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Characterization and Estimation of Digestibility of Phosphorus Forms in Rendered Animal Protein Ingredients and Fish Meals (03B-2)

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SCIENTIFIC ABSTRACT

Phosphorus (P) is present in different chemical compounds in animal feeds, and the solubility and digestibility of these different compounds are known to differ significantly. Animal protein ingredients generally have a high P content and are major contributors to total P of feeds for fish and other domestic animals. Estimation of different P compounds in these ingredients could help to improve the accuracy of estimates of digestible P contents of feeds. Bone-P and organic P contents were quantified in 32 animal protein ingredients, including 10 fish meals, 14 meat and bone meals, and 8 poultry by-products meals, using a fractionation protocol. The total P contents of the ingredients ranged from 2.1% to 8.3% on a dry matter (DM) basis. Organic P contents varied between 0.3% and 1.3% of DM. Highly significant ($p < 0.001$) linear relationships were observed between total P and ash and between bone-P and ash for all ingredients combined: total P (%) = 0.185 * ash (%) ($r^2 = 0.88$), and bone P (%) = 0.188 * ash (%) – 0.852 ($r^2 = 0.94$). These results suggest that bone-P can be easily and reliably estimated on the basis of ash content in animal protein ingredients.

A digestibility trial was conducted to validate a mathematical model that estimates the digestible phosphorus (P) content of salmonid feeds. The P digestibility model estimates digestible P contents of fish feed based on the dietary inclusion level of different P chemical compounds, which were characterized into five categories: bone-P, phytate-P, organic P, Ca monobasic / Na / K Pi supplement, Ca dibasic Pi supplement. The model suggests that the digestibility of these P compounds differs significantly, and the digestibility of bone-P is not additive. Test diets were formulated with two types of fish meal, two types of poultry by-product meal, two types of soy protein concentrates, and one type of meat and bone meal. The digestibility trial was carried out with rainbow trout (initial body weight ~ 48 g/fish) using the Guelph feces collection system. Comparison between experimental observed and model predicted values suggested that the model well predicted digestible P content of diets formulated with a wide variety of ingredients used in practical feed formulation. The model can be a useful tool in practical feed formulation.

1. INTRODUCTION

Phosphorus is a component of several different types of chemical compounds found in ingredients and feeds. These compounds include hydroxyapatite (bone-P), *myo*-inositol hexaphosphate (phytate-P), P compounds covalently linked to protein, lipid, and sugar (organic P), and various inorganic phosphate supplements. These compounds are present in various amounts in animal feeds depending on feed formulation and the compositional variability of the ingredients used. Differences in the chemical characteristics and solubility of these compounds are likely to result in different digestion dynamics of P within the animal gastrointestinal tract, and this, in turn, can significantly affect P digestibility. It is, consequently, necessary to quantify the different P forms in ingredients to better understand and/or predict the digestibility of P in feeds.

Animal protein ingredients (fish meal, poultry by-products meal, and meat and bone meal) generally have high P contents and often contribute a significant proportion of the total P of feeds for fish and, occasionally, other domestic animals. Animal protein ingredients are produced from a wide variety of raw materials, and manufacturing techniques and equipment (Prokop, 1996; Bureau et al., 1998). Consequently, P content and the proportion of chemical compounds in these ingredients may be highly variable, even for a given type of ingredient. A survey of the literature indicates that there are between 16 and 42 g/kg of P in fish meal, from 25 to 56 g/kg of P in meat and bone meal, and from 17 to 35 g/kg of P in poultry by-products meal (NRC, 1993, 1994, 1998; Sugiura et al., 1998b, 2000c; Sugiura and Hardy, 2000). Very little information on the proportion of P chemical compounds in these ingredients is available in the literature, although it is well-known that in the body of vertebrates, the majority of P (85 - 88%) exists as bone-P, about 10-15% is organic P, and only a small amount is present as free ions or soluble inorganic P phosphates (P_i) (Lall, 1991; Berner, 1997).

Estimates of the digestibility of P for animal protein ingredients are highly variable even for similar ingredients. For example, estimates of apparent digestibility of P in fish meal vary between 17% and 81% for rainbow trout (Ogino et al., 1979; Riche and Brown, 1996; Sugiura et al., 1998, 2000; Sugiura and Hardy, 2000). Differences in the levels of different P chemical forms could explain part of the variability in the estimates of apparent digestibility of P. Information on the contents of various chemical forms of P in animal protein ingredients would enable better prediction of digestibility of P in feed and/or P waste output by animal production operations (Cho and Bureau, 2001). There have been attempts to estimate bioavailability of P in ingredients and feeds based on chemical extractions (Petterson, 1988; Satoh et al., 1992, 1997; Buyukates et al., 2000). A fractionation method was also used for estimates of composition of animal manures (Petterson, 1988; Garcia-Ruiz and Hall, 1996; Dou et al., 2002; Wienhold and Miller, 2004). However, limited work has been carried out to quantify specific chemical compounds in animal protein ingredients. There is also a need for simple methods of estimating total P and bone-P contents of feed ingredients based on routine chemical analyses (e.g. proximate analysis).

A mathematical model was constructed to predict digestible P content of salmonid fish feeds by integrating available literature information. The digestibility model classified P chemical compounds into bone-P, phytate-P, organic P, Ca monobasic / Na / K Pi supplement, Ca dibasic Pi supplement. Digestible P content of diets was estimated based on contents of P chemical compounds. The model was described as follows:

Digestible P = 0.68 bone-P + 0 phytate-P + 0.84 organic P + 0.89 Ca monobasic / Na / K Pi supplement + 0.64 Ca dibasic Pi supplement + 0.51 phytase/phytate – 0.02 (phytase/phytate)^2 - 0.03 (bone-P)^2 - 0.14 bone-P * Ca monobasic / Na / K Pi supplement.

The units for all variables are g/kg diet, except for phytase/phytate ratio the unit is 100 FTU phytase/g phytate. The model suggests that the digestibility of different P chemical compounds differs significantly and digestibility of bone-P is not additive, as indicated by the significant quadratic function and its negative interaction with Ca monobasic / Na / K Pi supplement.

The mathematical model was constructed through integration of data from unrelated studies focusing on different ingredients or chemical compounds and using different experimental approaches. There is a need to validate the model *in vivo* using diets formulated with ingredients containing different levels of P chemical compounds fed to fish reared under the same experimental conditions. A digestibility experiment, therefore, was conducted to evaluate/validate the P digestibility model.

