Research Article

SUPPLEMENTATION OF CORN-SOYBEAN BASED LAYERS DIETS WITH DIFFERENT LEVELS OF ACID PROTEASE

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ABSTRACT

An experiment was conducted at the Animal Science Farm, Tuntungin, Putho of the Institute of Animal Science, University of the Philippines, Los Baños, College, Laguna, Philippines, during 2001-2002. Ninety 24-weeks old individually caged pullets were used in this study. Five treatments were randomly assigned to the 90 pullets following a completely randomized design. Each treatment was replicated 18 times with one pullet each. The dietary treatments were: basal diet (18% CP and 2800 kcal /kg, ME) reduced (red). CP diet (17% CP and 2800 kcal /kg, ME), red CP diet + 0.05% protease, red CP diet + 0.075% protease and red CP diet + 0.10% protease. Apparent digestibility of each nutrient in relation to egg production, egg weight, feed consumption, feed efficiency, body weight gain, shell thickness and yolk pigmentation, mortality, and income over feed cost were calculated All the data collected were subjected to statistical analysis using the general linear model (GLM) procedure of the SAS Institute (1988). One percent reduction of crude protein of diet of pullet significantly decreased the digestibility of all nutrients. Feed efficiency was significantly improved with 0.1% protease, while eggshell thickness was significantly improved with 0.075% protease supplementation of diets. Income over feed cost increased with dietary supplementation of protease. Increased level of protease supplementation of reduced CP diets consistently improved the digestibility of nutrients, overall average egg production and decreased feed consumption. Egg weight and feed efficiency were slightly improved with 0.075% and 0.1% protease supplementation of diet. Eggshell thickness was significantly improved with 0.075% protease Yolk color was not significantly influenced by increased protease supplementation of the diet. Income over feed cost was consistently increased with protease supplementation. It was concluded and recommended that crude protein of layer diets could be reduced by 1% provided that they are supplemented with protease, optimum level of inclusion of protease is 0.1% for layers and enzyme activity need to be accurately determined before it is used.

Key words: Layer, corn-based, acid protease, enzyme

INTRODUCTION

It has been well established that supplementing feeds with enzymes improves the efficiency of production, enhances the use of lower quality feedstuffs and reduces the excretion and waste of nutrients. (Close, 1996). The nutritional problems associated with cereal grains have been overcome by addition of feed enzymes. Studies have shown that the nutritive value of barley, oats, rye, corn and wheat can be considerably improved by the dietary inclusions of commercially available crude enzyme preparations (Marquardt *et al.*, 1994). Enzyme supplementation can break down anti-nutritional substances found in feed raw materials, thus augmenting the digestive capacity of the animal, improving availability of the feed nutrients, and increasing release of nutrients in the upper part of the gastrointestinal tract. Improvements in the efficiency of utilization of poultry diets and nutrients of about 10% have been reported to be achieved as a result of supplementation with enzymes (Cowieson *et al.*, 2000). A study was conducted to find out the effects of supplementation of corn-soy based layers diet with acid protease on its performance mainly on growth, feed consumption, nutrients digestibility and economics of production.

MATERIALS AND METHODS

Ninety 24-weeks old individually caged pullets were used in this study. Five treatments were randomly assigned to the 90 pullets following a completely randomized design. Each treatment was replicated 18 times with one pullet each. The dietary treatments were: basal diet (18% CP and 2800 kcal/kg, ME), red CP diet (17% CP and 2800 kcal/kg, ME) red CP diet+0.05% protease, red CP diet+0.075% protease, and red CP diet

+0.10 % protease. A basal layer diet was formulated to contain 18% crude protein and 2800 kcal/kg, ME supplemented with required vitamins, minerals and amino acids. The diets with reduced CP was formulated to contain 17% CP and 2800 kcal/kg, ME and likewise supplemented with required vitamins, minerals and amino acids. The different levels of protease were added to the diet with the reduced CP at the desired level (0.00%, 0.05%, 0.075%, and 0.1%). The ingredient composition and calculated nutrient contents of the diets are presented in Table 1.

