly some hair and blood. Compared to conventional MBM, the new MBM contains more hard offal, resulting in a high content of ash, Ca and P. Due to the variation in raw material sources and processing conditions, it is well known that the nutritive value of MBM varies greatly. Using tabulated data to extrapolate nutritive value of different MBM is of limited practical meaning and consequently a new database is required to characterize the high ash MBM.

Chemical characteristics of high ash meat and bone meal

Proximate analyses of five batches of high ash MBM showed stable contents of GE, protein, Ca and P with small variations in fat and ash content (Manuscript 1, Table 1), suggesting a consistent quality. On a DM basis, the high ash MBM contained GE, 4249 kcal/kg; protein, 53%; fat, 11.5%; ash, 29%; Ca, 9%; and P, 5%; TMEn, 2800 kcal/kg. Its proximate composition is very close to NRC (1994) table values for MBM with 50.4% CP except TMEn value, suggesting that the nutrient levels of the high ash MBM are in the normal range. High ash MBM has protein content similar to soybean meal, but ash, consisting mainly of Ca and P is 5 to 25 times higher than that for soybean meal. Although levels of Ca and P in high ash MBM are relatively well balanced, the high level may become a limiting factor restricting its usage in animal rations in terms of excessive P excretion causing pollution of the environment and negative effects of high Ca intake on fat and energy utilization.

The value for TMEn of the high ash MBM is close to that of wheat, barley (NRC, 1994) and about 200 kcal/kg higher than that in NRC (1994). Several authors have pointed out that NRC may underestimate ME value of MBM (Martorsiswoyo and Jensen, 1988; Dolz and Blas, 1992), Our estimate supports this finding and indicates that the new MBM is a potential energy source for monogastric animals in addition to being a source of protein, Ca and P.

Protein and amino acids in high ash meat and bone meal

No difference in total essential amino acid concentration between low and high ash MBM was observed when compared on a mg/gram protein (Chapter III, Manuscript 1, Table 5). This implies that the protein quality of high ash MBM is as good as that of low ash MBM. Current data for AA profile of high ash MBM is similar to that in NRC (1994) for MBM containing 50.4% CP.

Protein quality evaluation results indicated that *in vitro* protein digestibility of high ash MBM was 67%. Most amino acids were around 80-85% digestible, Cys had the lowest value at 63%, next to Try, Lys and Met (72-78%). There were no significant differences in AA digestibility determined either by intact or by cecectomized roosters between low and high ash MBM ($P>0.05$). *In vitro* protein digestibility results agreed well with data from *in vivo* tests for AA digestibility in that no differences were observed.

For MBM it is generally thought that the higher ash, the poorer the protein quality, since ash level is an indicator of bone added in a MBM product. It has been reported that 85% bone protein is collagen, being deficient in Try and other EAA (Eastoe and Long, 1964; Atkinson and Carpenter, 1970a). High dietary Ca may disturb protein absorption and decrease protein digestibility. Therefore high ash MBM may theoretically have poor protein/amino acid digestibility even though TEAA content is apparently not different between both MBM. However, the current study by *in vitro* and *in vivo* assays demonstrated that protein quality of high ash MBM was similar to that of low ash MBM, and was not affected by its high ash/Ca content. These results support findings of Partanen (1994) who reported high (33%) and low ash (21%) MBM had similar protein digestibility (75 vs. 76%), and Johnson and Parsons (1998) who reported that there was no difference in AA digestibility for high ash (34%) and low ash (24%) MBM.

Feeding value of the high ash meat and bone meal

In general, it is thought that MBM is nutritionally inferior to soybean meal. Increasing MBM inclusion rate correlates to declining animal performance (Sath and McClymont, 1965; Evans and Leibholz, 1979a). Our chick growth data displayed the same tendency as the findings from previous authors. Five to 10% inclusion levels of high ash MBM in replacement for soybean meal in male Leghorn chick diets (from post-hatching 10 to 20 days of age) resulted in 5-9% decrease ($P<0.05$) (Chapter IV, Manuscript 2, Figure 1) when Lys concentration was 90% NRC (1994) specification in comparison to those chicks fed the same level of MBM diets, but with Lys requirement met. Ten or 15% inclusion depressed broiler chick performance ($P<0.05$) compared to a soybean meal control diet (Chapter IV, Manuscript 2, Table 5 and Chapter V, Manuscript 3, Table 4) without AA supplemented or with formulation based on TAA at 90% NRC (1994) specifications. Causes for the depression may be related to amino acid imbalance due to low digestibility of Lys (77.5%), TSAA (71.3%) and Try (73.2%) in high ash MBM.

