Effect of a multienzyme complex in wheat-soybean meal diet on digestibility of broiler chickens under different rearing conditions

Josefa Madrid, Pablo Catalá-Gregori, Victoria García, Fuensanta Hernández
Departamento de Producción Animal, Universidad de Murcia, Spain

Abstract

This study investigated the effects of a multienzyme complex containing protease and carbohydrase enzymes on the performance and nutrient digestibility of broiler chickens under different rearing conditions from 1 to 42 d of age. Two experimental starter and finisher wheat-soybean meal based diets were used in a two-phase feeding programme, as follows: control diet (C); control diet plus 0.05% multienzyme complex of protease and carbohydrase enzymes (Vegpro) (VP). At location 1, broilers were raised in battery cages located in an experimental pen, without contact with litter. At location 2, broilers were raised in floor pens in a commercial farm, and had contact with litter. At location 3, broilers were raised in commercial farms. In general, no differences in growth performance, feed intake or feed conversion ratio were observed in broiler chickens fed on the different diets at location 1. Only, the average daily feed intake from 22 to 42 d was significantly higher in the VP group compared with the C treatment (P<0.01). In addition, the final body weight of the VP group was numerically greater than that of the birds fed with the control diet, although the inclusion of the multienzyme complex did not cause significant effects. However, the ileal digestibilities of dry matter and crude protein were enhanced (P<0.05) by VP in the case of broilers in floor pens (+2.5% and +2.7% for DM and CP, respectively) and in the commercial farm (+4.4% and +6% for the DM and CP, respectively) at 42 d of age, as well as faecal digestibility of the VP diet in experimental pen. It seemed that the effectiveness of the multienzyme complex on digestibility was higher when conditions of hygiene were poorer and stress was frequent. Based on the results of this study it can be concluded that the multienzyme complex of protease and carbohydrase enzymes might be effective for improving nutrient digestibility in broilers fed with a wheat-soybean meal based diet under commercial farm conditions.

Materials and methods

Experimental diets

The feeding program consisted of a starter diet until 21 days of age and a finisher diet until 42 days of age. In each period, two treatments were compared: control diet (C) and the same diet supplemented (VP) with 0.05% of a multienzyme complex (Vegpro, Alltech Inc.), a blend of proteases, amylases, cellulases and other residual enzyme activities (α-galactosidases and xylanases), produced by Aspergillus niger, Trichoderma viride and Aspergillus oryzae. All diets for each period were prepared with the same batch of ingredients. The diets were formulated to meet or exceed recommendations set out by the National Research Council (1994) for broiler chickens for each dietary period. In addition, all the diets included Diasol (Adivetor, Barcelona, Spain) as an acid-insoluble ash digestibility marker.

Introduction

The cell wall components of the cereals and protein-rich vegetable ingredients often used in poultry diets are poorly digested. The enzymatic breakdown of the non-starch polysaccharides (NSP) present in the cell wall (mainly arabinoxylans and β-glucans) is known to decrease the viscosity in the gastrointestinal tract (Bedford, 1995) and to increase ileal crude protein digestibility (Marsman et al., 1997). Soybean meal is the main protein-rich ingredient used in poultry diets. In early stages of life of chicks, the rapid food passage rate and the deficiency of innate enzymes could lead to a lower digestibility of the protein (Uni et al., 1999) which is required in high amounts for the young growing birds. However, industry has not been able to produce commercially viable products that consistently improve the digestibility of vegetable proteins (Choct, 2006).

Also, certain markets, such as Spain, prefer white carcasses rather than yellow ones, so the main cereal used for broilers is wheat instead of corn. The protein content of wheat is higher than that of corn (14.1 and 8.5%, respectively; National Research Council, 1994), as is NSP content (11.4 and 8.1%, respectively; Choct, 1997). In addition, despite the low protein content in cereals, the inclusion of a high percentage of cereal in diets provides the major part of the protein in the diet (Odelliteh et al., 2005). Therefore, the effectiveness of proteases and carbohydrases on broilers fed with wheat-soybean meal based diets could be higher than that with corn-soybean meal based diets. Vegpro is a multienzyme complex (Alltech Inc., Nicholasville, KY, USA) that has the potential to improve the nutritional value of protein-rich vegetable feed ingredients (Schutte and Pereira, 1998). However, few studies concerning this multienzyme complex generally show inconsistent results as regards broiler performance and nutrient digestibility (Schang et al., 1997; Ebert et al., 2000; García et al., 2000; Fischer et al., 2002). Such studies were conducted with broilers fed with corn-soybean based diets and did not consider different rearing conditions. However, it is known that differences in environmental conditions (e.g. housing) could affect the response of broilers to enzymes (particularly, the microbial challenge of being in contact with litter and the stress status of farm conditions).

