MEASUREMENT OF TRUE ILEAL CALCIUM DIGESTIBILITY OF FEED INGREDIENTS FOR BROILER CHICKENS

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ABSTRACT

The recent interest towards the use of digestible phosphorus (P) in poultry feed formulations necessitates the measurement of true calcium (Ca) digestibility of feed ingredients because of the close relationship between these two minerals for their absorption and post absorptive utilisation. When this thesis research was initiated, no published data were available on Ca digestibility of feed ingredients for broiler chickens. The major objective of the studies reported in this thesis was to determine the true Ca digestibility of feed ingredients for broiler chickens. In total, nine studies were conducted.

The first study (Chapter 4) was conducted to determine the effect of methodology on ileal endogenous Ca losses. Three methods, namely feeding a Ca- and P-free diet, maize gluten meal based diet and egg albumen based diet, were used. Ileal endogenous Ca losses differed among different methodologies. The highest ileal endogenous losses of 125 mg/kg dry matter intake (DMI) were recorded on the Ca- and P-free diet, followed by 77 and 43 mg/kg DMI on maize gluten meal and egg albumen diets, respectively.

In the second and third studies (Chapters 5 and 6), regression and direct methods, respectively, were used to determine the true Ca digestibility of meat and bone meal (MBM). The true Ca digestibility coefficient of MBM samples were ranged from 0.41 to 0.60. No difference was observed between true Ca digestibility coefficients of MBM determined by regression and direct methods. Since the direct method is less laborious and cost effective compared to regression method, this method was used in subsequent studies (Chapters 7 to 10) to determine the true Ca digestibility of a range of Ca sources.

In fourth and fifth studies (Chapters 7 and 8), the influence of dietary P, particle size and Ca to non-phytate P ratio was investigated on the true Ca digestibility of limestone for broiler chickens. The true Ca digestibility of three limestone samples varied from 0.56 to 0.62. Supplementation with recommended dietary P (4.5 g/kg) increased the true Ca digestibility of limestone when compared to diets without P. An increase in particle size from <0.5 to 1-2mm improved the true ileal Ca digestibility of

limestone. Widening the Ca to non-phytate P ratio reduced the true Ca digestibility of limestone for broiler chickens.

The sixth study (Chapter 9) was conducted to determine the effect of Ca source and particle size on the true Ca digestibility and total tract retention. Limestone and oyster shell were used as Ca sources. No difference was observed between the true Ca digestibility of limestone and oyster shell. An increase in particle size from <0.5 to 1-2 mm increased both the Ca digestibility and retention of both Ca sources, and increased the Ca concentration of gizzard contents.

The study reported in Chapter 10 was conducted to determine the true Ca digestibility of dicalcium phosphate (DCP), monocalcium phosphate (MCP), canola meal, poultry by-product meal and fish meal, and to compare the effect of dietary adaptation length on true Ca digestibility of DCP and MCP. The true Ca digestibility coefficients of these feed ingredients were lower than MBM, limestone and oyster shell, and ranged from 0.24 to 0.33. It was speculated that the length of adaption to the assay diets may be responsible for the lower than expected estimates. The effect of dietary adaptation length (24, 48 or 72 hrs) was subsequently examined, but had no effect on true Ca digestibility of DCP and MCP.

In the final study (Chapter 11), the true Ca digestibility of DCP was determined using different methodologies (regression, difference and direct methods). The true Ca digestibility coefficients of DCP were 0.34 and 0.21 with direct and different methods, respectively. A very low digestibility coefficient of 0.13 was determined by the regression method.

In conclusion, the true Ca digestibility coefficient of major Ca sources (limestone, oyster shell and MBM) is not high and varied from 0.40 to 0.70. Particle size of limestone and oyster shell influenced Ca digestibility, with coarser particles having higher digestibility. The direct method appears to be suitable for the determination of true Ca digestibility of limestone, oyster shell and MBM, but may not be appropriate for other Ca sources with intrinsic imbalance of Ca and P.

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Publications

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

Referred scientific papers

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Anwar, M. N., Ravindran, V., Morel, P. C. H., Ravindran, G. and Cowieson, A. J. (2016). Effect of limestone particle size and calcium to non-phytate phosphorus ratio on true ileal calcium digestibility of limestone for broiler chickens. *British Poultry Science*, **57**:707-713.

Anwar, M. N., Ravindran, V., Morel, P. C. H., Ravindran, G. and Cowieson, A. J. (2016). Apparent ileal digestibility of calcium in limestone for broiler chickens. *Animal Feed Science and Technology*, **213**:142-147.

Anwar, M. N., Ravindran, V., Morel, P. C. H., Ravindran, G. and Cowieson, A. J. (2016). Measurement of true ileal calcium digestibility in meat and bone meal for broiler chickens using direct method. *Poultry Science*, **95**:70-76.

