Journal of Animal and Veterinary Advances 10 (20): 2696-2698, 2011
ISSN: 1680-5593

Evaluation of the Combination of Vitamin D_3 and Papaya Leaf on Muscle Antioxidant Activity of Spent Chicken

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Abstract: Eighty spent chickens were employed in this study to assay the effect of combination of vitamin D_3 and papaya leaf on antioxidant activity of meat in spent layer hens. Diets were a corn-soybean meal based diet for finisher layer with and without vitamin D_3 which was supplemented with different levels of 0, 0.5, 1 and 2% for papaya leaf meal. Experiment lasted for 21 days. At day 0, 7, 14 and 21, the birds were scarified and breast muscle was obtained to determine antioxidant activity. Antioxidant activity was measured using 1, 1-Diphenyl-2-Picrylhydrazyl (DPPH). Result obtained from this study demonstrated that antioxidant activity of meat showed remarkable improvement between dietary treatments fed mix of vitamin D_3 and papaya leaf and control group. In conclusion, vitamin D_3 and papaya leaf when combined indicated an improvement in antioxidant activity of the spent meat.

Key words: Vitamin D_3 and papaya leaf combination, antioxidant activity, spent meat, breast muscle, corn soybean, Malaysia

INTRODUCTION

The poultry industry is faced with a large number of spent layer hens which are normally sold as old chickens and carry lower prices than the broiler chickens. Globally, there are about 2.6 billion spent hens that are used in the pet food industry and not much for human consumption. Meat from spent hens tends to be tough, non-juicy and low in fat. If the spent hens can be improved in terms of quality, farmers can sell the spent chickens at a better price. Thus, it was considered essential and economically viable to improve the meat quality of the birds. To achieve this goal, some approaches were applied such as additives, calcium chloride marinating, infusion or injection (Koohmaraie *et al*., 1988; Pringle *et al*., 1999) and other methods. Attempts to improve meat quality through improving tenderness through post-slaughter manipulations such are either costly, labor intensive, need large storage area and require longer storage time. Therefore, they are impractical and not economically workable. So recently, the method of improving meat tenderness before slaughtering is of great interest. Along with meat tenderness, its quality such as antioxidant activity, fatty acid content and other characteristics are strongly emphasized as well. This study mainly focused on the application of vitamin D_3 and papaya leaf on antioxidant activity of spent chicken meat.

MATERIALS AND METHODS

The experiment was undertaken with eighty spent chickens, ISA-brown which were taken from layer farm of University Putra Malaysia after a period of laying of 80 weeks. Chickens were kept in an individual cage on an optimal condition. Feeding 10 days adaptation was conducted and thereafter, the experiment lasted 21 days. The diets were fed individually in the feeders with a specified weight every day. Eight diets which served in this research were diet 1; control diet (which met or exceeded NRC (1994) recommendations for the bird at finisher stage) with and without vitamin D_3 which was supplemented with different levels of 0, 0.5, 1 and 2% Papaya Leaf Meal (PLM).

Papaya leaves were collected from local plants and separated from the stems, dried in a 65°C oven until constant weight. The dry leaves were grinded, passed through a sieve of 1 mm and properly mixed with the diet (Table 1). The sample of PLM was analyzed for crude protein, crude fibre, fat, dry matter, ash (AOAC, 1995) and calcium content using atomic absorption spectrophotometer (Table 2). Experimental period was 21 days. At day 0, 7, 14 and 21, the birds were slaughtered and left and right breast muscles of each bird were taken to assay antioxidant activity. Samples were kept in -80°C for later analysis.

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Table 1: Composition of basal diet

Ingredients (%)	Values
Corn grain	65.40
Soybean meal	22.01
Palm oil	1.50
Limestone	7.90
Dicalcium phosphate	2.04
Salt	0.40
Mineral premix[1]	0.25
Vitamin premix[2]	0.25
Choline Chloride	0.15
DL Met	0.10
Chemical composition	
ME (kcal kg^{-1})	2850.00
Crude protein (%)	16.60
Ca (%)	3.80
Crude fiber (%)	3.04
Ether extract (%)	3.62

Supplied per kg diet; Fe, 35 mg; Mn, 70 mg; Cu, 8 mg; Zn, 70 mg; I, 1 mg; Se, 0.25 mg; Co, 0.2 mg; calcium-D-pantothenate, 8 mg; folic acid, 0.5 mg; D-biotin, 0.045 mg; vitamin C, 50 mg; vitamin A, 8000 IU; vitamin D_3, 1×10^5 IU; vitamin E, 30 IU; vitamin K_3, 2.5mg; vitamin B_1, 2 mg; vitamin B_2, 5 mg; vitamin B_6, 2 mg; vitamin B_{12}, 0.01 mg and niacin, 30 mg; *Diet without vitamin D_3 excluded vitamin D_3 in the vitamin premix

Table 2: The chemical analysis of the papaya leaf

Parameters (%)	Values
Dry matter	21.47
Crude protein	26.21
Fat	6.55
Ash	11.51
Fiber	7.39
Ca	0.04

1, 1-Diphenyl-2-Picrylhydrazyl (DPPH) assay: To determine DPPH, a method of Blois (1958) was used. 1, 1-Diphenyl-2-Picrylhydrazyl (DPPH) radical scavenging activity was measured with the aqueous supernatant obtained from breast meat as follows: a 200 μL diluted aqueous supernatant (1%) was supplemented to 800 μL of water and 1 mL of methanolic DPPH solution (0.2 mM). The mixture was vortexed and left to stand at room temperature for half an hour.