2. EXPERIMENTAL PROCEDURES

2.1 Trial #1 Determination of Phosphorus Fractions in Animal Protein Ingredients

2.1.1 Sources of Samples

Thirty-two animal ingredients, including 10 fish meals, 8 poultry by-products meals, and 14 meat and bone meals, were obtained from various suppliers in North America. These ingredients were selected to cover a wide range of raw materials and finished products for each ingredient type. Details of these ingredient samples are presented in Appendix 1.

2.1.2 Chemical Analyses

Duplicate samples of ingredients were analyzed for proximate composition. Dry matter (DM) was analyzed by heating samples at 105°C for 24 h. Ash was analyzed according to AOAC gravimetric method 942.05 (AOAC, 1995). Crude protein (%N x 6.25) was analyzed according to the Kjeldahl method using a Kjeltech 1030 autoanalyzer (Tecator, Höganäs, Sweden). Lipid was analyzed according to AOAC acid hydrolysis method 954.02 (AOAC, 1995) by a commercial laboratory (AgriFood, Guelph, ON, Canada). A coefficient of variation (CV) of replicates below 5% was considered to be acceptable.

The P fractionation protocol was carried out as detailed in Ruban et al. (2001 a; b) but with slight modifications (Figure 1). Triplicate ingredient samples (0.4 g) were incubated in 1 N NaOH overnight with shaking, and then centrifuged. An aliquot of supernatant was incubated in 3.5 N HCl overnight, whereas pellets were incubated in 1 N HCl overnight with shaking, and then centrifuged. The supernatants and pellets were evaporated to dryness on a hot plate. The resulting P fractions included bone-P, organic P, and residual P (P resistant to acid and alkaline extraction, and thus unaccounted for in analysis). P contents in animal protein ingredients and fractioned samples were analyzed according to the colorimetric method of Heinonen and Lahti (1981).

2.1.3 Calculations and Statistical Analyses

The total P content of each ingredient analyzed was compared to the sum of bone-P, organic P and residual P by *t* test. Relationships between all analyzed variables were subjected to linear regression using SAS software (SAS Institute, 1999). Probability (p) of < 0.05 was considered to be significant.

2.2 Trial #2 Validation of a Phosphorus Digestibility Model For Salmonid Fish

2.2.1 Ingredients and Diets

A low digestible P reference diet meeting nutrient requirements recommended by NRC (1993) was formulated with herring meal, wheat middlings, corn gluten meal, and fish oil (Table 1). A series of test diets was formulated with 20% of two fish meals, two soybean protein concentrates, one meat and bone meal, and two poultry by-products meals that substituted 20% of corn gluten meal in reference diet (Table 1). Acid-washed diatomaceous silica (Celite AW521, Celite Corp., Lompoc, California) was included in diets to serve as a digestion indicator. The diets were mixed using a Hobart mixer (Hobart Ltd, Don Mills, Ontario) and pelleted using a laboratory steam pellet mill (California Pellet Mill Co., San Francisco, California). The feed pellets were dried in a current of air at room temperature for 24h, and were kept at 4°C until used. Amount of diets required was measured out weekly and then kept at room temperature.

2.2.2 Fish, Feeding, Sample Collection

Rainbow trout were obtained from a commercial hatchery (Rainbow Springs Trout Hatchery, Ontario, Canada). Initial fish body weight averaged 48 g. The fish were stocked in an aquatic system equipped with feces settling columns (the Guelph System) described by Cho et al. (1982). Maximum loading was kept below 3.5 kg of fish for each tank during the experiment. The velocity of the water flow was adjusted to minimize settling of the feces in the drainpipe and maximize recovery of the feces in the settling column. The fish were treated in accordance with the guidelines of the Canadian Council on Animal Care (CCAC, 1984) and the University of Guelph Animal Care Committee.

The experimental diets were randomly allocated to collection units. The fish were acclimated to both the tanks and the dietary regime for six days before collection of feces began. A total of four fecal samples were collected. Two fecal samples per diet were collected over a 3-week period. The experimental diets were randomly re-allocated to new tank collection units for a second period and two additional fecal samples per diet were collected in the following 2 weeks.

The fish were hand fed to apparent satiation three times daily between 0930 and 1600 hours. One hour after the last meal, the drainpipe and the settling column were brushed out to remove feed residues and feces from the system. One-third of the water in the tanks was drained to ensure that the cleaning procedure was complete. At 0900 hours the following day, the settled feces and surrounding water were gently withdrawn from the base of the settling column into a large centrifuge bottle. These feces were free of uneaten feed particles and considered a representative sample of the feces produced throughout the 24-hour period. The feces were centrifuged at 5,000 g for 15 min and the supernatant discarded. The feces were then freeze-dried, ground, and stored at -20°C until analysis.

2.2.3 Chemical Analyses

Samples of ingredients, diets and feces were analyzed for dry matter and ash according to AOAC (1995). Crude protein (N x 6.25) was analyzed by the Kjeldahl method using a Kjeltech 1030 autoanalyzer (Tecator, Höganäs, Sweden). Acid insoluble ash was analyzed according to Atkinson et al. (1984).

Bone-P content of animal ingredients and diet samples was analyzed following the P fractionation protocol. Phosphorus content was determined according to the spectrophotometric method of Heinonen and Lahti (1981).

2.2.4 Calculations and Statistical Analyses

The apparent digestibility coefficients (ADC) for the nutrients and energy of the test diets were calculated as follows (Cho et al., 1982):

$$ADC = 1-(F/D \times Di/Fi)$$

where D = % nutrient (or kJ/g gross energy) of diet

F = % nutrient (or kJ/g gross energy) of feces
Di = % digestion indicator (AIA) of diet
Fi = % digestion indicator (AIA) of feces

Validation of P fractionation protocol was carried out by regressing predicted bone-P content (based on bone-P contents of individual ingredients) on analyzed bone-P content of diet and by subsequently examining the slope and intercept of the regression. Similarly, validation of the model was carried out by comparison of the slope and intercept of the regression of model predicted digestible P contents on experimental observed values. The statistical analysis was performed by software GraphPad Prism (version 3.0, GraphPad Software, San Diego, CA) and S-Plus (Insightful Corporation, Seattle, WA).

