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Comparative effects of dietary sea urchin shell powder and feed additives on meat quality and fatty acid profiles of broiler breast meat

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ABSTRACT. This study was a small pen trial in which we investigated comparative effects of dietary sea urchin shell powder and feed additives on meat quality and fatty acid profiles of broiler breast meat. A total of 108 male broilers were assigned to 3 groups (control, 1% sea urchin shell powder, and 1% feed additives) with 3 replicates of 12 chicks per pen in a completely randomized design for 28 days. The following parameters have been investigated: proximate composition (DM, CP, EE, and ash), physicochemical properties (pH, TBARS, cooking loss and DPPH radical scavenging), meat color and fatty acid profiles. No remarkable effects between treatment and storage day were observed for proximate composition, physicochemical properties, meat color and fatty acid profiles. In conclusion, diets with 1% sea urchin shell powder have the ability to increase DPPH radical scavenging and unsaturated fatty acid, indicating an opportunity for partial diet substitution in comparison with 1% feed additives.

Keywords: sea urchin shell, proximate composition, physicochemical properties, meat color, fatty acid.

Efeitos comparativos do pó da casca do ouriço-do-mar e suplementos de dieta na qualidade da carne e no perfil de ácidos graxos da carne do peito de frangos de corte

RESUMO. Nesse ensaio investigaram-se os efeitos comparativos do pó da casca do ouriço-do-mar e suplementos alimentares sobre a qualidade de carne e o perfil de ácidos graxos de carne de peito de frangos de corte. Cento e oito frangos de corte machos foram distribuídos em 3 grupos (controle, 1% pó da casca do ouriço-do-mar, 1% aditivos alimentares), com 3 repetições, com 12 frangos por gaiola, num esquema aleatório, durante 28 dias. Foram investigados os seguintes parâmetros: composição aproximada (MS, PB, EE e cinzas), propriedades físico-químicas (pH, TBARS, perda no cozimento e o radical livre DPPH), cor da carne e ácidos graxos. Não foi observado nenhum efeito significativo entre o tratamento e o dia de armazenagem para a composição aproximada, propriedades físico-químicas, cor da carne e ácidos graxos. Os resultados mostram que rações com pó da casca do ouriço-do-mar são capazes de aumentar o radical livre DPPH e os ácidos graxos não saturados e revelam uma oportunidade para a substituição parcial da ração com 1% de aditivos alimentares.

Palavras-chave: ouriço-do-mar, composição aproximada, propriedades físico-quimicas, cor da carne, ácido graxo.

Introduction

Improving meat quality and reducing lipid oxidation has become one of the major concerns in today's meat industry. Thus, the development of cost-effective agents as animal feed is responsible for reducing loss of meat quality and extending shelf-life, which in turn contributes towards stability in meat color and consumer preference. Although many different additives have been tested to increase the quality of meat and its products, the use of marine by-products has been shown to have antioxidant activities (Shankarlal et al., 2011). For example, sea urchins (urchins) are well known as small, spiny, globular invertebrate animals that live in all of the world's oceans (Shankarlal et al., 2011). In particular, sea urchin shells have been reported to contain certain compounds with antioxidant and anti-bactericidal effects including echinochrome A and polyhydroxylated naphthoquinone pigments (Anderson et al., 1969; Lebedev et al., 2001; Service & Wardlaw, 1984). Moreover, other authors have suggested that the phenolic hydroxyl groups found in these shells could have antioxidant activity (Shankarlal et al., 2011).

In terms of nutritional value and pharmaceutical effects, sea urchins provide a good source of protein, polyunsaturated fatty acids, minerals (Ca, Fe, Mg, and Ca), vitamins (B and C groups), and are used in medicinal remedies for phlegm, tuberculosis, and neuralgia (Kim et al., 2002; Kim, 2005a, b). In addition, consumption of sea urchin has increased markedly in recent years in many countries. According to other reports, the gonads (edible part) are estimated to comprise approximately 20% of the sea urchin, while the remaining 80% consists of the shell (Kim et al., 2002). Thus, one common strategy for utilizing sea urchin shell is as a dietary supplement for poultry. Most studies have focused on the nutritional value, composition, and processing of sea urchin products, but little research has been carried out on the effects of sea urchin shell powder on overall meat quality.