A tube containing 1 mL of distilled water and 1 mL of methanolic DPPH solution (0.2 mM) served as the control. The absorbance of the solution was measured at 517 nm. The percentage of DPPH radical scavenging was gotten from the equation as:

$$\text{Radical-scavenging activity} = \left[\left(\frac{\text{1-absorbance value of testing solution}}{\text{Absorbance value of control solution}}\right)\right]\times 100$$

Statistical analysis: This experiment was a 2 (with and without vitamin D_3)×4 (four levels of PLM) factorial arrangement with a basis of Completely Randomized Design (CRD) with 10 replicates $treatment^{-1}$. Statistical analyses were performed using the procedure indicated the SAS statistical package (SAS, 1991). The significance of differences between means will be tested using the Duncan's multiple-range test (Duncan, 1955) of the GLM procedure. The $p<0.01$ were considered to be statistically significant.

RESULTS AND DISCUSSION

The interaction effects of vitamin D_3 and papaya leaf meal on antioxidant activity is shown in Table 3. For antioxidant activity, treatments contained 0.5, 1 and 2% PLM with vitamin D_3 had significant improvement ($p<0.01$) in antioxidant activity of meat compared to the group fed no vitamin D_3 at days 7, 14 and 21. For groups contained no vitamin D_3 as the level of PLM increased, the antioxidant activity decreased ($p<0.01$) over the experimental period. Interaction between vitamin D_3 and PLM was significant ($p<0.01$) at day 7, 14 and 21.

Some studies proved that there are close connection between the pre slaughter feeding regimen and meat tenderness. It appears that intensive pre slaughter feeding exerts an indirect influence on meat tenderness (Schroeder *et al*., 1982; Navid *et al*., 2010; 2011). Result of a study indicated that vitamin D_3 had similar reduction in the extent of lipid peroxidationas with vitamin E supplementation (Sardar *et al*., 1996). Other research conducted to study Vitamin D_3 antioxidant activity and its mechanism that showed Vitamin D_3 has a membrane antioxidant activity to inhibit iron-dependent lipid peroxidation in liposomes compared to cholesterol, ergosterol and tamoxifen (Wiseman, 1993). On the other hand, studies on the papaya seed's antioxidant property demonstrated that total phenolic content of the seed extracts was found to have a positive linear correlation with the total antioxidant activity.

This research revealed that chloroform-methanol extract of papaya seeds was found to have antioxidant activity due to having high phenolic content indicating contribution of phenolic phytoconstituents towards antioxidant activity (Kothari and Seshadri, 2010). Same results were obtained from other parts of this plant as well. Meat, seed and pulp of papaya showed significant ability to scavenge 1, 1-Diphenyl-2-Picrylhydrazyl, Hydroxyl (DPPH) and superoxide radicals. Vitamin C, malic acid, citric acid and glucose are some of the possible antioxidative components in papaya which can contribute to antioxidant property of this fruit (Osato *et al*., 1993). To the knowledge, there is a paucity of literature regarding

Table 3: The interaction effect of PLM and vitamin D_3 (1×10^5 IU) on antioxidant activity of meat >3 weeks of the experimental period in spent layer hens

	Vitamin D_3										
	PLM (+)				PLM (-)						
Antioxidant activity (days)	0	0.5	1	2	0	0.5	1	2	Vitamin D_3	PLM	Vit.×PLM
0	0.16±0.005^b	0.26±0.003^a	0.24±0.008^a	0.27±0.004^a	0.29±0. 01^a	0.21±0.001^a	0.21±0.001^a	0.13±0.005^b	***	***	-
7	0.17±0.003^b	0.27±0.003^a	0.27±0.004^a	0.28±0.001^a	0.30±0.002^a	0.22±0.001^b	0.21±0.004^c	0.14±0.003^d	***	***	***
14	0.17±0.002^b	0.28±0.003^a	0.28±0.003^a	0.28±0.001^a	0.31±0.003^a	0.23±0.002^b	0.22±0.005^c	0.15±0.002^d	***	***	***
21	0.18±0.002^b	0.29±0.003^a	0.29±0.003^a	0.29±0.003^a	0.33±0.004^a	0.35±0.103^a	0.23±0.003^a	0.16±0.000^b	***	***	***

The results are representative of ten spent chickens and are expressed as mean±SEM; ***Significant ($p<0.01$)

the antioxidant activity of papaya leaf along with vitamin D_3 however, according to our result papaya leaf can show its antioxidative a bility when diets included vitamin D_3.

CONCLUSION

It could be resulted that the dietary supplementation papaya leaf up to 2% of dry matter in the diets included vitamin D_3 might have good effect on muscle antioxidant activity of spent chicken if this additive is supplemented with the diet few weeks before slaughtering.

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