

# Supplementation of Corn-Soybean Based Layer Diets with Different Levels of Acid Protease

SATRIJO WIDI PURBOJO<sup>1</sup>, EDWIN S. LUIS<sup>2</sup>, RENY DEBORA TAMBUNAN<sup>3</sup> and DICKY PAMUNGKAS<sup>4</sup>

<sup>1</sup>Universitas Jenderal Soedirman, Purwokerto

<sup>2</sup>Institute of Animal Science, College of Agriculture University the Philippines Los Banos

<sup>3</sup>Balai Pengkajian Teknologi Pertanian Lampung

<sup>4</sup>Loka Penelitian Sapi Potong, Grati

(Diterima dewan redaksi 15 Oktober 2003)

## ABSTRAK

PURBOJO S. W., E. S. LUIS, R. D. TAMBUNAN and D. PAMUNGKAS. 2004. Suplementasi beberapa level asam protease dalam ransum ayam petelur berbahan dasar jagung-kedelai. *JITV* 9(1): 5-11.

Penelitian yang dilaksanakan di kandang percobaan milik *Institute of Animal Science, University of the Philippines Los Banos*, bertujuan mengetahui pengaruh suplementasi protease asam di dalam ransum ayam petelur. Sebanyak 95 ekor petelur dikandangkan secara individu. Lima perlakuan secara acak diujicobakan dengan menggunakan pola rancangan acak lengkap. Setiap perlakuan berisi 19 ekor sebagai ulangan. Percobaan dilakukan selama 16 minggu. Pola manajemen yang diberikan adalah sama, yakni pemberian pakan sekali sehari dan air minum tersedia *ad-lib*. Ransum basal petelur mengandung protein kasar 18% dan 2800 Kcal ME/kg ditambahkan vitamin, mineral dan asam amino. Ransum dengan penurunan protein 17% dan 2800 kcal ME/kg juga telah disusun. Ransum yang mengandung penurunan protein kasar disuplementasi protease dengan level yang berbeda (0,05; 0,075; dan 0,1%). Enam ekor ayam dari setiap perlakuan secara acak dipilih dan ditempatkan dalam kandang metabolis individu dan masing-masing diberi makan dengan ransum yang ditambahkan 0,2% chromic oxide sebagai indikator selama 7 hari. Pada hari ke-3 dan ke-6, sampel feses dari setiap perlakuan dikumpulkan dan dikeringkan sehingga siap untuk analisis proksimat dan determinasi chromic oxide. Hasil menunjukkan bahwa tidak ada perbedaan konsumsi pakan selama dua minggu dan sampai akhir penelitian. Pengurangan kadar protein kasar dengan penambahan 0,1% protease menunjukkan angka HDP yang tertinggi (93,7%) dan angka konversi terendah (1,85). Sementara itu, pengurangan kadar protein kasar melalui penambahan 0,075% protease menunjukkan angka tertinggi pada berat telur (58,8 g) dan ketebalan kerabang telur (0,392 mm). Tidak ada perbedaan nyata terhadap koefisien pencernaan.

**Kata kunci:** Asam protease, ransum petelur, suplementasi

## ABSTRACT

PURBOJO S. W., E. S. LUIS, R. D. TAMBUNAN and D. PAMUNGKAS. 2004. Supplementation of corn-soybean based layer diets with different levels of acid protease. *JITV* 9(1): 5-11.

The aim of this research was held in Institute of Animal Science farm, University of the Philippines Los Banos, was to know the effects of acid protease supplementation in layer diets. Ninety-four-week old pullets were caged individually. Five treatments were randomly arranged to 95 pullets following a completely randomized design. Each treatment was replicated 19 times. The feeding trial lasted for 16 weeks. The same management practices were provided to all treatments throughout the feeding period. Pullets were fed once a day in the morning and clean drinking water was available to the pullets at all times. A basal layer diet that contained 18% crude protein (CP) and 2800 Kcal ME/kg supplemented with required vitamins, minerals and amino acids were formulated. The diets with reduced protein of 17% and 2800 kcal ME/kg was also formulated. The diet with the reduced crude protein was supplemented with different levels of protease (0.05, 0.075 and 0.1%). Six birds from each treatment were randomly selected and placed in individual digestion cages. They were fed with their respective diets with chromic oxide as indicator for 7 days. Chromic oxide was added to the different diets at 0.2%. On the 3<sup>rd</sup> to 6<sup>th</sup> day of feeding, feces were collected using stainless fecal trays installed under each cage. At the end of the collection period, fecal samples collected from each replicate of treatment were dried then subjected to proximate analysis and chromic oxide determination. Result showed that no significant difference on biweekly feed consumption and overall observations. Reduced CP + 0.1% protease was the highest on the hen day production (93.75%) and the lowest of feed conversion (1.85) while reduced CP + 0.075% protease was the highest of egg weight (58.82 g) and eggshell thickness (0.392 mm). There was no significant difference on digestibility coefficient.

