MEASUREMENT OF TRUE ILEAL PHOSPHORUS DIGESTIBILITY IN FEED INGREDIENTS FOR POULTRY

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This Thesis is Dedicated to My Father (Mr. U.I. Mutucumarana), Mother (Late Mrs. A.L.Y. De Silva), and All the Teachers Who Led the Way and Expected Me to Achieve Such a Goal One Day……..!!!

Abstract

Global interest in improving the utilisation of phosphorus (P) by poultry has recently increased due to concerns over environmental pollution through excess P excretion, depletion of non-renewable inorganic phosphate deposits, and increasing price of inorganic phosphate supplements. Use of a sound criterion, preferably based on P digestibility, to assess P availability is needed to enable greater efficiency of utilisation of dietary P. No established methodology is currently available to measure the true digestible P contents in common feed ingredients for poultry.

The first experiment of this thesis (Chapter 3) investigated the effects of dietary calcium (Ca) concentrations (6, 9 and 12 g/kg) on the digestibility of P, Ca, nitrogen, fat and starch in different intestinal segments and on the apparent metabolisable energy of diets in young broiler chickens. The results showed that the digestion of P and Ca was completed by upper ileum and jejunum, respectively. The site of digestion of P and nitrogen was found to shift depending on the dietary Ca concentrations. The digestibility coefficients of P in low, normal and high Ca diets at the lower ileum were determined to be 0.417, 0.379 and 0.325, respectively. The overall data showed that increasing dietary Ca concentrations negatively influenced the digestion of P, nitrogen and fat, but had no effect on those of Ca, starch and apparent metabolisable energy.

The second experiment (Chapter 4) was conducted to determine endogenous losses of P and Ca in broiler chickens. The data showed that the ileal endogenous P losses in birds differed depending on the methodology employed. The ileal endogenous flow of P in birds fed P-free, gelatin-based and casein-based diets were 25, 104 and 438 mg/kg dry matter intake (DMI), respectively. Ileal endogenous flow of Ca in birds fed casein-based diet was estimated to be 321 mg/kg DMI.

The next three experiments (Chapters 5, 6 and 7) investigated the potential usefulness of regression method to evaluate true ileal P digestibility of seven feed ingredients. True ileal P digestibility coefficients of maize, canola meal, wheat, sorghum, soybean meal and maize-distiller's dried grains with solubles for broiler chickens were determined to be 0.676, 0.469, 0.464, 0.331, 0.798 and 0.727, respectively. For plant-based ingredients, the determined true digestible P values were consistently higher than corresponding non-phytate P values (Maize, 1.72 vs. 0.75; canola meal, 4.55 vs. 2.82; wheat, 1.49 vs. 1.11; sorghum, 0.78 vs. 0.55; soybean meal, 5.16 vs. 2.15; maize-distiller's dried grains with solubles, 5.94 vs. 4.36 g/kg, as fed

basis, respectively). Phytate P in maize (54.25%), soybean meal (69.7%) and maize-distiller's dried grains with solubles (41.5%) were well digested by broilers compared to canola meal (25.2%), wheat (18.1%) and sorghum (13.0%). True ileal P digestibility coefficients of three meat and bone meal (MBM) samples ranged from 0.420 to 0.693. Total and true digestible P contents of three MBM samples (MBM-1, MBM-2 and MBM-3) were determined to be 37.5 and 26.0; 60.2 and 36.6; and 59.8 and 25.1 g/kg, as fed basis, respectively, suggesting that P in MBM is not highly digestible. The overall data suggested that the use of regression approach to estimate true ileal P digestibility in feed ingredients has number of limitations. Overestimation as a result of using Ca- and P-deficient diets and the negative endogenous P losses observed for some ingredients (canola meal, sorghum and MBM-3) were main concerns. Negative ileal endogenous P losses were also shown to be associated with low true ileal P digestibility in these ingredients.

In the final experiment (Chapter 8), two regression-based methodologies were compared for the measurement of true ileal P digestibility in maize and soybean meal. The results showed that the methodology influenced P digestibility in maize and soybean meal. The use of assay diets containing a narrow Ca:total P ratio yielded higher P digestibility for both ingredients.

In this thesis research, the regression method was used to determine true ileal P digestibility of ingredients, but this approach suffers from several drawbacks. The data reported in this thesis also demonstrated that high dietary Ca concentrations were detrimental to the digestibility of nutrients, particularly of P, nitrogen and fat.