3. RESULTS AND DISCUSSION

3.1 Trial #1 Determination of Phosphorus Fractions in Animal Protein Ingredients

Table 2 summarizes the results of crude protein, lipid, ash, total P, bone-P, organic P, and residual P on a DM basis in fish meals, poultry by-products meals, and meat and bone meals. Overall, the total P contents of all ingredients samples varied from 2.1% to 8.3%, and ash contents varied from 10% to 37% on a DM basis. The total P contents of fish meals ranged from

2.5% to 4.7% on a DM basis, whereas bone-P contents were between 1.4% and 3.5%. Bone-P accounted for 53% to 79% of total P in fish meal. In poultry by-products meals, total P contents and bone-P contents ranged from 2.1% to 3.6% and from 1.2% to 3.1% on a DM basis, respectively. This translated into 60% to 91% of the total P being present as bone-P in poultry by-products meals. In meat and bone meals, total P content varies from 2.2% to 8.3% of DM, of which between 71% and 93% was bone-P. On a DM basis, bone-P contents of the 14 meat and bone meals varied between 1.6% and 7.0%. Organic P varied between 0.3% and 1.3% in all ingredients. Residual P represented < 2.5% of total P in all ingredients. The difference between total P and the sum of bone-P, organic P and residual P did not exceed 10% in all ingredients and was not significantly different ($p > 0.05$).

Figure 2 illustrates the relationship between the analyzed variables. Highly linear relationships ($p < 0.0001$) were observed among bone-P (%), total P (%), ash (%), and protein (%) as follows:

bone-P = 0.980 * total P – 0.711 ($r^2 = 0.97, p < 0.0001$)

total P = 0.185 * ash ($r^2 = 0.88, p < 0.0001$)

bone-P = 0.188 * ash – 0.852 ($r^2 = 0.94, p < 0.0001$)

Relationship between proportion of bone-P in total P (%) and ash (%) appeared to be asymptotic and could be in practice described by the following quadratic equation:

bone-P/total P = -0.057 * ash^2 + 3.749 * ash + 26.839 ($R^2 = 0.76, p < 0.0001$)

A significant linear equation was obtained to describe the relationship between bone-P (%), protein (%) and lipid (%) content as illustrated by Figure 3 and the following equation:

bone-P = 13.520 - 0.139 * protein -0.150 * lipid ($R^2 = 0.82, p < 0.0001$).

In the present study, bone P accounted for 53% to 93% of total P in the animal protein ingredients analyzed, reflecting the variability of the types and proportion of raw materials used in the manufacturing of these ingredients. Bone is a prominent raw material component in high-ash animal protein ingredients. Bone P content was negatively correlated with protein and lipid contents (Figure 3) and positively correlated with ash content (Figure 2). Bone-P/total P ratio approached an asymptote at high ash levels (Figure 2). Organic P content represented a minor proportion of total P content, especially at high ash levels. Residual P represented < 2.5% of total P in all ingredients. Contents of organic P were analyzed as organic P – I and organic P – II, with organic P – I representing the portion more easily hydrolyzed and/or dissolved, and thus probably more digestible to fish. The results suggest that organic P – I accounted for a major portion of total organic P. However, organic P represents several organic phosphate compounds, such as phosphoprotein, phospholipid, phosphosugar and nucleic acid. The employed fractionation scheme could not further differentiate organic phosphate compounds within organic P – I and II. The subsequent analysis of relationships between total P, bone-P, and organic P suggested that there was no specific advantage in separating out organic P – I and II, or measuring organic P directly instead of estimating it as the difference between total P and bone P. Within each ingredient type, the relationships between analyzed variables were also explored separately; it appeared that the relationships with all ingredients combined are representative of each ingredient type.

Relationship between proportion of bone-P in total P (%) and ash (%) was described by a quadratic equation in this study (Figure 2). True asymptotic relationship was also explored by fitting a Michaelis-Menten equation and was described as follows:
bone-P/total P = 115.8/(1+9.6/ash) ($R^2 = 0.75, p < 0.0001$)

The Michaelis-Menten equation did not improve the goodness of fit of the data, as there was no improvement in R^2 (0.75 for the Michaelis-Menten equation versus 0.76 for the quadratic equation) or residual standard error (5.15 for the Michaelis-Menten equation versus 5.12 for the quadratic equation). Therefore, the presented quadratic function is an adequate description of the asymptotic relationship. Although a quadratic equation is only asymptotic within a certain range of independent variable, ash contents of the animal ingredients tested in this study were from 9.9 to 37.3 g/kg, which fall in a wide range and are representative of ingredients used in practical feed formulation. A quadratic equation is also more readily applicable than a true asymptotic equation such as the Michaelis-Menten equation in practice.

Very little information on the proportion of P chemical compounds in these ingredients is available in the literature. The information of P content and the proportion of bone-P to organic P in whole animal body cannot provide reliable estimates of bone-P and organic P contents in animal protein ingredients because the compositions of the raw material vary significantly. While filleting by products in addition to whole fish can be used to produce fish meals, meat and bone meals and poultry by-products meals are often composed of offal, fats, feet, legs, bones, etc, from beef, pork, poultry slaughter houses (Prokop, 1996; Bureau et al., 1999). The heterogeneity of ingredient compositions is reflected in this study by the wide variations in contents of total P, bone-P and organic P in these animal protein ingredients.

The wide variation of bone P content appears to explain the variation of P digestibility of animal by-products reported in the literature. For salmonid fish, P digestibility ranges from 17% to 81% for fish meal, from 22% to 45% for meat and bone meal, and from 15% to 64% for poultry by-product meal (Ogino et al., 1979; Riche and Brown, 1996; Sugiura et al., 1998, 2000; Sugiura and Hardy, 2000; Cheng and Hardy, 2002). For swine, P digestibility was in the range of 66%-85% for meat and bone meal and 85%-90% for fish meal (Jongbloed and Kemme, 1990; Rodehutscord et al., 1997). In poultry, P digestibility was reported to be 74% for fish meal and 66% for meat and bone meal for 3-week-old broilers (Van der Klis and Versteegh, 1996). Because bone-P is generally believed to be less digestible than organic P to fish (Lall, 1991) and that its digestibility is not additive (Sugiura et al., 2000), the content of bone P in ingredients and the inclusion level of ingredients in experiment diets will greatly affect P digestibility of an ingredient. The depressing effect of dietary P level on P apparent digestibility in fish (Vielma and Lall, 1998; Rodehutscord et al., 2000; Sugiura et al., 2000) may be primarily due to the limited capacity of the fish gastrointestinal tract to solubilize hydroxyapatite, when diets were formulated with high level of animal ingredients, rather than through down-regulation of intestinal active transport by high P_i concentration (Avila et al., 2000). Therefore, quantification of different dietary P forms in feeds is needed to better understand and predict apparent digestibility of P.