Thus, the objective of this research was to evaluate the effect of a multienzyme complex of protease and carbohydrate enzymes on nutrient digestibility in broilers raised under different experimental conditions.
ingredients and composition of the control diet are presented in Table 1. The starter feed was offered in crumbles and the finisher diet was steam pelleted using a 4 mm die.

**Animal and experimental design**

The study was simultaneously conducted in three different locations with Ross 308 broiler chickens from a commercial hatchery up to 42 d of age. In location 1, one hundred and twenty male one-day-old broiler chickens were homogeneously distributed according to mean weight into laboratory cages (experimental pen). Groups of 10 broilers were placed in twelve 70x50 cm cages (6 replicates per treatment in a completely randomized design) with a 1x1 cm wire mesh bottom. All broilers were weighed at 1, 21 and 42 d of age. Feed intake was monitored weekly and weight gain was recorded at 21 and 42 d of age, so that the feed conversion ratio was calculated from 1 to 21 d and from 22 to 42 d on a cage weight basis. In location 2, two hundred and forty male one-day-old broiler chickens were homogeneously distributed by mean weight into sixteen 1x1 m floor pens (15 broilers per pen) built in a commercial farm. This provided eight replicates per treatment in a completely randomized design. The floor pens had wood shavings for litter.

In location 3, ninety-three thousand broiler chickens of both sexes were placed in four commercial farms. Two farms containing 21,000 broilers each were assigned to treatment VP and the other two farms containing 21,000 and 30,000 broilers, respectively, were assigned to control group. Farm was considered as the experimental unit.

Feed and water were provided ad libitum at the three locations. The temperature was controlled and gradually reduced from 32°C for the first 5 d of life to 20°C on d 40. The lighting cycle was 24 h/d from day 1 to 3, 18 h/d from day 4 to 20, 21 h/d from day 21 to 35 and 23 h/d from day 36 to 42. The animal care and experimental procedures used in this study conformed to regulations and guidelines from BOE (2005).

**Experimental and analytical procedure**

At the end of each experimental feeding period (d 21 and 42), excreta from each cage at laboratory location were collected for faecal apparent digestibility determination. In addition, samples of feed and ileal digesta were collected in each location at 21 and 42 days of age. Five chickens from each replicate in locations 1 (thirty per treatment) and 2 (forty per treatment), and twenty male chickens from each farm in location 3 (forty per treatment) were sacrificed by cervical dislocation and the small intestine was immediately exposed. In location 3, there were animals of both sexes, so the number of slaughtered animals was higher in order to select only male broilers to obtain the sample of ileal digesta. The number of animals was limited for animal welfare reasons. The ileum was defined as that segment between the Meckel’s diverticulum and a point 40 mm proximal to the ileocecal junction. The contents of the ileum were collected into plastic containers, stored at -20°C, lyophilized and ground to pass through a 0.5 mm mesh. Excreta samples were weighed and dried at 80°C until constant weight in a forced-air oven and then ground to pass through a 1 mm sieve.

Feed, excreta, and ileal digesta were analyzed for N content using the Kjeldahl method (AOAC, 1990), crude protein (CP) (N×6.25), and acid insoluble ash (AIA) (Vogtmann et al., 1975). All values were expressed on a dry matter (DM) basis. Coefficients of faecal or ileal apparent digestibility of DM and CP were calculated using AIA as an indigestible marker.

**Statistical analysis**

Data from growth performance, daily feed intake, feed conversion ratio and faecal or ileal apparent digestibilities were analysed by Student’s t-test using SPSS (1997) software. All statements of significance were based on a probability of less than 0.05.

