Estimation of standardised ileal threonine equivalency values of a multi-enzyme and its effects on broiler chick’s performance

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Abstract

An experiment was conducted to estimate standardised ileal threonine (SID Thr) equivalency of a commercial multi-enzyme and its effects on broiler chick’s performance. The 10 treatments included a basal diet containing 0.56% and 0.46% SID Thr in starter and grower periods; treatments 2 to 5 were supplemented with graded levels (0.07%, 0.14%, 0.21% and 0.28%) of L-threonine and treatments 6 to 10 were basal diet supplemented with 0.1, 0.2, 0.3, 0.4 and 0.5 g/kg of multi-enzyme. Supplementing diets with multi-enzyme significantly (P<0.01) improved the feed conversion ratio at 28 and 42 d of age. Multi-enzyme addition linearly increased body weight gain (P<0.05) at 28 d of age and the immune response (P<0.05) at 36 d of age. Breast, thigh and abdominal fat relative weights were not affected by supplementing the multi-enzyme. Nonlinear and linear equations were generated for the multi-enzyme and graded levels of threonine. Based on an assessment for the r² and P values of the equation, body weight gain, immune response and feed conversion ratio were sensitive measurements of responses to multi-enzyme addition. The nonlinear or linear response equations to the higher r² values for added L-threonine and the equation for added multi-enzyme were set to be equal and were solved. The body weight gain and feed conversion ratio responses from 0.5 g multi-enzyme per kg of diet at 28 and 42 d of age was therefore equal to 0.08%, 0.05%, 0.16% and 0.07% SID Thr, respectively, and the immune response from 0.5 g multi-enzyme per kg of diet was equal to 0.14% SID Thr.

Introduction

Feed ingredients of plant origin contain a number of components that cannot be digested by monogastric species because of the lack of or insufficiency of endogenous enzyme secretion. In addition to being unavailable to the animal, these components also lower the utilisation of other dietary nutrients, leading to depressed performance. Examples of such anti-nutritive components include pentosans in wheat, β-glucans in barley, and phytic acid, which is found in all plant feed ingredients. Over the past two decades, the use of enzymes in animal feeds has received much attention by the animal industry. Early studies indicated that the inclusion of supplemental proteases, α-amylases, β-glucanases, and mixed enzyme might have a positive influence on animal growth (Merstad and McNab, 1975; Pettersson and Aman, 1989). Recently, enzymes including endoxylanases and endomannanases have produced significant improvements in growth performance of poultry when supplemented to diets high in wheat and soybean meal (Odetallah, 2000; Odetallah et al., 2002). The inclusion of exogenous xylanases (xylanases and glucanases) to improve the performance of broiler chickens fed diets based on wheat and barley has become routine practice. The relevance of phytate-protein complexes in lowering protein utilisation in monogastric animals and the potential of microbial phytase phytase alone and together with other exogenous enzymes to release the phytate-bound protein has attracted considerable attention in recent years (Kies et al., 2001). Although the P-equivalency values for microbial phytase are well established (Colelho and Kornegay, 1996), corresponding data on amino acid release with added phytase and multi-enzymes are limited. The lysine equivalency of phytase has been determined (Ravindran et al., 2001) but the other amino acid effect of phytase alone or together with other exogenous enzyme is of considerable practical significance and needs to be quantified to enable its inclusion in least-cost diet formulation. Studies with complete diets have shown that methionine is generally the least and threonine the most responsive amino acid to phytase supplementation. Across these studies, phytase increased methionine digestibility by an average of 0.9% and threonine by 4% (Ravindran et al., 1999).

In our study, the effects of adding a multi-enzyme or threonine on the performance of broiler fed diet that is adequate in all amino acids but deficient in threonine were investigated. Our aim was to generate equations for performance responses obtained by supplementing the broiler diet with an enzyme (Natuzyme containing phytase: 1500 U/g, β-glucanase: 700 U/g, α-amylase: 700 U/g, cellulase: 6,000 U/g, pectinase: 700 U/g, xylanase: 10,000 U/g, lipase: 30 U/g and protease: 3,000 U/g) and threonine and to use these diets to calculate the equivalency values of multi-enzyme for threonine.

