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The effect of a mono-component exogenous protease and graded concentrations of ascorbic acid on the performance, nutrient digestibility and intestinal architecture of broiler chickens

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ABSTRACT

A total of 640 male Ross 308 broilers were used in a growth performance and digestibility study to explore the interactive effects of a mono-component exogenous protease and ascorbic acid. A total of 7 dietary treatments were generated by formulation of a nutritionally-marginal wheat/soy-based 'negative control' diet and supplementation of the same with three concentrations of ascorbic acid, without or with exogenous protease addition. A further 'positive control' diet was fed as a reference and was formulated to be nutritionally adequate. Diets were fed ad libitum from d1-35 post-hatch and water and environmental controls were as per breeder recommendations. At the termination of the experiment ileal digesta was collected for assessment of digestibility and the ileum and jejunum were excised for morphological measurements. There were no interactions ($P > 0.05$) between ascorbic acid and exogenous protease for any of the measurements taken during the study. Overall (d1-35) addition of protease resulted in a significantly lower FCR compared with birds fed the negative control diet. There was no effect of ascorbic acid on performance of the birds with the exception of a small reduction in FCR at the highest inclusion concentration only. Ascorbic acid addition significantly increased gut integrity and exogenous protease reduced ($P < 0.05$) the presence of sialic acid in ileal digesta. Exogenous protease significantly increased the ileal digestibility of nitrogen and all amino acids but had no effect ($P > 0.05$) on dry matter digestibility. Ascorbic acid addition had only modest effects on ileal nutrient digestibility but this was confirmed as significant for several amino acids including threonine and cysteine. Finally, addition of ascorbic acid linearly decreased crypt depth in the jejunum and exogenous protease increased villus height, reduced epithelial thickness and goblet cell number in the jejunum. It can be concluded that ascorbic acid and exogenous protease improve intestinal integrity in growing broiler chickens and offer potential as a strategic dietary intervention to enhance performance, intestinal resilience and nutrient recovery.

1. Introduction

There is growing interest on a global basis in nutritional interventions capable of improving intestinal resilience and function in commercial broiler production, especially as various prophylactic antibiotic treatments are phased out (Sneeringer et al., 2015). Our laboratory has previously demonstrated potential of a combination of exogenous protease and ascorbic acid in enhancing production

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efficiency and intestinal integrity in broiler chicken production (Cowieson et al., 2017a). The mechanisms involved appear to be associated with reduction in the severity of the effect of various proteinaceous antinutrients e.g. antigenic proteins in soybean meal (Rooke et al., 1998), reduced endogenous protein flow and enhanced intestinal strength, perhaps mediated via an improvement in collagen architecture (Cowieson and Roos, 2016; Cowieson et al., 2017b). Furthermore, the use of exogenous protease enables formulation of poultry diets to meet bird requirements at a lower protein and amino acid concentration that in turn has a beneficial effect on litter conditions (Oxenboll et al., 2011), the environment in the hind gut (Cowieson and Roos, 2016) and the sustainability of poultry production in general (Leinonen and Williams, 2015).

Exogenous proteases have been used in commercial poultry production for several years, both as mono-component products and as part of various enzyme admixtures (Cowieson and Adeola, 2005). These enzymes elicit beneficial effects on dietary amino acid digestibility (Cowieson and Roos, 2014). However, whilst these effects may be of focal interest, recent work has shown that exogenous proteases exert a range of effects that extend beyond dietary protein and amino acid digestibility. For example, Fru-Nji et al. (2011) noted improvements in ileal energy digestibility and Kalmendal and Tauson (2012) observed a significant increase in ileal starch and fat digestibility where a mono-component exogenous protease was added to a broiler diet. Furthermore, Cowieson et al. (2017a,b) noted beneficial effects of exogenous protease on mucin secretion, tight junction integrity and various other immune and morphological traits in broilers that are highly suggestive of effects that extend beyond 'simple' changes in dietary protein solubility and digestion.

Ascorbic acid is perhaps best known, nutritionally, as an anti-oxidant but is also a vital co-factor to enable hydroxylation of lysine and proline for collagen synthesis (Chaterjee et al., 1975; Murad et al., 1981; Whitehead and Keller, 2003). Although poultry possess rudimentary capacity to synthesise ascorbic acid it is possible that this is insufficient for optimal collagen integrity in fast-growing modern genotypes as it has been demonstrated that supplemental ascorbic acid is advantageous to bird growth, especially under stressful conditions (Whitehead and Keller, 2003; Cowieson et al., 2017a).