The aim of this study was to evaluate the comparative effects of dietary sea urchin shell powder and feed additives on meat quality during storage and fatty acid profiles of broiler breast meat.

Material and methods

Sample preparation

Sea urchin (*Hemicentrotus pulcherrimus*) used for supplementing the feed was collected from local markets. After eliminating the soft body and spines from the urchins, the shells were dried and finely ground to powder. Feed additives (Vital Gold®, soluble complex nutrition supplement) utilized in this study were purchased from Dae Sung Microbiological Labs. Co. Ltd (Euiwang, South Korea). The composition of the feed additives used in this study was as follows: 6,000,000 IU vitamin A, 2,000,000 IU cholecalciferol, 3000 IU tocopherol acetate, 3.0 g thiamine, 5.0 g riboflavin, 1.0 g pyridoxine, 2.0 mg cyanocobalamin, 2.0 g ascorbic acid, 1.0 g menadione sodium bisulfite, 0.01 g biotin, 5.0g Ca. pantothenate, 5.0 g nicotinamide, 0.1 g folic acid, 30.0 g choline bitartrate, 10.0 g DL-methionine, and 2.0 g L-lysine.

Animals and experiment procedures

All experimental procedures were carried out in accordance with the guidelines of an experimental poultry farm in Gunwi (South Korea). One hundred and eight 1-day-old male broiler chickens (Arbor Acres) were purchased from a commercial hatchery and randomly divided into 3 groups with 3 replicates of 12 chicks per pen in a completely randomized design. The experimental groups were as follows: control (basal diet); 1% sea urchin shell powder; and 1% feed additives. All experimental groups were fed a starter diet containing 23% crude protein, 13.00 MJ ME kg^{-1} energy, 1% Ca, and 0.45% available P up to 21-d of age; from 22 to 28 days of age birds were provided finisher diets containing 21% crude protein, 13.00 MJ ME kg^{-1} energy, 0.90% Ca, and 0.35% available P. The broiler facility had an automatically controlled light, temperature, ventilation and heating system. Birds were housed in a pen (providing 11.1 birds m^{-2}) covered with rice hulls and wood shavings; diet and water *ad libitum* were provided.

Measurement and analysis

At the end of this experiment (28 days), 3 chickens from each pen were used for preparation and storage of breast meat. After stunning, birds were killed by ventral neck cutting and exsanguinated at a local slaughterhouse. To obtain breast meat, the individual carcasses were plucked and eviscerated, and all skin, subcutaneous fat, and visible connective tissues were removed. For evaluation of quality parameters, breast meat was packed inside re-sealable plastic bags and immediately refrigerated at 4°C until analysis.

Dry matter (DM), crude protein (CP), ether extract (EE), and ash contents of broiler breast meat were measured according to the procedure of AOAC (2005). pH, 2-thiobarbituric acid reactive substances (TBARS), cooking loss, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay, and coloration were analyzed in samples of breast meat stored for 0, 3, and 7 days at 4°C. For pH measurement, about 10 g of breast meat was homogenized with 90 mL of distilled water for 10 min. using a homogenizer.

pH value was determined using a digital pH meter (691 pH meter, Metrohm, Swiss). TBARS were measured by following the procedures of Sinnhuber and Yu (1977). A 0.5 g sample was added to 3 mL of 1% thiobarbituric acid (TBA) plus 0.3% NaOH solution and 17 mL of 0.25% trichloroacetic acid solution plus 3.6 mM HCl solution, heated in a water bath for 30 min. at 98°C, and then cooled in ice water for 15 min. Further, 5 mL of reaction solution were homogenized with 3 mL of chloroform in a test tube and centrifuged at 3500 rpm for 30 min. The absorbance was observed at a wavelength of 532 nm using a UV-Vis spectrophotometer (UV-24D, Shimadzu, Tokyo, Japan).

