Biological Value of Papain Synthetic in Layer Ration at Various Protein Contents

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ABSTRACT: Two ways of utilizing papain synthetic in layer rations, supplemented directly and inoculated to soybean oil meal (SBOM-hydrolyzate) before being mixed in the ration, have been studied. Three hundred 24-week old layers were randomly allocated to 15 dietary treatments to measure the effect of ration containing 17, 15, and 14% CP and two levels of papain (0.075 and 0.15%) either directly supplemented in ration or in form of SBOM-hydrolyzate, derived from inoculating SBOM with 0.5 and 1.0% papain before being mixed in the rations. All dietary treatments factorially (3x5) were plotted in Completely Randomized Design and replicated to 5. Accumulated data for 4 x 28 days of egg production performances were statistically analyzed with analysis of variance, and followed by DMRT to compared the different effect among single dietary treatments. Results indicated that directly supplemented papain or SBOM-hydrolyzate in the rations produced significantly ($P<0.01$) higher and more efficient as compare to dietary control. However, both parameters statistically were not affected by the levels of papain. The significant interaction effect ($P<0.05$) between levels of protein and papain treatments were observed on feed intake, hen day production, and egg weight. It was, generally concluded that supplementation papain in the ration produced better performance as compared either with dietary control or diet containing hydrolyzate of SBOM, specially for low protein (14%) layer diet.

Key Words : Papain, Supplementation, Hydrolyzate, Soybean oil meal, Layers

Introduction

In the poultry farming, feed is a dominant factor at production. To increase the feed efficiency lately researchers have been interested to employ enzyme in the poultry feed.

One of many kinds of enzyme mostly employed is enzyme ß-glucanase to overcome ß-glucan that is different for chicken to digest and this enzyme found in some cereals. The use of enzyme ß-glucanase in the feed containing barley and oats significantly increases the growth and feed efficiency of broilers (Cantor et al., 1989; Ferket et al., 1989; Cantor et al., 1990; Farrell, 1991; Raudati et al., 1991).

Up to now, research on the use of enzyme supplementation for layer feed have been very limited. Pescatore et al. (1990) use "Allzyme BG" in layer feed and this can increase egg production. In addition, Brake (1992) uses Kemzyme, that is the combination of α-amylase, ß-glucanase, pullulanase, protease and gluconamylase as much as 0.1% and this tend to increase egg production ($P<0.1$), whereas the use of Kemzyme-AG (α-galactosidase) 0.05 to 0.1% is able to increase egg production significantly ($P<0.05$).

The use of enzyme for hydrolyzing feed or feedstuff of poultry has not yet much been involved by the researchers. Former publication that recorded is the Kratzer (1944) report stating that addition of protein that has been hydrolyzate could increase, fairly high the level of nitrogen amino in chicken's blood compared if added in the form of pure protein. In addition, Lee et al. (1989), report stating that the use of *feather-lysate*, that is hydrolyzed by *Bacillus licheniformis* enzyme as protein sources, could give growth curve of broilers identical to the use of soybean protein.

Papain is enzyme extracted from papaya. It includes in the classification of thiol protease and is formed by 212 amino acid (Fersht, 1985). Papain has monomeric structure with molecule weight of 21,000 and is fairly stabil to the temperature changes. Peptide bond which is formed by α-amino carboxy close to arginin and lysine residue is the most sensitive to papain. Papain, too, can hydrolyze peptide bond specifies for chymotrypsin, pepsin, aminopeptidase, and Carboxypeptidase. (Hill, 1965).

The research aim is to know the benefit of papain used/added in layer feed at some levels of protein on the basis of the production performances.

Experimental Procedurs

Three hundred Lohmann layers 24 weeks old were randomly divided into 15 groups of dietary treatments using factorial design 3x5; 3 levels of protein and 5 treatments of papain. Each group of treatment was replicated to 5 times, and each replication used 4 layers. Levels of protein use were 17% (CP-17), 15% (CP-15) and 14% (CP-14), whereas treatments of papain were as control groups (C), feed supplementation were 0.075% (S0.075) and 0.15% (S0.15) and for hydrolyzing soybean oil meal (SBOM) with papain levels of 0.5% (H0.5) and 1.0% (H1.0). SBOM for all ration were determined as much as 15%, therefore if calculated on the basis of total feed weight, papain in the treatment S0.075 is as much as H0.5 and S0.15 is as much as H1.0.

Hydrolysis of SBOM was conducted at substrate levels at 12% (w/v), temperature 40-50°C, pH 6.5 - 7.5 for three hours. To absorb the water after finishing the hydrolysis process, other feedstuff were used corn and rice polishing. This process dried under sunlight.