Materials and methods

One-day-old male broiler chicks were obtained from a commercial hatchery and were randomly allotted to 40 pens (seven birds per pen) in electrically heated, raised battery cages in an environmentally controlled room. Each dietary treatment was randomly assigned to four pens of seven chicks each. The diets, in mash form, were fed during the experimental period. Feed and water were available ad libitum. Ten experimental diets containing varying levels of standardised ileal threonine (SID Thr) or multi-enzyme were formulated. A diet contains 0.56% and 0.46% SID Thr in starter (0 to 28 d) and grower (28 to 42 d) periods, served as the basal diet (diet 1). The composition of the basal diet is shown in Table 1. These diets meet or exceed the recommended standardised ileal digestible amino acid levels for broilers based on optimum dietary lysine content and the ideal protein concepts for all amino acids, except threonine, similarly to those employed by other studies to calculate nutrient equivalency of enzymes (Ravindran et al.,
Diets containing 0.63%, 0.70%, 0.77% and 0.84% SID Thr levels in starter periods (diets 2 to 5) and diets containing 0.53%, 0.6%, 0.67% and 0.74% SID Thr in grower periods (diets 2 to 5) were formulated by addition of L-threonine to diet 1. Diet 1 was supplemented with 0.1, 0.2, 0.3, 0.4 and 0.5 g multi-enzyme per kg of diet, resulting in diets 6 to 10. To formulate the diets, total crude protein and amino acids of feed ingredients were multiplied to standardised ileal crude protein and amino acid digestibility coefficients of feedstuffs for broilers, which have been determined by Hoehler et al. (2006) in Degussa Corporation.

Effect of threonine

The influence of dietary treatments on broiler performance is summarised in Table 2. Addition of graded levels of L-threonine to the basal diet had no effect on body weight gain at 28 d of age but increased body weight gain at 42 d of age (quadratic effect, P < 0.05). The feed conversion ratio was not influenced by the addition of SID threonine. The immune response to PHA-P increased linearly and quadratically (P < 0.009) to increasing levels of SID Thr at 36 d of age. The absence of a significant influence of added graded levels of synthetic threonine on feed conversion ratio in the overall period and body weight gain at 28 d of age may be a result of the high endogenous losses of this amino acid in the diets containing wheat and barley. On the other hand, the requirements for threonine to body weight gain and feed conversion ratio are lower than that of the immune response and, for this reason, increasing SID Thr in contrast to the basal diet did not influence feed conversion ratio and body weight gain.

Effect of enzyme

The response in weight gain to the addition of graded levels of multi-enzyme reached a plateau at 0.3 g multi-enzyme per kg of diet at 28 d of age. The feed conversion ratio decreased quadratically (P < 0.05) at the 28 and 42 d of age as multi-enzyme supplementation increased. The immune response to PHA-P increased linearly and quadratically (P < 0.009) to increasing levels of multi-enzyme supplementation at 36 d of age. Zaghari et al. (2008) determined the crude protein equivalency of this multi-enzyme. Their study showed that the multi-enzyme can improve protein utilisation. Many studies with complete diets have shown that methionine is generally the least and threonine the most responsive amino acid to phytase supplementation. Across the studies, phytase increased methionine digestibility by an average of 0.9% and threonine by 4%. The studies indicated conclusively that wheat is more responsive to multi-enzyme supplementation than maize in terms of amino acid digestibility; for this reason we used wheat and barley in experimental diets.