Limiting amino acids in practical broiler diets with 10% high ash meat and bone meal

Study on AA supplementation provided evidence that the chick growth depression from high ash MBM inclusion was mainly a result of AA deficiency. Addition of Lys and Met to 10% high ash MBM diet significantly improved chick weight gain and feed conversion ratio (FCR) ($P<0.05$), and brought these two parameters back to those chicks fed a soybean meal control diet ($P>0.05$). Further addition of Thr and Try to the diet already containing Lys and Met yielded a small extra response, but did not reach significance ($P>0.05$). These results indicated that Lys and Met are equally limiting in high ash MBM diets. Our data agreed well with many previous findings (Sathe and McClymont, 1968; Skurray and Cumming 1974b), but differed from that of Stockland et al. (1971), and Wang et al. (1997) who found Try was the first limiting amino acid in diet either when MBM contributed 60% protein for growing pigs or when MBM was used as a sole protein source for chicks. The disagreement may be related to the amount of MBM included and the cereal type in the test diet. Wilson (1967) tested the order of limiting amino acids in cereal-MBM diets, and found that when MBM and cereal contributed equal amounts of protein (50:50) to a diet, the limiting amino acids were Lys and Met. Johri et al. (1981) further showed that cereal type affects AA deficiency order. In our studies, high ash MBM contributed 25% protein, cereals (corn and barley) contributed 26%, and soybean meal made up the other half of protein in the diet. Thereby, a deficiency order may be decided by MBM, soybean meal and cereals altogether. The deficiency of Try in MBM may be compensated by soybean meal.

Potential effect of high Ca and P in MBM on chick performance

Formulation of broiler diets with over 10% high ash MBM would lead to Ca and P 30% greater than the normal requirements. The high dietary Ca and P from MBM are considered to decrease feed intake and consequently depress chick growth rate. However, there is disagreement in the literature on whether the Ca and P at these levels would be a major factor responsible for the alleged performance depression or whether a poor AA balance would be the major factor affecting chick performance. Summers et al. (1964) thought that excessive Ca and P were responsible for the poor chick performance when using MBM, whereas Runnels (1968) indicated that 2% dietary Ca would not affect chick growth when energy and essential amino acids met the requirement of birds. Evans and Leibholz (1979a) reported that high (3.2%) dietary Ca content from MBM did not reduce pig performance. Johnson and Parsons (1997) found that high contents of Ca (2.31%) and P (0.87%) provided by limestone and dicalcium phosphate to the levels equivalent to those from high ash MBM did not retard performance in growing chicks.

Our results agree favorably with most of the literature data. Inclusion of 10 or 15% high ash MBM resulted in diets containing Ca at 1.34 and 1.89%, respectively, which were 30 and 89% higher than the normal requirement. In these cases, chick performance was impaired in comparison to a corn-soybean meal diet ($P<0.05$, Chapter V, Manuscript 3, Table 4 and Figure 3). However, it seemed that the depression was not due primarily to the high Ca and P contents in the diet since when limestone and mono-dicalcium phosphate were added to a soybean meal diet to equal the levels of Ca and P present in 15% MBM diet no adverse effect on feed intake and chick growth was observed. Chicks receiving the diet with additional Ca and P from inorganic sources grew as well as those fed on the soybean meal control diet containing normal levels of Ca and P ($P>0.05$). When dietary AA was supplemented on a digestible AA basis, performance of chicks fed the same level of MBM was significantly improved ($P<0.05$, Chapter V, Manuscript 3, Table 4, Diets 8 vs. 9). These results indicate that AA imbalance, particularly Lys and Met due to their low digestibility, was the primary reason for the performance depression. However, excessive dietary Ca and P may be a secondary factor as FCR of the soybean meal diet with additional Ca and P was not equal to that of soybean meal control diet ($P<0.05$).