**Results and discussion**

Table 2 presents the performance from 1 to 21 and 22 to 42 d of age at location 1 (laboratory cages). In general, there were no differences (P>0.05) in body weight, weight gain, feed intake or feed conversion among treatments. Only the average daily feed intake from 22 to 42

---

**Table 1. Composition of experimental diets.**

| Protein Source | Starter (1 to 21 d) | Finisher (22 to 42 d) |
|----------------|---------------------|-----------------------|
| Ingredients, % |                     |                       |
| Wheat          | 33.18               | 49.85                 |
| Soybean meal   | 33.45               | 27.30                 |
| Corn           | 20.0                | 10.0                  |
| Fish meal      | 4.01                | 1.87                  |
| Fat            | 4.48                | 6.35                  |
| Diaisol*       | 1.0                 | 1.0                   |
| Calcium carbonate | 0.83               | 0.87                  |
| Calcium phosphate | 1.34               | 1.36                  |
| Sodium bicarbonate | 0.1               | 0.3                   |
| Sodium chloride | 0.26                | 0.12                  |
| DL-methionine  | 0.34                | 0.3                   |
| L-Lysine       | 0.3                 | 0.31                  |
| L-Threonine    | 0.03                | 0.03                  |
| Choline chloride | 0.09               | 0.07                  |
| Vitamin premix | 0.05                | 0.03                  |
| Mineral premix | 0.1                 | 0.1                   |

| Calculated composition, %<sup>§</sup> |                     |                       |
|----------------------------------------|---------------------|-----------------------|
| ME, MJ/kg                              | 11.93               | 12.56                 |
| Crude protein (N×6.25)                 | 23.87               | 20.82                 |
| Lysine                                 | 1.45                | 1.22                  |
| Methionine + cystine                   | 1.09                | 0.95                  |
| Threonine                              | 0.92                | 0.79                  |
| Ca                                     | 1.00                | 0.90                  |
| Total P                                | 0.62                | 0.73                  |
| Available P                            | 0.50                | 0.45                  |

<sup>1</sup>Acid-insoluble ash digestibility marker. <sup>2</sup>Supplied per kilogram of Starter diet: vitamin A (retinyl acetate), 15,000 U; vitamin D₃, 5000 U; vitamin E (DL-α-tocopheryl acetate), 80 mg; vitamin K, 5 mg; thiamin, 3 mg; riboflavin, 10 mg; pyridoxine, 5 mg; vitamin B₁₂, 0.02 mg; niacin, 70 mg; folic acid, 2 mg; biotin, 0.4 mg; pantothenic acid, 20 mg; Finisher diet: vitamin A (retinyl acetate), 9000 U; vitamin D₃, 3000 U; vitamin E (DL-α-tocopheryl acetate), 40 mg; vitamin K, 3 mg; thiamin, 1.8 mg; riboflavin, 6 mg; pyridoxine, 5 mg; vitamin B₁₂, 0.012 mg; niacin, 42 mg; folic acid, 1.2 mg; biotin, 0.24 mg; pantothenic acid, 12 mg. <sup>3</sup>Supplied per kilogram of Starter diet: manganese, 120 mg; zinc, 100 mg; iron, 80 mg; copper, 20 mg; iodine, 2 mg; selenium, 0.3 mg; cobalt, 0.2 mg. <sup>4</sup>According to FEDA (FEDINA, Fundación Española para el Desarrollo de la Nutrición Animal, 2003).
d was significantly higher in the VP group (P<0.01), which agrees with other reports concerning enzyme supplementation (Vahjen et al., 2005). In addition, the body weight of broilers from the VP group was numerically greater than that of animals feed with the C diet at 42 d of age, although no significant effect was observed.

In previous studies, some authors showed that Vegpro multienzyme complex did not improve broiler performance in battery cages (Ebert et al., 2000) or in floor pens (Garcia et al., 2000; Fischer et al., 2002) when included in a corn-soybean meal based diet. Others suggested that the effect of Vegpro multienzyme complex on performance seemed to be related to the type of diet, as an improvement of performance was noticed in broilers fed with a corn-soybean meal, full-fat soy and wheat bran based diet but not when broilers were fed a corn-soybean meal diet (Schang et al., 1997) in battery cages. This effect could suggest that the effect of Vegpro multienzyme complex is more noticeable in diets based on ingredients with a high content of the targeted molecules.