The simultaneous inclusion of phytase and various exogenous enzymes in wheat-based diets has been shown to generate synergistic increases in digestibility of some amino acids (Ravindran et al., 1999; Selle et al., 2003). It is possible that the combination of phytase and various exogenous enzymes may be similarly advantageous for other feed ingredients. Ravindran et al. (1999) suggested that phytate may exacerbate endogenous amino acid losses and Cowieson et al. (2004) provided support for this suggestion, as they reported that phytate significantly increased the excretion of total endogenous amino acids in the broiler by 28% and phytase tended to reduce this loss by

| Ingredient | Basal Diet 1 | Basal Diet 2 |
|------------|-------------|-------------|
| Soybean meal, % | 16.88 | 8.75 |
| Corn gluten meal, % | 8.28 | 39.91 |
| Soybean meal, % | 43.99 | 8.75 |
| Wheat, % | 15 | 25 |
| Dicalcium phosphate, % | 2.27 | 2.05 |
| NaHCO₃, % | 0.63 | 0.49 |
| Oyster shell, % | 1.2 | 1.1 |
| Vitamin-trace mineral, % | 0.5 | 0.5 |
| DL-Methionine, % | 0.25 | 0.16 |
| L-Lysine HCl, % | 0.68 | 0.59 |
| L-Arginine, % | 0.32 | 0.3 |
| Glutamic acid, % | - | 1.7 |
| AME, kcal/kg | 2900 | 3000 |

Table 1. Composition of basal diet.

| Ingredients | Starter (28 d) | Grower (42 d) |
|------------|-------------|-------------|
| Soybean meal, % | 16.88 | 8.75 |
| Corn gluten meal, % | 8.28 | 39.91 |
| Soybean meal, % | 43.99 | 8.75 |
| Wheat, % | 15 | 25 |
| Dicalcium phosphate, % | 2.27 | 2.05 |
| NaHCO₃, % | 0.63 | 0.49 |
| Oyster shell, % | 1.2 | 1.1 |
| Vitamin-trace mineral, % | 0.5 | 0.5 |
| DL-Methionine, % | 0.25 | 0.16 |
| L-Lysine HCl, % | 0.68 | 0.59 |
| L-Arginine, % | 0.32 | 0.3 |
| Glutamic acid, % | - | 1.7 |
| AME, kcal/kg | 2900 | 3000 |

SID, Standardised ileal digestibility. *Vitamin and mineral premix supplied the following per kilogram of diet: vitamin A, 11,000 U; vitamin D₃, 1800 U; vitamin E, 11 mg; vitamin K₃, 2 mg; vitamin B₁₂, 5 µg; vitamin B₆, 2 mg; vitamin B₂, 0.024 mg; niacin acid, 28 mg; folic acid, 0.5 mg; pantothenic acid, 12 mg; choline chloride, 250 mg; Mn, 100 mg; Zn, 65 mg; Cu, 5 mg; Se, 0.22 mg; I, 0.5 mg; Co, 0.5 mg.
20%. The increased losses of amino acids generated by phytate were attributed to phytate stimulating secretion of gastrointestinal mucoproteins, especially mucin. Mucin is reduced by threonine, therefore use of exogenous enzymes reduces the secretion of mucin and threonine losses, and increased threonine digestibility.

The multifaceted effects of multi-enzymes in practical diets are being appreciated increasingly and it is possible that the observed performance responses may reflect the release of P-available amino acids, and decrease secretion of gastrointestinal mucoproteins or energy by added multi-enzyme. In the case of multi-enzyme in our experiment, improvements in body weight gain, feed conversion ratio and immune response may be a result of the release of threonine from anti-nutrient protein complexes or a reduction in the secretion of gastrointestinal mucoproteins, so in this way, multi-enzyme has reduced the gastrointestinal maintenance requirements and resulted in performance improvements. On the other hand, the experimental diets had the same ME and met or exceeded the recommended standardised ileal digestible amino acid levels for broilers, based on optimum dietary lysine content and the ideal protein concepts for all amino acids, except threonine, and are similar to those employed by other studies (Ravindran et al., 2001; Yi et al., 1996; Zaghari et al., 2008). Thus, performance improvement is assumed to be the result of improved threonine availability.

