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# Impact of Exogenous Enzymes in Sorghum- or Wheat-Based Broiler Diets on Nutrient Utilization and Growth Performance

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**Abstract:** The impact of two enzyme preparations in either sorghum- or wheat-based broiler diets on nutrient utilization and growth performance was determined. One preparation (Enzyme A) combined protease, xylanase and ß-glucanase activities and the second (Enzyme P) contained xylanase activity. Sorghum- or wheat-based starter (1-14 days), grower (15-28) and finisher (29-42) diets without or with either Enzyme A or Enzyme P were offered to broilers from 1-42 days post-hatch. Each of the six dietary treatments was offered to six replicates of six birds per cage. Total excreta collections were completed in the grower and finisher phases to determine the effects of dietary treatments on nutrient utilization as assessed by Apparent Metabolizable Energy (AME), Nitrogen (N) retention and N-corrected AME (AMEn). Both preparations contained similar levels of xylanase activity and enhanced nutrient utilization in wheat-based broiler diets with more pronounced responses in the finisher phase. In this phase, Enzyme A significantly increased AME by 0.98 MJ, N retention by 4.80 percentage units and AMEn by 0.95 MJ/kg. Similarly, Enzyme P increased AME by 1.21 MJ, N retention by 4.25 percentage units and AMEn by 1.24 MJ/kg. In contrast, enzyme inclusions in sorghum-based grower and finisher diets did not influence nutrient utilization and this is reflected in significant treatment interactions ($p<0.001$) for AME and AMEn in the finisher phase. In broilers offered wheat-based diets, both enzymes similarly improved growth performance; Enzyme A and Enzyme P significantly improved feed efficiency by 7.0% and 7.1%, respectively, from 1-42 days post-hatch. In sorghum-based diets, Enzyme P numerically depressed feed efficiency; whereas Enzyme A marginally enhanced feed efficiency and increased weight gain by 6.7%, which closely approached significance ($p<0.06$). Kafirin is the dominant protein fraction in sorghum and the possibility that the protease component in Enzyme A, subtilisin, has the capacity to degrade kafirin is considered.

**Key words:** Broilers, exogenous enzymes, growth performance, nutrient utilization, sorghum, wheat

## INTRODUCTION

In Australia, diets for broiler chickens are typically based on wheat, sorghum or wheat-sorghum blends. Sorghum has a more consistent and higher energy density than wheat and is usually priced at an advantage; however, sorghum-based diets have been associated with inferior broiler performance in comparison to wheat-based diets. Robertson and Perez-Maldonado (2006) reported that sorghum was inferior to wheat in terms of feed efficiency and breast-meat yield, both crucial parameters for sustainable chicken-meat production. However, this difference may be due in part to the positive impact of Non-Starch Polysaccharide (NSP) degrading enzymes on performance of broilers offered wheat-based diets. NSP-degrading enzymes with predominantly xylanase activity are routinely included in wheat-based diets and their capacity to enhance growth performance and nutrient utilization is well-established. In contrast, because sorghum is a 'non-viscous' grain, similar responses are not evident in broilers offered sorghum-based diets. This difference was recently demonstrated by Shakouri *et al*. (2009). The addition of an enzyme with xylanase and ß-glucanase activities increased 28-day weight gain by 19.3% in broilers offered wheat-based diets but did not alter growth rates of birds offered sorghum-based diets. The purpose of this study was to compare nutrient utilization and growth performance responses in broilers offered either wheat- or sorghum-based diets following the inclusion of two enzyme preparations containing either a combination of protease, ß-glucanase and xylanase activities or solely xylanase activity.

