



## Estimation of endogenous calcium and phosphorus loss in layer parent cockerels

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### ABSTRACT

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A study was carried out to estimate the endogenous loss of calcium (Ca) and phosphorus (P) in white leghorn parent cockerels. P is one of the most important elements both in feeding and as well as environmental point of view. It is considered as one of the most polluting nutrients in areas of intensive rearing of animals because they excrete high levels of P in their faeces. Ca and P serve a variety of functions including structure and strength of bones and cell walls, buffering systems and also energy transfer mechanisms. While calculating the factorial requirement of Ca and P for layers, the knowledge on the maintenance requirement and as well as amount of minerals excreted through the eggs. For this, thirty parent cockerels were divided into three replicates each consisting of ten birds. The birds were starved and their faeces are collected and analysed for Ca and P contents. The endogenous faecal excretion of Ca and P was found to be about 245.24±6.94 and 101.95±1.21 mg/kg of metabolic body weight, respectively.

**Keywords:** Calcium, Cockerels, Endogenous, Maintenance, Phosphorus

### INTRODUCTION

Nowadays digestible phosphorus in poultry feed formulation is more focused to determine to phosphorus digestibility (Mutucumarana *et al.*, 2014, 2015; Rodehutsord *et al.*, 2017; Verardi *et al.*, 2019). Calcium (Ca) and phosphorus (P) are the important elements that are essential for the both plants and animals' growth. Maintaining proper Ca and P ratio is very critical for absorption and utilization of minerals. P serves a variety of functions including structure and strength of bones and cell walls, buffering systems and energy transfer mechanisms. While Ca plays an important role in muscle contraction, nutrient transport, blood coagulation, enzyme and hormone release, etc. The laying hen requires Ca and P for the production of the egg, replacement of tissue metabolites as nucleotides and phospholipids and to maintain skeletal integrity. The release of P from the skeleton into the blood accompanies any skeletal mobilisation of calcium associated with egg shell formation during the dark period when the hens were not fed (Mongin and Sauveur, 1979). This P is lost in the urine (Taylor and Kirkley, 1967) and ultimately must be replaces if skeletal mass is to be maintained. The role of P is important in laying hen feeding, but the higher P concentration there will be increased excretion through the faeces and higher feeding cost. Inappropriate levels of dietary Ca and P have detrimental effects on both the bird's health and also egg shell quality (Hamilton and Sibbald, 1977; Miles and Harms, 1982).

There was always an uncertainty the availability of P from the plant sources due to the presence of phytic acid (Ravindran *et al.*, 1995) which demands the nutritionist to go for generous safety margins. Poultry did not have an insufficient endogenous secretion of phytase enzymes (Maenz and Classen, 1998), hence there will be need to assess the precision utilization of P. Unfortunately, P is one of expensive nutrient in the poultry diet and it is also a potent environmental pollutant (Edwards and Daniel, 1992). Hence, the phosphorus requirement should be calculated in such a manner to have less effect on the environment. Therefore, instead of going for conventional method of estimating the mineral requirement factorial method of determination will provide the exact requirement for maintenance and egg production. Bravo *et al.* (2003) observed that one of the important questions concerning P metabolism is how to determine P excretion, particularly, endogenous faecal P. The primary role of gastro intestinal tract is nutrient digestion and absorption as well as secretion of certain amount of endogenous nutrients (Ravindran *et al.*, 2016). Various authors have studied the rate of endogenous phosphorus loss in different farm animals such as pigs (Lopes *et al.*, 1999), ruminants (Salviano and Vitti, 1998) and equines (Furtado *et al.*, 2000). Bile, secretion of enzymes and sloughed cells are primary sources of endogenous phosphorus (Fan *et al.*, 2001),

For layers the P is excreted through the faeces and urine constituted endogenous P loss (maintenance requirement). Literature on endogenous P loss in chickens

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is scarce, but estimates have generally ranged from 250-450 mg of endogenous P/ kg of dry matter intake (DMI) (Rutherford *et al.*, 2004). The knowledge on endogenous P loss is important in the study of P because these obligatory losses are used to determine mineral requirements (Scott *et al.*, 1995). Endogenous P has been estimated by numerous methods, including feeding P-free diets (Peterson and Stein, 2004) and radiolabeled P (Fernandez, 1995). Another method that has been used to estimate the endogenous excretion of P or P at zero P intakes, is the regression method (Fan *et al.*, 2001). The present study was carried out to estimate the endogenous P loss in cockerels.

## MATERIALS AND METHODS

### Ethical approval

The experimental procedure performed in adult cockerels was as per the approved protocols of Institute Animal Ethical Committee (IAEA).

### Experimental design

Thirty six cockerels were divided into three replicates of twelve each and housed in individual cages. Birds were weighed at the beginning of and at the conclusion of the experiment. They were maintained off feed for 24 hours but given free access to deionized water before and during the period of excreta collection. Polythene sheets (pre-weighed) were spread over the dropping trays for the collection of mixed excreta and weighed to calculate the excreta voided.

### Collection of data

Representative samples of the excreta were taken, finely ground and stored in polythene bottles for estimation of dry matter, calcium and phosphorus. The dry matter, phosphorus and calcium content of the excreta were estimated as per the method of AOAC (2002). The constant weight of a sample after complete removal of moisture or water is the dry matter. The moisture content of a sample is estimated by heating it in an oven to a constant weight at 105-110°C under atmospheric pressure. The moisture is removed as vapour. Phosphorus in the faecal sample is converted to phospho-molybdo-vanadate complex and the intensity of colour is measured at 400 nm in a UV-visible spectrophotometer. The developed regression equation of  $0.0848x - 0.0652$  with a  $R^2$  value of 0.9906 for

standard curve using Potassium di-hydrogen Phosphate ( $KH_2PO_4$ ) was used for estimation of phosphorus. The calcium in the sample is precipitated as calcium oxalate using ammonium oxalate in acidic medium. The precipitated calcium oxalate is filtered out, washed with ammonium hydroxide to free ammonium oxalate from the precipitate and dissolved in hot sulphuric acid and the liberated oxalic acid is estimated by potassium permanganate titration.