**Calculation of standardised ileal equivalency of multi-enzyme**

Our study was designed to estimate the SID Thr equivalence values of a multi-enzyme based on the premise that the addition of various exogenous enzymes simultaneously to a SID threonine-deficient basal diet will release threonine from anti-nutrient protein complexes and decrease endogenous losses of threonine, therefore result in performance improvements in broilers. Standardised ileal threonine equivalency values of multi-enzyme were calculated using equations for body weight gain, feed conversion ratio and immune response (Table 3). This data set was used to estimate SID Thr equivalency values for multi-enzyme by using the procedure described by Yi et al. (1996). Linear and nonlinear functions that gave the best fit to the data set (weight gain, feed conversion ratio and immune response) were derived for SID Thr and multi-enzyme (Table 3). The nonlinear or linear response equations with the higher $r^2$ values for SID Thr and the equation for multi-enzyme were then set to be equal and were solved. Based on the equations obtained for weight gain ($y$) response to graded levels of dietary SID Thr and added multi-enzyme, and by setting these equations for SID Thr and multi-enzyme to be equal and solving them, the level of SID Thr equivalents to 0.5 g multi-enzyme per kg of diet addition were 0.64% and 0.51% at 28 and 42 d, respectively.

### Table 2. Effects of multi-enzyme supplementation and standardised ileal threonine on body weight gain, feed conversion ratio and immune response of broiler.

| Trait             | Enzyme, g/kg | Body weight gain, g | Feed conversion ratio, g/g | Immune response to PAH-P, mm |
|-------------------|--------------|---------------------|---------------------------|------------------------------|
|                   | (0 to 28 d)  | (28 to 42 d)        | 28 d                      | 42 d                        | 28 d                      | 42 d                      | 36 d                      |
|                   | 0.56         | 0.46                | 0                         | 1238.73                     | 2273.63                  | 1.56                       | 1.74                       | 0.965^d                   |
|                   | 0.63         | 0.53                | 0                         | 1336.02                     | 2312.11^ab               | 1.61                       | 1.75                       | 1.137^cd                  |
|                   | 0.70         | 0.60                | 0                         | 1302.15                     | 2414.20^ab               | 1.54                       | 1.73                       | 1.375^bc                  |
|                   | 0.77         | 0.67                | 0                         | 1300.36                     | 2437.59^a                | 1.54                       | 1.74                       | 1.703^ab                  |
|                   | 0.84         | 0.74                | 0                         | 1243.79                     | 2430.71^ab               | 1.57                       | 1.73                       | 1.778^a                   |
| Mean              | 1264.21      | 2355.70             | 1.56                      | 1.74                       | 1.390                    |
| SE                | 13.50        | 23.11               | 0.01                      | 0.01                        | 0.086                    |
| P                 | 0.20         | 0.04                | 0.28                      | 0.90                        | 0.001                    |
|                   | 0.56         | 0.46                | 0.1                       | 1217.50^c                   | 2303.30                  | 1.58^a                     | 1.75^b                     | 0.875^c                   |
|                   | 0.56         | 0.46                | 0.2                       | 1225.60^f                   | 2266.76                  | 1.62^a                     | 1.81^b                     | 1.120^c                   |
|                   | 0.56         | 0.46                | 0.3                       | 1268.70^j                   | 2327.32                  | 1.53^b                     | 1.74^b                     | 1.253^bc                  |
|                   | 0.56         | 0.46                | 0.4                       | 1258.60^ab                  | 2339.46                  | 1.55^b                     | 1.74^b                     | 1.025^bc                  |
|                   | 0.56         | 0.46                | 0.5                       | 1250.60^abc                 | 2323.01                  | 1.54^b                     | 1.72^b                     | 1.527^a                   |
| Mean              | 1244.10      | 2311.90             | 1.57                      | 1.75                       | 1.160                    |
| SE                | 6.70         | 11.67               | 0.01                      | 0.01                        | 0.067                    |
| P                 | 0.02         | 0.20                | 0.03                      | 0.04                        | 0.009                    |

SID, standardised ileal threonine. ^Means within a column with different superscripts differ (P<0.05), (P<0.001).