Whilst the effects of ascorbic acid or exogenous protease on broiler performance, nutrient digestibility and intestinal morphology appear positive and promising, there is scant information in the literature on possible dose sensitivity, especially for ascorbic acid. Previous work focused on a single dietary concentration of ascorbic acid (200 mg/kg of a stabilised ascorbic acid product that contains 350 g/kg ascorbic acid; i.e. 70 mg/kg ascorbic acid; Cowieson et al., 2017a). It was therefore the objective of the experiment that is described herein to investigate the effect of graded concentrations of ascorbic acid (35, 70 and 105 mg/kg; being 100, 200 or 300 mg/kg of the ascorbic acid product) without or with exogenous protease (200 mg/kg; 15,000 PROT/kg) on performance and intestinal health of broiler chickens fed a commercially-relevant wheat-based diet. It was hypothesised that both exogenous protease and ascorbic acid would improve production performance, nutrient digestibility and intestinal architecture, possibly via complementary mechanisms.

2. Materials and methods

The experimental procedures were approved by the Massey University Animal Ethics Committee and, complied with the New Zealand Code of Practice for the Care and Use of Animals for Scientific Purposes.

2.1. Birds and dietary treatments

Male broiler (Ross 308) chicks were obtained from a local hatchery as day-olds and reared in cages in an environmentally controlled room. On d0, chicks were individually weighed and 640 birds were allocated on weight basis to 80 cages (8 chicks per cage). The 8 dietary treatments were then randomly assigned to 10 replicate cages each. Experimental diets were fed from 0 to 21 (starter) and 22 to 35 (grower) days. During week 1, a broiler coccidiosis vaccine (Immucox, Pacificvet, Christchurch, New Zealand) was given via drinking water to all chicks. The temperature was maintained at 32 °C on d1 and then gradually reduced to 24 °C by d21. The birds received 20-h of fluorescent illumination and allowed free access to the diets and water. Birds were checked at least three times daily (9.00 a.m., 1.00 p.m. and 4.00 p.m.) and any unusual aspect of bird behaviour or condition was recorded. Sick or injured animals were weighed and removed from the study. The experimental design was a completely randomised design. Two control diets, positive (PC) and negative (NC), based on wheat and soybean meal, were mixed for each phase of the study (0–21 and 22–35 d) (Table 1). Full diet details can be found in Table 1 but briefly the NC diet was formulated to be equivalent to the PC in all nutrients other than protein and digestible amino acids (a reduction in crude protein and digestible amino acids of around 4% was targeted). The NC diet was then used to develop 7 dietary treatments by adding graded concentrations of ascorbic acid (ROVIMIX Stay-C 35, DSM Nutritional Products, Kaiseraugst, Switzerland) without or with an exogenous protease (RONOZYME ProAct, DSM Nutritional Products, Kaiseraugst, Switzerland). The protease used in the current experiment was a serine protease expressed in *Bacillus licheniformis*. One protease (PROT) unit is defined as the amount of enzyme that releases 1 mmol of *p*-nitroaniline from 1 mM substrate (Suc-Ala-Ala-Pro-Phe-pNA_ per minute at pH 9.0 and 37 °C). ROVIMIX Stay-C 35 contains 35% ascorbic acid and was included in the diets at concentrations of 100, 200 or 300 mg/kg (representing ascorbic acid concentrations of 35, 70 or 105 mg/kg respectively). For clarity the concentration of the added product will be used henceforth (100, 200 or 300 mg/kg). All the diets were fed in pellet form and contained a background activity of phytase (RONOZYME HiPhos GT) and xylanase (RONOZYME WX) to reflect commercial reality. The arrangement of treatments was as follows: PC, NC, NC + 100 mg/kg ascorbic acid, NC + 200 mg/kg ascorbic acid, NC + 300 mg/kg ascorbic acid, NC + 100 mg/kg ascorbic acid + 200 mg/kg protease, NC + 200 mg/kg ascorbic acid + 200 mg/kg protease and NC + 300 mg/kg ascorbic acid + 200 mg/kg protease.