TBARS values were calculated according to the following equation:

TBARS (mg malondialdehyde (MA) kg^{-1} sample) = ([absorbance sample-absorbance blank] × 46)/(meat sample weight [g] × 5).

Before cooking loss determination, the breast meats were weighted and boiled in a water bath for 15 min at 90°C. The samples were then cooled at room temperature for 15 min. and weighted (Boles & Swan, 1996).

Cooking loss was calculated as follows:

Cooking loss (%) = [(weight of raw breast after thawing – weight of cooked breast)/weight of raw breast after thawing] × 100.

DPPH radical scavenging assay was determined according to the method by Blois (1958), with a slight modification. Further, 20 μL of extract were blended with 195 μL of ethanolic DPPH solution. The mixture was shaken vigorously for 30 s and kept in the dark at room temperature. Ascorbic acid was used as a positive control to obtain a calibration curve. Absorbance was recorded at 517 nm using a UV-Vis spectrophotometer (UV-24D, Shimadzu, Tokyo, Japan).

DPPH radical-scavenging activity was determined from the following equation:

DPPH radical scavenging (%) = [1 - (absorbance of sample solution/absorbance of control)] × 100.

Color measurement of breast meat was determined using a Minolta colorimeter CR-300 (Minolta Camera Co. CR-300, Tokyo, Japan) with standard color plates (Y = 93.5, x = 0.3132, y = 0.3198) to measure L★ (lightness), a★ (redness), and b★ (yellowness) parameters.

Fatty acid analysis was performed by extraction with chloroform/methanol (2:1, vol/vol) according to Folch et al. (1957). Fatty acid methyl esters were determined by gas chromatography (GA-17A, Shimadzu, Tokyo, Japan) with a CP-Sil88 column (100 m × 0.25 mm × 0.2 μm; Chrompack, Middelburg, The Netherlands). The initial oven temperature was 40°C, increased to 230°C with 1.5°C $min.^{-1}$ Injector and detector temperatures were 240 and 260°C, respectively. Identification of the fatty acid peak from 14:0 to 24:1 was determined by comparing the relative retention times of the sample with those of standard.

Statistical analyses

Data were analyzed with analysis of variance (ANOVA) using GLM procedure of SAS (SAS, 2004). Significant differences among treatment means were tested using Duncan's multiple-range test at 5% probability level (Duncan, 1955).

Results and discussion

Proximate composition of chicken breast

There were no significant differences ($p > 0.05$) among the treatments in DM, CP and EE contents, whereas significant effects of sea urchin shell powder and feed additives on ash contents ($p < 0.05$) were noted (Table 1). Results showed that using sea urchin shell powder (T1) or feed additives (T2) in broiler diets did not affect the proximate composition of broiler breast meat. Similarly, Kim (2005b) reported no effect of dietary sea urchin shell powder supplementation (1, 3 and 5%) on proximate composition of broiler meat. At present, no comparison with other research on the exact mechanism of proximate composition is possible because evaluation of the use of sea urchin shell powder with respect to proximate composition has not yet been reported.

Table 1. Effects of dietary sea urchin shell powder and feed-additive supplementation on proximate composition of chicken breast after 4 weeks.

Item	Treatment[1]		
	Control	T1	T2
DM	25.52±0.02	25.07±0.19	24.63±0.55
CP	25.03±0.62	25.25±0.67	24.65±0.38
EE	1.08±0.19	0.64±0.18	0.39±0.18
Ash	2.58±0.13[a]	1.52±0.14[c]	2.18±0.14[b]

[a-c] Means with different superscript in the same row differ significantly ($p < 0.05$); [1]T1 = basal diets + 1% sea urchin shell powder; T2 = basal diets + 1% feed additive.

