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Apparent ileal digestibility of calcium in limestone for broiler chickens

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ABSTRACT

The objective of the study that is presented herein was to determine the apparent ileal calcium (Ca) digestibility of limestone for broiler chickens as influenced by limestone source and dietary phosphorus (P) concentration. Limestone from three commercial sources (coded as LM-1, LM-2 and LM-3) were obtained, ground to pass through a 0.2 mm sieve and analysed for mineral composition and *in vitro* solubility. Analysed Ca concentration and *in vitro* solubility coefficient of LM-1, LM-2 and LM-3 were 410, 390 and 420 g/kg, and 0.28, 0.29 and 0.27, respectively. Two experimental diets, containing 9 g/kg Ca, were developed from each limestone source with 0 and 4.5 g/kg dietary P. Titanium dioxide was incorporated as an indigestible marker. Each experimental diet was then randomly allotted to six replicate cages (eight birds per cage) and fed from day 21 to 24 post-hatch. Apparent ileal digestibility of Ca was calculated using the indicator method. Apparent ileal Ca digestibility coefficients of LM-1, LM-2 and LM-3 were determined to be 0.58, 0.61 and 0.54, respectively. Calcium digestibility of LM-3 was lower ($P < 0.05$) than LM-2 but similar ($P > 0.05$) to LM-1. There was no difference ($P > 0.05$) between the Ca digestibility of LM-1 and LM-2. Increasing the dietary P concentration from 0 to 4.5 g/kg in assay diets increased ($P < 0.05$) the average Ca digestibility from 0.56 to 0.60.

1. Introduction

Limestone is the major inorganic calcium (Ca) source in poultry diets and, on average, contains 380 g/kg Ca (NRC, 1994). However, Ca concentration of limestone varies from sample to sample depending upon the concentration of other minerals. Determination of Ca digestibility has not been considered in the past due to the low cost, abundant availability and surplus global reserves of limestone. The recent initiative towards the use of digestible phosphorus (P) values in feed formulations (WPSA, 2013) has attracted interest in the measurement of digestible Ca content of feedstuffs, because of the adverse effects of high dietary Ca on the availability of P (Ballam et al., 1984; Tamim and Angel, 2003; Plumstead et al., 2008). On the other hand, lower dietary P concentrations have also been reported to reduce the Ca availability (Sebastian et al., 1996; Viveros et al., 2002). The ratio between dietary Ca to P is critical for the absorption and post-absorptive utilisation of both minerals.

Abbreviations: DM, dry matter; Ca, calcium; P, phosphorus; LM, limestone; DMI, dry matter intake; AIDC, apparent ileal digestibility coefficient.

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Calcium in plasma is tightly regulated and Ca or P deficient diets can influence Ca homeostasis and can affect the estimation of digestible Ca content of the test ingredient (Proszkowiec-Weglarz and Angel, 2013). A Ca to non-phytate P ratio of 2:1 is generally used in poultry feed formulations (NRC, 1994), but it is possible that the move towards digestible values may shift digestible Ca to digestible P ratio more towards 1:1.

Historically Ca availability has been described in terms of bioavailability relative to a calcium carbonate standard. Although it is generally assumed that Ca from different Ca supplements is highly available (Blair et al., 1965; Peeler, 1972; Reid and Weber, 1976), our previous studies (Anwar et al., 2015, 2016) have demonstrated that the true ileal digestibility coefficient of Ca in meat and bone meal for broilers is not high, ranging between 0.45 and 0.60. A similar scenario may exist with limestone.

In vitro solubility has been used by the feed industry as an indicator of Ca availability in limestone (Cheng and Coon, 1990a). A number of techniques have been employed to determine the *in vitro* solubility of limestone and these include measurement of percentage weight loss, pH change, proton consumption, percentage hydrogen ion disappearance and pH plateau time (Cheng and Coon, 1990b). Of these, the percentage weight loss is the most commonly used method. An inverse relationship between *in vitro* and *in vivo* solubilities of limestone has been observed (Zhang and Coon, 1997a; De Witt et al., 2006), with limestones of low *in vitro* solubility staying longer in the gizzard and increasing the *in vivo* solubility and Ca availability in layer hens.

Currently there is no established method available for the determination of Ca digestibility in poultry. In our previous studies, regression (Anwar et al., 2015), and direct (Anwar et al., 2016), methods were used to determine the Ca digestibility in meat and bone meal for broiler chickens and it was observed that both methods yielded comparable Ca digestibility coefficients. The regression method is laborious, costly and time consuming because each ingredient needs to be tested at least three inclusion levels to develop a regression line. On the other hand, the direct method is simple, but determines the apparent digestibility and a correction for endogenous Ca losses is required to calculate true Ca digestibility. Recently, the direct method has been used to determine the apparent total tract Ca digestibility of meat and bone meal in growing pigs (Sulabo and Stein, 2013). In the present study, the influence of the source of limestone and dietary P concentration on the apparent ileal Ca digestibility of limestone for broiler chickens was determined using the direct method.

2. Materials and methods

The experiment was conducted according to the New Zealand Revised Code of Ethical Conduct for the use of live animals for research, testing and teaching, and approved by the Massey University Animal Ethics Committee.

2.1. Diets and experimental design

Limestone from three commercial sources (coded as LM-1, LM-2 and LM-3) were obtained, ground to pass through 0.2 mm sieve to ensure uniform particle size and, representative samples were analysed for mineral composition and *in vitro* solubility. Limestone samples used in this study were calcitic and mined in the north island of New Zealand. Two experimental diets were developed from each limestone with 0 and 4.5 g/kg dietary P (Table 1). Limestone served as the sole source of dietary Ca in the experimental diets. Inclusion level of limestone was set to maintain the recommended dietary Ca concentration (9 g/kg) for broiler growers (Ross, 2007). In diets supplemented with P, Ca:non-phytate P ratio was maintained at 2:1. Titanium dioxide (3 g/kg) was incorporated in all diets as an indigestible marker.

2.2. Birds

Day-old male broilers (Ross 308) were obtained from a local hatchery, raised on floor pens in an environmentally controlled room and fed a commercial starter crumble (220 g/kg CP, 9.0 g/kg Ca and 4.5 g/kg P). Temperature was maintained at 31 °C on day 1 and gradually reduced to 22 °C by 21 days of age. On day 14, birds were moved to grower cages and the crumbles were gradually changed to a mash diet as the assay diets were in mash form. On day 21, following overnight fasting, the birds were individually weighed and allotted to 36 cages (eight birds per cage) on weight basis so that the average bird weight per cage was similar. The six experimental diets were then randomly allotted to six replicate cages each. The diets, in mash form, were fed on an *ad libitum* basis for 72 h from day 21 to day 24 post-hatch and the birds had free access to water. A lighting schedule of 20 h light per day was provided. Group body weights and feed intake were recorded on days 21 and 24.

2.3. Digesta collection and processing

On day 24, all birds were euthanised by intravenous injection (1 ml per 2 kg body weight) of sodium pentobarbitone (Provet NZ Pty. Ltd., Auckland, New Zealand) and, ileal digesta were collected and processed as described by Ravindran et al. (2005). The ileum was defined as that portion of the small intestine extending from the Meckel's diverticulum to a point ~40 mm proximal to the ileo-caecal junction. The ileum was then divided into two halves and the digesta were collected from the lower half towards the ileo-caecal junction. Digesta from birds within a cage were pooled, frozen immediately and