Title of Doctoral Dissertation:

"Determination of prebiotic properties of whey lactose in broiler chicken diets"

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Abstract

Birds are unable to digest milk sugar lactose to any appreciable extent and the hydrolisation of this disaccharide that occurs in poultry is the result of the hindgut microflora activity. Some evidence exists within the literature demonstrating that lactose may promote the growth of lactose-utilizing bacteria, and that its protective action against different enteropathogenic colonisation can be assigned primarily to the acidic pH of the intestinal digesta resulting from fermentative conversion of lactose to short-chain organic acids. The research trials looking at the growth performance of broiler chickens fed diets supplemented with dried whey as a source of lactose are relatively scarce and have produced inconclusive results. In particular, there is insufficient knowledge concerning the dose-related efficacy of whey lactose as a feed additive for broilers grown to market age. There are also no clear data regarding dose-dependent side effects of dietary lactose, *e.g.*, increased free-water content of the excreta, which may have relevance to the use of lactose in chickens fed under field conditions.

With the aim to verify the hypotheses that: when included in moderate amounts in the diet lactose sourced from dried whey (LAC) will exert prebiotic effects which may be finally reflected in improvements in growth and slaughter performances, and that co-supplementation of whey lactose with bacteria of the genus *Lactobacillus* will amplify the potential beneficial effects of lactose presence in the feed, two consecutive experiments were conducted using Ross 308 broiler chickens. In both experiments, the excreta of chickens were visually evaluated for consistency according to a 5-point scale, where 1 was faecal material that was dry and well-formed and 5 was material that was very loose or liquid.

The objective of Experiment 1 was to determine the effect of dietary LAC at levels of 1, 2 and 3% fed from 8 to 21 or to 42 days of age on production performances, and selected indices of the caecal micro-environment and gross morphology in birds reared in wire-floor battery cages. Results indicated that only long-term feeding of 1%- and 2% LAC-diets resulted in higher body weight gains (BWG) and lower feed-to-gain ratios than those observed on the non-LAC control diet, with a clear tendency toward a larger share of breast muscle in carcass at the 2% LAC level. On the other hand, a provision of LAC at the 3% dietary level reduced BWG of chickens and decreased their feed efficiency during the grower phase and the whole period of rearing, compared with 1% and 2% LAC treatments but not with the control one. Increasing levels of dietary LAC produced gradual increases in the relative empty caecal weight and length, and this effect was accompanied by greater faecal score values indicating more loose excreta, especially in the case of long-term feeding with 3% LAC diet. Reduction in the pH of the caecal contents was confirmed at day 21 for birds fed the 1% and 2% dietary LAC. The lower caecal pH values were correlated to an increased overall sum of total volatile fatty acids (VFA) determined at this time point, and caused great increases in the concentration of undissociated forms of acetic, propionic and butyric acids. Feeding whey lactose at the 2% and 3% dietary doses significantly reduced plate counts of members of the coliform bacterial group, whereas the viable counts of lactic acid producing bacteria (LAB) were found to be significantly higher at these two dietary levels of LAC. Under the conditions of this experiment, the 2% dietary level of whey lactose has been recognized as having the ability to most beneficially alter production performances of broilers, with a relatively moderate occurrence of undesirable side effects (loose/wet droppings, enlarged caeca).

In Experiment 2, a 42-day growth assay utilizing chickens kept in a litter-floor pens was used to determine the effects of LAC fed at the 2% dietary inclusion level alone or in combination with *Lactobacillus agilis* bacteria (90 million viable cells/kg) on productive performance parameters, VFA concentrations and counts of several bacterial groups in the caeca determined by fluorescent *in-situ* hybridisation (FISH), lumen pH and gross morphometry of some digestive tract segments, thigh bone (*os femoris*) characteristics, and blood serum biochemistry. The influence of 2% whey lactose supplementation on the observed patterns in caecal VFA and pH changes was generally similar to that found in Experiment 1. The pH of crop and ileal contents was significantly reduced by the inclusion of LAC in the diet. The relative weights of empty caeca were increased, and the excreta of chickens fed supplemental LAC in this experiment contained more moisture. Femur relative

breaking strength, and ash content increased by the presence of LAC in the diet. The overall effect of LAC supplementation on the biochemical blood characteristics was statistically insignificant. Data obtained by the FISH technique revealed that counts of *Bacteroides sp./Prevotella sp.* group were higher after the addition of whey lactose. Compared with the control, all three dietary supplementations, *i.e.* with LAC, *L. agilis*, and with their combination, significantly reduced total counts of the family *Enterobacteriaceae* and decreased numbers of naturally occurring *Clostridium perfringens* bacteria. Overall, the additive effects of the simultaneous supplementation of LAC and *L. agilis* were found on the butyrate concentration and on *C. perfringens*, and *Enterobacteriaceae* counts in the caeca. No improvements in BWG and post-slaughter endpoints were observed due to uncombined and combined supplementation of 2% LAC and *L. agilis*, indicating that the shifts in the composition of caecal microbiota toward a healthier composition by using these additives were not large enough to create positive performance responses in broilers kept for 42 days on a litter floor.

In conclusion, significant differences in VFA concentrations in the caeca together with the decrease in the populations of undesirable and pathogenic bacteria determined in material obtained under different housing conditions are valuable indicators pointing to prebiotic properties of whey lactose included in broiler diets. However, further supplementation studies utilizing varying amounts of whey lactose and *L. agilis* bacteria are needed to clarify the existence of dose-dependent effects of feeding with these additives on the growth performance of chickens maintained in a litter-floor environment.

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