Physicochemical properties

No differences ($p > 0.05$) in physicochemical properties were observed in pH and TBARS for the control or in TBARS for sea urchin shell powder, as storage days increased (Table 2). However, there was an effect of sea urchin shell powder and feed additives (T1 and T2) on pH values over storage ($p < 0.05$), including an influence of feed additives (T2) on TBARS values. At 3 and 7 days of storage, all treatments had a significant ($p < 0.05$) effect on pH (but not at 0 days). Significant differences ($p < 0.05$) in TBARS values were found among all treatments at 0 and 3 days of storage, but not at 7 days of storage. This finding is comparable with results presented by Kim (2005b) in which no differences in pH values were observed in treatments with increasing quantities of sea urchin shell powder. In current study, control groups had the lowest pH and the highest TBARS values between treatments and storage. Also, the treatment with 1% sea urchin shell powder (T1) showed higher pH and TBARS values in comparison with 1% feed additives (T2). In general, the effectiveness of antioxidants gives more important roles depending on meat pH (Xiong et al., 1993). Phenolic compounds from herbal products or sea urchin shell have been reported to increase antioxidant activity, which could reduce lipid oxidation represented by TBARS (Pokorný, 1991; Shankarlal et al., 2011). As may be seen in pH and TBARS values, our results showed that, regardless of pH values, adding sea urchin shell powder or feed

additives to broiler diets did impart antioxidant activity when compared to controls. Cooking loss showed minor differences ($p < 0.05$) over storage days. In all treatments, storage had no remarkable effect on cooking loss. In the present study, we found that cooking loss for all treatments slightly increased or decreased from 0 to 3 days of storage, then abruptly decreased at 7 days of storage. In particular, cooking loss is expressed as the reduction in weight of meat during the cooking process, and has three major components: thawing, dripping and evaporation (Barbanti & Pasquini, 2005; Jama et al., 2008). Eventually, an increase in cooking loss has a negative impact on the meat industry because it leads to deterioration of nutritional quality (Jama et al., 2008). Overall, our result indicated that use of sea urchin shell powder and feed additives did not cause any cooking loss. As storage days increased, DPPH radical-scavenging activity was influenced ($p < 0.05$) by dietary sea urchin shell powder (T1), but not by control treatments or those with feed additives (T2). After 0, 3, and 7 days of storage, there were no remarkable differences ($p < 0.05$) in DPPH radical-scavenging activity among treatments. In addition, DPPH radical scavenging activity in all treatments tended to decrease as storage time increased. DPPH radical-scavenging activity was higher in the treatment with sea urchin shell powder at 0 and 3 days of storage and feed additives at 7 days of storage. The trend toward higher DPPH radical-scavenging could be a result of sea urchin shell powder and feed additives possessing antioxidant activities (phenolic hydroxyl groups) (Shankarlal et al., 2011). In a study by Shankarlal et al. (2011), purple sea urchin shell (*Salmacis virgulata* L) showed DPPH radical-scavenging effects at a concentration of 100 μg mL^{-1} when compared with standard ascorbic acid.

Table 2. Effects of dietary sea urchin shell powder and feed-additive supplementation on pH, TBARS, cooking loss, DPPH, and shear force in chicken breast meat during storage.

Item		Treatment[1]		
	Storage days	Control	T1	T2
pH	0	5.99±0.08^{aX}	6.05±0.03^{aY}	5.94±0.01^{aZ}
	3	5.99±0.02^{aX}	6.16±0.01^{aX}	6.02±0.03^{bY}
	7	5.97±0.02^{aX}	6.19±0.01^{aX}	6.09±0.02^{bX}
TBARS (mg MA 100 g^{-1})	0	0.44±0.06^{aX}	0.34±0.06^{aX}	0.21±0.06^{bY}
	3	0.42±0.04^{aX}	0.37±0.06^{abX}	0.29±0.04^{bY}
	7	0.48±0.04^{aX}	0.43±0.03^{aX}	0.40±0.06^{aX}
Cooking loss (%)	0	29.60±2.05^{aX}	27.04±0.58^{aX}	29.54±0.24^{aX}
	3	28.82±0.59^{aX}	28.03±4.09^{aX}	31.52±1.24^{aX}
	7	15.34±2.24^{aY}	10.35±1.14^{aY}	13.95±4.90^{aY}
DPPH (%)	0	30.23±15.88^{aX}	41.37±0.86^{aX}	39.67±4.97^{aX}
	3	25.22±7.51^{aX}	33.46±4.16^{aXY}	28.20±2.98^{aX}
	7	25.75±4.08^{aX}	24.19±7.05^{aY}	26.29±12.08^{aX}