## MATERIALS AND METHODS

Key feed ingredients were analyzed for protein, calcium, total phosphorus and phytate-phosphorus and sorghum- and wheat-based starter (1-14 days post-hatch), grower (15-28) and finisher (29-42) diets were

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Table 1: Composition and nutritional specifications of basal sorghum-based and wheat-based diets

| | Starter | | Grower | | Finisher | |
|---|---|---|---|---|---|---|
| Items | Sorghum | Wheat | Sorghum | Wheat | Sorghum | Wheat |
| **Composition (g/kg)** | | | | | | |
| Sorghum (92 g/kg protein) | 605.97 | - | 655.86 | - | 649.84 | - |
| Wheat (135 g/kg protein) | - | 631.14 | - | 695.56 | - | 690.88 |
| Soyabean meal (473 g/kg protein) | 273.52 | 213.00 | 193.87 | 120.81 | 200.87 | 126.89 |
| Canola meal (369 g/kg protein) | 70.00 | 70.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| Vegetable oil | 9.25 | 41.43 | 8.63 | 41.18 | 12.05 | 44.17 |
| Dicalcium phosphate | 18.97 | 18.78 | 17.27 | 17.15 | 15.44 | 15.34 |
| Limestone | 7.77 | 10.88 | 7.88 | 8.12 | 8.94 | 9.17 |
| Salt | 1.61 | 1.26 | 1.34 | 0.87 | 1.84 | 1.38 |
| Sodium bicarbonate | 3.18 | 3.73 | 3.02 | 3.74 | 2.24 | 2.97 |
| Lysine monohydrochloride | 2.73 | 3.38 | 3.99 | 4.94 | 2.12 | 3.07 |
| Methionine | 3.47 | 2.68 | 3.92 | 3.10 | 3.29 | 2.46 |
| Threonine | 0.98 | 1.17 | 1.67 | 1.98 | 0.82 | 1.12 |
| Vitamin-mineral premix | 2.50 | 2.50 | 2.50 | 2.50 | 2.50 | 2.50 |
| Phytase feed enzyme | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| **Specifications (g/kg)** | | | | | | |
| Metabolizable energy (MJ/kg) | 12.35 | 12.35 | 12.50 | 12.50 | 12.55 | 12.55 |
| Protein | 220.0 | 222.0 | 200.0 | 200.5 | 200.0 | 200.0 |
| Calcium | 9.40 | 10.50 | 9.00 | 9.00 | 9.00 | 9.00 |
| Total phosphorus | 8.73 | 8.13 | 8.34 | 7.68 | 8.04 | 7.38 |
| Phytate phosphorus[1] | 2.83 | 2.25 | 2.85 | 2.20 | 2.87 | 2.22 |
| Nonphytate phosphorus[2] | 5.89 | 5.88 | 5.49 | 5.48 | 5.17 | 5.16 |
| Lysine | 13.34 | 13.34 | 12.63 | 12.62 | 11.35 | 11.32 |
| Methionine | 6.62 | 5.99 | 6.83 | 6.16 | 6.24 | 5.56 |
| Threonine | 8.83 | 8.83 | 8.64 | 8.64 | 7.91 | 7.89 |

[1]Calculated from analyzed phytate-P contents of plant-sourced feed ingredients. Wheat 1.45, Sorghum 2.10, Soyabean meal 3.85, Canola meal 7.30 g/kg. [2]By subtraction

formulated on this basis in accordance with commercial practice. The composition and nutritional specifications of the six basal diets are presented in Table 1. Each of the six basal diets were mixed and divided into three batches. One batch served as the control and either Enzyme A or Enzyme P was added to the remaining batches to provide a total of 18 experimental diets, which were fed as mash.

Enzyme A (Avizyme® 1202) is a granular preparation derived from *Trichoderma longibrachiatum* and *Bacillus subtilis* and was included in diets at 350 g per tonne. Enzyme A contains 200 U/g endo-1,3(4)-beta-glucanase (EC 3.2.1.6), 5000 U/g endo-1,4-beta-xylanase (EC 3.2.1.8) and 1600 U/g subtilisin (EC 3.4.21.62), the protease component. Enzyme P (Porzyme® 93010) is a granular preparation derived from *Trichoderma longibrachiatum*, which contains 40000 U/g endo-1,4-beta-xylanase (EC 3.2.1.8) and was included in diets at 50 g per tonne.