Ingredients	Basal diet (%)	Reduced CP diet (%)
Corn yellow	50.50	50.90
Soybean yellow, US	27.60	24.70
Rice bran DI	7.61	10.10
Lime stone, fine	5.70	5.70
Limestone, coarse	4.00	4.00
Crude coco oil	2.00	2.00
Biophos/TG21	1.70	1.70
Salt	0.30	0.30
Chorine chloride	0.25	0.25
DL-Methionine	0.17	0.18
Vitamin premix	0.125	0.125
Mineral premix	0.100	0.100
Total	100.00	100.00
Calculated Analysis		
Crude protein (%)	18.00	17.00
M.E. (kcal/kg)	2800	2800
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
Lysine (%)	1.02	0.94
Methionine +Cystine (%)	0.79	0.77

Table 1. Ingredient composition and calculated nutrient contents of basal diet and diets with reduced crude protein of layers

Digestion trial was conducted to determine the digestibility of the feed nutrients. Six birds from each treatment were randomly selected and placed in individual cages. They were fed their respective diets with 0.2% chromic oxide as indicator for 7 days. On the 3rd to 6th day of feeding, feces were collected using stainless trays installed under each cage. At the end of the collection period, fecal samples collected from each replicate of each treatment were dried, and subjected to proximate analysis and chromic oxide determination. Apparent digestibility of each nutrient was calculated in relation to egg production, egg weight, feed consumption, feed efficiency, body weight gain, shell thickness and yolk pigmentation, mortality, and income over feed cost calculated. All the data collected were subjected to statistical analysis using the general linear model (GLM) procedure of the SAS Institute (1988). Tests of significant differences among treatment means were done using the Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Chemical analysis of the layer diet

The proximate, calcium and phosphorus analyses of the basal layer diet and diet with reduced crude protein supplemented with different levels of acid protease is shown in Table 2. The actual analyses of the diets confirm closely with their calculated analysis in terms of crude protein, ether extract and calcium. Crude fiber and phosphorus analyses are slightly higher than the calculated.

	Perc	cent in diet
Chemical components	Basal	Reduced CP Diet
Dry Matter	90.28	90.52
Crude Protein	18.28	17.12
Ether extracts	5.61	5.50
Crude fiber	4.71	4.73
Nitrogen-free-extract	64.24	65.69
Ash	7.16	6.96
Calcium	3.88	3.90
Total phosphorus	0.87	0.82

Table 2. Proximate, calcium and phosphorus analyses of layer's basal diet and diet with reduced protein used in the study

Digestibility study

Coefficients of digestibility of crude protein (CP), ether extract (EE), crude fiber (CF) and nitrogen-freeextract (NFE) of basal layer diet and diets with reduced CP supplemented with different levels of acid protease are shown in Table 3. A significant decrease in digestibility coefficients of CP, EE, CF, and NFE were observed when crude protein of the diet was reduced by 1% relative to the basal diet. Protease supplementation of reduced CP diet with increasing levels significantly increased the digestibility coefficients of CP and EE but not CF. Digestibility coefficients of NFE significantly increased with 0.075% and 0.1% protease but not with 0.05% protease supplementation of the reduced CP diets.

 Table 3. Digestion coefficients (%) of crude protein, ether extract, crude fiber and nitrogen-free- extract of layers fed basal diet and diet with reduced crude protein supplemented with different levels of acid protease

Treatments		Digestion coefficie	nts (%)	
	Crude protein	Ether extract	Crude fiber	Nitrogen-free- extract
Basal diet	79.80a	77.78a	64.88a	87.98a
Diet with reduced crude protein	74.16b	74.65b	56.42b	84.05b
Diet with reduced crude protein				
+0.05%protease	78.97a	78.40a	60.79ab	84.28b
Diet with reduced crude protein				
+0.075%protease	78.77a	78.98a	62.31ab	87.35a
Diet with reduced crude protein				
+0.1%protease	80.24a	79.71a	63.39ab	86.67a

Means within column having different superscripts are significant at P<0.05

In general, increasing the levels of protease in diets with reduced crude protein increased the digestibility of nutrients to the level of the basal diet. Results demonstrate that the reduction of CP in diet decreased the digestibility of nutrients, while protease supplementation of diets with the reduced crude protein enhanced the digestibility of all the nutrients. The enhancement in the digestibility of nutrients could be attributed to the exogenous protease, which acted together with the endogenous proteolytic enzymes. The results are in conformity with the findings of Nasi (1988) who observed improved protein utilization of hens fed enzyme supplemented barley and oats diets. Brenes *et al.* (1993) observed an improvement in the crude protein digestibility of enzyme supplemented raw, autoclaved and dehulled lupins in chicken diets. Addition of enzymes to the layer diets increased availability of energy found significant differences in protein, starch and ME content of corn samples with different densities when supplemented with enzymes (Baido *et al.*, 1997).