$^{a-b}$Means with different superscript in the same row differ significantly ($p < 0.05$); $^{X-Z}$Means with different superscript letters in the same column differ significantly ($p < 0.05$); [1]T1 = basal diet + 1% sea urchin shell powder; T2 = basal diet + 1% feed additive.

Meat color

In all treatments, there was an increase in L★, a★, and b★ values for up to 3 days of storage and then a decrease or increase in L★, a★, and b★ values until 7 days of storage (Table 3). All treatments showed changes in a★ values at storage day 3. Both treatment and storage day (0, 3, and 7) had an effect on b★ values. However, no significant differences were observed among any treatments for L★ values at 0, 3, and 7 days, or a★ values at 0 and 7 days. According to other reports (Dirinck et al., 1996), the main criterion for meat to be readily accepted by consumers is color: more brightly red-colored meat (not brown or darker meat) is considered as fresh. Therefore, the redness and yellowness values provide more important information in relation to meat color. In spite of the ability of sea urchin shell powder to have antioxidant activity, our overall results showed no improvement in color of breast meat; however, other authors did find an influence of diet with increasing levels of sea urchin shell powder (Kim, 2005a).

Table 3. Effects of dietary sea urchin shell powder and feed-additive supplementation on color in chicken breast meat during storage.

Item		Treatment[1]		
	Storage days	Control	T1	T2
L★ (lightness)	0	49.73±1.37^{aY}	49.68±1.01^{aY}	47.11±3.22^{aY}
	3	54.38±1.15^{aX}	51.97±1.14^{aX}	51.84±2.41^{aX}
	7	49.97±2.07^{aY}	50.58±1.03^{aXY}	52.10±1.41^{aX}
a★ (redness)	0	3.22±1.23^{aX}	1.75±0.45^{aY}	2.87±0.76^{aY}
	3	3.21±1.49^{abX}	2.45±0.56^{bY}	4.72±1.17^{aX}
	7	4.18±0.46^{aX}	3.78±0.55^{aX}	3.45±0.79^{aXY}
b★ (yellowness)	0	9.65±1.20^{aY}	7.26±0.51^{abY}	5.78±1.89^{bY}
	3	12.11±0.61^{aX}	10.30±1.07^{bX}	6.66±1.19^{cY}
	7	10.86±0.72^{aXY}	9.31±0.68^{bX}	9.17±0.39^{bX}

$^{a-c}$Means with different superscript in the same row differ significantly ($p < 0.05$); $^{X-Y}$Means with different superscript in the same column differ significantly ($p < 0.05$); [1]T1 = basal diet + 1% sea urchin shell powder; T2 = basal diet + 1% feed additive.

Fatty acid profiles

Some differences ($p < 0.05$) in all treatments were found in the percentages of oleic acid (C18:1), linoleic acid (C18:2), alpha-linolenic acid (C18:3n-3), saturated fatty acid (SFA) and (MUFA+PUFA):SFA (Table 4). However, no significant differences were observed for the percentages of other fatty acids. In this study, the most common fatty acids were unsaturated fatty acid (MUFA and PUFA), followed by saturated fatty acids. Remarkably, sea urchin shell powder supplementation (1%) corresponded to the highest (numerically) unsaturated fatty acid contents and (MUFA+ PUFA):SFA ratios, and to lower saturated fatty acid contents. This may be explained by the fact that the reduction of SFA was related to the reduction of peroxide-scavenging enzyme activity.

Table 4. Effects of dietary sea urchin shell powder and feed-additive supplementation on fatty acid profiles in chicken breast meat after 4 weeks.