**Key words:** Acid protease, layer diets, supplementation

## INTRODUCTION

Poultry industry requires the use of high-energy and high protein feeds. The energy was supplied from wheat, barley and maize. It has long been recognized that broiler and layer diet based on wheat, barley or high fat feeds, present a difficult challenge for digestive system of the broilers and layers. This is frequently manifested as wet litter syndrome that in turn results in increased ammonia production in the broiler houses and increased incidence of breast blisters and hock burns on broiler carcasses. These problems have been overcome by addition of feed enzymes. Improvements in the efficiency of poultry diets and nutrients of about 10% has been reported to be achieved a resulted supplementation with enzymes (ANKRAH *et al.*, 1999; BERGH *et al.*, 1999; COWAN and KORSBAK, 1996; SILVERSIDES and BEDFORD, 1999; COWIESON *et al.*, 2000). CHOT (1997) stated that ammonia emission reduces substantially by nutritional management, such as by improvement in the utilization of dietary protein. For every percentage of unit reduction of dietary crude protein, ammonia concentration could be reduced by 11% and ammonia emission by 10 to 12%.

Cereals are well known as sources of non-starch polysaccharides that are unable to be digested by poultry and which can form viscous gels in the aqueous environment, such as the one found in the gastrointestinal tract. Addition of carbohydrases to poultry feed rapidly reduced the viscosity caused by the digestion of the long-chain molecules (ADAM, 2000). Hemicellulase, xylanase or arabinase to reduce viscosity of wheat, rye and other cereal and Pentosan enzymes to reduce viscosities other grain or viscous carbohydrates (MARQUADT *et al.*, 1987).

In the modern poultry industry most feed manufacturers are currently using enzyme additives to lower their feed cost, improve animal performance, and reduce the problems associated with wet manure. Digestion and absorption of nutrients in pigs and poultry will occur if the enzymes secreted by the gut act directly on the feed constituents. Some of the nutrients may escape by endogenous enzymes if they are protected by anti-nutritional properties. Moreover, CLOSE (1996) mentioned that poultry do not secrete endogenous enzymes to breakdown non-starch polysaccharides that present in most raw materials of plant origin. As a consequence, these compounds can interfere with and limit the digestion and absorption of nutrients from feeds in the gastro-intestinal tract.

The exogenous enzymes enhance production performance through better digestion and absorption of

nutrients and less excretion in the feces along with the reduction in the overall costs of production. Hence, general objective of this research was to observe the effects of acid protease supplementation of poultry.

## MATERIALS AND METHODS

### Time and place of study

This experiment was held in Institute of Animal Science Farm, College of Agriculture, University of the Philippines Los Banos, Laguna (14° 10' North; 121° 15' East and 14 meter above sea level) from November 2001 until April 2002.

### Feeding trial

Ninety-four-weeks old pullets caged individually were used in this study. Five treatments were randomly arranged to 95 pullets following a completely randomized design. Each treatment was replicated 19 times. The feeding trial lasted for 16 weeks. The same management practices were provided to all treatments throughout the feeding period. Pullets were fed once a day in the morning and clean drinking water was available to the pullets at all times.

### Formulation of diets

A basal layer diet containing 18% crude protein (CP) and 2800 Kcal ME/kg supplemented with required vitamins, minerals and amino acids were formulated. The diets with reduced protein of 17% and 2800 kcal ME/kg was also formulated and it was listed in Table 1 and 2.

