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Influence of the Combination of Vitamin D_3 and Papaya Leaf on Meat Quality of Spent Layer Hen

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Abstract: The study was conducted to improve meat quality of spent layer hens through supplementing the old layer hens feed with vitamin D_3 in combination with papaya leaf meal applied 3 weeks prior to slaughter. Eighty spent chickens were served in this study after a period of laying for 80 weeks. 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; breast muscle was obtained for meat and skin color, shear force, drip loss, cooking loss, crude protein, fat and calcium. Most of parameters showed significant improvement when diet supplemented with papaya leaf meal and vitamin D_3. It can be concluded that for some characteristics related to meat quality, combination of papaya leaf meal and vitamin D_3 has synergistic effect.

Key words: Vitamin D_3 and papaya leaf combination, meat quality, spent layer, tenderness, juiciness, Malaysia

INTRODUCTION

Meat tenderness is probably, the most important factor influencing consumer's eating satisfaction. Other sensory properties such as juiciness and flavor can only be evaluated after the product has been prepared and is consumed. Consumers have color opportunity based on regional preferences and previous experience. Some consumers prefer light meat and others like dark meat. The consumers will select the products which best meet their expectation for color and uniformity.

The problem of inconsistency in the meat tenderness is partly due to inability to regularly produce tender meat and to identify carcasses producing tough meat. Meat of spent layer fowls becomes tough with increasing age and this toughness limits, its use in whole muscle foods, resulting in considerable economic losses to the poultry industry which produces spent fowl as a by-product of table and hatching egg production.

For this reason, spent fowl meat has been traditionally used in less profitable, comminuted or retorted products in which small particle size or thermal dispensation is used to reduce toughness. Thus, the potential economic benefit to be gained by tenderizing this meat is tremendous. One of the means of improving meat quality of spent layers is to improve its tenderness. Traditionally, meat is tenderized by marinating it with pastes made from papaya leaf, skins of green papaya and pineapple skin. Now-a-days, Papain or Bromelin which are photolytic enzymes obtained from papaya and pineapple, respectively are available in groceries as meat tenderizers under different trade names and either in liquid or powder forms (Krishnaiah *et al*., 2002). Koohmaraie *et al*. (1988) and Hamm (1975) confirmed that post mortem meat tenderization could be manipulated and improved through the elevation of intracellular calcium level at post mortem either by calcium chloride marinating, infusion or injection. However, all these methods are laborious, time-consuming and not practicable on large scale operation. A more appropriate method would be a pre-slaughter treatment on the animals that would induce tenderization post mortem. Findings from other research conducted by Dransfield (1981) and Dutson *et al*. (1981) agree that a high ultimate muscle pH ($\geq$6.0) is related to a more tender muscle. Currently, the improvement in meat tenderness is achieving by addition of the natural products. This experiment aimed to evaluate, the combination effect of vitamin D_3 and papaya leaf meal on some meat quality parameters including meat color, shear force, pH, CP, fat, Ca and antioxidant activity.

MATERIALS AND METHODS

The study was conducted with eighty spent chickens, ISA-brown which were provided from layer farm of University Putra Malaysia after a period of laying

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of 80 weeks. Hens were kept in an individual cage on an optimal condition. Feeding 10 days adaptation and thereafter, 21 days for experiment have conducted and the diets were fed individually in the feeders with a specified weight every morning. Eight diets which served in this research were diet one: control diet (based on NRC recommendation level to meet or exceed the bird requirements at finisher stage) with and without vitamin D_3 which was supplemented with different levels of 0, 0.5, 1 and 2% for papaya leaf meal. Papaya Leaf Meal (PLM) 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 (PLM) was analyzed for crude protein, crude fibre, fat, dry matter, ash (AOAC, 1995) and calcium content using atomic absorption spectrophotometer. Experimental period was 21 days at day 0, 7, 14 and 21, the birds were slaughtered and left and right breast muscle of each bird were taken to assay shear force, meat color, CP, fat, Ca, pH of the muscle, drip loss, cooking loss and antioxidant activity. Samples were kept in -80°C for later analysis. To measure the cooking loss, 50 g breast muscle was weighed before chilling, vacuumed immediately, packed and cooked in 80°C water bath. A 21-gauge needle was used to insert the thermometer probe into the geometric centre of a steak. Thereafter when the sample's core temperature reached 80°C, the thermometer was removed. The sample was taken out of the water bath and weighed. The cooking loss was then calculated based on the following formula:

$$\text{Cooking loss (\%)} = \frac{\left(\begin{array}{c}\text{Pre-cook meat weight} - \\ \text{Post-cook meat weight}\end{array}\right)}{\text{Pre-cook meat weight}} \times 100\%$$

