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ORIGINAL PAPER

Pea (*Pisum sativum* L.) Seeds as an Alternative Dietary Protein Source for Broilers: Influence on Fatty Acid Composition, Lipid and Protein Oxidation of Dark and White Meats

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Abstract An experiment was conducted to evaluate the production parameters, breast and leg muscle fatty acid composition and lipid and protein oxidative stability of broilers fed peas (*Pisum sativum* L.). The trial involved 120 birds (Hubbard strain) allotted to two groups: group I—control group, fed a basal diet containing soybean meal (195 g kg^{-1}) as the main protein source; whereas group II—experimental group fed diet containing peas (400 g kg^{-1}) as a substitute for conventional soybean. No significant differences were observed for body weight, feed intake or feed conversion ratio ($P > 0.05$). The total lipids were lower ($P < 0.05$) in the breast and leg muscles of broilers fed peas. The content of total n-3 polyunsaturated fatty acids were higher ($P < 0.05$) in the white and dark meat of birds fed the pea diet compared with soybean control diet. After 7 days of refrigerated storage, the levels of thiobarbituric acid-reactive substances, lipid hydroperoxides and carbonyl proteins expressed as dinitrophenylhydrazine were similar ($P > 0.05$) in white and dark meat of chicks fed either diet. The data indicates that dietary pea inclusion does not cause detrimental changes in lipid and protein oxidation of poultry dark and white meats, suggesting the possibility of replacing soybean meal with peas.

Keywords Broiler · Peas · Meat quality · Lipid and protein oxidation

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Introduction

Peas (*Pisum sativum* L.) are widely produced specially in the Mediterranean area and could be used as an alternative protein source to soybean because of their good nutritive value [1–3]. The interest in peas as a valuable constituent of functional food is increasing and has led to studies involving the antioxidant activity in pea seeds [4]. Many studies have investigated the beneficial effects of supplementing natural antioxidants in poultry diets on different factors including meat quality and oxidative stability [5]. In fact, dietary supplementation or replacement with alternative ingredients has proved to be a simple method of introducing a natural antioxidant into chicken meat [6].

Oxidation of lipids and proteins can affect meat quality [5, 6]. The onset of oxidative reactions in muscle foods during processing and storage leads to undesirable sensory changes and serious health concerns [7]. The influence of lipid oxidation on quality traits such as meat color and odor is well recognized [6, 7]; however the impact of protein oxidation on meat quality requires further attention. Amongst other effects, the oxidative degradation of muscle proteins involves modification of the amino acid side chains, with the formation of carbonyl compounds being the most marked change in oxidized proteins [8]. There are many reports on the biochemical changes in different meats, most of which have focused on lipid oxidation [9], but not on protein oxidation [10]. However, despite many studies having been conducted to evaluate the effect of dietary peas on growth performance of broiler chickens [1, 3, 11], limited research on the effect of a basal diet containing non-conventional protein sources on poultry meat oxidative stability have been completed [12]. Therefore, the objective of this research was to determine the effects on fatty acids composition, lipid and protein oxidation in white

(breast) and dark (leg) meat of broiler chickens fed diets in which soybean meal was replaced with peas.

Materials and Methods

Animals and Diets

A trial with 120 Hubbard strain female broiler chicks was conducted from 14 to 49 days of age following two dietary treatments. Broilers, from a commercial hatchery, were raised in a conventional environment and fed a common starter diet suppling 20.5% of crude protein and 12.3 MJ of ME/kg of diet [13] (MJ megajoules; ME metabolizable energy) until 14 days of age. On day 14, birds were individually weighed and randomly divided among 12 pens in a commercial poultry facility located in Province of Bari, Italy. Each diet was replicated six times, with each replicate comprising one pen of ten birds. Birds were fed one of two wheat middlings-based diets containing soybean or micronized-dehulled pea (*Pisum sativum* cv. Spirale) meal as the main protein sources [3] from day 14 to 49 (the slaughtering age). Feed (pelleted form) and water were provided ad libitum. Ingredient and chemical composition of the diets are reported in Table 1. The trial was carried out observing the animal welfare Directive No. 91/629/EEC (received in Italy by D.L. 533/92 and modified by D.L. 331/98).

Sample Collection

On day 49 of the trial, three broilers from each pen were selected according to the average body weight within the pen following a 12 h fasting. Broilers were weighed individually, killed by cervical dislocation and then immediately bled. Breast and leg meat of each animal was immediately taken and placed on a fibre board tray, wrapped in air-permeable low density polyethylene film and stored for 7 days in darkness at 4 °C to mimic commercial conditions of meat storage.