**Table 1.** Dietary treatments

Treatment (T)	Description
T1	18% CP and 2800 Kcal ME/kg diet (basal diet)
T2	17% CP and 2800 Kcal ME/kg
T3	17% CP and 2800 Kcal ME/kg + 0.05% Protease
T4	17% CP and 2800 Kcal ME/kg + 0.075% Protease
T5	17% CP and 2800 Kcal ME/kg + 0.10% Protease

**Table 2.** Ingredient composition and calculated nutrient contents of basal and reduced crude protein of layer diets

Ingredient (%)	Basal diet	Reduced CP diet
Yellow corn	50.50	50.90
Yellow soybean, U.S. 46 %	27.60	24.70
Rice bran DI	7.61	10.10
Limestone, fine	5.70	5.70
Limestone, coarse	4.00	4.00
Crude coco oil	2.00	2.00
Biofos/TG21	1.70	1.70
Salt	0.30	0.30
Vitamin premix	0.125	0.125
Mineral premix	0.100	0.100
Chlorine chloride 25	0.25	0.25
DL- Methionine	0.17	0.18
Total	100	100
<b>Calculated nutrient contents (% as fed):</b>		
Crude protein	18.00	17.00
Crude fat	5.15	5.47
Crude fiber	2.44	2.56
Calcium	3.75	3.75
Total phosphorus	0.78	0.80
Available phosphorus	0.50	0.50
Kcal ME/kg	2800	2800

### Digestion trial

Digestion trial was conducted to determine digestibility of feed nutrients. Six birds from each treatment were randomly selected and placed in individual digestion cages. They were fed with their respective diets with chromic oxide as indicator for 7 days. Chromic oxide was added to different diets at 0.2%. On the 3<sup>rd</sup> to 6<sup>th</sup> day of feeding, feces were collected using stainless fecal trays installed under each cage. At the end of the collection period, fecal samples collected from each replicates of treatment were dried then subjected to proximate analysis and chromic oxide determination. Apparent digestibility (COD) of each nutrient was calculated.

$$\text{COD} = 100 - 100 \frac{\% A}{\% B} \times \frac{\% X}{\% Y}$$

where:

A = Indicator in feed      X = Nutrient in feces  
 B = Indicator in feces      Y = Nutrient in feed

### Data gathered

The data gathered were: feed consumption, feed efficiency, total egg mass, egg production, egg weight, shell thickness and yolk pigmentation.

### Data analyzed

The data were analyzed by using completely randomized design.

## RESULTS AND DISCUSSION

### Proximate composition

The result of proximate analysis of layer diets used in the study is presented in Table 3. Dry matter, ether extract and crude fiber among basal diets and diets with reduces CP were not significantly different but crude protein, total ash and nitrogen free extract contents on the diets with reduces CP was lower than basal diets.

### Coefficient of digestibility (COD)

The coefficient of digestibility of dry matter, crude protein and ether extract of diets used in the study were presented in Table 4

The average of COD of dry matter, crude protein and ether extract of diets used in the study among the treatments were not significantly different although there is a tend of increase the COD's of diets supplemented with acid protease (T-3, T-4 and T-5) than those diets without acid protease supplementation (T-1 and T-2). This indicates that enzyme supplementation to pullet diets was able to improve its digestibility. HUBILLA (1994) reported that a marked

improvement in the apparent coefficient digestibility and an increase in metabolizable energy of the different feed grains when their feed were supplemented with commercial enzymes.

### Feed consumption

Data in Table 5 showed that the average of feed consumption among treatments were not significantly different ( $P>0.05$ ) but feed consumption by pullets with lowered crude protein with no enzyme supplementation (T-2) showed higher than other treatments. This may be due to the respon of the pullets, in order to get more nutrients, pullet will consume more feeds.

**Table 3.** Proximate analysis of layer diets used in the study

Chemical composition (%)	Basal diet	Reduced CP diet
Dry matter	92.3	92.6
Crude protein (CP)	18.5	17.0
Ether extract	5.1	5.8
Crude fiber	4.3	5.5
Total ash	7.1	7.0
Nitrogen-free extract	64.9	64.6

**Table 4.** Coefficient of digestibility (COD) of dry matter, crude protein and ether extract of diets used in the study

COD (%)	Treatment				
	T-1	T-2	T-3	T-4	T-5
Dry matter	66.7	65.0	67.3	68.0	67.7
Crude protein	74.9	74.2	78.6	80.7	80.9
Ether extract	84.3	83.2	87.6	88.3	87.9