Egg production

Table 4 presents the biweekly and overall average egg production of layers fed basal diet and diets with reduced crude protein supplemented with different levels of acid protease from 24 to 40 weeks of age. Results showed that except on first and sixth biweekly feeding periods, reduction of the crude protein of the layer diet by 1% generally depressed the biweekly and overall average egg production of the pullets. Significant reduction in egg production was observed only on the third biweekly period.

Protease supplementation of reduced crude protein diet generally increased the biweekly egg production of the pullets in most periods of the trial. Increased level of protease supplementation of the reduced crude protein diet generally but not significantly increased the biweekly egg production of the pullets. The overall average egg production of the pullets was slightly depressed with reduction of crude protein in the diet. Supplementation of diet with 0.1% protease significantly increased the overall egg production of the pullets relative to those fed reduced crude protein diet with no enzyme supplement. Lower levels of inclusion of protease (0.05% and 0.075%) showed no significant improvement on the overall egg production of the pullets.

Table 4. Biweekly and overall average egg production of pullets fed basal diet and diets with reduced crude protein supplemented with
different level of acid proteases from 24 to 40 weeks of age

				Perce	nt egg produ	uction			
Treatments	Feeding period bi-weekly								
	1	2	3	4	5	6	7	8	Overall
Basal diet	85.61	96.43	94.84a	93.65	93.65	92.46	94.84ab	92.46	92.73ab
Diet with reduced crude protein	89.30	92.06	86.42b	90.47	90.47	93.65	92.14ab	89.28	90.62b
Diet with reduced crude protein +0.05% protease	91.50	92.46	89.28ab	91.26	91.26	90.49	93.65ab	88.57	90.85b
Diet with reduced crude protein+0.075%protease	88.49	94.44	88.49ab	92.06	92.06	90.87	89.68b	91.66	91.00b
Diet with reduced crude protein+0.1%protease	86.55	96.03	94.44a	95.23	95.23	94.44	95.63a	95.24	94.05a

Means within column having different superscripts differs significantly p<0.05

The result suggests that egg production was slightly depressed with 1% reduction of CP in diet. Supplementation of diets with 0.1% protease significantly improved the egg production of the pullets. The decrease in egg production rate of pullets with reduction of crude protein in diet could be due to the decreased digestibility of nutrients while the significant improvement in egg production rate of pullets at 0.1% protease could be attributed to the increased digestibility of nutrients as shown in Table 3. The results are supported by the report of Nasi (1988) who observed significant improvement in egg production of hen fed diets supplemented with cellulase, beta-glucanase and protease. Jacob *et al.* (2000) noted that the enzyme supplementation of reduced crude protein diet did not adversely affect egg production. An improvement in egg production of pullets was reported with enzyme supplementation of cereal based diets (Luis,1998).

Egg weight

Table 5 presents the average biweekly and overall average egg weights of pullets fed basal diet and diets with reduced crude protein supplemented with different levels of acid protease from 24 to 40 weeks of age. One percent reduction of crude protein of the diet showed no significant effect on the average biweekly as well as overall average egg weight of eggs produced by pullets. No consistent trends in egg weights were seen during the entire biweekly periods with the reduction of crude protein in diet. Overall average egg weight was slightly increased with reduction of crude protein in diet of pullets. Except on biweekly periods seventh and eighth at 0.075% protease, no consistent and significant effect on egg weights of pullets was seen during the entire biweekly periods. Likewise except with pullets fed 0.05% protease, egg produced by pullets fed diets with 0.075% and 0.1% protease supplementation was slightly heavier than those fed similar diet with no protease.

Results showed that neither the reduction in crude protein nor protease supplementation of reduced CP diets influenced the size of egg produced by the pullets. This observation is in consonance with the findings of Nasi (1988) and Dong (1997), who found no significant effect on the weight of eggs laid by pullets fed diets supplemented with enzyme. Pullets fed cereal- based diets supplemented with multi enzymes produced eggs of heavier weights than those fed diets without enzymes supplement (Brufau *et al.*, 1993). This difference in egg weight could be due to the different diets and kind and level of enzymes used.