The basal diets were consits of SBOM (determined 15%), fish meal, yellow corn rice polishing, and vitamin-mineral premix until up to 100% with three of protein levels, 17, 15 and 14%, metabolizable energy is about 2750 kcal/kg, calcium 3.5% and phosphore 0.47%. Parameter recorded included of feed consumption, egg production (HDA), egg weight and feed conversion. Data obtained will be analyzed by analysis of variance using factorial design and if significantly then continued to test between means using DMRT (Steel and Torrie, 1980).

Results and Discussion

Feed Consumption

Feed consumption of the chicken group given feed containing protein 17, 15 and 14%, was statistically not different (Table 1). This is similar to the results obtained by some former researchers, that is chickens given feed with different levels of protein did not result in feed consumption differences (Calderon and Jensen, 1990; Jensen et al. 1990, Penz and Jensen, 1991; Keshavarz and Jackson, 1992).

Levels or treatments of papain statistically did not affect on feed consumption. However, further statistic analysis indicated that feed consumption was significantly effected ($P<0.05$) by the interaction between protein levels and papain treatments. Papain treatments indicated the effect on protein level of 14% significantly, that was chicken having treatments S0.075 indicated higher feed consumption ($P<0.05$) compared with C, S0.15, and H0.5. Further study showed that feed consumption of chickens having treatment CP-14 with S0.075 resulted in the highest feed consumption (118.8 g/day), whereas for treatment CP-14 with H0.5 showed the lowest feed consumption (108.4 g/day).

Egg Production

Effect of papain treatments on egg production from all treatments were shown on Table 2. The results of this research showed that papin treatments ($P<0.01$) and levels of feed protein indicated different effect significantly $P<0.05$) on egg production. In addition, the two treatments also indicated significant interaction ($P<0.05$).

Table 1. Feed consumption (g/day)

Papain treatments (B)	Protein levels (A) CP-17	CP-15	CP-14	Average (B)
C (Control)	113.8^{abc}	112.9^{abc}	110.8^{bc}	112.5
S0.075	110.7^{bc}	113.1^{abc}	118.8^{a}	114.2
S0.15	113.1^{abc}	112.6^{abc}	109.04^{bc}	111.6
H0.5	116.7^{ab}	109.5^{bc}	108.4^{c}	111.5
H1.0	114.1^{abc}	110.2^{bc}	114.12^{abc}	111.5
Average (A)	113.7	111.7	112.2	

The different superscripts at the columns and row of interaction indicate significant difference ($P<0.05$)

Table 2. Egg production (HDA, %)

Papain treatments (B)	Protein levels (A) CP-17	CP-15	CP-14	Average (B)
C (Control)	69.69bcd	64.11d	63.69d	65.83i
S0.075	78.33ab	80.24a	84.36a	81.01g
S0.15	78.90ab	80.89a	74.91abc	78.91g
H0.5	79.57a	69.87bcd	63.36d	70.91h
H1.0	77.45ab	74.87abc	67.32cd	73.21h
Average (A)	76.79e	73.99ef	71.07f	

The different superscript at the columns and row of interaction, at the row of the average of protein levels (A), and at the columns of the average of papain treatment (B) indicate significant different ($P<0.05$).

The results of this research were similar with some former researches, that protein levels between 16 to 17% were proper protein content for maximum production. Feeding with lower protein content than above value, tends to lower egg production (Keshavarz, 1984; Calderon and Jensen, 1990; Colnago and Jensen, 1990; Dobson, 1990; Jensen et al., 1990; Keshavarz dan Jackson, 1992). The difference of egg production of chickens which got feed with some levels of protein was most presumably effected by the content of essential amino acid in the feed. The content of critical amino acid for all treatments have fulfilled the standard needs according NRC (1984), except the content of methionine. The content of metionin in the treatments CP-17, CP-15 and CP-14, was 0.30, 0.26 and 0.23%, whereas the standard from NRC (1984) is 0.32%.

So as the effect of papain treatment, the effect of enzyme supplementation in the feed on egg, production was similar with former research result, although the enzyme supplementated was different (Pescatore et al. 1990; Pan and Guenter 1992). And the increase of egg production caused by the use of hydrolyzate of SBOM in this research was presumably caused by the higher amino acid obtained from SBOM that was absorbed by the chickens. This has been proved by Kratzer (1944), chickens that were feed casein hydrolyzate have higher blood nitrogen amino content compared to those fed pure casein. Another research conducted by Lee et al. (1989), prove that addition of featherlyzate, results in better broiler growth compared with commercial feather meal.

It was been stated that there was significant interaction ($P<0.05$). On treatment CP-17, there was no different effect on egg production between supplementation treatment and hydrolysis, or levels of papain involved, except treatment H0.5 which showed significantly higher egg production compared to control group. On CP-15, only hydrolyzed treatment with papin level 0.5% resulted in significantly lower egg production ($P<0.05$) compared to supplementation treatment. On CP-14 papain treatment showed more various egg production. On S0.075, resulted in the highest egg production of all treatments.

