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# Effect of calcium source and particle size on the true ileal digestibility and total tract retention of calcium in broiler chickens

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#### ABSTRACT

The influence of calcium (Ca) source and particle size on true ileal digestibility coefficient (TIDC) of Ca and apparent total tract retention coefficient (ATTRC) of Ca in broilers was assessed. Four experimental diets containing limestone and oyster shell, each as fine (<0.5 mm) and coarse (1–2 mm) particles, were fed to broiler chickens from 21 to 24 days of age. A Ca- and phosphorus-free diet was used to determine the ileal endogenous Ca losses. Limestone and oyster shell were obtained from commercial sources and ground to pass through a set of sieves to obtain the fine and coarse particles. Titanium dioxide was incorporated in all diets as an indigestible marker. Each experimental diet was randomly allotted to six replicate cages (eight birds per cage). Apparent ileal digestibility coefficient and ATTRC of Ca were calculated using the indicator method and the TIDC values were calculated by correcting for endogenous Ca losses. Ileal endogenous Ca losses were determined to be 115 mg/kg of dry matter intake. The main effect of Ca source and the interaction between Ca source and particle size were not significant (P>0.05) for the TIDC and ATTRC of Ca. Both TIDC and ATTRC of Ca were influenced (P < 0.05) by particle size, with coarser particles increasing the digestibility and retention values. Increased particle size increased the TIDC of Ca in limestone from 0.38 to 0.62 and that of oyster shell from 0.33 to 0.56. The corresponding increases in ATTRC of Ca were from 0.44 to 0.66 in limestone and from 0.40 to 0.60 in oyster shell. Calcium concentration in the gizzard contents of birds was higher (P < 0.05) for limestone as compared to ovster shell and increased with increase in particle size. Calcium concentration in the gizzard contents was strongly correlated with the TIDC (r=0.81; P<0.01) and ATTRC (r=0.82; P<0.01) of Ca. A strong correlation (r=0.80, P<0.01) was also observed between TIDC and ATTRC of Ca.

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Abbreviations: AIDC, apparent ileal digestibility coefficient; ATTRC, apparent total tract retention coefficient; Ca, calcium; DM, dry matter; DMI, dry matter intake; GMD, geometric mean diameter; GSD, geometric standard deviation; P, phosphorus; Ti, titanium dioxide; TIDC, true ileal digestibility coefficient.

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#### 1. Introduction

Limestone and oyster shell are major sources of calcium (Ca) used in poultry diets, and contain approximately 380 g/kg Ca (NRC, 1994). However, the Ca concentration of limestone has been reported to vary from 360 to 428 g/kg in different studies (Reid and Weber, 1976; Ajakaiye et al., 2003; Browning and Cowieson, 2013; Wilkinson et al., 2013). Calcium is present in the form of calcium carbonate in both sources, but limestone is inorganic Ca of calcitic origin while oyster shell is organic Ca of marine origin. In the past, Ca availability has been described in terms of relative bioavailability to a calcium carbonate standard, which was considered to be 100% available. The relative bioavailability of Ca in limestone and oyster shell for broiler chickens has been reported to be high, varying between 73 and 109% and 87–108%, respectively (Blair et al., 1965; Reid and Weber, 1976; Augspurger and Baker, 2004). However, data from our previous study (Anwar et al., 2016a) indicate that the apparent ileal Ca digestibility coefficient (AIDC) of Ca in limestone was not high, ranging from 0.51 to 0.62. A similar scenario may exist for oyster shell. Currently no published data are available for the true ileal digestibility coefficient (TIDC) of Ca in oyster shell for poultry.

Source and particle size of limestone and oyster shell have been reported to influence the Ca availability, measured in terms of growth and tibia ash, in broiler chickens (McNaughton et al., 1974; Reid and Weber, 1976). In layers, shell weight, shell thickness and bone breaking force have been found to increase with increasing particle size of limestone (Cheng and Coon, 1990a). Large particles have been speculated to stay longer in the gizzard, thus increasing their *in vivo* solubility and utilisation in laying hens (Zhang and Coon, 1997a). Guinotte et al. (1991) reported an increase in Ca retention in broiler chickens fed diets with fine Ca carbonate (0.15 mm) as compared to medium (0.6–0.8 mm) and coarse (>1.18 mm) Ca carbonate. However, Safaa et al. (2008) did not observe any difference of particle size of limestone in layers for Ca availability in terms of egg shell quality and tibia Ca concentration.

In a previous study (Anwar et al., 2016b), TIDC of Ca in limestone with larger particles (1–2 mm) was observed to be markedly higher (0.71 vs. 0.43) compared to fine particles (<0.5 mm). These findings might be due to differences in the retention in the gizzard of different sized particles, and the possible slow release and increased absorption. The objective of the present work was to determine the effect of particle size of limestone and oyster shell on TIDC, apparent total tract retention coefficient (ATTRC) of Ca and Ca retention in the gizzard of broiler chickens.

#### 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. Particle size distribution

Limestone and oyster shell samples were ground to pass through a 8-mm sieve. To determine the particle size of grounded limestone and oyster shell, a set of sieves (Endocott, London, UK) sized 2, 1, 0.5, 0.212, 0.125 and 0.075 and a sieve shaker were used as described by Baker and Herman (2002). The samples were passed through the sieve stack on shakers for 10 min. The amount of sample retained on each sieve was determined and, the geometric mean diameter (GMD) and geometric standard deviation (GSD) were calculated for each sample. These calculations were based on the assumption that the weight distribution of the samples is logarithmically normal. Limestone and oyster shell particles that passed through 2 mm sieve and retained on 1 mm sieve were considered as coarse, while that passed through 0.5 mm sieve were considered as fine particles for the current study.

#### 2.2. Diets and experimental design

Limestone and oyster shell were obtained from commercial sources and ground to pass through a set of sieves to obtain fine (<0.5 mm) and coarse (1-2 mm) particles as described above. Representative samples were analysed for mineral composition and *in vitro* solubility. Two experimental diets, with fine (<0.5 mm) and coarse particle size (1-2 mm), were developed using each Ca source, which served as the sole source of dietary Ca (Table 1). Inclusion levels of limestone and oyster shell were set to maintain the recommended dietary Ca concentration (9 g/kg) for broiler growers (Ross, 2014) with a Ca to non-phytate phosphorus (P) ratio of 2:1. A Ca- and P-free diet was also developed to determine the ileal endogenous Ca losses. Titanium dioxide (3 g/kg) was incorporated in all diets as an indigestible marker.

#### 2.3. 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 (formulated to contain 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. Between days 14 and 20, the crumbles were gradually changed to mash as the experimental diets were in mash form. On day 21, the birds were individually weighed and allocated to 30 cages (eight birds per cage) on weight basis so that the average bird weight per cage was similar. The five experimental diets were then randomly allotted to six replicate cages each. The diets, in mash

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