THE NUTRITIVE VALUE OF BLACK GRAM, LUPINS AND SOYBEAN MEAL IN BROILER CHICKENS AS AFFECTED BY ENZYME SUPPLEMENTATION

K.G. Wiryawan[1]

ABSTRACT

Three different enzyme preparations; a xylanase (EA), a multi-enzyme product containing xylanase, α-amylase and protease (EB) and a protease (EP) were assayed according to a precision feeding method for their effects on true metabolizable energy (TME) value, digestibility of protein and digestibility of amino acids of black gram (*Phaseolus mungo*), lupins (*Lupinus angustifolius*) and soybean meal (SBM). The TME values of black gram, lupins and SBM were 9.33, 11.18 and 12.11 MJ kg^{-1} respectively. EA significantly (P<.01) improved the TME value of lupins, but not black gram and SBM. On the other hand, EB increased (P<.05) the TME value of black gram by 9%, but did not improve the TME value of either SBM or lupins. The TME values of the three legumes did not improve by EP supplementation. Overall, enzyme supplementation increased the apparent protein digestibility (APD) by 6%, however only with the multi enzyme product was the increase significant. The true protein digestibility (TPD) values were not significantly improved and in some cases were significantly decreased by enzyme supplementation. The mean apparent amino acid digestibility (AAAD) and true amino acid digestibility (TAAD) coefficients were 77.4 and 85.5% for SBM, 73.7 and 85.3% for lupins and 59.4 and 69.4% for black gram respectively. Both lupins and SBM showed positive responses in amino acid digestibility for all enzymes tested, however, the greatest improvement (P<.05) was observed after addition of protease to SBM and after the addition of xylanase to lupins. This study indicates that the nutritive value of black gram, lupins and SBM can be improved by enzyme supplementation.

Key words : Broiler chickens, Enzyme, Black gram, Lupins, Metabolizable Energy, Digestibility

INTRODUCTION

Low net protein ratio (NPR) of black gram and lupins (Wiryawan and Dingle, 1995) may be associated with a relatively high concentrations of non-starch polysaccharides (NSP) which are resistant to endogenous enzymic digestion in the alimentary tract (Brillouet *et al.*, 1988; Smits and Annison, 1996; Choct, 1996; Choct, 1997). Studies with lupins showed that adding a crude enzyme preparation containing β-galactanase and a minor α-galactosidase (Bryden *et al.*, 1994), or a mixture of hemicellulase, xylanase, pentosanase and cellulase (Annison *et al.*, 1995), to lupin diets increased the apparent metabolisable energy (AME) value by 10.7% and 14% respectively. Application of a carbohydrase to a pea containing diet resulted in a 14% increase in AME value (Jeroch *et al.*, 1995). Black gram and lupins are high in carbohydrate and protein, therefore application of carbohydrases, protease or enzyme mixture containing carbohydrases and protease may improve their nutritive value.

The aim of this study was to determine whether enzyme supplementation would improve the true metabolizable energy (TME) value and the digestibility of protein and amino acids of black gram and lupins, and to compare them with that of SBM, the most commonly used source of plant protein in poultry diets.

[1] Faculty of Animal Husbandry, University of Mataram, Mataram 83125. Indonesia

MATERIALS AND METHODS

Three different enzyme prepara-tions, a xylanase 'Avizyme 1300' (EA), a multi-enzyme product containing xylanase, α amylase and protease 'Avizyme 1500' (EB), and a protease (EP) were assayed according to the modified method of Sibbald (1979), for their effects on the TME value, digestibility of protein and digestibility of amino acids of black gram and lupins. SBM was also assayed for comparison.