Analysis of bone P and total P contents of different batches of animal protein ingredients is an expensive and tedious process. The heterogeneous nature of animal protein ingredients, in particular, high-ash meat and bone meal, further complicates analysis. Given the very good relationships between contents of bone P, total P and ash, our study suggests that bone P content in animal protein ingredients can be easily and reliably estimated on the basis of total P content or ash content of the ingredients. Our study also suggests that there is no advantage in measuring organic P directly instead of estimating it as the difference between total P and bone P.

3.2 Trial #2 Validation of a Phosphorus Digestibility Model For Salmonid Fish

The chemical composition of the test ingredients are presented in Table 3. All the experimental diets were well accepted by the fish. Growth rates, expressed as thermal-unit growth coefficients (Cho, 1992), ranged between 0.19 and 0.25, whereas feed efficiency (gain/feed) ranged between 0.9 and 1.2.

Good agreement was observed between predicted bone-P content of the diets based on bone-P contents of ingredients and analyzed bone-P content directly from diets (Figure 4), suggesting that the P fractionation protocol is not only suitable for analysis of animal ingredients, but also suitable for compounds diets. Contents of P fractions in experimental diets are presented in Table 4. ADC values of dry matter, crude protein and ash of the diets are reported in Table 5. Comparisons of ADC of P and digestible P content (g/kg) from experimental observations and model predictions are reported in Table 6 and Table 7, respectively. There was no statistical difference ($p > 0.05$) between model prediction and experimental observations of ADCs of P and digestible P contents of the diets, except for Diet 1, and 7 (Table 6, 7).

The slight underestimate of P digestible contents by the model could be a result of an overestimation of phytate-P contents in these diets. Nevertheless, the overall model prediction was accurate and non-biased across all experimental diets, suggested by the slope and intercept of the linear regression of the model predicted values on experimental observed values (Figure 5).

Phosphorus contents and digestibility values of individual ingredients are highly variable, depending on quality of raw materials and processing method. For rainbow trout, apparent digestibility of P of fish meal varies from 17 to 81%, meat and bone meal from 22 to 45%, poultry by-product meal from 15 to 64%, soybean meal from 20 to 35% (Ogino et al., 1979; Riche and Brown, 1996; Sugiura et al., 1998, 2000; Sugiura and Hardy, 2000; Cheng and Hardy, 2002). Dietary P levels have variable effects on digestibility of P (Satoh et al., 1996; Vielma and Lall, 1998; Rodehutscord et al., 2000; Sugiura et al., 2000). This large amount of information has not been integrated through a quantitative modeling approach.

The P digestibility model is the first mathematical model to estimate P digestibility and digestible P content of fish feeds. The experiment results suggested that P digestibility model well predicted P digestible P contents of the experimental diets formulated with a variety of ingredients used in practical feed formulation, and it adequately describes the non-additivity of P

digestibility of fish diets. Although empirical in nature, the model incorporated mechanistic elements and its prediction of P digestibility is based on differentiation of P compounds rather than aggregates of total dietary P. The feature of an empirical model with mechanistic elements enables this model to be more physiologically relevant and biologically meaningful than a pure empirical model, yet it is more readily applicable in practical feed formulation than a pure mechanistic model.

This current digestibility trial proves that the model can be a useful tool in practical feed formulation containing bone-P, phytate-P and organic P. Future validation of model with respect of various types of inorganic P supplement is necessary.

CONCLUSION

The experiment results suggest that P digestibility model accurately estimated P digestibility and digestible P contents of the experimental diets formulated with a wide variety of ingredients used in practice. The P digestibility model is the first mathematical model developed to estimate digestible P content of fish feeds and it can be a useful tool in practical feed formulation.

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Table 1. Formulation and composition of diets used in the digestibility trial.

Ingredients	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
				%				
Fish meal, herring	15	35	15	15	15	15	15	15
Fish meal, menhaden			20					
Meat-bone meal 56% CP				20				
Poultry by-product meal (low ash)					20			
Poultry by-product meal (regular)						20		
Soy protein concentrate							20	
Soy protein concentrate, dephytinized								20
Wheat middlings	20	20	20	20	20	20	20	20
Corn gluten meal	45	25	25	25	25	25	25	25
Vitamin premix[1]	1	1	1	1	1	1	1	1
Mineral premix[1]	1	1	1	1	1	1	1	1
Lysine	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Celite	1	1	1	1	1	1	1	1
Fish oil	15.5	15.5	15.5	15.5	15.5	15.5	15.5	15.5
Total	100	100	100	100	100	100	100	100
Analyzed Composition (DM basis)								
				%				
Dry matter	95.9	96.3	96.3	96.2	96.1	96.3	96.3	96.2
Crude protein (N x 6.25)	40.5	43.4	42.7	41.4	44.7	41.2	40.1	40.6
Ash	4.5	6.7	7.8	9.0	7.1	8.1	5.4	5.4

[1]Composition of vitamin and mineral premixes were as reported in Bureau et al. (1998).

Table 2. Contents of dry matter (DM), crude protein (CP), lipid, ash, total P and bone-P in fish meals (FM), poultry by-products meals (PBM), and meat and bone meals (MBM).