Chemical Analysis

Compound feed samples were ground in a hammer mill provided with a 1-mm pore size screen and analyzed in triplicate for their content of dry matter (method 945.15), ash (method 942.05), crude protein ($N \times 6.25$, method 990.03), crude fiber (method 973.18) and ether extract (method 945.16) according to the procedures outlined by the AOAC [14]. Meat total lipids were extracted according to the chloroform–methanol (2:1 v/v) method of Folch et al. [15]. The phospholipid content was calculated ($P \times 25$) after phosphorus was determined in the total lipid extract by the method of Bartlett [16].

Table 1 Ingredient, chemical and fatty acid composition of pea and soybean meal diet fed to broilers

Item	Diet	
	Soybean	Pea
Ingredients, g/kg as-fed		
Durum wheat middlings	741.5	538.5
Soybean meal, 48% CP	195.0	–
Pea	–	400.0
Soybean oil	17.0	15.0
Calcium carbonate	14.0	16.0
Dicalcium phosphate	13.0	12.0
Sodium chloride	2.5	2.5
Sodium bicarbonate	2.0	2.0
Vitamin–mineral premix[a]	5.0	5.0
L-Lysine HCl	4.4	2.5
Enzyme[b]	2.0	2.0
DL-Met	1.6	2.5
Choline chloride	1.0	1.0
Coccidiostat[c]	1.0	1.0
Chemical analysis, %		
Dry matter	89.75	90.21
Crude protein	20.56	20.49
Crude fiber	3.21	2.68
Crude fat	3.93	3.54
Ash	5.20	4.99
Calculated analysis		
Metabolizable Energy (MJ/kg)	12.1	12.1
Lys, %	1.15	1.15
Calcium, %	1.02	1.01
Met + cys, %	0.79	0.78
Available P, %	0.31	0.31
Fatty acids, %		
Σ Saturated fatty acids	31.03	34.75
Σ Monounsaturated fatty acids	33.64	25.01
Σ Polyunsaturated fatty acids	35.33	39.86
Total n-6	31.54	36.97
Total n-3	1.68	2.03

[a] Supplied per kilogram of diet: vitamin A 12,000 IU; vitamin E, 10 mg; vitamin D 2,200 IU; niacin 35.0 mg; D-pantothenic acid 12 mg; riboflavin 3.63 mg; pyridoxine 3.5 mg; thiamine 2.4 mg; folic acid 1.4 mg; biotin 0.15 mg; vitamin B 0.03 mg; Mn 60 mg; Zn 40 mg; Fe 1,280 mg; Cu 8 mg; I 0.3 mg; Se 0.2 mg

[b] Provided per kilogram of product: *endo*-1,4-beta-glucanase, 800,000 U; *endo*-1,3(4)-beta-glucanase 1,800,000 U; *endo*-1,4-beta-xylanase 2,600,000 U (Roxazyme® G2-G, Roche, Switzerland)

[c] Provided 33 g of robenidine hydrochloride/kg of feed

Determination of Fatty Acid Composition

In preparation for the analysis of fatty acid composition, samples of breast (Pectoralis major) and leg (Peroneous longus) meat (5 g each) were freeze-dried and then ground.

Methyl heptadecanoate (Fluka, USA) was dissolved into *n*-hexane (1 mg/ml) as an internal standard. Methyl esters of the FA were prepared by incubating samples (300 mg each) and 5 ml internal standard 2 h at 80 °C with methanolic acetyl chloride in a total volume of 9 ml [17]. After cooling to room temperature, 7 ml of 7% (w/v) K_2CO_3 was added with mixing, and then the organic phase was collected after centrifuging at 1,500*g* for 2 min at 4 °C. Fatty acid methyl esters were separated over a CP-SIL883 column (100 m × 0.25 mm i.d., film thickness 0.20 μm fused silica; Varian, Palo Alto, CA, USA) in a Shimadzu (Model 2GC17A, Kyoto, Japan) gas chromatograph with a HP GC Chem Station Rev. A.05.04 data handling system and using flame ionization detection. Helium was used as the carrier gas at a constant flow rate of 1.7 ml/min. The oven temperature was programmed as follows: 175 °C, held for 4 min; 175–250 °C at 3 °C/min; and then maintained for 20 min at 250 °C. The injector port and detector temperature were 250 °C. Samples (1 μl) were injected with an auto-sampler. Output signals were identified and quantified from the retention times and peak areas of known calibration standards. Composition was expressed as weight percentages of the total fatty acids.