Anwar, M. N., Ravindran, V., Morel, P. C. H., Ravindran, G. and Cowieson, A. J. (2015). Measurement of true ileal calcium digestibility in meat and bone meal for broiler chickens. *Animal Feed Science and Technology*, **206**:100-107.

Conference proceedings

Anwar, M. N., Ravindran, V., Morel, P. C. H., Ravindran, G. and Cowieson, A. J. (2016). Effect of particle size and calcium to non-phytate phosphorus ratio on true calcium digestibility of limestone for broiler chickens. *The Proceedings of 25th World Poultry Congress.* pp. 17 (Abstract). Beijing, China.

Anwar, M. N., Ravindran, V., Morel, P. C. H., Ravindran, G. and Cowieson, A. J. (2016). Digestible calcium in feedstuffs: Methodology and challenges. In M. R. Abdollahi and V. Ravindran (Eds.) *Proceedings of the Massey Technical Update Conference.* Vol. 18, pp. 73-80. Monogastric Research Centre, Massey University, Palmerston North, New Zealand.

Anwar, M. N., Ravindran, V., Morel, P. C. H., Ravindran, G. and Cowieson, A. J. (2015). Measurement of true ileal calcium digestibility of limestone for broiler chickens. *20th European Symposium on Poultry Nutrition*, pp. 210 (Abstract). Prague, Czech Republic.

Anwar, M. N., Ravindran, V., Morel, P. C. H., Ravindran, G. and Cowieson, A. J. (2015). Measurement of true ileal calcium digestibility of meat and bone meal for broiler chickens using the direct method. *Poultry Science*, **94** (E-Suppl.1), pp. 157 (Abstract). PSA Annual Meeting, Kentucky, USA.

Anwar, M. N., Ravindran, V., Morel, P. C. H., Ravindran, G. and Cowieson, A. J. (2015). Measurement of true ileal calcium digestibility in meat and bone meal and limestone for broiler chickens. In V. Ravindran (Ed.) *Proceedings of the Massey Technical Update Conference.* Vol. 17, pp. 96-106. Monogastric Research Centre, Massey University, Palmerston North, New Zealand.

Anwar, M. N., Ravindran, V., Morel, P. C. H., Ravindran, G. and Cowieson, A. J. (2015). Measurement of true ileal calcium digestibility of meat and bone meal for broiler chickens. *Proceedings of the Australian Poultry Science Symposium.* Vol. 26, pp. 137. Sydney, New South Wales, Australia.

Anwar, M. N., Ravindran, V., Morel, P. C. H., Ravindran, G. and Cowieson, A. J. (2013). Determination of digestibility and availability of calcium from different sources in broiler chickens. In V. Ravindran (Ed.) *Proceedings of the Massey Technical Update Conference.* Vol. 15, pp. 100-104. Monogastric Research Centre, Massey University, Palmerston North, New Zealand.

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List of Abbreviations

%	Percent
µg	Micro gram
µl	Micro litre
1, 25-$(OH)_2D_3$	1, 25-dihyroxycholicalciferol
AAFCO	Association of American Feed Control Officials
AIDC	Apparent ileal digestibility coefficient
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
Ca	Calcium
Ca_E	Calcium in excreta
Ca_I	Calcium in diet
Ca_O	Calcium in digesta
CM	Canola meal
CO_2	Carbon dioxide
CP	Crude protein
CT	Calcitonin
Cu	Copper
DCP	Dicalcium phosphate
DM	Dry matter
DMI	Dry matter intake
Fe	Iron
FM	Fish meal
g	Gram
g/b/d	Gram per bird per day
GLM	General linear model
GMD	Geometric mean diameter
GSD	Geometric standard deviation

H_2O	Water
HCl	Hydrochloric acid
I	Iodine
IECaL	Ileal endogenous calcium losses
IU	International unit
kg	Kilogram
M	Molar
MBM	Meat and bone meal
MCP	Monocalcium phosphate
mg	Milligram
MJ	Mega joule
mm	Millimetre
Mn	Manganese
Mo	Molybdenum
N	Nitrogen
N_2	Nitrogen gas
NRC	National Research Council
NS	Non-significant
O_2	Oxygen
ºC	Degree centigrade
P	Phosphorus
P	Probability
PBPM	Poultry by-product meal
PTH	Parathyroid hormone
SAS	Statistical analysis software
SE	Standard error
Se	Selenium
SEM	Standard error of mean
Ti	Titanium dioxide

TIDC	True ileal digestibility coefficient
Ti_E	Titanium dioxide in excreta
Ti_I	Titanium dioxide in diet
Ti_O	Titanium dioxide in digesta
UV	Ultra violet
WPSA	World's Poultry Science Association
Zn	Zinc