Effect on chick performance of diet formulation with 10 and 15% levels of high ash meat and bone meal based on total versus digestible amino acid values

Since the low digestibility of Lys and TSAA is the primary factor causing a depression in chick performance, we further investigated the effect on chick performance of formulating diets with high ash MBM on a digestible vs. total AA basis. Theoretically, formulation on digestible AA instead of total AA value should be more accurate and economic. However, our results provided useful evidence that there are other preconditions governing such a conclusion. When all AA met NRC (1994) requirements, inclusion of 10% high ash MBM with AA based on total or digestible values (Chapter V, Manuscript 3, Table 4, Diets 2 vs. 6) displayed no differences in chick performance, both performed similar to that of corn-soybean meal control diet ($P>0.05$), suggesting no advantage gained from DAA formulation. However, when all AA were at 90% of NRC (1994) specifications, inclusion of 10 or 15% high ash MBM on a TAA basis (Chapter V, Manuscript 3, Table 4, Diets 4 and 5) resulted in lower chick performance ($P<0.05$). Chicks fed a diet containing 15% MBM showed the lowest feed intake, weight gain and FCR ($P<0.05$). However, performance of all chicks fed diets containing 10 or 15% high ash MBM (Chapter V, Manuscript 3, Table 4, Diets 7 and 8) was significantly improved ($P<0.05$) to the level of chicks fed on corn-soybean meal control diets when diets were formulated on a DAA basis. Our results demonstrated formulating on a DAA basis is certainly superior to on a TAA basis. These results indicate that dietary AA level plays a key role on the conclusion of dietary formulation on DAA vs. TAA values. Lack of adequate DAA requirement data is probably one of several factors conducting to the controversy. At present, research has indicated that for broiler dietary formulation based on DAA values the requirement for Lys and TSAA should be reduced by 8 to 10% of NRC specifications (Parsons, 1990; Dalibard and Paillard, 1995). Our study confirmed that a reduction of 10% for all AA did not result in performance reduction during 14 d observation for both corn-soybean meal control diets, and diets with high ash MBM formulated on a DAA basis with 90% NRC specification showed high performances (weight gain 520 g and FCR 1.42 during 14 days). It suggests, for 0-3 week broilers, a reduction of 10% AA requirement is possible if formulating on a DAA basis.

Chapter VII

General Summary

The objective of the study was to characterize the chemical composition of a new high ash meat and bone meal (MBM) and to evaluate the feeding value of the new product for poultry. Experiments were conducted to compare high ash MBM with conventional low ash MBM with regard to available energy and digestible amino acid contents. Protein quality was also assessed using an in vitro protein digestibility assay. The ability of high ash MBM diets to support chick growth in comparison to soybean meal control diets and the effect of amino acid supplementation of high ash MBM diets formulated on a total vs. digestible amino acid basis was also studied.

Five batches of high ash MBM and two batches of low ash MBM were analyzed for chemical composition, available energy content and protein quality. Results indicated that the contents of most nutrients in high ash MBM were consistent from batch to batch except for a small variation in fat and ash. On a DM basis, high ash MBM contained: GE, 4250 $\pm$ 200 kcal/kg; protein, 53 $\pm$ 3%; ash, 29 $\pm$ 2% with relatively balanced Ca (9%) and P (5%); fat, 11.5% with 50% as unsaturated fatty acids. True metabolizable energy corrected to zero nitrogen retention was as 2800 kcal/kg for high ash MBM which was significantly lower than the value (3321 kcal/kg) of low ash MBM ($P<0.05$). *In vitro* protein digestibility was 66%, true digestibility of TEAA was 83%, and digestibilities of Lys, TSAA and Try were 77, 71 and 73%, respectively for high ash MBM. There were no statistical differences in protein quality for both MBM measured either by AA digestibility or by in vitro protein digestibility despite the difference in ash content. Overall the results suggest that high ash MBM is an effective source of supplemental protein, Ca, P and energy.

Results of growth trials with Leghorn and broiler chickens showed that the nutritional value of high ash MBM was inferior to that of soybean meal. When inclusion levels of MBM increased from 7.5 to 15% in substitution for soybean meal, chick performance declined linearly ($P<0.05$). Amino acid deficiency caused by a low digestibility of Lys and TSAA was the primary factor causing a depression in chick performance, whilst excessive dietary levels of Ca and P provided by high ash MBM were a secondary factor causing a poor chick performance which was noted in FCR. Supplementation of Met and Lys in combination to diets containing 10% high ash MBM significantly improved chick performance, while the further addition of Thr and Try to diets already containing Lys and Met yielded only a small non-significant response. The results suggested Lys and Met to be equally limiting in corn-based diets containing 10% high ash MBM.