On the basis of individual analyses of relevant feed ingredients, sorghum diets contained an average of 2.85 g/kg and wheat diets contained 2.22 g/kg phytate-P. All experimental diets were supplemented with a phytate-degrading enzyme (Phyzyme® XP at 500 FTU/kg) because phytase is commonly included in poultry diets primarily to increase phytate-bound phosphorus utilization irrespective of the cereal grain base (Selle and Ravindran, 2007). The exogenous enzymes used in this experiment were supplied by Danisco Animal Nutrition. Day-old chicks (Cobb) were purchased from a commercial hatchery and vent-sexed males were retained for the feeding study. A total of 216 identified (wing-bands) chicks were allocated to 36 cages on the basis of body-weight. Each of the six dietary treatments was offered at random to 6 pens containing 6 chicks. Feed and water was provided *ad libitum* in an environmentally controlled facility. The birds were individually weighed at 1, 14, 28 and 42 days post-hatch and feed consumption was recorded on a per cage basis. The body-weights of dead or culled birds were recorded daily in order to adjust feed conversion ratio calculations.

In the grower phase (20-23 days post-hatch) total excreta output from each cage was collected for 96 hours and feed intakes recorded to calculate AME on a Dry Matter (DM) basis and N retention by determining gross energy and N contents of the diets and excreta. This procedure was repeated for a 72 h period in the finisher phase (34-36 days post-hatch). AME was determined as MJ/kg on a dry matter basis and metabolizable energy intakes (MJ/day) were calculated from feed intakes for the entire grower and finisher phases. AMEn was calculated by applying the factor of 36.54 kJ per gram nitrogen retained in the body (Hill and

Anderson, 1958). The following equations were used to calculate AME and N retention:

$$\text{AME diet [MJ/kg DM]} = \frac{\text{(Feed intake x GE-diet) - (Excreta output x GE-excreta)}}{\text{Feed intake}}$$

$$\text{N retention [\%]} = \frac{\text{(Feed intake x N-feed) - (Excreta output x N-excreta)}}{\text{(Feed intake x N-feed)}} \text{ x } 100$$

Experimental data was analyzed by a general linear model (SPSS® Inc.) as a two by three factorial array of treatments of two grain types (sorghum, wheat) and three enzyme inclusions (nil, Enzyme A, Enzyme P). When considered relevant, the significance of pair-wise comparisons between specific treatments was taken into consideration. The conduct of the experiment complied with specific guidelines set down by the Animal Ethics Committee of Sydney University.

## RESULTS

The treatment effects on nutrient utilization are shown in Table 2. In the grower phase, there were no statistically significant treatments effects on dietary AME; however, wheat-based diets had numerically higher AME values (14.25 versus 14.03 MJ/kg; p<0.16) than sorghum. In wheat-based diets, Enzyme P increased AME by 0.58 MJ (p<0.04) and Enzyme A tended to increase AME by 0.46 MJ (p<0.10) on the basis of pair-wise comparisons, Energy intakes (MJ/day AME) were not influenced by grain type or enzyme inclusion in both the grower or finisher phases. N retention was significantly higher in sorghum- than wheat-based diets in the grower phase by 4.31 percentage units (64.50 versus 60.19%; p<0.001). Enzyme inclusions significantly enhanced N retention (p<0.02) by an average of 3.25 percentage units or 5.4% but responses in wheat-based diets were more pronounced. N-corrected AME values were 0.50 MJ higher in wheat-based diets (13.04 versus 12.54 MJ/kg; p<0.01) but the overall impact of enzyme addition was not significant (p>0.30). Although, Enzyme P increased AMEn by 0.56 MJ in wheat-based diets and this was significant (p<0.04) from a pair-wise comparison.

In the finisher phase, wheat-based diets tended to have higher AME values (p<0.10) and the effect of enzyme inclusion was significant (p<0.01). However, there was a significant treatment interaction (p<0.001) because, on average, enzyme addition tended to depress AME (14.02 versus 14.18 MJ/kg) in sorghum-based diets but increase AME by 1.095 MJ (14.645 versus 13.55 MJ/kg) in wheat-based diets. Again, N retention was significantly higher in sorghum-than wheat-based diets in the finisher phase (61.33 versus 58.70%; p<0.02). Overall, enzyme inclusions significantly increased N retention (61.13 versus 57.79%; p<0.02) but, as in the grower phase, responses in wheat-based diet were more pronounced. AMEn values were significantly higher in wheat-based diets by 0.49 MJ (13.04 versus 12.55 MJ/kg; p<0.001) but enzyme inclusions had a significant impact (p<0.01) increasing AMEn from 12.52 to an average of 12.935 MJ/kg. However, this increase was despite numerical reductions in AMEn of sorghum-based diets and because of robust responses in wheat-based diets such that the treatment interaction (p<0.001) was highly significant.