Seven-day-old male-broiler chickens were weighed and randomly allocated into individual cages in a temperature-controlled room at 30 ± 0.5°C. They were fed a commercial starter diet for two weeks. After a 24 h fasting period, they were hand-fed 25 - 35g of black gram, lupins or SBM meals in a 1:1 slurry of water: legume meal without or with 1 g kg^{-1} EA, EB or 0.45 g kg^{-1} EP, by using a plastic syringe to place the slurry directly into the crop. A plastic tray under the cage collected excreta for the following 24 h period. The excreta of two birds was pooled, stored at -20°C, freeze-dried, left at room temperature for 24 h, and weighed and ground to pass a 0.5 mm screen for analyses. There were six replicates of two birds per treatment. Because analysis of amino acids is expensive, two replicates of excreta samples were proportionally pooled, so that three samples per treatment were analyzed for amino acids.

The endogenous and metabolic energy and nitrogen used in calculating TME and TPD were obtained from three pooled samples, each collected from two chickens, whilst the amino acid values used in calculation of TAAD was measured from a pooled sample of four chickens as described for the precision-fed birds (Sibbald, 1979). The TME and digestibility were calculated according to the following formulas:

$$TME(MJ/kg) = \frac{EI - FE + EEL}{FI} \quad \text{........ (1)}$$

$$APD(\%) = \frac{PI - PF}{PI} X100 \quad \text{.................... (2)}$$

$$TPD(\%) = \frac{PI - (PF - EPL)}{PI} X100 \quad \text{.. (3)}$$

$$AAAD(\%) = \frac{AAI - AAF}{AAI} X100 \quad \text{...... (4)}$$

$$TAAD(\%) = \frac{AAI - (AAF - EAAL)}{AAI} X100 \quad \text{(5)}$$

where:

EI	= energy intake
FE	= fecal energy
FI	= feed intake in kg
EEL	= endogenous energy lost by unfed birds
PI	= protein intake
PF	= fecal protein
EPL	= endogenous protein loss
AAI	= amino acid intake
AAF	= fecal amino acid and
EAAL	= endogenous amino acid loss

Chemical Analyses. Both legumes were hand-cleaned to remove wastes, then ground to pass a 0.8 mm screen. The gross energy (GE) content of legume meals and fecal samples were determined with a Parr Oxygen Bomb Calorimeter, using benzoic acid as standard and correcting for total acid production by titration with 0.0709 N sodium carbonate solution. Nitrogen (N) contents were analyzed in an automatic nitrogen analyzer using the combustion method (Sweeney, 1989). Crude protein (CP) content was calculated as N x 6.25.

Quantitative analyses of individual amino acids were carried out using HPLC after hydrolysis with 6M HCl for 18 h at 110°C (Cohen *et al.*, 1989). Because cystine and methionine are destroyed by acid hydrolysis, they were oxidized to cysteic acid and methionine sulphone prior to hydrolysis.

Statistical Analysis. The data were analyzed by using the General Linear Model (GLM) procedure of SAS® (SAS Institute, Inc. 1990) for a 3 legume (black gram vs lupin vs SBM) x 4 treatment (control vs EA vs EB vs EP) experimental design (Mead *et al.*, 1993). Treatment means were compared using the Duncan's new multiple range tests.

Table 1. True metabolizable energy (TME) and apparent protein digestibility (APD) and true protein digestibility (TPD) of black gram, lupins and soybean meal in growing chickens.

Legume	Enzyme	TME (MJ kg^{-1})	APD (%)	TPD (%)
Black gram	Control	9.33^{de}	51.0^{ef}	70.9^{d}
	EA	9.12^{e}	48.8^{f}	68.1^{d}
	EB	10.30^{cd}	53.2^{def}	72.4^{d}
	EP	8.56^{e}	39.1^{g}	58.6^{e}
Lupins	Control	11.18^{bc}	54.8^{def}	74.1^{cd}
	EA	12.68^{a}	61.9^{cd}	77.6^{bcd}
	EB	11.95^{ab}	59.6^{cde}	75.6^{cd}
	EP	11.18^{ab}	56.7^{cdef}	75.6^{cd}
SBM	Control	12.11^{ab}	64.7^{bc}	82.6^{abc}
	EA	12.28^{ab}	70.9^{ab}	86.2^{ab}
	EB	12.04^{ab}	72.7^{ab}	87.6^{a}
	EP	11.75^{ab}	76.6^{a}	90.1^{a}
Pooled SEM	-	0.367	2.9	3.1