	DM	CP	Lipid	Ash	Total P	Bone-P	Organic P - I	Organic P - II	Residual P
	%	%DM							
Fish meals									
FM – 1	90.1	78.3	12.4	10.7	2.6	1.4	0.7	0.3	0.0
FM – 2	85.4	74.0	11.1	12.0	2.5	1.5	0.8	0.2	0.0
FM – 3	75.6	71.9	10.2	14.5	2.6	1.7	0.8	0.1	0.0
FM – 4	93.4	66.7	13.6	17.6	3.7	2.7	0.7	0.1	0.0
FM – 5	92.9	68.3	10.5	19.8	4.7	3.5	1.1	0.2	0.1
FM – 6	91.1	68.0	9.2	20.8	3.8	2.8	0.7	0.1	0.1
FM – 7	90.6	68.6	6.1	20.6	3.8	3.0	0.7	0.1	0.1
FM – 8	92.0	68.1	14.0	17.8	3.4	2.5	0.8	0.1	0.0
FM – 9	94.2	73.6	10.0	16.2	2.5	1.7	0.6	0.2	0.0
FM – 10	92.0	73.1	8.8	15.8	2.7	1.8	0.9	0.1	0.0
Poultry by-products meals									
PBM – 1	96.2	67.6	13.4	14.4	2.7	1.8	0.6	0.1	0.0
PBM – 2	93.7	68.2	14.7	12.7	2.5	1.8	0.6	0.1	0.0
PBM – 3	94.1	70.1	16.8	9.8	2.1	1.2	0.7	0.1	0.0
PBM – 4	98.5	61.4	15.0	18.9	3.4	3.1	0.6	0.1	0.1
PBM – 5	94.2	68.3	14.9	13.6	2.6	2.0	0.6	0.1	0.0
PBM – 6	93.6	64.6	10.9	19.7	3.6	3.1	0.5	0.1	0.1
PBM – 7	96.3	72.0	14.9	13.1	2.6	1.7	0.7	0.1	0.0
PBM – 8	93.9	69.8	9.4	14.4	2.7	1.9	0.6	0.1	0.0
Meat and bone meals									
MBM – 1	95.0	54.8	13.6	22.3	4.2	3.5	0.5	0.1	0.1
MBM – 2	96.3	61.8	10.0	22.5	3.5	3.0	0.4	0.0	0.1
MBM – 3	96.1	54.0	12.8	27.7	4.7	3.9	0.4	0.0	0.1
MBM – 4	95.1	49.0	11.8	35.5	6.3	5.9	0.3	0.0	0.1
MBM – 5	96.5	57.0	12.7	23.5	3.5	3.2	0.4	0.1	0.1
MBM – 6	90.5	57.0	14.3	23.1	4.0	3.3	0.4	0.1	0.1
MBM – 7	94.5	50.9	12.8	27.8	5.0	4.3	0.5	0.1	0.1
MBM – 8	95.2	55.2	12.5	24.9	4.0	3.2	0.5	0.1	0.1
MBM – 9	96.0	45.7	12.1	37.3	8.3	7.0	0.8	0.2	0.2
MBM – 10	95.6	49.6	11.8	26.9	5.5	4.3	0.9	0.2	0.1
MBM –11	95.0	59.8	19.7	13.2	2.2	1.6	0.6	0.1	0.0
MBM – 12	94.3	50.5	12.0	30.8	5.4	5.0	0.4	0.1	0.1
MBM – 13	92.2	55.6	10.7	23.8	3.8	3.2	0.4	0.1	0.1
MBM – 14	95.2	63.7	12.3	21.4	4.0	3.3	0.4	0.0	0.1

Table 3. Chemical composition of the test ingredients used in the digestibility trial.

Ingredients	DM	Composition (Dry matter basis)[1]		
		CP	Ash	Total P
	%	%	%	%
Fish meal, herring	93.5	71.0	13.4	2.5
Fish meal, menhaden	93.4	66.7	17.6	3.7
Meat-bone meal 56% CP	96.5	57.0	23.5	3.5
Poultry by-product meal (low ash)	97.8	59.5	14.3	2.6
Poultry by-product meal (regular)	98.5	61.4	18.9	3.4
Soy protein concentrate	92.4	59.9	6.9	1.0
Soy protein concentrate, dephytinized	91.0	59.3	6.5	0.8

[1]DM, dry matter; CP, crude protein (N x 6.25)

Table 4. P chemical compounds in experimental diets (g/kg dry matter)[1].

Diet No.	Diet	Total P	Bone-P	Phytate-P	Organic P
1	Reference	7.3	2.9	2.6	1.8
2	Fish meal, herring	12.0	6.3	1.9	3.8
3	Fish meal, menhaden	13.7	8.0	2.0	3.8
4	Meat-bone meal 56% CP	14.6	8.7	1.9	3.9
5	Poultry by-product meal (low ash)	12.6	6.7	1.9	4.0
6	Poultry by-product meal (regular)	14.5	8.4	1.9	4.2
7	Soy protein concentrate	8.7	3.1	3.1	2.6
8	Soy protein concentrate, dephytinized	8.5	3.0	2.2	3.3

[1]Total P and bone-P contents were analyzed, phytate-P contents were estimated from diet formulae, and organic P contents were calculated by difference.

Table 5. Apparent digestibility coefficients (%) of dry matter (DM), crude protein (CP), and ash of the experiment diets (Mean ± SE)[1,2].

Diet No.	Diets	DM	CP	Ash
		%	%	%
1	Reference	75 ± 0.7	92 ± 0.4	42 ± 1.2
2	Fish meal, herring	74 ± 0.5	91 ± 0.3	38 ± 1.0
3	Fish meal, menhaden	71 ± 0.2	90 ± 0.2	35 ± 0.8
4	Meat-bone meal 56% CP	68 ± 0.9	88 ± 0.4	38 ± 0.9
5	Poultry by-product meal (low ash)	75 ± 0.4	92 ± 0.2	43 ± 0.9
6	Poultry by-product meal (regular)	70 ± 1.0	89 ± 0.4	37 ± 0.9
7	Soy protein concentrate	74 ± 0.8	93 ± 0.2	43 ± 1.3
8	Soy protein concentrate, dephytinized	72 ± 0.6	93 ± 0.3	45 ± 0.2

[1]Mean (n= 4 replicates)
[2]CP, crude protein (N x 6.25)

Table 6. Comparison of apparent digestibility (%) of P from experimental observation (n = 4) with model prediction (Mean ± SE)[1].

Diet No.	Diet	Observation	Prediction
1	Reference	53.4 ± 1.4[a]	45.1 ± 1.6[b]
2	Fish meal, herring	53.4 ± 0.8	51.6 ± 1.1
3	Fish meal, menhaden	50.8 ± 0.5	48.1 ± 1.0
4	Meat-bone meal 56% CP	48.2 ± 1.0	46.8 ± 1.0
5	Poultry by-product meal (low ash)	56.8 ± 1.2	51.5 ± 1.0
6	Poultry by-product meal (regular)	52.1 ± 1.3	48.2 ± 0.9
7	Soy protein concentrate	53.3 ± 1.3[a]	46.1 ± 1.2[b]
8	Soy protein concentrate, dephytinized	59.3 ± 1.3	53.9 ± 1.3

[1]Values within row with different subscript letters are significantly different ($p < 0.05$).

Table 7. Comparison of digestible P (g/kg DM) from experimental observation (n = 4) with model prediction (Mean ± SE)[1].