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Publications

Studies completed during candidature, some of which are reported in this thesis, have been presented in the following communications:

Refereed scientific papers:

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Mutucumarana R.K., Ravindran V., Ravindran G., Cowieson A.J. (2013) Measurement of phosphorus digestibility in maize and canola meal for broiler chickens, in: J. Roberts (Ed.), Proceedings of the Australian Poultry Science Symposium, Sydney, New South Wales, Australia. pp. 56-59.

Mutucumarana R.K., Ravindran V., Ravindran G., Cowieson A.J. (2012) Influence of dietary calcium concentrations on the digestion and absorption of calcium and phosphorus along the intestinal tract of broiler chickens. Poster session presented at the meeting of 46th Annual Conference of the Nutrition Society of New Zealand. Auckland, NZ.

Mutucumarana R.K., Ravindran V., Ravindran G., Cowieson A.J. (2012) Absorption of calcium and phosphorus along the small intestine of broiler chickens. Proceedings of the Advancing Poultry Production Massey Technical Update Conference, Monogastric Research Centre, Massey University, Palmerston North, New Zealand. pp. 52-59.

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List of Abbreviations

%	Percent
˚C	Degree Celsius
$1,25(OH)_2D_3$	1,25-dihydroxyvitamin D_3/calcitrol
$25\text{-}(OH)_2D_3$	25-hydroxycholecalciferol
^{91}Y	Yttrium
AAFCO	Association of American Feed Control Officials
ADP	Adenosine di-phosphate
AIDC	Apparent ileal digestibility coefficient
AME	Apparent metabolisable energy
AMP	Adenosine mono-phosphate
ANOVA	Analysis of variance
AOAC	Association of analytical chemists
ATP	Adenosine tri-phosphate
ATTRC	Apparent total tract retention coefficient
BW	Body weight
BWG	Body weight gain
Ca/Ca^{2+}	Calcium/Calcium ion
$Ca_{10}(PO_4)_6(OH)_2$	Calcium hydroxyapatites
$Ca_3(PO_4)_2$	Calcium orthophosphate
$CaCo_3$	Calcium carbonate
c-GMP	Cyclic guanine mono-phosphate
Co_3^{2-}	Carbonate ion
Corp.,	Corporation
CP	Crude protein
DL	Dextrorotatory and levorotatory
DDGS	Distiller’s dried grains with solubles

df	Degree of freedom
dl	Decilitre
DM	Dry matter
DMI	Dry matter intake
DNA	Deoxy-ribonucleic acid
EPL	Endogenous phosphorus loss
FGF	Fibroblast growth factor
FI	Feed intake
g	Gram
g/b/d	Grams per bird per day
GE	Gross energy
GLM	General linear model
GMD	Geometric mean diameter
GSD	Geometric standard deviation
h	Hours
h^2	Heritability
$H_2PO_4^-$	Dihydrogen phosphate ion
HCl	Hydrochloric acid
HDL	High density lipoproteins
HPO_4^{-2}	Hydrogen phosphate ion
IdP	Ileal digestible phosphorus
IP_6	Phytate/Myo-inositol 1,2,3,4,5,6-hexakis dihydrogen phosphate
IU	International units
K^+	Potassium ion
kg	Kilogram
KH_2PO_4	Potassium dihydrogen phosphate

L-tryptophan	Levorotatory form of tryptophan
LDL	Low density lipoproteins
M	Molar
MBM	Meat and bone meal
mg	Milligram
min	minute
MJ	Mega joule
mm	Millimetre
mM	millimolar
mRNA	Messenger ribonucleic acid
N	Nitrogen
Na/Na^+	Sodium/Sodium ion
Na_2HPO_4	Disodium hydrogen phosphate
nm	Nano metre
NPP	Non-phytate phosphorus
NRC	National Research Council
NS	Not significant
P	Phosphorus
P	*P*-value
P_D	Ileal phosphorus output
P_E	Excreta phosphorus output
P_I	Dietary phosphorus content
P_i	Inorganic phosphorus
PO-DMI	Total output of phosphorus per dry matter intake
PTH	Parathyroid hormone
Q	Quadratic
r^2	Coefficient of determination, simple

RNA	Ribonucleic acid
SAS	Statistical analysis software
SD	Standard deviation
SE	Standard error
SEM	Standard error of mean
SLC	Solute carrier family
Ti	Titanium
TPI	True phosphorus indigestibility
TPUC	True phosphorus utilisation coefficient
U	Phytase units
UV	Ultraviolet
VLDL	Very low density lipoproteins
WPSA	World Poultry Science Association
α	Alpha
µ	Microns
µmoles	Micromoles