The progressive growth performance results are shown in Table 3. From 1-42 days post-hatch, broilers offered sorghum-based diets tended (p<0.10) to have higher feed intakes (4.0%) and weight gains (3.8%) than their wheat-based counterparts but, at 1.84, feed efficiency was identical. Broilers on sorghum-based diets had significantly (p<0.05-0.001) higher feed intakes (12.1 and 5.4%) and weight gains (10.1 and 4.6%) from 1-14 and 1-28 days post-hatch. However, broilers on wheat-based diets tended to have enhanced feed conversion ratios (1.335 versus 1.365; p<0.08) from 1-14 days post-hatch.

Enzyme inclusions significantly improved (p<0.04) feed efficiency from 1-14 days post-hatch; Enzyme A by 3.8% (1.329 versus 1.381) and Enzyme P by 3.0% (1.340 versus 1.381). From 1-42 days post-hatch the two enzymes tended to improve (p<0.10) feed efficiency; Enzyme A by 4.5% (1.803 versus 1.887) and Enzyme P by 3.2% (1.826 versus 1.887). However, in wheat-based diets, Enzyme A enhanced feed efficiency by 7.0% (p<0.03) and Enzyme P by 7.1% (p<0.02), which were significant improvements on the basis of pair-wise comparisons. In contrast, in sorghum-based diets, Enzyme A numerically improved feed efficiency (1.8%) but Enzyme P numerically depressed feed efficiency (0.8%). This contrasting responses to enzymes between grains is reflected in the trend towards a treatment interaction (p = 0.153).

The pattern of feed intake responses to enzyme inclusions differed from 1-42 days post-hatch. Enzymes tended to increase feed intakes by an average of 5.2% in sorghum-based diets but in wheat-based diets intakes were depressed by an average of 4.8%. Again, this is reflected in the trend towards a treatment interaction (p = 0.104).

From 1-42 days post-hatch, enzyme inclusions tended (p<0.16) to increase weight gains; Enzyme A by 4.3% and Enzyme P by 4.1% in both sorghum- and wheat-based diets. Interestingly, in broilers offered sorghum-based diets Enzyme A increased weight gain by 6.7% (2626 versus 2507 g/bird), which closely approached significance (p<0.06).

## DISCUSSION

Broilers offered non-supplemented sorghum-based diets had slightly higher weight gains (1.8%) and tended to have lower feed conversion ratios (1.845 versus 1.929; p<0.15) from 1-42 days post-hatch than their

Table 2: Effects of enzyme inclusions on nutrient utilization of broilers offered either sorghum- or wheat-based diets in grower and finisher phases