To determine drip loss, 10 g breast muscle was weighed immediately after slaughtering. After 3 days, the breast muscle was weighed again and based on the recorded weight the drip loss was calculated:

$$\text{Drip loss (\%)} = \frac{\left(\begin{array}{c}\text{Pre-chilling meat weight} - \\ \text{Post-chilling meat weight}\end{array}\right)}{\text{Pre-chilling meat weight}} \times 100\%$$

Postmortem muscle pH was measured by using a pH meter (Mettler Toledo, model 320). Colour quantity was obtained by a Hunter-Lab model D-25 color and color difference meter (Hunter Associates Laboratory, Fairfax, VA readings were taken on both the bottom (the bone side) and upper (the skin side) surface of each sample).

Table 1: Composition of basal diet with vitamin D_3 (1×10^5 IU)

Ingredients (%)	Values
Corn grain	63.80
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, 2.5 mg; 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 vit D_3 in the vitamin premix

The colorimeter was standardized with a standard plate (# 6274; L = 92.4, a = -0.7, b = -0.9). After calibration, the sample was put on the plate and at three different angles, the color was measured. Meat tenderness was determined by Warner-Bratzler shear force analysis which was according to the mechanical force necessary to shear, the muscle fibres of a cooked meat sample. The Atomic Absorption Spectrometer (AAS) was employed to measure calcium after digestion of the sample with 6 M HCl. Shear force analysis was based on the mechanical force necessary to shear, the muscle fibres of a cooked meat sample and the instrumental meat tenderness measurement was determined by Warner-Bratzler. Muscle fat and crude protein was determined by using AOAC (1995) method.

This experiment was a 2×4 factorial arrangement with a basis of Completely Randomized Design (CRD) with ten replicates per treatment. Statistical analyses were performed using the procedure indicated the SAS statistical package (SAS, 1990). The significance of differences between means will be tested using the Duncan multiple-range test (Duncan, 1955) of the GLM procedure ($p<0.01$).

RESULTS AND DISCUSSION

The interaction effects of vitamin D_3 and papaya leaf meal on shear force, cooking loss and drip loss are showed in Table 2. The interaction between vitamin D_3 and PLM was significant ($p<0.01$) for shear force. When diet was not supplemented with PLM, shear force was highest among treatments ($p<0.01$). However, added

Table 2: The interaction effect of PLM and vitamin D_3 on shear force, cooking loss and drip loss in spent layer hen

Vitamin	+ve (%)				-ve (%)						
PLM	0	0.5	1	2	0	0.5	1	2	Vit. D_3	PLM	Vit×PLM
Shear force (g cm^{-2})	4.10±0.10^b	4.36±0.09^a	4.11±0.10^b	4.05±0.10^b	6.17±0.11^a	3.79±0.08^b	3.72±0.10^b	3.51±0.10^b	NS	***	***
Cooking loss (%)	35.20±0.09^b	34.90±0.24^c	35.27±0.20^b	35.10±0.10^b	34.70±0.20^b	35.40±0.10^b	35.06±0.12^b	37.90±0.18^a	***	***	***
Drip loss (%)	1.26±0.06^b	1.29±0.01^b	1.30±0.08^a	1.22±0.01^c	1.25±0.02^a	1.25±0.06^b	1.28±0.01^{ab}	1.30±0.04^a	***	***	***

Table 3: The interaction effect of PLM and vitamin D_3 on meat and skin colour, muscle Ca, fat and CP in spent layer hen

Vitamin	+ve (%)				-ve (%)						
PLM	0	0.5	1	2	0	0.5	1	2	Vit. D_3	PLM	Vit×PLM
Meat colour (skin)	65.10±1.06^a	50.10±1.03^d	51.60±1.10^c	63.7±1.07^b	65.60±1.10^a	63.10±1.20-^{b}	65.6±1.00^a	43.80±1.38^c	***	***	***
Meat colour (muscle)	49.42±0.09^c	41.01±0.03^d	62.20±0.58^a	57.3±0.05^b	49.45±0.09^c	62.42±0.01^a	62.5±0.17^a	63.50±51.20^a	***	***	***
Muscle Ca (mg g^{-1})	2.90±0.10^{ab}	3.31±0.20^a	2.52±0.20^b	2.6±0.20^{ab}	2.00±0.10^c	2.80±0.20^b	3.2±0.10^b	4.00±2.25^a	NS	***	***
Muscle CP (%)	79.90±0.09^b	81.90±0.10^a	81.00±0.10^a	81.9±0.06^a	79.50±0.01^b	82.14±0.10^a	81.9±0.10^a	81.90±0.10^a	NS	***	***
Muscle fat (%)	4.23±0.08^d	7.10±0.20^c	7.20±0.03^c	7.2±0.05^c	2.45±0.11^e	7.50±0.03^b	7.5±0.09^b	7.88±0.94^a	NS	***	***