Fatty acids (%)	Treatment1		
	Control	T1	T2
Myristic acid (C14:0)	0.74±0.01	0.67±0.04	0.73±0.05
Myristoleic acid (C14:1)	0.21±0.02	0.21±0.03	0.21±0.02
Pentadecanoic acid (C15:0)	0.11±0.01	0.09±0.01	0.09±0.01
Palmitic acid (C16:0)	23.35±0.76	21.80±0.47	23.71±0.31
Palmitoleic acid (C16:1)	4.62±0.29	4.61±0.87	4.74±0.20
Heptadecanoic acid (C17:0)	0.17±0.01	0.15±0.02	0.14±0.01
Heptadecenoic acid (C17:1)	0.95±0.06	0.95±0.13	0.99±0.03
Stearic acid (C18:0)	11.42±0.58	10.21±1.33	11.17±0.25
Oleic acid (C18:1)	32.03±0.49^{b}	36.18±2.98^{a}	32.63±0.68^{b}
Linoleic acid (C18:2)	16.20±0.23^{a}	14.57±0.41^{c}	15.92±0.53^{b}
α-linolenic acid (C18:3n-3)	0.87±0.01^{a}	0.62±0.10^{c}	0.78±0.01^{b}
Eicosenoic acid (C20:1n-9)	0.12±0.01	0.09±0.02	0.12±0.01
Eicosadienoic acid (C20:2)	0.51±0.04	0.42±0.06	0.38±0.03
Eicosatrienoic acid (C20:3)	1.60±0.15	1.35±0.10	1.48±0.09
Arachidonic acid (C20:4n-6)	4.81±0.18	5.59±1.65	4.72±0.24
Eicosapentaenoic (C20:5n-3, EPA)	0.34±0.02	0.28±0.02	0.34±0.02
Nervonic acid (C24:1)	0.89±0.04	1.03±0.34	0.86±0.10
Docosapentaenoic acid (C22:5n-3, DPA)	0.52±0.01	0.56±0.12	0.53±0.02
Docosahexaenoic (C22:6n-3, DHA)	0.55±0.02	0.62±0.22	0.46±0.02
Saturated fatty acid (SFA)	35.80±0.40^{a}	32.92±0.98^{b}	35.83±0.44^{a}
Mono unsaturated fatty acid (MUFA)	38.81±0.66	43.06±3.42	39.56±0.76
Poly unsaturated fatty acid (PUFA)	25.39±0.55	24.02±2.53	24.61±0.91
PUFA:SFA	0.71±0.02	0.73±0.05	0.69±0.03
(MUFA+PUFA):SFA	1.79±0.03^{b}	2.04±0.09^{a}	1.79±0.03^{b}
n-6	21.01±0.36	20.16±2.06	20.65±0.76
n-3	2.27±0.04	2.08±0.38	2.10±0.60
n-6:n-3 ratio	9.24±0.05	9.99±0.89	9.81±0.13

$^{a-c}$Means with different superscript letters in the same row differ significantly ($p < 0.05$); [1]T1 = basal diet + 1% sea urchin shell powder; T2 = basal diet + 1% feed additive.

Similar results were obtained in a study conducted by Kim (2005a), where the addition of sea urchin shell powder to the diet affected oleic acid, total saturated fatty acid (TS), total unsaturated fatty acid (TU), and TS/TU ratios. Additionally, considering n-6, n-3 and n-6:n-3 ratios, our data give no direct evidence of an increase in fatty acid contents using sea urchin shell powder (1%). Thus, more specific research on lipid oxidation and fatty acid profiles in relation to meat quality from sea urchin shell powder should be carried out.

Conclusion

The use of 1.0% sea urchin shell powder or 1% feed additives in broiler diets shows no physicochemical properties or meat color during storage, or proximate composition and fatty acid profiles in broiler breast meat. Overall, DPPH radical-scavenging activity and unsaturated fatty acids were higher in treatments with 1% sea urchin shell powder; the urchin shell could act as an antioxidant by directly reducing free radicals and enzyme activity. Furthermore, the use of sea urchin shell powder in animal diets requires more experimental evidence by direct investigations to understand its mechanisms of action and beneficial effects.

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