Diet No.	Diet	Observed	Model Prediction
1	Reference	3.9 ± 0.1^{a}	3.3 ± 0.1^{b}
2	Fish meal, herring	6.4 ± 0.1	6.2 ± 0.1
3	Fish meal, menhaden	7.0 ± 0.1	6.6 ± 0.1
4	Meat-bone meal 56% CP	7.0 ± 0.2	6.8 ± 0.1
5	Poultry by-product meal (low ash)	7.2 ± 0.2	6.5 ± 0.1
6	Poultry by-product meal (regular)	7.6 ± 0.2	7.0 ± 0.1
7	Soy protein concentrate	4.6 ± 0.1^{a}	4.0 ± 0.1^{b}
8	Soy protein concentrate, dephytinized	5.1 ± 0.1	4.6 ± 0.1

[1]Values within row with different subscript letters are significantly different ($p < 0.05$).

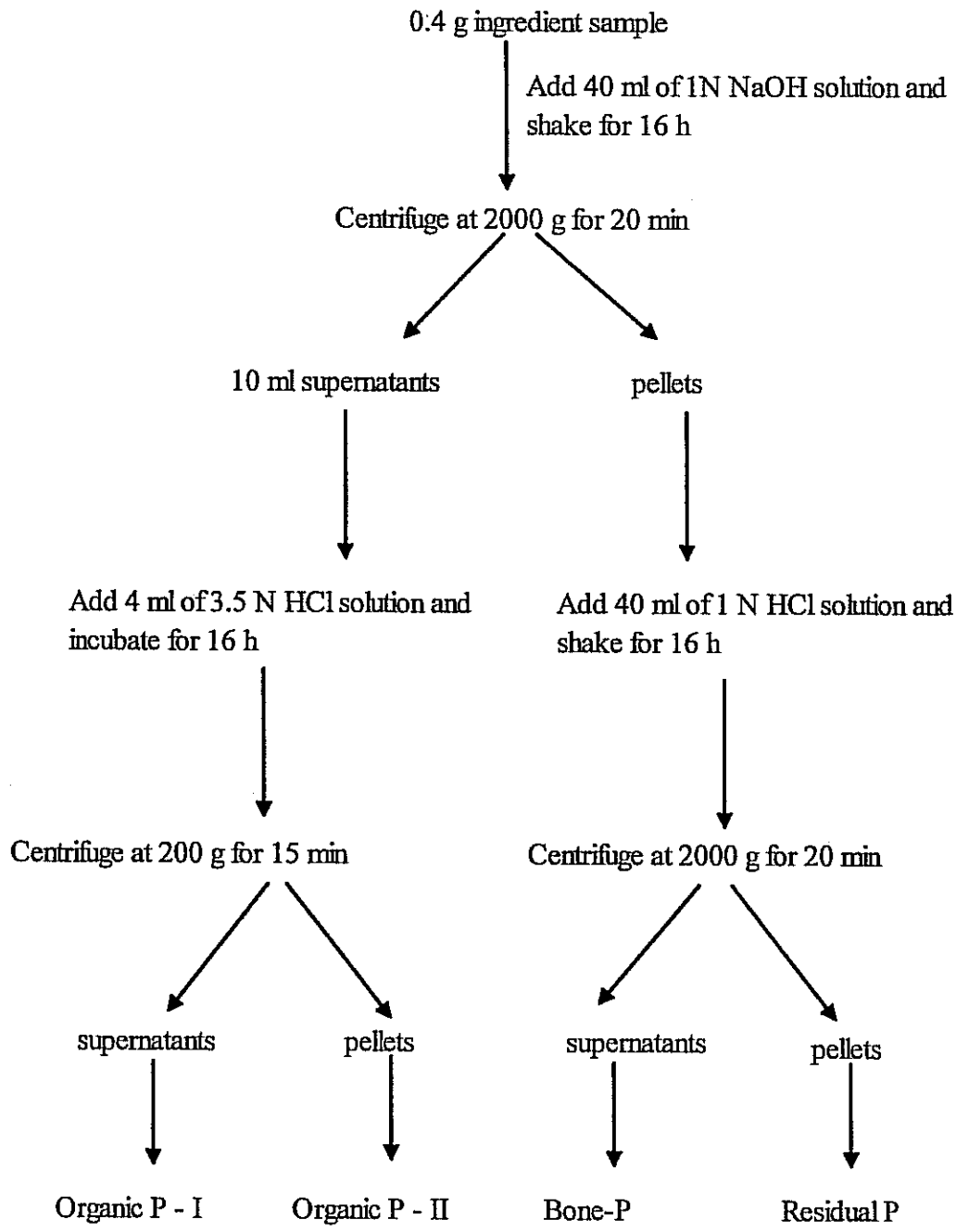

Figure 1. P fractionation protocol.