The results are representative of ten spent chickens and are expressed as mean±SEM

papaya leaf meal at levels 0.5, 1.0 and 2.0 did not change shear force. Supplementation of diet with vitamin D_3 reduced cooking loss significantly ($p<0.01$). However, vitamin D_3 supplementation at levels of 0.5, 1.0 and 2.0% did not change cooking loss. Vitamin D_3 at 0.5 level of papaya leaf meal, reduced drip loss significantly ($p<0.01$). There were no significant differences among supplemented and non-supplemented diets with vitamin D_3.

The interaction influences of vitamin D_3 and papaya leaf meal on meat and skin colour, muscle Ca, CP and fat are shown in Table 3. Meat colour (skin) was reduced in those treatments that supplemented with vitamin D_3. However, meat colour (skin) was not affected by vitamin D_3 supplementation at 2.0% papaya leaf meal. For meat colour (muscle) at 0.05 and 2.0% PLM, there were significant differences between vitamin D_3 supplemented and non-supplemented groups ($p<0.01$). However at levels 0 and 1.0, no significant differences was observed among treatments. Vitamin D_3 supplementation increased calcium of the muscle when combined with 0.5% of papaya leaf meal but Ca reduction was observed at 1.0 and 2.0% PLM ($p<0.01$).

Papaya leaf meal mixed with vitamin D_3 and also alone had significant effect on muscle CP and all levels of PLM (0.5, 1 and 2%) showed significant increase in CP content of the muscle. Papaya leaf meal increased muscle fat ($p<0.01$) when it did not supplemented with vitamin D_3 and the value of 2% PLM displayed the highest fat percentage among others.

Study conducted by some researchers, demonstrated that there is a 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, 1978). As observed in the present study, addition of papaya leaf meal in lower values to the diet improved shear force however, the high values failed to affect this characteristic. Regarding the drip loss, PLM was not so successful to improve this characteristic in meat (with or without vitamin D_3). An important factor in the further processing of poultry meat is water retention. Meat including poultry, contains about 70-75% water at slaughter (Honikel, 1987). Water holding capacity contributes to the palatability (Miller and Harrison, 1965), microbial quality (Jay, 1967) and mechanized potential (Saffle, 1968) of meat. It has been positively correlated with tenderness and negatively correlated with drip loss of meat (Wierbicki and Deatherage, 1958; Hamm, 1975). Fluid loss during meat processing and storage disturbs producers, processors and consumers that should be improved by useful approaches (Honikel, 1987).

Related to skin colour, PLM supplemented with vitamin D_3 failed to improve this trait. While 1 and 2% of PLM without vitamin D_3 improved the skin colour. For meat colour, PLM combined with vitamin D_3 was also efficient in improving the muscle colour. Pigments in muscle tissue consist primarily of two proteins, myoglobin and hemoglobin. Myoglobin is the major pigment of muscle and hemoglobin is the major pigment of blood. Myoglobin is created and found within the muscle cells, depending on different muscles and it contributes 50-80% of the meat pigments. Hemoglobin is created in the bone marrow and exists in blood and meat which comprise 20-50% of the pigments (Fox Jr, 1987). The combined concentrations of these two pigments determine muscle colour. Other pigments such as cytochrome, flavin and vitamin B_{12} are also found in the muscle but they are present in such small quantities that they contribute little or nothing to meat colour. By increasing these pigments, the meat colour will improve that consequently will affect customers attraction.

Muscle calcium increased by addition of combination of PLM and vitamin D_3. Similar to this, result reported by Sazili that supplementation of vitamin D_3 at 1×10^5 IU for 3 consecutive days, prior to slaughter enhanced tenderness of spent chicken. In addition, oral administration of calcium propionate to cattle 3-6 h before slaughter had also increased cooked meat tenderness at 4 and 7 days post mortem (Duckett *et al.*, 1998).

CONCLUSION

It can be said that there are some merits in the dietary supplementation of 2% papaya leaf meal with vitamin D_3 in spent layer hens for few weeks before slaughtering and this combination can improve the meat quality. Obviously, this will also improves the utilization of spent hens from egg production and poultry breeding industry to human consumption, thereby providing an opportunity for human food industry to utilize spent layer hens.

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