Dietary AA level, per se, plays an important role in the decision to base diet formulation on either digestible AA or TAA values. Results of the current study showed that no significant advantage was gained for diets with 10% MBM based on total vs. digestible AA values when AA specifications were 100% NRC (1994). However, when all AA specifications were placed at 90% NRC, formulation of diets with 10 or 15% high ash MBM based on DAA was superior to that based on TAA values, and growth performance of broiler chickens fed these diets were equivalent to that of soybean meal control.

It is concluded that commercial high ash MBM is a valuable source of supplemental protein, Ca, P and energy for poultry. The protein quality of high ash MBM is similar to that for low ash MBM, and it can be incorporated up to a level of 10% in broiler chicken diets using DAA values in diet formulation.

References

Anderson, J. O., and R. E. Warnick, 1971. Relative value of three animal protein supplements in chick rations. Poultry Sci. 50 (Suppl.): 1545 (Abstr).

Andrews, R. P., and N. A. Baldar, 1985. Amino acid analysis of feed constituents. Science Tools, Vol. 32, 2:44-48.

Association of Official Analytical Chemists, 1990. Official Methods of Analysis. 15th ed. Association of Official Analytical Chemists, Inc., Arlington, V.A.

Atkinson, J., and K. J. Carpenter, 1970a. Nutritive value of meat meals. II. Influence of raw materials and processing on protein quality. J. Sci. Food Agric. 21:366-373.

Atkinson, J., and K. J. Carpenter, 1970b. Nutritive value of meat meals. III. Value of meat meals as supplements to a cereal basal diet: Limiting amino acid in these diets. J. Sci. Food Agric. 21:373-376.

Baker, D. H., and Y. Han, 1994. Ideal amino acid profile for broiler chicks during first three weeks posthatching. Poultry Sci. 73:1441-1447.

Batterham, E. S., M. B. Manson, and H. C. Kirton, 1970. A nutritional evaluation of diets containing meat meal for growing pigs. IV. Differences in growth of pigs on different meals and in chemical and chick tests of these meals. Aust. J. Exp. Agric. Anim. Husb., 9:384-390.

Batterham, E. S., C. E. Lewis, R. F. Lowe, and the late C. J. McMillan, 1980. Digestible energy content of meat meals and meat and bone meals for growing pigs. Anim. Prod. 31:273-277.

Batterham, E. S., R .F. Lowe, and R. E. Darnell, 1986. Effect of pressure and temperature on the availability of lysine in meat and bone meal determined by slope-ratio assay with growing pigs, rats and chicks and by chemical techniques. Br. J. Nutr. 55:441-453.

Batterham, E. S., 1992. Availability and utilization of amino acids for growing pigs. Nutr. Res. Rev. 5:1-18.

Brooks, P. 1991. Meat and bone meal: The underutilized raw material. July, Feedstuffs. 13-15.

Canola Council of Canada, 1997. Canola meal feed industry guide, Poultry. Edited by Dave Hickling.

Cromwell, G. L., 1989. An evaluation of the requirements and biological availability of calcium and phosphorus for swine. Pages 1-15 in: Proc. Texas Gulf Nutr. Symposium, Raleigh, NC.

Cromwell, G. L., T. S. Stahly, and H. J. Monegue, 1991. Amino acid supplementation of meat meal in lysine-fortified, corn-based diets for growing-finishing pigs. J. Anim. Sci. 69:4898-4906.

Dale, N., 1997. Metabolizable energy of meat and bone meal. J. Appl. Poultry Res. 6:169-173.

Dalibard, P., and E. Paillard, 1995. Use of the digestible amino acid concept in formulating diets for poultry. Anim. Feed Sci. Tech. 53:189-204.

Dolz, S., and C. DeBlas, 1992. Metabolizable energy of meat and bone meal from Spanish rendering plants as influenced by level of substitution and method of determination. Poultry Sci. 71:316-322.

Duncan, D. B., 1955. Multiple range and multiple FF-tests. Biometrics 11, 1-42.