| Treatments | | Grower phase | | | | Finisher phase | | | |
|---|---|---|---|---|---|---|---|---|---|
| Grain type | Enzyme addition | AME (MJ/kg DM) | AME (MJ/day) | N retention (%) | AMEn (MJ/kg DM) | AME (MJ/kg DM) | AME (MJ/day) | N retention (%) | AMEn (MJ/kg DM) |
| Sorghum | Nil | 14.00 | 1.48 | 63.56$^{bc}$ | 12.51$^{a}$ | 14.18$^{bc}$ | 2.16 | 59.89$^{b}$ | 12.72$^{b}$ |
| Sorghum | Enzyme A | 14.14 | 1.50 | 64.43$^{bc}$ | 12.68$^{ab}$ | 14.08$^{b}$ | 2.07 | 61.77$^{b}$ | 12.55$^{ab}$ |
| Sorghum | Enzyme P | 13.94 | 1.43 | 65.53$^{c}$ | 12.43$^{a}$ | 13.95$^{ab}$ | 2.12 | 62.32$^{b}$ | 12.38$^{ab}$ |
| Wheat | Nil | 13.90 | 1.48 | 56.80$^{a}$ | 12.75$^{ab}$ | 13.55$^{a}$ | 2.18 | 55.69$^{a}$ | 12.31$^{a}$ |
| Wheat | Enzyme A | 14.36 | 1.49 | 61.64$^{b}$ | 13.06$^{bc}$ | 14.53$^{cd}$ | 2.13 | 60.49$^{b}$ | 13.26$^{c}$ |
| Wheat | Enzyme P | 14.48 | 1.53 | 62.12$^{bc}$ | 13.31$^{c}$ | 14.76$^{d}$ | 2.16 | 59.94$^{b}$ | 13.55$^{c}$ |
| SEM | | 0.1853 | 0.0432 | 1.226 | 0.1821 | 0.1443 | 0.0813 | 1.2768 | 0.1408 |
| **Main effects. Grain type** | | | | | | | | | |
| Sorghum | | 14.03 | 1.47 | 64.50 | 12.54 | 14.07 | 2.12 | 61.33 | 12.55 |
| Wheat | | 14.25 | 1.50 | 60.19 | 13.04 | 14.28 | 2.15 | 58.70 | 13.04 |
| **Enzyme addition** | | | | | | | | | |
| Nil | | 13.95 | 1.48 | 60.18 | 12.63 | 13.87 | 2.17 | 57.79 | 12.52 |
| Enzyme A | | 14.25 | 1.50 | 63.03 | 12.87 | 14.30 | 2.10 | 61.13 | 12.90 |
| Enzyme P | | 14.21 | 1.48 | 63.82 | 12.87 | 14.36 | 2.14 | 61.13 | 12.97 |
| **Significance** | | | | | | | | | |
| Grain type (G) | | 0.158 | 0.358 | 0.000 | 0.002 | 0.086 | 0.571 | 0.017 | 0.000 |
| Enzyme addition (E) | | 0.232 | 0.860 | 0.015 | 0.328 | 0.003 | 0.723 | 0.019 | 0.007 |
| G x E interaction | | 0.253 | 0.366 | 0.236 | 0.191 | 0.000 | 0.981 | 0.519 | 0.000 |

$^{abc}$Mean values within columns not sharing common superscripts are statistically different at the 5% level of probability

Table 3: Effects of enzyme inclusions on growth performance of broilers offered either sorghum- or wheat-based diets from 1-14, 1-28 and 1-42 days post-hatch