One of the bases that maybe used to explain the above phenomena was presumably the exact ratio between papain as an enzyme and feed as a substrate, as some former researches show that one of the factors effected the success of hydrolysis process by means of enzyme was enzyme ratio : substrate (Hill, 1965; Friesen et al., 1991; Brenes et al., 1991).

As has been known that on the process of protein hydrolysis, each enzyme has different characteristic in its ability to break down amino acid bonds in the protein structure. Therefore, it can be understood that the difference an protein content will affect on all hydrolysis product, because each level of protein has specific number of amino acid bond in the protein structure that can be broken down by the enzyme involved. Moreover, feed containing similar protein level, if feed source for protein intake is different, was assumed to be different in the amount of protein digested.

Egg Weight

Papain treatment and protein levels did not show the significantly effect on egg weight, either on papain supplementation treatment or hydrolysis of SBOM. But there was significant interaction

Table 3. Egg weight (g/egg)

Papain Treatment (B)	Protein Levels (A) CP-17	CP-15	CP-14	Average (B)
C (Control)	62.8^{a}	62.4^{ab}	60.6^{ab}	61.9
S0.075	61.2^{ab}	60.8^{ab}	62.9^{a}	61.6
S0.15	62.9^{a}	61.9^{ab}	60.3^{b}	61.7
H0.5	61.8^{ab}	60.6^{ab}	60.3^{b}	60.9
H1.0	60.2^{b}	62.0^{ab}	61.2^{ab}	61.1
Average (A)	61.8	61.6	61.06	

The different superscripts at the columns and row of interaction indicate significant difference ($P<0.05$)

($P<0.05$) between papain and protein levels on supplementation treatment (Table 3).

By papain treatments, either in the form of supplementation or hydrolysis, only as papain supplementation 0.15%, protein level showed significantly different effect on egg weight ($P<0,05$), that was between protein level of 17% and 14%. The other papain treatments, protein level did not show different effect on egg weight.

If investigated on the basis of each protein level, papain treatment showed different effect significantly ($P<0.05$) at protein level of 17 and 14%, whereas at protein level of 15% did not show different effect on egg weight. At protein level of 17%, hydrolysis treatment with papain 1.0% resulted in lower egg weight ($P<0,05$) compared to control and papain supplementation treatment 0.15%, whereas among the other treatments howed indifferent egg weight. At protein level 14%, papain suppelementation treatment 0.075% resulted in higher egg weight ($P<0,05$) compared with treatment of papain hydrolysis 0.5% and papain supplementation 0.15%.

Feed Conversion

Based on statistical analysis, treatment of papain supplementation on SBOM hydrolyzate in layers' feed could result in significantly lower feed conversion ($P<0.01$) compare to the control. From this research, it can be seen that papain use, either through supplementation on hydrolysis of feedstuff could increase feed efficiency. Of the two papain treatments, however, papain supplementation could result in significantly better feed efficiency than hydrolysis of SBOM through *in vitro*.

The increase of feed efficiency, is presumably connected to a report stating that broilers given feed containing enzyme, their digestive system will relatively be smaller, and their weight will be higher compared to those given control feed (Cantor et al., 1990; Brenes et al., 1991). This shows presumably the decrease of activity of digestive system for enzyme secretion so that its size becomes smaller, and wholly, digestion efficiency becomes better. Generally, it can be seen than feed conversion for experiment of SBOM hydrolysis shows worse figure compared to treatment of papain supplementation.

Table 4. Feed Conversion

Papain Treatments (B)	Protein Levels (A) CP-17	CP-15	CP-14	Average (B)
C (Control)	2.62	2.85	2.87	2.78^{d}
S0.075	2.32	2.34	2.24	2.31^{c}
S0.15	2.27	2.26	2.38	2.30^{c}
H0.5	2.38	2.37	2.93	2.55^{e}
H1.0	2.45	2.34	2.77	2.50^{e}
Average (A)	2.41^{b}	2.44^{b}	2.64^{a}	

The different superscript at the row (A) and column (B) indicate significant difference ($P<0.05$).

From the research results above, it generally can be proved that papain supplementation will more effective if added to feed containing low protein and at relatively low level (0.075%). At that condition, it proves that egg production, egg weight and feed conversion ratio were better than with the other treatments. The use of SBOM hydrolyzate product only effected well on high protein level (17 and 15%). This indicated by low feed conversion figure. However, at low protein level (14%), feed conversion figure has not significantly different.

Conclusion and Suggestion

Conclusion

Papain supplementation on layers feed results in better production performance (egg production, feed conversion and egg weight) compared to control or the use of SBOM hydrolyzate, especially at low protein level (14%). Papain level 0.075 and 0.15% of the total feed weight doesn't show different effect on the investigated parameters.

Suggestion

It needs further research on enzyme addition at chicken feed, including the use of hydroyzate product as a supporting research before these research results are recommended for its application although from these research results can be proved that enzyme addition can improve layers productivities.

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