Eastoe, J. E., and J. E. Long, 1960. The amino acid composition of processed bones and meat. J. Sci. Food Agric., 11:87-92.

Evans, D. F., and J. Leibholz, 1979a. Meat meal in the diet of the early-weaned pig. I. A comparison of meat meal and soya bean meal. Anim. Feed Sci. Tech. 4:33-42.

Evans, D. F., and J. Leibholz, 1979b. Meat meal in the diet of the early-weaned pigs. II. Amino acid supplementation. Anim. Feed Sci. Tech. 4:43.

FAO. 1998. Food and Agriculture Organization of the unite Nations. FAO Statistics Series. No. 11, 89-100.

Farrell, D. J., 1978. Rapid determination of metabolizable energy of food using cockerels. Br. Poultry Sci. 19:303-308.

FDA, 1996. Section 5: Rendering and processing practice in the United States. Docket 96N-0135. Dockets Management Branch (HFA-305), Food and Drug Administration, Rockville, MD.

Fernandez, J. A., 1992. Calcium and phosphorus metabolism in growing pigs studied by the balance technique and simultaneous radio-calcium and radio-phosphorus kinetics. Ph.D Thesis, The Royal Veterinary and Agricultural University, Frederiksberg, Denmark, 148 pp.

Fernandez, S. R., S. Aoyagi, Y. Han, C. M. Parsons, and D. H. Baker, 1994. Limiting order of amino acids in corn and soybean meal for growth of the chick. Poultry Sci. 73:1887-1896.

Fernandez, S. R., Y. Zhang, and C. M. Parsons, 1995. Dietary formulation with cottonseed meal on a total amino acid versus a digestible amino acid basis. Poultry Sci. 74:1168-1179.

Foxcroft, P. D., 1984. The feed compound. 8-11.

Hugli, T. E., and S. Moore, 1972. Determination of tryptophan content of protein by ion exchange chromatography of alkaline hydrolysates. J. Biological Chem. 247, 9:2828-2834.

Johnson, M. L., and C. M. Parsons, 1997. Effect of raw material source, ash content and assay length on protein efficiency ratio and net protein ratio values for animal protein meals. Poultry Sci. 76:1722-1727.

Johnson, M. L., C. M. Parsons, G. C. Fahey, Jr., N. R. Merchen, and C. G. Aldrich, 1998. Effect of species raw material source, ash content and processing temperature on amino acid digestibility of animal by-product meals by cecectomized roosters and ileally connulated dogs. J. Anim. Sci. 76:1112-1122.

Johnston, J., and C. N. Coon, 1979. A comparison of six protein quality assays using commercially available protein meals. Poultry Sci. 58: 919-927.

Johri, T. S., Pran Vohra, F. H. Kratzer, and Leslie Earl, 1980. The evaluation of nutritional value of meat and bone meals as influenced by cereals or corn starch. Poultry Sci. 59:1832-1838.

Knabe, D. A., D. C. LaRue, E. J. Gregg, G. M. Martinez, and T. D. Tanksley, Jr. 1989. Apparent digestibility of nitrogen and amino acids in protein feedstuffs by growing pigs. J. Anim. Sci. 67:441-458.

Kratzer, F. H., and P. N. Davis, 1959. The feeding value of meat and bone meal protein. Poultry Sci. 1389-1393.

Leeson, S., and J. D. Summers, 1997. Commercial poultry production. 2nd ed. University books. Guelph, Ontario, Canada.

Lessire, M., and B. Leclercq, 1983. Metabolizable energy content of meat meal for chickens. Arch. Gefluegelkd, 47:1-3.

Lessire, M., B. Leclercq, L. Conan, and J. M. Hallouis, 1985. A methodological study of the relationship between the metabolizable energy values of two meat meals and their level of inclusion in the diet. Poultry Sci. 64:1721-1728.

March, B. E., J. Biely, and R. J. Yong, 1950. Supplementation of meat scraps with amino acids. Poultry Sci. 28:718.

Martosiswoyo, A. W., and L. S. Jensen, 1988. Available energy in meat and bone meal as measured by different methods. Poultry Sci. 67:280-293.