| Treatments | | 1-14 days post-hatch | | | 1-28 days post-hatch | | | 1-42 days post-hatch | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Grain type | Enzyme addition | Weight gain (g/bird) | Feed intake (g/bird) | Feed efficiency (g/g) | Weight gain (g/bird) | Feed intake (g/bird) | Feed efficiency (g/g) | Weight gain (g/bird) | Feed intake (g/bird) | Feed efficiency (g/g) |
| Sorghum | Nil | 335 | 460$^{bc}$ | 1.374$^{ab}$ | 1227 | 1906$^{bc}$ | 1.554 | 2507 | 4622 | 1.845 |
| Sorghum | Enzyme A | 344 | 469$^{c}$ | 1.361$^{ab}$ | 1276 | 1965$^{c}$ | 1.541 | 2675 | 4846 | 1.812 |
| Sorghum | Enzyme P | 339 | 460$^{bc}$ | 1.360$^{ab}$ | 1241 | 1940$^{bc}$ | 1.564 | 2626 | 4883 | 1.860 |
| Wheat | Nil | 301 | 416$^{ab}$ | 1.388$^{a}$ | 1168 | 1848$^{ab}$ | 1.584 | 2462 | 4752 | 1.929 |
| Wheat | Enzyme A | 305 | 403$^{a}$ | 1.297$^{c}$ | 1176 | 1791$^{a}$ | 1.523 | 2508 | 4484 | 1.794 |
| Wheat | Enzyme P | 319 | 420$^{ab}$ | 1.321$^{bc}$ | 1235 | 1872$^{ab}$ | 1.519 | 2549 | 4566 | 1.792 |
| SEM | | 13.653 | 16.793 | 0.0197 | 27.957 | 38.333 | 0.0210 | 60.466 | 122.707 | 0.0387 |
| **Main effects. Grain type** | | | | | | | | | | |
| Sorghum | | 339 | 463 | 1.365 | 1248 | 1937 | 1.553 | 2603 | 4783 | 1.839 |
| Wheat | | 308 | 413 | 1.335 | 1193 | 1837 | 1.542 | 2507 | 4601 | 1.838 |
| **Enzyme addition** | | | | | | | | | | |
| Nil | | 318 | 438 | 1.381 | 1197 | 1877 | 1.569 | 2485 | 4687 | 1.887 |
| Enzyme A | | 324 | 436 | 1.329 | 1226 | 1878 | 1.532 | 2591 | 4665 | 1.803 |
| Enzyme P | | 329 | 450 | 1.340 | 1238 | 1906 | 1.541 | 2588 | 4724 | 1.826 |
| **Significance** | | | | | | | | | | |
| Grain type (G) | | 0.009 | 0.000 | 0.079 | 0.023 | 0.003 | 0.520 | 0.061 | 0.078 | 0.983 |
| Enzyme addition (E) | | 0.734 | 0.969 | 0.033 | 0.343 | 0.092 | 0.210 | 0.153 | 0.887 | 0.096 |
| G x E interaction | | 0.756 | 0.796 | 0.150 | 0.262 | 0.263 | 0.209 | 0.582 | 0.104 | 0.153 |

$^{abc}$Mean values within columns not sharing common superscripts are statistically different at the 5% level of probability

wheat-based counterparts. Although not conclusive, this outcome does not support the perception that sorghum-based diets are inferior to wheat-based broiler diets. Predictably, this study confirmed that nutrient utilization of broilers offered wheat-based broiler diets is advantaged by the inclusion of xylanase-based enzyme preparations; whereas, this is not the case with sorghum-based diets. This was best illustrated by the highly significant ($p<0.001$) treatment interactions for AME and AMEn in the finisher phase where the energy densities of wheat-based diets, but not sorghum-based diets, were enhanced by enzyme inclusions. In this context, the statement by Choct (2006) that the search for effective enzymes for broiler diets based on non-viscous cereal grains including maize and sorghum is ongoing assumes relevance.

Enzyme A (1750 units/kg feed) and Enzyme P (2000 units/kg feed) provided similar levels of xylanase activity; therefore, it is not surprising that responses in nutrient utilization and growth performance to both enzymes in wheat-based diets were comparable. For example, Enzyme A significantly improved feed efficiency by 7.0% and from 1-42 days post-hatch and increased AME by 0.98 MJ in the finisher phase. Similarly, Enzyme P improved feed efficiency by 7.1% and energy utilization by 1.21 MJ/kg. It is well-established that the inclusion of NSP-degrading, xylanases in wheat-based diets can enhance broiler performance, particularly when the

wheat is of poor quality ('low-ME' wheat). Typically, wheat contains 114 g/kg NSP, which is predominantly arabinoxylan and 21% of wheat NSP is soluble (Choct, 2006). As reviewed by Cowieson *et al*. (2006), it is accepted that xylanases have two prime modes of action. One is that increased gut viscosity triggered by soluble NSP is counteracted by xylanase which facilitates digestive and absorptive processes in the gut. The second is that by degrading insoluble NSP, xylanase disrupts the integrity of cell walls thereby permitting the release of 'caged' nutrients.

Sorghum is a 'non-viscous' grain as it contains 48 g/kg NSP, including arabinoxylan, but only 4% of sorghum NSP is soluble (Choct, 2006). Moreover, the disruption of insoluble NSP in sorghum endosperm cell walls by NSP-degrading enzymes is considered to be limited, which is attributed to the extent of arabinose substitution and high levels of glucuronic acid in sorghum arabinoxylan (Taylor, 2005). In the present study Enzyme P, with essentially only xylanase activity, did not generate tangible responses in sorghum-based diets.