In our study, basal diets had a high level of wheat (>30% and >45% of wheat for starter and finisher diets, respectively), and although wheat has a higher CP and NSP content than to corn (National Research Council, 1994; Choct, 1997), no improved growth performance was found with the addition of multienzyme complex. However, C and VP diets were formulated to meet or exceed recommendations by the National Research Council (1994) for broiler chickens for each dietary period. Table 2 also shows the effect of the Vegpro multienzyme complex on nutrient digestibility. In the laboratory location, faecal apparent digestibility of DM and CP were improved by +3.5% (P<0.05) and +11.4% (P<0.001) respectively in the multienzyme complex supplementated group at 42 d of age, whereas ileal apparent digestibilities of DM and CP were enhanced (P<0.05) by VP treatment at 42 d of age. In floor pens (location 2), the increase of ileal apparent digestibilities was +2.5% for the DM and +2.7% for the CP in the VP group.

**Table 2. Effect of Vegpro multienzyme complex on performance and digestibility (faecal and ileal) at location 1 (laboratory cages), and ileal digestibility at location 2 (floor pens) and at location 3 (farms).**

| Item                        | Age | C± | VP | P values $^\$ |
|-----------------------------|-----|----|----|---------------|
| Location 1 (laboratory cages) |     |    |    |               |
| Body weight g               | 21 d| 949±37.3 | 967±30.4 | ns            |
|                             | 42 d| 2931±74  | 3011±89  | ns            |
| Average daily weight gain, g/d| 1-21 d| 43.2±1.77 | 44.1±1.44 | ns            |
|                             | 22-42 d| 94.4±3.72 | 97.3±3.80 | ns            |
| Average daily feed intake, g/g| 1-21 d| 51.4±1.40 | 52.5±0.86 | ns            |
|                             | 22-42 d| 157±3.35  | 165±3.78  | **            |
| Feed conversion ratio, g/g  | 1-21 d| 1.19±0.02 | 1.19±0.03 | ns            |
|                             | 22-42 d| 1.67±0.08 | 1.69±0.05 | ns            |
| Faecal apparent digestibility, % |     |    |    |               |
| Dry matter                  | 21 d| 65.0±3.78 | 66.9±2.52 | ns            |
|                             | 42 d| 71.4±1.86 | 74.9±2.95 | *             |
| Crude protein               | 21 d| 58.1±4.78 | 59.3±2.28 | ns            |
|                             | 42 d| 54.7±3.00 | 66.1±1.65 | ***           |
| Ileal apparent digestibility, % |     |    |    |               |
| Dry matter                  | 21 d| 67.8±1.39 | 66.8±0.71 | ns            |
|                             | 42 d| 71.8±2.44 | 72.1±4.44 | ns            |
| Crude protein               | 21 d| 78.2±1.70 | 76.1±2.72 | ns            |
|                             | 42 d| 78.8±4.40 | 82.0±2.98 | ns            |
| Location 2 (floor pens)°  |     |    |    |               |
| Ileal apparent digestibility, % |     |    |    |               |
| Dry matter                  | 21 d| 70.5±6.29 | 67.4±4.80 | ns            |
|                             | 42 d| 69.8±2.21 | 72.3±1.50 | *             |
| Crude protein               | 21 d| 81.8±4.81 | 81.0±3.61 | ns            |
|                             | 42 d| 77.2±2.83 | 78.9±1.54 | *             |
| Location 3 (farms)#         |     |    |    |               |
| Ileal apparent digestibility, % |     |    |    |               |
| Dry matter                  | 21 d| 63.7±9.86 | 69.0±3.28 | ns            |
|                             | 42 d| 71.6±3.36 | 76.0±0.95 | **            |
| Crude protein               | 21 d| 77.9±6.26 | 82.1±0.92 | ns            |
|                             | 42 d| 76.0±2.37 | 82.0±1.95 | ***           |