^{abc}Means in column bearing different superscripts are significantly different (P<.05)
EA = Enzyme A: xylanase (Avizyme 1300)
EB = Enzyme B: multi enzyme (Avizyme 1500)
EP = Enzyme P: protease (unnamed product of Finnfeeds International Ltd.)

RESULTS AND DISCUSSION

Table 1 shows the effects of enzyme supplementation on the true metabolizable energy (TME), and the apparent digestibility (APD) and true digestibility (TPD) of protein of the three legumes. There was no significant diffe-rence between the TME values of SBM and lupins and both of these legumes had higher TME values (P<.05) than that of black gram.

Overall, enzyme addition increased (P<.05) TME values, but the response was not consistent, and there was a significant interaction (P<.05) between legumes and enzymes. The multi-enzyme product (EB) increased (P<.05) the TME value of black gram by 9%, but did not improve the TME value of either SBM or lupins. On the other hand, xylanase (EA) addition significantly (P<.01) improved the TME value of lupins, but not black gram and SBM. This was consistent with results reported earlier (Wiryawan *et al*., 1995) that supplementation of legume meals with carbohydrases (primarily xylanase) significantly increased the TME values of lupins and faba beans. The positive effect of the addition of EA on TME value was probably due to an increase in digestibility of the NSP component of the lupins (Choct *et al*., 1995). All enzymes tested failed to improve the TME value of SBM, possibly because of the highly digestible nature of the SBM protein, starch and lipid by gastrointestinal enzymes.

There was a highly significant (P<.001) difference between the three legumes studied in both apparent protein digestibility (APD) and true protein digestibility (TPD). The average APD and TPD for the non-supplemented legumes were 64.7 and 82.6% for SBM, 54.8 and 74.1% for lupins and 51.0 and 70.9% for black gram. Overall, enzyme supplementation increased APD by 6%, however only with the multi enzyme product was the increase significant. The TPD values were not significantly improved and in some cases

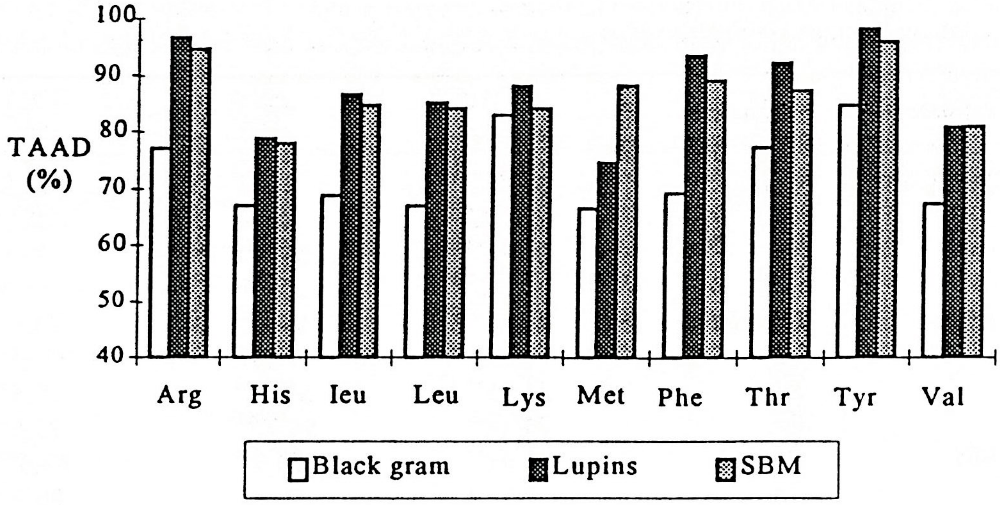

Figure 1. True digestibilities of amino acids of black gram, lupins and SBM in growing chickens.

were significantly decreased by enzyme supplementation. For example, protease improved the APD (P<.01) of SBM but significantly depressed (P<.01) the APD and the TPD of black gram. These results suggest that the enzyme or enzyme mixture needed for enhancement of the nutritional value of a particular grain legume is specific.