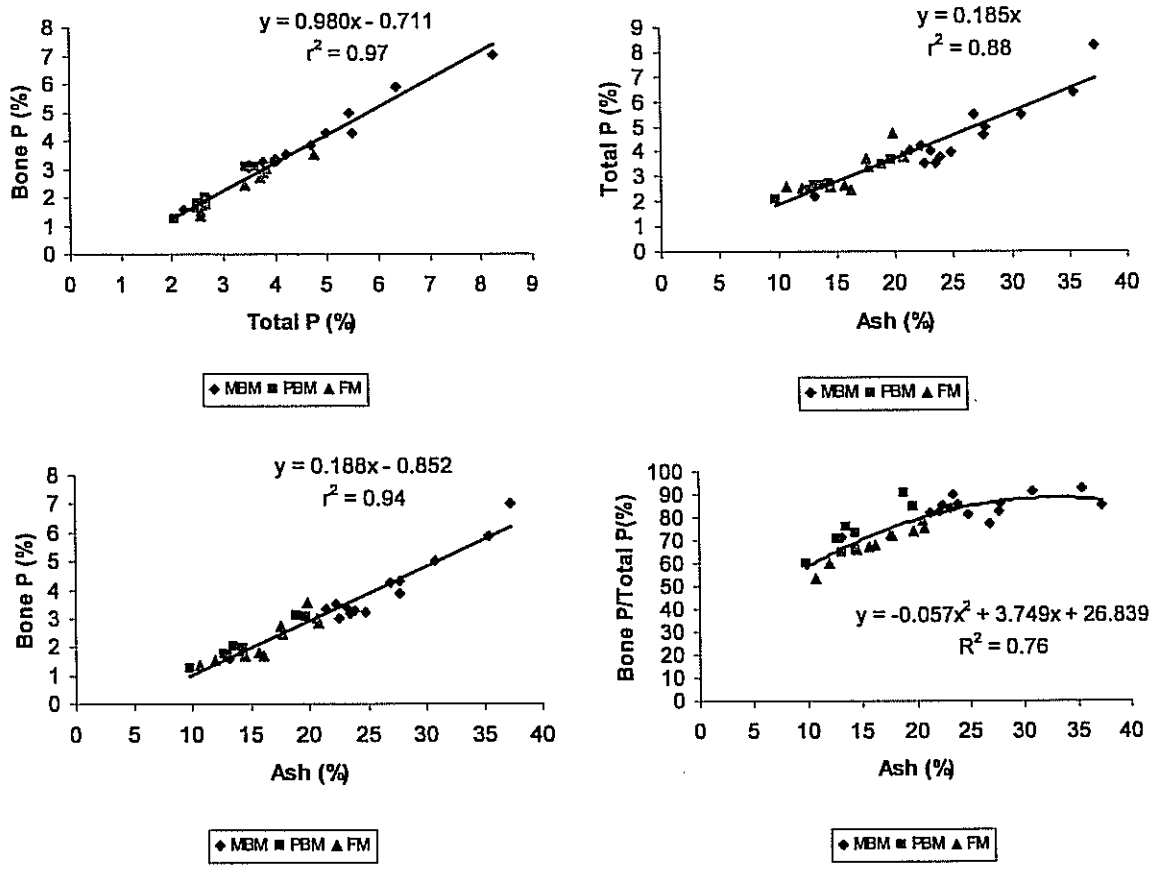

Figure 2. Relationship among bone-P, total P, ash, and bone-P/total P in meat and bone meal (MBM), poultry by-product meal (PBM), fish meal (FM).

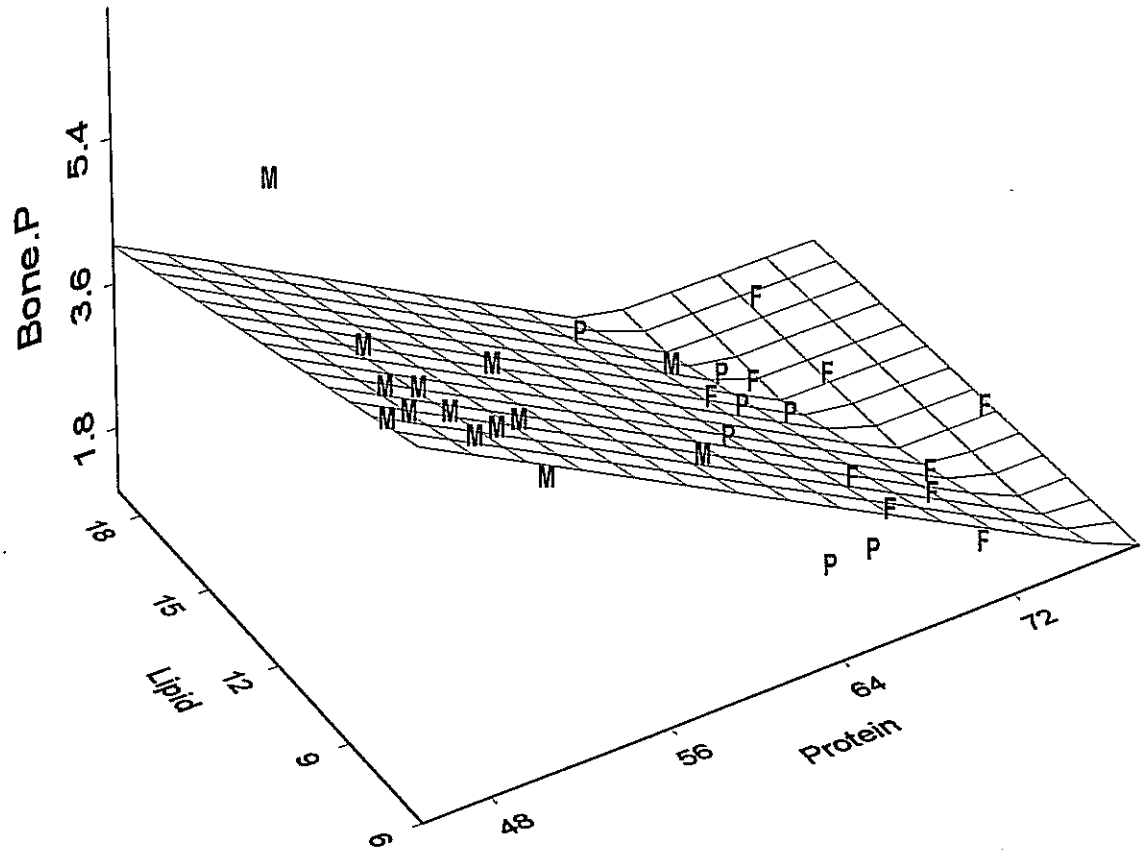

Figure 3. Relationship among bone-P (%), protein (%), and lipid (%) in meat and bone meal (M), poultry by-product meal (P), fish meal (F). The linear relationship was described as bone-P = 13.520 - 0.139 * protein -0.150 * lipid ($R^2 = 0.82$).

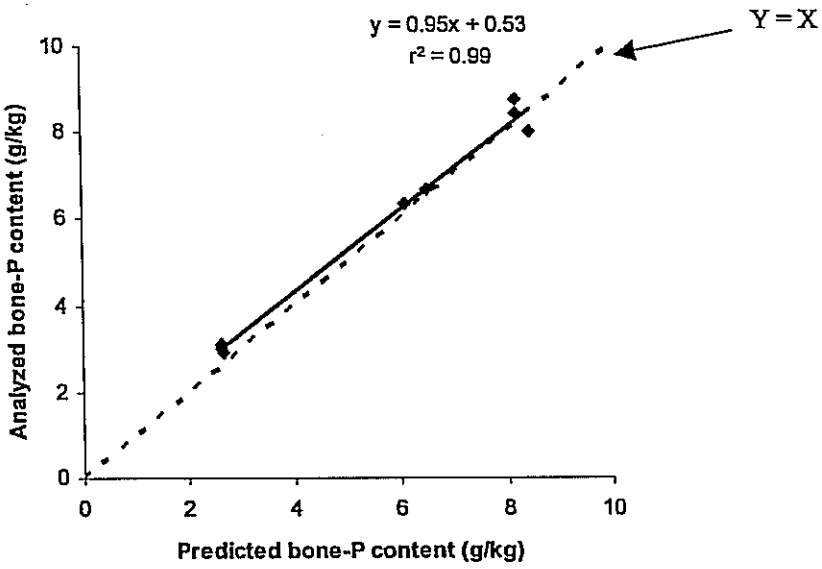

Figure 4. Predicted and analyzed bone-P content of the experiment diets (g/kg DM).