$^1$Means represent 6 battery cages. $^2$Means represent 8 floor pens. $^3$Means represent 2 farms. $^4$C: control diet, VP: control diet plus 0.05 % of Vegpro. $^5$Means: standard deviation. $^*$ns: not significant, *P<0.05, **P<0.01, ***P<0.001.
(P<0.05) at 42 d of age. In the same way, the effect of dietary Veggro multienzyme complex on the nutrient digestibility in broilers raised in commercial farms (location 3) showed that VP substantially improved the ileal apparent digestibility of DM (+4.4%) and CP (+6%) compared with C group (P<0.01) at 42 d of age (Table 2). Thus, under farm raising conditions (when conditions of hygiene were poorer and stress was frequent), the beneficial effect of multienzyme complex on ileal apparent digestibility was more noticeable. In addition, it seemed that the Veggro multienzyme complex produced improvements in digestibility at the older age. It has been reported that intestinal enzyme production increases with age (Uni et al., 1998). Probably the higher endogenous intestinal enzyme content of 42 d-old broilers, together with the addition of Veggro multienzyme complex supplementation, resulted in better DM and CP faecal and ileal digestibilities compared with the control group. This could be due to the effect of Veggro, the greater maturity of the intestinal tract at that age, and a greater resident bacteria population that could hydrolyze some substrates. Moreover, the feed passage rate tends to be slower in the older birds (Bedford, 2000), which allows a greater opportunity for enzyme activity of resident bacteria and endogenous digestive secretions.

However, this digestibility improvement was not reflected in a significant increase in performance, since both C and VP groups showed high performances in laboratory conditions, and the diets were formulated with ranges above security requirements. Hernández et al. (2006) indicated that little or no response of some additives can be expected when performance is high; in addition the growth-promoting effect depends on the sanitary conditions of the place where tests are developed, since it seems that any effect is not so evident in good environmental condition.

Accordingly, in a study concerning proteases and carbohydrases added to corn-soybean meal based diets for broilers raised in cages, CP and NSP ileal digestibilities were improved, but broiler performance was not (Marsman et al., 1997). In contrast, the first report concerning Veggro multienzyme complex in broilers (Charlton and Pugh, 1995) raised in battery cages indicated that the addition of this enzyme to wheat-soybean or wheat-barley-soybean based diets increased true metabolizable energy corrected for nitrogen, amino acid digestibility and performance.

The enhancement in digestibility produced by Veggro in this study agrees with the recent results of Sefton and Leeson (2006), who found that the digestibility of soybean meal energy, protein and amino acids, improved by at least 7% when Veggro was added to the diet of 19 to 72 week-old layers (birds with a higher age). Adding exogenous proteases to the diet could improve the nutritional value of soybean meal by degrading its proteins and inactivating potentially anti-nutritional factors (Ghazi et al., 2002; Hong et al., 2002). Most oligosaccharides in soybean meal are α-galactosides which could potentially impair digestibility (Vahjen et al., 2005). The effect of cellulases, xylanases and α-galactosidases could help to hydrolyze cell wall components of soybean meal and wheat, thereby improving their digestibility by increasing the accessibility of proteases and amylases to their substrates (proteins and starch granules). Other authors have suggested that cellulases affect the fat metabolism in broilers by an unknown mechanism (Saleh et al., 2005).

Conclusions

These results indicate that the addition of Veggro multienzyme complex to wheat-soybean meal based diets formulated to exceed nutrient recommendations did not significantly improve performance when birds were kept in ideal conditions. However, the nutrient ileal digestibility in 42 day-old broilers raised in floor pens or on commercial farms was improved by supplementation of the multienzyme complex. It seemed that the effect of this multienzyme complex on digestibility was more consistent under poorer conditions (floor pens or commercial farm).

References

AOAC, 1990. Official Methods of Analysis. 15th ed. Association of Official Analytical Chemists, Washington, DC, USA.

Bedford, M.R., 1995. Mechanism of action and potential environmental benefits from the use of feed enzymes. Anim. Feed Sci. Tech. 53:145-155.

Bedford, M.R., 2000. Exogenous enzymes in monogastric nutrition—their current value and future benefits. Anim. Feed Sci. Tech. 86:1-13.

BOE, 2005. Real Decreto Español 1201/2005, de 10 de octubre, sobre protección de los animales utilizados para experimentación y otros fines científicos. Boletín Oficial del Estado 252:34367-34391.

Charlton, P., Pugh, R., 1995. Expanding enzyme applications: higher amino acid energy values for vegetable proteins. Feed-Compounder 15:30-32 (abstr.).

Choct, M., 1997. Feed non-starch polysaccharides: Chemical structures and nutritional significance. Feed Milling International 191(June issue):13-26.

Choct, M., 2006. Enzymes for the industry: past, present and future. World Poultry Sci. J. 62:5-15.