**Table 5.** Average feed consumption of pullets supplemented with different level of protease

Treatment	Feed consumption (g/day)								
	Week								
	2	4	6	8	10	12	14	16	Average
T-1	98.1	98.2	97.4	99.0	99.0	102.0	102.6	102.2	98.1
T-2	98.4	97.9	98.1	98.8	100.1	102.9	103.4	103.9	100.4
T-3	98.7	98.4	97.7	98.5	98.1	101.3	101.9	101.6	99.6
T-4	96.9	97.2	96.9	98.6	99.3	100.8	101.3	100.5	98.2
T-5	96.9	97.0	97.1	98.3	98.6	101.2	101.3	100.2	98.8

### Egg production

The average percentage of egg production in T5 was significantly higher ( $P<0.05$ ) other treatments (T2, T3, and T4) but was similar with T1 (Table 6). This indicates that enzyme supplementation in pullets is beneficial even though crude protein (CP) level is lowered but not beneficial when protein level is lowered and no enzyme supplementation.

### Feed efficiency

Average feed efficiency of pullet fed with diets supplemented with different level of acid protease was presented in Table 7. Average feed efficiency was significantly ( $P<0.05$ ) better in treatment with reduced crude protein and 0.1% enzyme supplementation than T-2 and T-3 with reduced crude protein and with reduced crude protein plus 0.05% enzyme supplementation. This shows the 0.1% enzyme supplementation is better.

### Egg weight

Average weight of eggs was presented in Table 8. Average egg weight was significantly higher ( $P<0.05$ ) in T4 (58.8 g) than other treatments. This indicates that the supplementation of acid protease affected to the weight of egg lasted 16 week, especially when the diet was lowered in CP and supplemented with 0.075% of protease.

### Shell thickness and yolk color

Average shell thickness of egg was presented in Table 9. Average shell thickness in T4 (0.392 mm) was significantly higher ( $P<0.05$ ) than T3 (0.389 mm) and T5 (0.382 mm). Average yolk color was presented in Table 10. The average yolk color was significantly higher ( $P<0.05\%$ ) in T5 (8.30) and T3 (8.25) than T2 (8.03) and T4 (8.02).

**Table 6.** Average percentage of egg production (hen-day basis) of pullet

Treatment	Hen-day egg production (%)								
	Week								
	2	4	6	8	10	12	14	16	Average
T-1	86.1	96.6	94.4	93.6	92.9	92.1	94.7	92.5	92.9 <sup>ab</sup>
T-2	89.5	92.5	86.2	90.5	91.7	93.6	92.1	89.3	90.2 <sup>b</sup>
T-3	91.3	92.1	89.1	91.4	89.8	90.6	93.6	88.8	90.8 <sup>b</sup>
T-4	89.1	93.6	88.3	92.5	91.0	89.8	88.3	91.3	90.5 <sup>b</sup>
T-5	86.9	94.7	94.4	94.7	94.7	94.4	95.1	95.1	93.7 <sup>a</sup>

Treatments with the different superscripts were significantly different ( $P<0.05$ )

**Table 7.** Average feed efficiency of pullet

Treatment	Feed efficiency (kg feed/kg egg)								
	Week								
	2	4	6	8	10	12	14	16	Average
T-1	2.2	1.8	1.8	1.8	1.9	1.9	1.0	1.9	1.9 <sup>ac</sup>
T-2	2.0	1.9	2.0	1.9	1.8	1.9	1.1	2.0	1.9 <sup>ab</sup>
T-3	2.0	2.0	2.0	1.9	2.0	2.0	1.9	2.0	2.0 <sup>a</sup>
T-4	2.0	1.9	1.9	1.8	1.9	1.9	1.9	1.8	1.9 <sup>bc</sup>
T-5	2.0	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8 <sup>c</sup>

The average of treatment with the different superscripts were different significantly ( $P<0.05$ )

**Table 8.** Average weight of egg produced by pullets fed diets supplemented with different level of protease

Treatment	Weight of egg (g)								
	Week								
	2	4	6	8	10	12	14	16	Average
T-1	54.4	55.3	57.4	57.5	57.9	57.9	57.1	57.5	56.9 <sup>b</sup>
T-2	55.9	56.3	56.4	57.2	58.8	58.4	58.6	58.4	57.5 <sup>ab</sup>
T-3	53.9	54.7	56.4	57.5	57.3	57.6	58.0	58.6	56.7 <sup>b</sup>
T-4	54.9	55.9	58.3	59.2	60.3	59.9	60.9	61.5	58.8 <sup>a</sup>
T-5	54.9	58.0	57.4	58.1	58.5	59.0	58.8	59.4	58.0 <sup>ab</sup>