				E	Egg weight (g	g)			
Treatments				Feedir	ng period bi-	weekly			
_	1	2	3	4	5	6	7	8	Overall
Basal diet	54.33	55.41	57.57	57.53	57.55	57.79	57.52b	57.55b	56.87ab
Diet with reduced crude protein	56.00	56.46	56.36	57.21	58.66	58.39	58.38b	58.38b	57.50ab
Diet with reduced crude protein +0.05%protease	53.91	54.63	56.18	57.49	57.39	57.57	58.12b	58.79b	56.75b
Diet with reduced crude protein+0.075%protease	54.60	56.14	58.50	59.24	59.75	59.68	60.92a	61.41a	58.82a
Diet with reduced crude protein+0.1%protease	54.73	58.44	57.53	58.16	58.66	59.33	58.97ab	59.59ab	58.19ab

Table 5. Biweekly and overall average egg weight of pullets fed basal diet and diets with reduced crude protein supplemented with different level of acid proteases from 24 to 40 weeks of age

Means within column having different superscripts differs significantly p<0.05

Feed consumption

Table 6 presents the average biweekly and overall average feed consumption of pullets fed basal diet and diets with reduced crude protein supplemented with different levels of acid protease from 24 to 40 weeks of age. Results showed that except on the second and fourth biweekly feeding periods, reduction of crude protein of the diet generally increased the biweekly as well as the overall average feed consumption of the pullets. Significant increase in feed consumption of pullets with reduction of dietary protein was observed only during the eighth biweekly feeding period. Protease supplementation of reduced crude protein diets generally decreased the feed consumption of pullets during the entire feeding periods except the feeding period eighth, in which it significantly decreased at all levels of protease supplementation relative to those fed the reduced CP diet with no protease. Likewise, protease supplementation of diets with reduced crude protein decreased consistently but not significantly the overall average feed consumption of pullets.

 Table 6. Biweekly and overall average feed consumption (g/day) of pullets fed basal diet and diets with reduced crude protein supplemented with different level of acid proteases from 24 to 40 weeks of age

				Feed co	onsumption	(g/day)					
Treatments	Feeding period bi-weekly										
	1	2	3	4	5	6	7	8	Overall		
Basal diet	98.12	98.28	97.74	99.23	99.90	102.09	102.70	102.29 ^b	99.92		
Diet with reduced crude protein	98.31	97.77	98.43	98.92	100.08	102.76	102.88	103.87 ^a	100.41		
Diet with reduced crude protein +0.05%protease	98.52	98.29	97.58	98.34	99.00	101.12	101.74	101.56 ^{bc}	99.52		
Diet with reduced crude protein+0.075%protease	96.62	97.05	96.71	98.20	99.06	100.62	101.19	100.48 ^{cd}	98.75		
Diet with reduced crude protein+0.1%protease	96.71	96.88	97.14	98.16	98.40	100.98	101.16	100.06 ^d	98.69		

Means within column having different superscripts differs significantly p<0.05

Results showed that pullets tended to consume more feeds when fed reduced crude protein diet and tended to consume less feeds when diets were supplemented with protease. The slight increase in feed consumption of pullets fed diets with reduced crude protein could be due to the tendency of the birds to eat more to compensate for lower crude protein content of the diet. In contrast, the slight decrease in feed consumption of pullet fed protease-supplemented diets could be due to the increased digestibility of nutrients as presented in Table 3. The results are supported by the findings of Luis (1998) who did not find significant effect on feed consumption of pullets fed enzyme-supplemented diets. A decrease in feed consumption of

pullets was observed when diets were supplemented with multi enzymes (Dong, 1997). Similarly, Reddy (1999) reported decreased feed consumption of pullets when corn-soya based diets were supplemented with protease.