Ebert, A.R., Kessler, AM., Penz Jr., A.M., Pophal, S., Ribeiro, A.M.L., 2000. Effect of adding Veggro in two energy level diets on the performance of broilers exposed to heat stress. Poultry Sci. 79(S1):19-20 (abstr.).

Fischer, G., Maier, J.C., Rutz, F., Bermudez, V.L., 2002. Desempenho de frangos de corte alimentados com dietas à base de milho e farelo de soja, como u sem adição de enzimas. Rev. Bras. Zootecn. 31:402-410.

Fundación Española para el Desarrollo de la Nutrición Animal, 2003. Tablas FEDNA de composición y valor nutritivo de alimentos para la formulación de pienso compuestos. FEDNA Publ., C. De Blas, G.G. Mateos and P.G. Rebollar eds., Madrid, Spain.

García, E.R.M, Murakami, A.E., Branco, A.F., Furlan, A.C., Moreira, I., 2000. Efeito da suplementação enzimática em rações com farelo de soja e soja integral extrusadas sobre a digestibilidade de nutrientes, o fluxo de nutrientes na digesta ileal e o desempenho de frangos. Rev. Bras. Zootecn. 29:1414-1426.

Ghazi, S., Rooke, J.A., Galbraith, H., Bedford, M.R., 2002. The potential for improvement of the nutritive value of soya-bean meal by different proteases in broiler chicks and broiler cockerels. Brit. Poultry Sci. 43:70-77.

Hernández, F., García, V., Madrid, J., Orenjo, G., Catalá, P., Megiás, M.D., 2006. Effect of formic acid on performance, digestibility, intestinal histomorphology and plasma metabolite levels of broiler chickens. Brit. Poultry Sci. 47:50-56.

Hong, D., Burrows, H., Adeola, O., 2002. Addition of enzyme to starter and grower diets for ducks. Poultry Sci. 81:1842-1849.

Marsman, G.J.P., Gruppen, H., Van der Poel, A.F.B., Kwakkel, R.P., Verstegen, M.W.A., Voragen, A.G.J., 1997. The effect of thermal processing and enzyme treatments of soybean meal on growth performance, ileal nutrient digestibilities, and chyme characteristics in broiler chicks. Poultry Sci. 76:864-872.

National Research Council, 1994. Nutrient Requirements of Poultry. 9th rev. ed. National Academy Press, Washington, DC, USA.

Odetallah, N.H., Wang, J.J., Garlich, J.D., Shih,
J.C.H., 2005. Versazyme supplementation of broiler diets improves market growth performance. Poultry Sci. 84:858-864.
Saleh, F., Ohtsuka, A., Hayashi, K., 2005. Effect of dietary enzymes on the ileal digestibility and abdominal fat content in broilers. Anim. Sci. J. 76:475-478.
Schang, M.J., Azcona, J.O., Arias, J.E., 1997. Effects of a soya enzyme supplement on performance of broilers fed corn/soy or corn/soy/full-fat soy diets. Poultry Sci. 76(Suppl. 1):132(abstr.).
Sefton, A.E., Leeson, S., 2006. Improving soybean meal digestion during the full first cycle of lay. Page 228 (abstr.) in Poultry Science Association Annual Meet., Edmonton, Canada.
Schutte, J.B., Pereira, A.S., 1998. Effect of an enzyme preparation (Vegpro) on broiler chick performance. Zootecnica International 21(5):44-47.
SPSS, 1997. SPSS Base 7.5 for Windows. SPSS, Chicago, IL, USA.
Uni, Z., Ganot, S., Sklan, D., 1998. Posthatch development of mucosal function in the broiler small intestine. Poultry Sci. 77:75-82.
Uni, Z., Noy, Y., Sklan, D., 1999. Posthatch development of small intestinal function in the poult. Poultry Sci. 78:215-222.
Vahjen, W., Busch, T., Simon, O., 2005. Study on the use of soya bean polysaccharide degrading enzymes in broiler nutrition. Anim. Feed Sci. Tech. 120:259-276.
Vogtmann, H., Pfirter H.P., Prabucki, A.L., 1975. A new method of determining metabolisability of energy and digestibility of fatty acids in broiler diets. Brit. Poultry Sci. 16:531-534.