Marked differences were observed between digestibility of individual amino acids (Figure 1). The least digestible essential amino acids were methionine for black gram, methionine and histidine for lupins and valine for SBM. This was in agreement with the results reported by Perez *et al.*, (1993), Fan *et al.*, (1994) and Fickler *et al.*, (1996). Although the mean AAAD of SBM was higher (P<.05) than lupins and black gram, there was no significant difference between the TAAD of lupins and SBM, but both of them were higher (P<.01) than the TAAD of black gram. The mean AAAD and TAAD coefficients were 77.4 and 85.5% for SBM, 73.7 and 85.3% for lupins and 59.4 and 69.4% for black gram respectively. The differences in AAAD of SBM and lupins might be due to lower endogenous amino acid excreted by the chickens' fed SBM meal compared to those fed lupins meal.

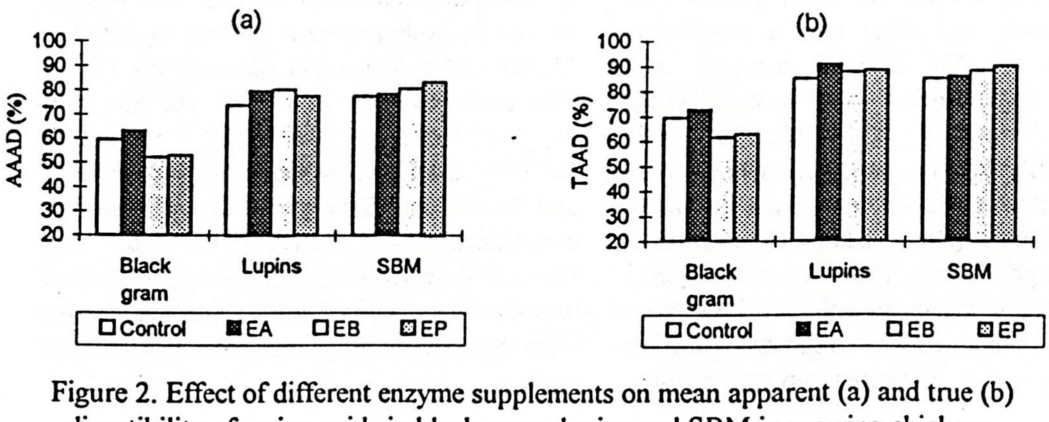

Figure 2. Effect of different enzyme supplements on mean apparent (a) and true (b) digestibility of amino acids in black gram, lupins and SBM in growing chickens.

Overall, AAAD and TAAD were increased by enzyme supplementation (Figure 2). The magnitude of the response, however, was not consistent and there was a significant interaction between legume and enzyme and between legume and amino acid. Both lupins and SBM showed positive responses in amino acid digestibility for all enzymes tested, however, the greatest improvement (P<.05) was observed after addition of protease to SBM and after the addition of xylanase to lupins. Although the TAAD of black gram increased after addition of xylanase (4.7%), this was not significantly greater (P=.095).

CONCLUSION

The TME, protein and AA digestibilities of black gram, lupins and SBM responded positively to supplementation with xylanase, protease and α-amylase. Lupins, for almost all parameters responded most to xylanase, and SBM, for almost all parameters, responded most to protease. The TME and protein digestibility of black gram did not respond to xylanase or protease but there was a tendency of a positive response to multi-enzyme preparation that also contained amylase in addition to xylanase and protease. The results of this study suggest that the nutritive value of black gram, lupins and SBM can be improved by enzyme supplementation.