### Table 3. Regression equation and estimated standardised ileal threonine equivalency values of multi-enzyme.

| Trait             | Regression equation between responses and SID Thr | $r^2$ | Regression equation between responses and multi-enzyme | $r^2$ | Estimated SID Thr equivalency of 0.1 g multi-enzyme, % |
|-------------------|--------------------------------------------------|------|------------------------------------------------------|------|-----------------------------------------------------|
| Body weight       | 28 d $Y=-30036.4x^3+60516.5x^2-39962.3x+912.5$ | 0.84 | $Y=-2738.3x^3+1865.2x^2-187.4x-1218.4$ | 0.39 | 0.016                                                |
|                   | 42 d $Y=-5093.4x^3+6483x^2+349$                  | 0.31 | $Y=-57.61x^2+146.5x+2274.4$                          | 0.19 | 0.010                                                |
| Feed conversion ratio | 28 d $Y=34.5x^2-71.81x^2+49.16x-9.5$            | 0.31 | $Y=7.07x^2-6.36x^2+1.513x+1.493$                     | 0.42 | 0.032                                                |
|                   | 42 d $Y=-0.6655x^3+1.198x^2-0.74x+1.896$        | 0.15 | $Y=9.076x^2-8.68x^2+2.33x+1.6$                       | 0.40 | 0.014                                                |
| Immune response   | 36 d $Y=-69.349x^3+123.3x^2-68.173x+13$         | 0.73 | $Y=70.20x^3-62.08x^2+17.11x-0.305$                   | 0.51 | 0.028                                                |
42 d of age, respectively. Released SID Thr was calculated by subtracting obtained values from SID Thr content of the basal diet. Therefore, the gain response from 0.5 g multi-enzyme per kg of diet was equal to 0.08% and 0.05% SID Thr at 28 and 42 d of age, respectively. Similarly for the feed conversion ratio, the level of SID Thr equivalent to 0.5 g multi-enzyme per kg of diet addition was 0.72% and 0.53% at 28 and 42 d of age, respectively; thus, the feed conversion ratio response from 0.5 g multi-enzyme per kg of diet was equal to 0.16% and 0.07% SID Thr at 28 and 42 d of age, respectively. Based on the immune response, the level of SID Thr equivalent to 0.5 g multi-enzyme per kg of diet addition was 0.6%; therefore, the immune response from 0.5 g multi-enzyme per kg of diet was equal to 0.14% SID Thr. Breast, thigh and abdominal fat were not affected by supplementing the multi-enzyme; thus, these data were not used to calculate equivalency.

Mortality during the experimental period was within acceptable levels (less than 2%) and was not related to dietary treatments. The average of the three estimates was 0.1% for 0.5 g of enzyme and the addition of the 0.5 g multi-enzyme to the SID Thr-deficient diet was calculated to be equivalent to 0.016%, 0.01%, 0.032%, 0.014% and 0.028% SID Thr, as measured by responses in body weight gain, feed conversion ratio and immune response. Based on the assumption that the observed response in weight gain, feed conversion ratio and immune response to added multienzyme were a result of the release of threonine alone, Denbow et al. (1995), Harper et al. (1997) and Ravindran et al. (2001) used $r^2$ as the criterion for selecting either linear and nonlinear regression models to describe the changes in certain response criteria to graded levels of phytase and supplemental inorganic phosphorus. The $r^2$ values of linear and nonlinear models generated from the growth performance data in this experiment were lower than those observed in the literature (Adedokun et al., 2004; Denbow et al., 1995; Ravindran et al., 2001; Yi et al., 1996). The differences may be attributed to the duration of study and age of birds. Denbow et al. (1995), Yi et al. (1996) and Ravindran et al. (2001) conducted 21-d trials, whereas our trial was 42 d in duration. Adedokun et al. (2004) used only the linear regression equation and obtained higher $r^2$ values using phytase. However, the duration of their study was 14 d after allowing ad libitum access to a standard P-adequate starter diet for seven days. In our study, we had four more weeks for variance to increase as the birds grew. The data of our experiment were in agreement with that of Jendza et al. (2006), which showed $r^2$ values for the performance prediction equations generated from the weanling pig and broiler growth performance data at the older ages to be lower than those at younger ages.

**Conclusions**

Results of this experiment showed that addition of 0.5 g of this multi-enzyme released, on average, 0.1% threonine from anti-nutrient protein complexes.

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