Matterson, L. D., L. M. Potter, M. W. Stutz, and E. P. Singsen, 1965. The metabolizable energy of feed ingredients for chicks. Storrs Agric. Exp. Stn, Res, Rep. 7. Univ. Coun., Storrs, CT.

Meade, R. J., and W. S. Teter, 1957. The influence of calcium pantothenate, tryptophan and methionine supplementation and source of protein upon performance of growing swine fed corn-meat and bone scrap rations, J. Anim. Sci. 16:892.

Metz, S. H. M, and J. M. Van der Meer, 1985. Nylon bag and in vitro techniques to predict on vivo digestibility of organic matter in feedstuffs for pigs. In: Proceedings of the 3rd international seminar on digestive physiology in the pig. Eds Just A., Jorgenesen H & Fernandez J. A; National Institute of Animal Science. Copenhagen. pp 373-376.

Mills, P. A., R. G Potter, and R. R. Marquardt, 1989. Modification of the glucosamine method for the quantification of fungal contamination. Can. J. Anim. Sci. 69:1105-1106.

Moughan, P. J., J. Schrama, G. A. Skilton, and W. C. Smith, 1989. In vitro determination of nitrogen digestibility and lysine availability in meat and bone meals and comparison with in-vivo ileal digestibility estimates. J. Sci. Food Agric. 47:281-292.

NRC, 1994. Nutrient Requirements of Poultry. 9th rev. ed. National Research Council. National Academy Press, Washington, DC.

NRC, 1998. Nutrient Requirements of Swine. 10th rev. ed. National Research Council. National Academy Press, Washington, DC.

Orban, J. I., and D. A. Roland, Sr., 1992. The effect of varying bone meal sources on Phosphorus utilization by 3-week old broilers. J. Appl. Poultry Res. 1:75-83.

Parsons, C. M., 1990. Digestibility of amino acids in feedstuffs and digestible amino acid requirements for poultry. St. Louis, MO. Biokyowa, Inc..

Parsons C. M., F. Castanon, and Y. Han. 1997. Protein and amino acid quality of meat and bone meal. Poultry Sci. 76:361-68.

Partanen, K., 1994. The effect of ash content on the nutritive value of meat & bone meal for growing pigs. Acta. Agric. Scand., Sect. A. Animal Sci. 44:152-59.

Partrick, H., 1953. Supplements for a "meat-scraps" type chick ration. Poultry Sci. 32:570-572.

Prokop, W. H., 1985. Rendering systems for processing animal by-product materials. J. American Oil Chem. Sci. 62:805-811.

Rhone-Poulenc, 1989. Nutrition guide, 1st Edition, Rhone-Poulenc Animal Nutrition, Commentry, France.

Rostagno, H. S., J. M .R. Pupa, and M. Pack, 1995. Diet formulation for broilers based on total versus digestible amino acid. J. Appl. Poult. Res. 4:293-299.

Runnels, T. D., 1968, Meat and bone meal as an ingredient in broiler diets. Feedstuffs. Oct. 19, 27-33.

Salmon, R. E., 1977. Fish meal, meat and bone meal, fermentation byproducts and ingredient combinations in diets of young turkeys. Can. J. Anim. Sci., 57:751-754.

SAS Institute, 1985. SAS ® User's Guide: Statistics. Version 5 Edition. SAS Institute Inc., Cary, NC.

Sathe, B. S., and G. L. McClymont, 1965. Nutritional evaluation of meat meals for poultry. III. Association of chick growth with the bone, calcium and protein contributed by meat meals to diets, and the effect of mineral and vitamin plus antibiotic supplementation. Aust. J. Agric. Res., 16, 234-55.

Sathe, B. S., and G. L. McClymont, 1968. Nutritional evaluation of meat meals for poultry. VI. Association of growth promotion, protein digestibility, and hot-water-soluble protein content, and effect of amino acid supplementation. Aust. J. Agric. Res,. 19:171-179.

Schang, M. J., and R. M. G. Hamilton, 1982. Comparison of two direct bioassays using adult cocks and four indirect methods for estimating the metabolizable energy content of different feedingstuffs. Poultry Sci. 61:1344-1353.

Sell, L. J., 1996. Influence of dietary concentration and source of MBM on performance of turkeys. Poultry Sci. 75:1076-1079.