Feed efficiency

Table 7 presents the average biweekly and overall average feed efficiency of pullets fed basal diet and diets with reduced crude protein supplemented with different levels of acid protease from 24 to 40 weeks of age. One percent reduction of crude protein in diet generally depressed the biweekly and overall feed efficiency of the pullets during the entire feeding periods. Statistical analysis of the data showed no significant differences between the two treatments in the biweekly and overall feed efficiency except on the third biweekly period. Protease supplementation of diets with the reduced crude protein generally improved the feed efficiency of pullets. Statistical analysis of the data showed no significant differences among treatments in feed efficiency of pullets. Statistical analysis of the data showed no significant differences among treatments in feed efficiency of pullets. Statistical analysis of the data showed no significant differences among treatments in feed efficiency of the reduced crude protein diet with 0.1% significantly enhanced the overall average feed efficiency of pullets relative to those fed reduced crude protein diet with no supplementary protease. Lower levels (0.05% and 0.075% protease) of protease supplementation of the diets gave no significant improvement in the feed efficiency of the pullet.

The results indicate that reduction in crude protein of diet by one percent slightly depressed the feed efficiency of the pullets. On the other hand, protease supplementation generally improved the feed efficiency of the pullets. The depression in feed efficiency with reduction of CP in diet could be due to increased feed consumption and decreased egg production of pullets. The improvement in feed efficiency with protease supplementation of diets could be attributed to the increased egg production and decreased feed consumption of pullets. The results confirm the findings of Cresswell (1997) and Nasi (1988) who reported a significant improvement in feed efficiency of hens fed corn-based diets with multi-enzyme supplementation. An insignificant improvement in feed efficiency of pullets was reported with multi-enzyme supplementation of diets (Luis, 1997). Reddy (1999) reported a significant improvement in feed efficiency of layers fed diets supplemented with protease.

				F	eed efficien	cy					
Treatments	Feeding period bi-weekly										
	1	2	3	4	5	6	7	8	Overall		
Basal diet	2.08	1.84ab	1.81b	1.84ab	1.86	1.90ab	1.90	1.93ab	1.90abc		
Diet with reduced crude protein	1.99	1.90ab	1.98a	1.93a	1.88	1.91ab	1.90	2.02a	1.93ab		
Diet with reduced crude protein +0.05%protease	2.03	2.02a	1.98a	1.90ab	1.97	1.95a	1.88	1.98a	1.96a		
Diet with reduced crude protein+0.075%protease	1.97	1.84ab	1.89ab	1.81ab	1.85	1.87ab	1.89	1.79b	1.86ba		
Diet with reduced crude protein+0.1%protease	2.06	1.79b	1.79b	1.78b	1.82	1.81b	1.80	1.77b	1.83c		

 Table 7. Biweekly and overall average feed efficiency (kg feed/kg eggs) of pullets fed basal diet and diets with reduced crude protein supplemented with different level of acid proteases from 24 to 40 weeks of age

Means within column having different superscripts differs significantly $p{<}0.05$

Shell thickness and yolk color scores

Table 8 presents the average biweekly and overall average eggshell thickness of hens fed basal diet and diet with reduced crude protein supplemented with different levels of acid protease from 24 to 40 weeks of age. Results showed that 1% reduction of crude protein in diet had no significant effect on biweekly and overall average shell thickness of eggs produced during the entire feeding periods. Protease supplementation of the reduced crude protein diets likewise did not significantly affect the shell thickness of eggs produced during the entire feeding periods. Eggshell thickness was significantly improved at 0.05% during sixth and 0.075% protease supplementation of diets during seventh

biweekly feeding periods over those fed reduced CP diets with no protease. The overall average shell thickness of eggs was significantly improved at 0.075% but not at 0.05% and 0.1% protease supplement. In general, increased levels of protease in diets showed no consistent effect on the shell thickness of eggs produced.

The results indicate that reduction of crude protein of the diet failed to influence the shell thickness of eggs produced. Protease supplementation slightly improved the shell thickness of eggs produced. The results are in agreement with the findings of Luis (1997) and Dong (1997) who found no significant effect on the eggshell thickness of pullets fed diets with enzyme supplement. An improvement in eggshell thickness was observed with multi-enzyme supplementation of cereal-based diets of laying hens (Sirvydis *et al.*, 1998). The difference in eggshell thickness could be due to the kind and level of enzyme present in the supplement.