The dominant protein fraction in sorghum is kafirin, which is a relatively poor source of digestible amino acids due to disulphide cross-linkages and low solubility. As reviewed by Selle *et al*. (2010a), approximately 50% of sorghum protein consists of kafirin which is located in protein bodies in sorghum endosperm where it is intimately associated with starch granules. The quantification of kafirin concentrations in sorghum is not straightforward. However, from the relationship between kafirin and total protein levels in sorghum established by Taylor *et al*. (1984) it may be estimated that the 92 g/kg protein sorghum used in this study contained 41.2 g/kg kafirin. Both Taylor (2005) and Black *et al*. (2005) have suggested that the inclusion of proteases with the capacity to degrade kafirin in sorghum-based poultry diets may be beneficial.

An enzyme combination (Avizyme® 1500) with protease (4000 units per kg) plus "-amylase and xylanase activities has been evaluated in sorghum-based broiler diets. As reported by Cadogan *et al*. (2005), this combination significantly increased ($p<0.05$) weight gain by 3.7% and feed intake by 4.9% from 1-21 days but significant responses were not observed from 22-42 days post-hatch. In an earlier study, the enzyme combination improved feed efficiency by 6.5% (1.72 versus 1.84; $p = 0.01$) and tended to increase weight gain by 5.7% (2255 versus 2133 g/bird; $p = 0.07$) in male broilers offered sorghum-based diets from 1-42 days post-hatch (Pack *et al*., 1998).

In the present study, Enzyme A contained 560 units per kg protease in addition to xylanase and ß-glucanase activities and this combination increased weight gain by 6.7% ($p<0.06$) from 1-42 days post-hatch. This raises the possibility that the protease component, subtilisin, may have contributed towards this positive response in sorghum-based diets. Subtilisins, derived from *Bacillus subtilis*, have the capacity to hydrolyze keratin (Evans *et al*., 2000; Kim *et al*., 2001; Kim *et al*., 2004). Keratin is a highly indigestible protein source because of its insolubility and extensive disulphide cross-linkages and, intuitively, a protease with the capacity to degrade keratin would have the potential to degrade kafirin in the avian gastrointestinal tract.

Mahagna *et al*. (1995) evaluated the addition of exogenous protease (derived from *Bacillus subtilis*) and amylase in sorghum-based broiler diets. At the higher inclusion level, this enzyme preparation slightly, but consistently, improved the total tract digestibility of amino acids. However, the most pronounced response of approximately 4.0% was recorded for cystine and both beta-kafirin and gamma-kafirin are relatively rich in cystine (Shull *et al*., 1992). Thus the cystine response may indicate that subtilisin degraded and reduced the extent of disulphide cross-linkages in kafirin and that this was the genesis of the enhanced digestibility of amino acids.

Of relevance to the present study is that the protease component of Enzyme A (subtilisin *per se*) has been shown to degrade kafirin *in vitro* via protein gel electrophoresis procedures (Finn, 2008). Moreover, Sultan *et al*. (2010) reported that this subtilisin significantly increased apparent ileal crude protein digestibility by 4.5% (0.815 versus 0.780) and AME by 0.74 MJ (14.81 versus 14.07 MJ/kg DM) in 42-day old broilers.

The nutritive value of sorghum is vulnerable to wet-cooking or 'moist-heat', as this increases the extent of disulphide cross-linkages in kafirin (Duodu *et al*., 2003). This vulnerability has been mainly demonstrated in *in vitro* pepsin digestibility assays. However, in broilers, exposure of sorghum to moist-heat has been shown to reduce true ileal amino acid digestibility to a substantial extent (Mitaru *et al*., 1985) and significantly depress growth performance and N retention in young broilers (Selle *et al*., 2010b). As implied by Taylor (2005), there is the possibility that steam-pelleting sorghum-based diets at high temperatures may constitute sufficient 'moist-heat' to compromise the nutritive value of sorghum. In the present study the diets were fed as mash; however, it may prove instructive to determine the effects of graded inclusion levels of subtilisin *per se* in steam-pelleted, sorghum-based broiler diets.

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