Sibbald, I. R., 1979. A bioassay for available amino acids and true metabolisable energy in feedingstuffs. Poultry Sci. 58:668-675.

Sibbald, I. R., 1986. The TME system of feed evaluation: Methodology, feed composition data and bibliography. Bulletin 1986-4E, Agriculture Canada, Ottawa.

Skurray, G. R., and L. S. Herbert. 1974a. Batch dry rendering: influence of raw materials and processing conditions on meat meal quality. J. Sci. Food Agric. 25:1071-1079.

Skurray G. R., and R. B. Cumming, 1974b. Nutritional evaluation of meat meals for poultry. VIII. Nutritive value of and limiting amino acid in cereal diets supplemented with meat & bone meal. Aust. J. Agric. Res. 25:193-199.

Slominski, B. A., J. Simbaya, L. D. Campbell, G Rakow, and W. Guenter, 1999. Nutritive value for broilers of meals derived from newly developed varieties of yellow-seeded canola. Anim. Feed Sci. Tech. 78:249-262.

Steel, R.G.D, J. H. Torrie, and D. A. Diekey, 1997. Principle and Procedures of Statistics. 3rd ed. McGraw Hill.

Stockland, W. L., R. J. Meade, and J. W. Nordstrom, 1971. Lysine, methionine and tryptophan supplementation of a corn-meat and bone meal diet for growing swine. J. Anim. Sci. 32, 2:262-267.

Summers, J. D., S. J. Slinger, and G. C. Ashton, 1964. Evaluation of meat meal as a protein supplement for the chick. Can. J. Anim. Sci. 44:228-234.

Veldman A, H.A. Vahl, G. J. Borggreve, and D. C Fuller, 1995. A survey of the incidence of Salmonella species and enterobacteriaceae in poultry feeds and feed components. Vet. Rec. 1995, 136, 7: 169-72.

Waibel, P. E., N. A. Nahorniak, H. E. Dziuk, N. M. Walser, and W. G. Olson, 1984. Bioavailability of phosphorus in commercial phosphate supplements for turkeys. Poultry Sci. 63:730-737.

Waibel, P., J. Liu, and S. Noll, 1987. Utilization of feather meal, blood meal, and meat and bone meal in market turkey diets. Proc, Minnesota Conference on Turkey Research. Minn. Agric. Exp. Stn. Misc. Rep. 3-1987. St. Paul. MN.

Waldroup, P. W., C. B. Ammerman, and R. H. Harms, 1965. The utilization of phosphorus from animal protein sources for chicks. Poultry Sci. 44:1302-1306.

Waldroup, P. W. and M. H. Adams, 1994. Evaluation of the phosphorus provided by animal proteins in the diet of broiler chickens. J. Appl. Poultry Res. 3:209-218.

Wang, X., F. Castanon, and C. M. Parsons, 1997. Order of amino acid limitation in meat & bone meal. Poultry Sci. 76:54-58.

Wang, X., and C. M. Parsons, 1998a. Effect of raw material source, processing systems, and processing temperatures on amino acid digestibility of meat and bone meal. Poultry Sci. 77:834-841.

Wang, X., and C. M. Parsons, 1998b. Bioavailability of the digestible lysine and total sulfur amino acids in meat and bone meals varying in protein quality. Poultry Sci. 77:1003-1009.

Wang, X., and C. M. Parsons, 1998c. Dietary formulation with meat and bone meal on a total versus a digestible or bioavailable amino acid basis. Poultry Sci. 77:1010-1015.

Waring, J. J., 1969, The nutritive value of fish meal, meat and bone meal and field bean meal as measured by digestibility experiments on the adult colostomised fowl. Br. Poultry Sci., 10:155-163.

Wilder, O. R. M, R. Barbera Oregory, and Paul C. Oslby, 1957. Chemical composition of meat meal and tankage influence of composition on feeding value. American Institute Foundation. Bulletin, NO. 34.

Wilson M. N., 1967 Investigations into the protein quality of meat meal and of other protein concentrates. Proceedings 1967 Australian Poultry Science Convention. pp.105-114.

Zhang, W.J., L. D. Campbell, S. C. Stothers, 1994. An investigation of the feasibility of predicting nitrogen-corrected true metabolizable energy (TMEn) content in barley from chemical composition and physical characteristics. Can. J. Anim. Sci. 74:355-360.