			Egg sl	nell thickness	s (mm)				
Treatments		Feeding period bi-weekly							
	1	2	3	4	5	6	7	Overall	
Basal diet	0.398	0.395	0.380	0.386	0.373	0.371c	0.367c	0.381bc	
Diet with reduced crude protein	0.402	0.392	0.374	0.381	0.377	0.373bc	0.369c	0.82bc	
Diet with reduced crude	0.408	0.397	0.377	0.386	0.76	0.395a	0.381ab	0.389ab	
protein +0.05%protease									
Diet with reduced crude protein	0405	0.399	0.388	0.395	0.390	0.384ab	0389a	0.393a	
+0.075%protease									
Diet with reduced crude protein	0392	0.390	0.384	0.386	0.372	0.376bc	0.374bc	0.380c	
+0.1%protease									

 Table 8. Biweekly and overall average egg shell thickness (mm) of pullets fed basal diet and with reduced crude protein supplemented with different level of acid proteases from 24 to 40 weeks of age

Means within column having different superscripts differs significantly p<0.05

Table 9 presents the biweekly and overall average yolk color of eggs of pullets fed basal diet and diets with reduced crude protein supplemented with different levels of protease from 24 to 40 weeks of age. Except on second and fourth biweekly periods reduction of crude protein of diet decreased the biweekly as well as the overall average yolk color of eggs of pullets. Statistical analysis of the data showed that reduction of CP of diet significantly decreased yolk color of eggs only during the fifth and sixth biweekly periods. Protease supplementation of reduced CP diets showed no consistent trends in biweekly as well as overall average yolk color of eggs produced.

Table 9.	Biweekly and overall average egg yolk color (Roche color fan) of pullets fed basal diet and diets with reduced crude protein	L
	upplemented with different level of acid proteases from 24 to 40 weeks of age	

			I	Egg yolk col	or			
Treatments			Feedir	ng period bi-	weekly			
	1	2	3	4	5	6	7	Overall
Basal diet	8.16b	7.83b	8.11	8.27	8.38a	8.33a	8.38ab	8.21ab
Diet with reduced crude protein	8.11b	8.00b	7.88	8.38	7.77b	7.88b	8.16b	8.03ab
Diet with reduced crude protein +0.05%protease	8.85a	8.50a	8.33	8.27	7.83b	7.83b	8.22b	8.26ab
Diet with reduced crude protein +0.075%protease	7.83b	8.44a	8.11	8.05	7.66b	7.38c	8.50ab	7.99b
Diet with reduced crude protein +0.1%protease	8.27ab	8.66a	8.33	7.94	7.05ab	8.05ab	8.61a	8.13a

Means within column having different superscripts differs significantly $p{<}0.05$

The above results indicate that the yolk color of eggs was not influenced either by reduction of crude protein of diet or protease supplementation of reduced CP diet except during second biweekly feeding period. Significant increase in yolk color during some biweekly periods indicates that the protease was able to release pigments responsible for yolk color development. The observations are in agreement with the findings of Igbasan and Guenter (1996) who found no significant effect on yolk color of eggs fed wheat-peas based diets supplemented with enzymes. Sorensen (1996) also did not find significant effect on egg yolk color of eggs fed maize-sunflower diets supplemented with enzymes.

Initial body weight and body weight gain

Table 10 presents initial body weight and body weight gain of pullets fed basal diet and diets with reduced crude protein supplemented with different levels of protease from 24 to 40 weeks of age. Reduction of crude protein of diet slightly decreased the body weight gain of pullets. Body weight gain of pullets inconsistently decreased with increased level of protease supplementation to the reduced crude protein diet. No significant differences in initial, final and body weight gain of hens were observed among treatments either with the reduction of CP or protease supplementation of reduced CP diet.

Although not significantly different, results indicate that reduction of crude protein of diet decreased body weight gain of pullets. Likewise, enzyme supplementation of reduced CP diet also decreased the body weight gain of the pullets. The decrease in body weight gain of pullets with reduction of dietary protein could be due to decreased digestibility of nutrients as shown in Table 3. The decreased body weight gain in pullets fed protease-supplemented diets could be due to slightly increased egg production as shown in Table 4. The results confirm the findings of Luis (1998) and Pan *et al.* (1998), who observed no significant increase or decrease body weight gain in pullets fed diets supplemented with enzymes.

