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FACTORS INFLUENCING FAT DIGESTION IN POULTRY

A thesis presented in partial fulfilment of the requirements for the degree of

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Abstract

The first experiment in this thesis was conducted to understand the digestion of fat along the gastrointestinal tract and ileal endogenous fat losses. The second, third and fourth experiments investigated the factors influencing fat digestion in broilers, including age of birds, cereal type, fat source and dietary calcium concentrations. In the fifth experiment, influence of unsaturated to saturated fatty acid ratio (U:S ratio) on fat digestion was examined.

The first experiment reported in Chapter 4 showed that jejunum is the major intestinal site where majority of fat and fatty acids is digested and absorbed. Long chain fatty acids showed lower digestibility than short chain fatty acids, and unsaturated fatty acids were better digested than saturated fatty acids. The fatty acid profile of ileal endogenous fat was remarkably similar to that of the bile, suggesting that the reabsorption of fat and fatty acids in bile was incomplete.

Data reported in Chapter 5 showed that the apparent metabolisable energy (AME) and total tract digestibility of fats was influenced by the age of broilers. The AME of fat was markedly lower during the first week, increased rapidly during second week and then remained constant thereafter. Total tract fat digestibility was poor during the first week and then increased until the third week of age. No further improvement was observed after the third week. The AME and fat digestibility of soybean oil, poultry fat and palm oil were determined to be higher than those of tallow. Blending of tallow and soybean oil resulted in AME and fat digestibility estimates higher than the arithmetic averages of tallow and soybean oil.

The study reported in Chapter 6 showed that the supplementation of tallow in wheat- and maize-based diets resulted in lower weight gain than that of soybean oil, but fat source had no effect on the weight gain of broilers fed sorghum-based diets. Broilers fed soybean oil supplemented diets had lower feed per gain, higher total tract retention and ileal digestibility of fat compared to those fed tallow supplemented diets. In addition, supplementation of xylanase in wheat-based diets resulted in improved weight gain and feed efficiency of broiler starters irrespective of the fat source. Xylanase supplementation increased the AME of tallow supplemented diets, but had no effect on soybean oil supplemented diets.

Data reported in Chapter 7 indicated that high dietary calcium concentrations had negative impact on broiler performance, irrespective of tallow inclusion levels. High calcium concentrations resulted in higher excreta soap and, lowered the total tract retention of fat, calcium and phosphorus. Lower calcium concentrations resulted in higher ileal digestibility of fat, nitrogen and phosphorus.

Data from the final experiment (Chapter 8) showed that the U:S ratio influenced the performance of broilers during the starter period (1 to 21 day), but had no effect on the performance over the whole trial period (1 to 35 day). Increasing the U:S ratio decreased the AME of diet and increased the total tract retention of fat. A positive linear correlation between U:S ratio and the AME of fat blends was observed, with increasing U:S ratios improving the AME of fat blends.

In conclusion, the research reported in this thesis identified several factors that influence the digestion of fat in poultry. Age of broilers influenced the digestion and absorption of fat, particularly during the first week of age. The findings consistently demonstrated that tallow was more poorly digested than soybean oil. The utilisation of these two fat sources was influenced by the cereal base used in the diets, with the effect of fat source on weight gain differing between cereal types. High dietary calcium concentrations were detrimental to the digestibility of nutrients, especially of fat. A finding of practical interest was that digestion and absorption of animal fats, containing high concentrations of saturated fatty acids, can be improved by blending with soybean oil to increase the U:S ratio.

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- Tancharoenrat, P., Zaefarian, F., Ravindran, G., A.L. Molan and Ravindran, V. (2012). Calcium x fat interactions in broiler diets. Proceedings of the Massey Technical Update Conference, Volume 14, Pages 70-77. Monogastric Research Centre, Massey University, Palmerston North, New Zealand.
- Tancharoenrat, P., Zaefarian, F., Ravindran, G., A.L. Molan and Ravindran, V. (2011). Digestion of fat and fatty acids along the digestive tract of chickens. Proceedings of the Poultry Science Association 100th Annual Meeting, Volume 90, Pages 161-162. St.Louis, Missouri, USA.
- Tancharoenrat, P., Zaefarian, F., Ravindran, G.and Ravindran, V. (2011). Cereal type x fat source interactions in broiler diets. Proceedings of the Massey Technical Update Conference, Volume 13, Pages 93-99. Monogastric Research Centre, Massey University, Palmerston North, New Zealand.
- Tancharoenrat, P., Zaefarian, F., Ravindran, G.and Ravindran, V. (2010). Energy utilisation and digestibility of fats as influenced by age of broilers. Proceedings of the Australian Poultry Science Symposium 21: 59. Sydney, Australia.
- Tancharoenrat, P., Zaefarian, F., Ravindran, G.and Ravindran, V. (2010). Interactions between cereal type and fat type in broiler diets. Proceedings of the Massey Technical Update Conference, Volume 12, Pages 96-102. Monogastric Research Centre, Massey University, Palmerston North, New Zealand.
- Ravindran, V., Zaefarian, F. and Tancharoenrat, P (2010). Digestion of lipids in poultry: limitations and opportunities. Proceedings of 4th International Broiler Nutritionists' Conference, Poultry Beyond 2015, Pages 27-45. Convention Centre, Christchurch, New Zealand.
- Tancharoenrat, P., Zaefarian, F., Ravindran, G.and Ravindran, V. (2009). Energy utilisation of fats as influenced by age of broilers. Proceedings of the International Conference on the Role of Universities in Hands-On Education, Rajamankala University of Technology Lanna, Chiangmai, Thailand.
- Tancharoenrat, P., Zaefarian, F., Ravindran, G.and Ravindran, V. (2009). Energy utilisation of fats as influenced by age of broilers. Proceedings of the Massey Technical Update Conference, Volume 11, Pages 67-72. Monogastric Research Centre, Massey University, Palmerston North, New Zealand.

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List of Abbreviations

AME Apparent metabolisable energy

AMEn Nitrogen-corrected apparent metabolisable energy

ANOVA Analysis of variance

°C Degree Celsius

Ca Calcium

DM Dry matter

FFA Free fatty acid

g Gram

GE Gross energy

h Hours

K PotassiumKg Kilogram

meq Milliequivalent

mg Milligram
MJ Mega joule
ml Millilitre
mm Millimetre
N Nitrogen

Na Sodium

ng Nanogram

NRC National research council

NSP Non-starch polysaccharide

P Phosphorus

S Saturated
Ti Titanium

TME True metabolisable energy

U Unsaturated

μl Microlitre