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REFERENCES

Annison, G., Choct, M. and Hughes, R.J. 1995. Enzymes and the nutritive value of lupins. *Proc. Aust. Poult. Sci. Sym.*, 7:126.

Brillouet, J.M., Rovav, X., Hoebler, C., Barry, J.L., Carre, B. and Lorta, E. 1988. A new method for determination of insoluble cell walls and soluble nonstarchy polysaccharides from plant materials. *J. Agric. Food Chem.*, 36:969.

Bryden, W.L., Gill, R.J. and Balnave, D. 1994. Feed enzyme supplement improves the apparent metabolis-able energy of lupins for broiler chickens. *Proc. Aust. Poult. Sci. Sym.*,6:115.

Choct, M. 1996. The role of feed enzymes in animal nutrition toward 2000. *Proc. 20th World's Poult. Conf.*, 2:125.

Choct, M. 1997. Non-starch polysaccha-rides: Chemical structures and nutritional significance. *Feed Milling International*, 191:13.

Choct, M., Hughes, R.J., Wang, J., Bedford, M. R., Morgan, A.J. and Annison, G. 1995. Feed enzymes eliminate antinutritive effect of non-starch polysaccharides and modify fermentation in broilers. *Proc. Aust. Poult. Sci. Sym.*, 7:121.

Cohen, S.A., Tarven, T.L. and Meys, M. 1989. *The Pico-Tag Method.* A manual of advanced techniques for amino acid analysis. Millipore Corporation. Bedford, Massachusetts.

Fan, M.Z., Sauer, W.C. and Jaikaran, S. 1994. Amino acid and energy digestibility in peas (*Pisum sativum*) from white-flowered spring cultivars for growing pigs. *J. Sci. Food Agric.*, 64:249.

Fickler, J., Fontaine, J. and Heimbeck, W. (1996). *Degussa Amino Data 1.O.* Degussa Interactive PC-Software.

Jeroch, H., Hauschild, A and Muller, A. 1995. Influence of mechanical treatment and enzyme supplementation on nutritive value of peas

(*Pisum sativum* L.) for broiler chickens. *Bodenkultur*, 46:263.

Mead, R., Curnow, R.N. and Hasted, A.M.1993. *Statistical Methods in Agriculture and Experimental Biology*, 2nd edition. Chapman & Hall, London.

Perez, L., Fernandez-Figares, I., Nieto, R., Aguilera, J.F. and Prieto, C. 1993. Amino acid ileal digestibility of some grain legume seeds in growing chickens. *Anim. Prod.*, 56:261.

SAS Institute Inc.1990. *SAS/STAT® User's Guide, version 6*. 4th edition. Cary, NC.

Sibbald, I. R. 1979. A bioassay for available amino acids and true metabolizable energy in feeding-stuffs. *Poultry Sci.*, 58:668.

Smits, C.H.M. and Annison, G. 1996. Non-starch plant polysaccharides in broiler nutrition - towards a physiologically valid approach to their determination. *World's Poult. Sci. J.*, 52:203.

Sweeney, J. 1989. Generic combustion method for determination of crude protein in feeds: Collaborative study. *J. Assoc. Off. Anal. Chem.*, 72:770.

Wiryawan, K.G. and Dingle, J.G. 1995. Screening tests of the protein quality of grain legumes for poultry production. *Br. J. Nutr.*, 74:671.

Wiryawan, K.G., Dingle, J.G., Kumar, A., Gaughan, J.B. and Young, B.A. 1995. True metabolisable energy content of grain legumes: effects of enzyme supplementation. *Recent Advances in Animal Nutrition in Australia*, p. 196 [J.B. Rowe and J.V. Nolan, editors]. University of New England, Armidale.