Table 10. Mean initial, final, and body weight gain of pullets fed basal diet and diets with reduced crude protein supplemented with different levels of proteases from 24 to 40 weeks of age

Treatments	Body weight (g)						
	Initial	Final	Gain				
Basal diet	1368.42	1534.21	165.78				
Diet with reduced crude protein	1359.47	1505.78	146.31				
Diet with reduced crude protein +0.05%protease	1341.47	1477.63	132.36				
Diet with reduced crude protein+0.075%protease	1384.73	1500.00	115.26				
Diet with reduced crude protein+0.1%protease	1356.31	1485.52	129.21				

Means within column having different superscripts differs significantly p<0.05

Income over feed cost (IOFC)

Table 11 presents the IOFC of pullets fed basal diet and diets with reduced crude protein supplemented with different levels of acid protease from 24 to 40 weeks of age. Results showed that income over feed cost of pullets decreased with the reduction of crude protein in diet. Protease supplementation of reduced crude protein diets of pullets with increased levels of supplements improved consistently the IOFC.

The results indicate that IOFC decreased with reduction of crude protein of the diet. Moreover, IOFC increased with protease supplementation of reduced crude protein diet. The decrease in income over feed cost with reduction of crude protein in diet could be due to increased feed intake and feed cost relative to those fed basal diet. On the other hand, the increase in income over feed cost of pullets with protease supplementation of reduced crude protein diets could be attributed to the decreased feed intake and total feed cost relative to those pullets fed reduced crude protein diet. Results are in agreement with the findings of Dong (1997), who reported a slightly higher income over feed cost of layers fed corn-soybean based diets supplemented with enzymes. This result contradicts the findings of Luis (1997), who observed a lower IOFC of layers fed corn-based diets supplemented with enzymes. The decrease in income over feed cost in their study could be due to different diet composition and the kind and level of enzyme present in the supplement.

Treatments	0				
	Total feed	Total feed	Total eggs	Income from	IOFC
	consumed (kg)	cost (PhP)	(dozens)	sale of eggs	(PhP)
Basal diet	11.00	112.75	8.96	296.34	183.59
Diet with reduced crude protein	11.25	112.95	8.60	284.00	171.05
Diet with reduced crude protein	11.00	112.04	8.49	280.23	168.18
+0.05%protease					
Diet with reduced crude protein	11.10	110.55	8.80	290.51	179.96
+0.075%protease					
Diet with reduced crude protein	11.07	111.20	9.00	2.97.10	185.90
+0.1%protease					

Table 11. Income over feed cost (IOFC) of pullets fed basal diet and diets with reduced crude protein supplemented with different levels of acid protease from 24 to 40 weeks of age

0 Standard sized eggs calculated on the basis of 56.60 grams, 1 Price of protease 636.00 PhP per kg, 2 Sale of eggs 33.00 PhP per dozen, 3 Feed cost 9.94 and 9.73 PhP, respectively for basal and reduced crude protein diets

CONCLUSIONS

One percent reduction of crude protein of diet of pullet significantly decreased the digestibility of all nutrients. Both biweekly and overall average egg production, feed consumption, egg weight, feed efficiency, eggshell thickness, yolk color and body weight gain were not significantly affected by dietary reduction of crude protein. Income over feed cost decreased with reduction of crude protein of the diet. Protease supplementation of reduced CP diets significantly improved the CP and EE digestibility of diets and generally increased the biweekly and overall egg production in most periods of the trial. Egg weight, feed consumption, and yolk color were not significantly influenced by protease supplementation of the reduced CP diets. Feed efficiency was significantly improved with 0.1% protease, while eggshell thickness was significantly improved with 0.075% protease supplementation of diets. Income over feed cost increased with dietary supplementation of protease. Increased level of protease supplementation of reduced CP diets consistently improved the digestibility of nutrients, overall average egg production and decreased feed consumption. Egg weight and feed efficiency were slightly improved with 0.075% and 0.1% protease supplementation of diet. Eggshell thickness was significantly improved with 0.075% protease Yolk color was not significantly influenced by increased protease supplementation of the diet. Income over feed cost was consistently increased with protease supplementation. The crude protein of layer diets could be reduced by one percent provided that they are supplemented with protease. Optimum level of inclusion of protease is 0.1% for layers. Enzyme activity must be accurately determined before it is used.

ACKNOWLEDGMENTS

The author wishes deep sense of gratitude to the editor, Director of research, Dean of Institute of Agriculture and Animal Sciences, and all other staffs who directly or indirectly helped in finalizing and publishing this paper.

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