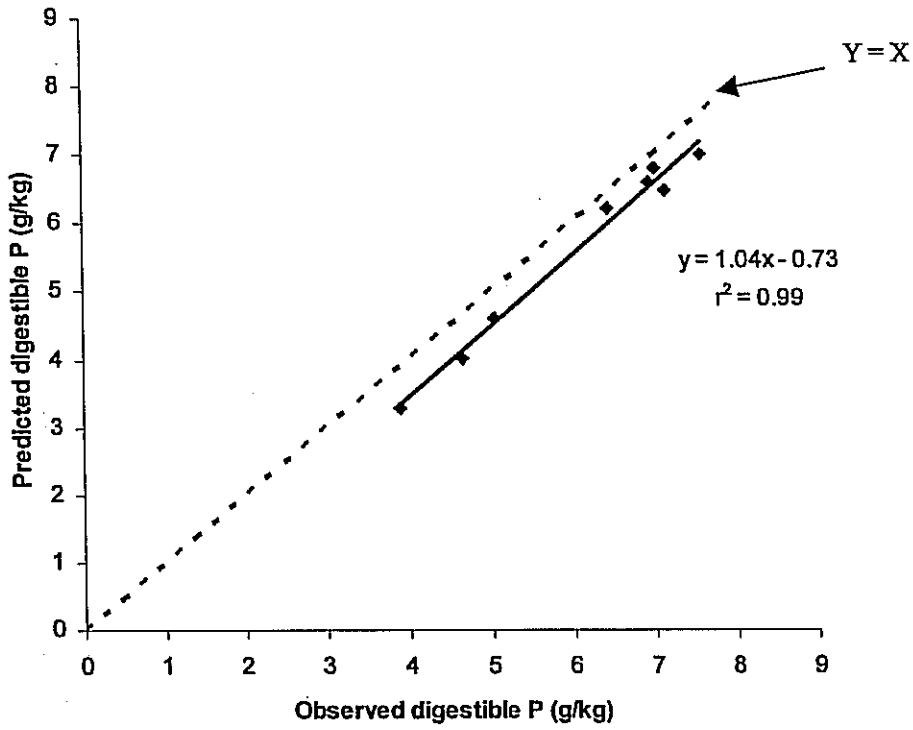

Figure 5. Predicted P and observed digestible P content of the experiment diets (g/kg DM).

Appendix 1. Samples of fish meals (FM), poultry by-products meals (PBM), and meat and bone meals (MBM), and their contents of crude protein (CP), lipid, ash, total P on % as is basis.

Sample	Description	Origin	DM	CP	Lipid	Ash	Total
				(% as is basis)			
Fish meals							
FM – 1	Herring Meal	Canada	90.1	70.5	11.2	9.6	2.3
FM – 2	Herring Meal	Canada	85.4	63.2	9.5	10.2	2.1
FM – 3	Herring Meal	Canada	75.6	54.4	7.7	11.0	2.0
FM – 4	Menhaden meal	USA	93.4	62.3	12.7	16.4	3.5
FM – 5	Mexican sardine meal	Mexico	92.9	63.5	9.8	18.4	4.4
FM – 6	Menhaden meal	USA	91.1	61.9	8.4	18.9	3.5
FM – 7	Ruminant grade Menhaden meal	USA	90.6	62.2	5.5	18.7	3.4
FM – 8	Fair average quality menhaden meal	USA	92.0	62.7	12.9	16.4	3.1
FM – 9	Herring meal	Canada	94.2	69.3	9.4	15.3	2.4
FM – 10	Peruvian fish	Peru	92.0	67.3	8.1	14.5	2.5
Poultry by-products meals							
PBM – 1	Poultry by-product meal	Canada	96.2	65.0	12.9	13.9	2.6
PBM – 2	Poultry by-product meal	USA	93.7	63.9	13.8	11.9	2.3
PBM – 3	Poultry by-product meal air classified	USA	94.1	66.0	15.8	9.2	2.0
PBM – 4	Poultry by-product meal	Canada	98.5	60.5	14.8	18.6	3.3
PBM – 5	Poultry by-product meal low ash	Canada	94.2	64.3	14.0	12.8	2.4
PBM – 6	Poultry by-product meal	USA	93.6	60.5	10.2	18.4	3.4
PBM – 7	Poultry by-product meal	Canada	96.3	69.3	14.3	12.6	2.5
PBM – 8	Poultry by-product meal	Canada	93.9	65.5	8.8	13.5	2.5
Meat and bone meals							
MBM – 1	Meat and bone meal	Canada	95.0	52.1	12.9	21.2	4.0
MBM – 2	Meat and bone meal 61%	USA	96.3	59.5	9.6	21.7	3.4
MBM – 3	Meat and bone meal 53%	USA	96.1	51.9	12.3	26.6	4.5
MBM – 4	Meat and bone meal 45%	USA	95.1	46.6	11.2	33.8	6.0
MBM – 5	Meat and bone meal 56%	USA	96.5	55.0	12.3	22.7	3.4
MBM – 6	Meat and bone meal	Canada	90.5	51.6	12.9	20.9	3.6
MBM – 7	Meat and bone meal	Canada	94.5	48.1	12.1	26.3	4.7
MBM – 8	Meat and bone meal	Canada	95.2	52.6	11.9	23.7	3.8
MBM – 9	Meat and bone meal 43%	USA	96.0	43.9	11.6	35.8	8.0
MBM – 10	Meat and bone meal	Canada	95.6	47.4	11.3	25.7	5.3
MBM –11	Meat and bone meal low ash	Canada	95.0	56.8	18.7	12.5	2.1
MBM – 12	Meat and bone meal	Canada	94.3	47.6	11.3	29.0	5.1
MBM – 13	Meat and bone meal	Canada	92.2	51.3	9.9	21.9	3.5
MBM – 14	Meat and bone meal	USA	95.2	60.6	11.7	20.4	3.8