The average of treatment with the different superscripts were different significantly (P<0.05)

**Table 9.** Average shell thickness of egg produced by pullets supplemented with different level of protease

Treatment	Shell thickness (mm)								
	Week								
	2	4	6	8	10	12	14	16	Average
T-1	0.399	0.399	0.377	0.387	0.373	0.370	0.366	-	0.381 <sup>bc</sup>
T-2	0.400	0.391	0.383	0.361	0.374	0.371	0.369	-	0.381 <sup>bc</sup>
T-3	0.406	0.401	0.378	0.387	0.376	0.393	0.381	-	0.389 <sup>ab</sup>
T-4	0.402	0.397	0.388	0.393	0.380	0.379	0.387	-	0.392 <sup>a</sup>
T-5	0.391	0.381	0.386	0.383	0.373	0.372	0.375	-	0.382 <sup>c</sup>

The average of treatment with the different superscripts were different significantly (P<0.05)

**Table 10.** Average yolk color of egg produced by pullets supplemented with different level of protease

Treatment	Yolk color (Roche Color Fan)								
	Week								
	2	4	6	8	10	12	14	16	Average
T-1	8.26	7.90	8.52	8.73	8.84	8.21	8.37	-	8.31 <sup>ab</sup>
T-2	8.10	8.00	7.88	8.38	7.77	8.83	8.17	-	8.03 <sup>b</sup>
T-3	8.84	8.47	8.32	8.26	7.78	7.84	8.21	-	8.25 <sup>a</sup>
T-4	7.78	8.89	8.10	8.10	7.63	7.52	8.53	-	8.02 <sup>b</sup>
T-5	8.26	8.68	8.36	8.05	7.52	7.73	8.58	-	8.30 <sup>a</sup>

The average of treatment with the different superscripts were different significantly (P<0.05)

## CONCLUSION

Reduced CP + 0.1% protease was the highest on the hen day production (93.75%) and the lowest of feed conversion (1.85), while reduced CP + 0.075% protease was the highest of egg weight (58.82 g) and eggshell thickness (0.392 mm).

## REFERENCES

- ADAM, C.A. 2000. Enzymes are Important Components in Antibiotic-Free Poultry Feeds. *Feed Mix Special*. pp. 16-18.
- ANKRAH, N.O., G. L. CAMPBELL, R.T. TYLER, B.G. ROSSNAGEL and S.R.T. SOKHANSANJ. 1999. Hydrothermal and beta-glucanase effects on the nutritional and physical properties of starch in normal

- and waxy hullless barley. *Anim. Feed Sci. Tech.* 81: 205-219.
- BERGH, M.O., A. RAJDAN and P. AMAN. 1999. Nutritional influence of broiler chicken diets based on covered normal, waxy and high amylase barleys with or without enzyme supplementation. *Anim. Feed Sci. and Tech.* 78: 215-226.
- CHOT, M. 1997. Enzymes in Animal Nutrition: The Unseen Benefit IDRC: Resources: Books: Catalogue: [Http://Www.Idrc.Ca/Books/Focus/821/Chp5.Html](http://www.idrc.ca/books/focus/821/Chp5.html).
- CLOSE, W.H. 1996. Enzymes in Action. *Pigs Missets* 12 (7): 21-24.
- COWAN, W. D. and A. KORSBAK. 1996. Enzymes for Layers Diets. *British Poult. Sci.* 37: S48.
- COWIESON, A.J., T. ACAMOIC and BEDFORD, M.R. 2000. Enzyme Supplementation of Diets Containing *Camelina sativa* Meal for Poultry. *British Poult. Sci.* 41: 689-690.
- HUBILLA, P.R.L. 1994. Enzymes Supplementation of Feed Grains in Broiler Diets. Graduate Thesis, UPLB.
- MARQUARDT, R.R., A.I. FENGLER and J. PAWLIK. 1987. Improvement of the nutritional value of rye and wheat grains through the use of crude enzymes of microbial origin. *In: Biotechnology in The Feed Industry*. T.P. LYONS (Ed.) Proc. of Alltech's Third Annual Symposium. Alltech Technical Pub., Nicholasville, Ky. USA.
- SILVERSIDES, F.G. and M.R. BEDFORD. 1999. Effect of Pelleting Temperature on The Recovery and Efficiency of A Xylanase Enzyme in Wheat-Based Diets. *Poult. Sci.* 78: 1184-1190.