### Statistical analysis

Data collected were analyzed and the results were correlated with body weight and the regression equations were derived to identify the endogenous loss of phosphorus in maintaining cockerels.

## RESULT AND DISCUSSION

The observations of body weight, excreta produced, excreta dry matter, calcium and phosphorus excreted are presented in table 1. The individual values are the average of three times and presented. The endogenous calcium and phosphorus values were in the range of 210.2 to 290.1 and 97.20 to 106.24 mg per kg of metabolic body weight (MBW) (Table 1). Regression equation on endogenous loss based on metabolic body weight were developed as Ca (mg/kg of MBW)  $464.37 + (-149.79 \times MBW)$  with a  $R^2$  value of 0.9781 and for phosphorus (mg/kg MBW)  $116.17 + (-9.885 \times MBW)$ ,  $R^2=0.9872$  (Table 2). The authors could not find any relevant research data available on about the endogenous loss of calcium and phosphorus under fasting or maintenance stage for chicken.

Dilger and Adeola (2006) reported that endogenous loss of phosphorus at the rate of 190.5 and 395.8 mg/kg of DMI in excreta in chicks fed conventional and low-phytate soybean meal. However, they also recorded a value of 208.5 and 144.5 mg/ kg DMI from pre-cecal samples in chicks. However, the reported  $R^2$  value is very low (0.50) to moderate (0.87) which indicates their repeatability and or reliability under different conditions. The observation on endogenous loss can be used for formulation of these minerals in chicken feeds.

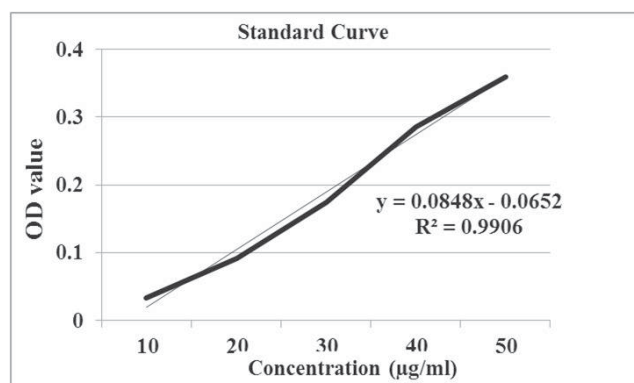
From the above study it was observed that the endogenous loss of calcium and phosphorus in the layer cockerels about  $245.24 \pm 6.94$  and  $101.95 \pm 1.21$  mg per

**Table 1:** Endogenous loss of calcium and phosphorus loss (Mean±SE) in layer parent cockerels

| Bird weight (kg) | Excreta produced/bird (g) | Dry Matter % | Calcium (mg/kg MBW) | Phosphorus (mg/kg MBW) |
|------------------|---------------------------|--------------|---------------------|------------------------|
| 1.626            | 5.11                      | 99.28        | 245.24±6.94         | 101.95±1.21            |

**Table 2:** Regression equation on the endogenous loss of calcium and phosphorus loss expressed per kilogram of metabolic body weight in layer cockerels

| Minerals   | Regression equation   | $R^2$ value |
|------------|---|-------------|
| Calcium    | $464.3727 + (-149.792 \times \text{metabolic body weight})$ | 0.9781      |
| Phosphorus | $116.17 + (-9.885 \times \text{metabolic body weight})$     | 0.9872      |



**Fig. 1:** Standard curve, regression equation with regression coefficient for phosphorus

kg of metabolic body weight respectively. These data will be helpful in formulation of non-phytate phosphorus levels in chicken and reduce environmental pollution. Endogenous losses of P also a diet-dependent one. Endogenous P losses in birds fed with casein-based diet were found to be higher than in those fed gelatin-based and P-free diets. Phosphorus-free diets are devoid of protein and the absence of protein will reduce enzyme secretions which in turn lowers the endogenous P secretion into the gut lumen (Mutucumarana and Ravindran, 2021). Higher endogenous P losses observed in higher phosphorus content diet (casein based diet). Due to the presence of higher protein diet ultimately increase the proteolytic enzyme secretion (Ravindran *et al.*, 2009)

Endogenous P losses were increased in the excreta in birds fed P-free and gelatin-based diets, but no difference was observed between the sites of measurement in those fed the casein-based diet. Ileal endogenous losses of P in birds fed P-free, gelatin-based and casein-based diets were estimated to be 25.1, 104 and 438 mg/kg DM intake, respectively. Ileal endogenous P losses in birds fed the casein-based diet were higher ( $P < 0.05$ ) than those in birds fed the P-free and gelatin-based diets. Excreta endogenous P losses in birds fed those two diets were lower ( $P < 0.05$ ) than that in the birds fed the P-free diet (Mutucumarana and Ravindran, 2021). The higher endogenous P in the excreta of bird's fed P-free and gelatin-based diets suggests an increased P output via urine when diets contain little or no Ca. The Ca-deficient diets result in lower P retention in broilers (Liu *et al.*, 2013).

## CONCLUSION

The endogenous faecal excretion of Ca and P was found to be about  $245.24 \pm 6.94$  and  $101.95 \pm 1.21$  mg/kg of metabolic body weight, respectively.

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