Determination of Lipid Oxidation

Thiobarbituric acid-reactive substances were determined on fresh and after 7 days of storage at 4 °C meat samples as described by McDonald and Hultin [18]. Tissue samples (2 g) were weighed into test tubes each with 18 ml of 3.86% perchloric acid; samples were homogenized with a Polytron (IKA Labortechnik T25-B, Selangor, Malaysia) 3 × 15 s at high speed. Fifty microliters of butylated hydroxyl anisole (BHA) (4.5% BHA in ethanol) was added to the sample prior to homogenization. The homogenate was filtered through a filter paper. The filtrate (2 ml) was mixed with 2 ml of 20 mM TBA in distilled water and incubated in a boiling water bath for 30 min. After cooling, the absorbance of filtrate was determined at 531 nm against a blank containing 2 ml of 3.86% perchloric acid and 2 ml of 20 mM thiobarbituric acid-reactive solution. The thiobarbituric acid-reactive substances values were expressed as milligrams of malonaldehyde per kg of meat.

Determination of Lipid Hydroperoxides

Around 1 ± 0.01 g of each muscle was homogenized in 5 ml of chloroform/methanol (1:1) for 30 s. Subsequently, the Polytron was washed for 30 s with 5 ml solvent. The homogenates and wash solutions were then combined. Three ml of 0.5% NaCl was added and the mixture was vortexed for 30 s before centrifugation for 10 min to separate the mixture into two phases. Ice cold chloroform/methanol (1:1) (1.3 ml) was added to 2 ml of the lower phase and briefly vortexed. Twenty-five microliters of ammonium thiocyanate (4.38 M) and 25 μl ferrous chloride (18 mM) were added to assay for lipid hydroperoxides according to Shantha and Decker [19]. Samples were incubated for 20 min at room temperature before the absorbances at 500 nm were determined.

Determination of Protein Oxidation

Protein oxidation, as measured by the total carbonyl content, was evaluated by derivatization with dinitrophenylhydrazine as described by Oliver et al. [20] with slight modifications. Burger patties (1 g) were minced and then homogenized 1:10 (w/v) in 20 mM sodium phosphate buffer containing 6 M NaCl (pH 6.5) using an Ultraturrax homogenizer (IKA-Werke, Staufen, Germany) 2 × 30 s. Two equal aliquots of 0.2 ml were taken from the homogenates and dispensed in 2 ml Eppendorf tubes. Proteins were precipitated by cold 10% TCA (1 ml) and subsequent centrifugation for 5 min at 4,200*g*. One pellet was treated with 1 ml 2 M HCl (protein concentration measurement) and the other with an equal volume of 0.2% (w/v) dinitrophenylhydrazine in 2 M HCl (carbonyl concentration measurement). Both samples were incubated for 1 h at room temperature. Afterwards, samples were precipitated by 10% TCA (1 ml) and washed three times with 1 ml ethanol:ethyl acetate (1:1, v/v) to remove excess dinitrophenylhydrazine. The pellets were then dissolved in 1.5 ml of 20 mM sodium phosphate buffer containing 6 M guanidine HCl (pH 6.5), stirred and centrifuged for 2 min at 4,200*g* to remove insoluble fragments. Protein concentration was calculated from the absorption at 280 nm using bovine serum albumin as the standard. The amount of carbonyls was expressed as nmol of carbonyl per mg of protein using an absorption coefficient of 21.0 nM^{-1} cm^{-1} at 370 nm for protein hydrazones.

Statistical Analysis

A completely randomized design was used with two treatments and six replicates (pens) per treatment and data were statistically analyzed using ANOVA with the General Linear Model procedure of SAS [21].

Results and Discussion

Growth Performance and Fatty Acid Composition of Leg and Breast Muscle

There were no differences in the final body weight, average daily gain, feed intake and feed conversion ratio among

treatments (Table 2), although feed intake ($P = 0.096$) tended to be higher for the pea treatment compared with the soybean meal diet.

The lack of differences in average daily gain, feed intake or the gain to feed ratio among treatments, although the lower trend in feed intake for the soybean group, is consistent with Igbasan and Guenter [1] and more recently with Laudadio and Tufarelli [3], who reported a decrease in feed intake in broiler fed a conventional diet containing soybean meal, compared with pea meal, although the final weights and average daily gains were similar ($P < 0.05$).

The influence of dietary treatment on total lipid and phospholipid content and the fatty acid composition of breast and thigh muscle is presented in Table 3. Meat from breast and drumstick of broilers fed pea diet exhibits a significant lower fat content ($P < 0.05$), whereas total phospholipid levels were similar among treatments, confirming the optimal nutritional value of meat produced when birds fed peas. The fatty acid content of human foods has become increasingly important, as several fatty acid have been implicated in health issues in humans. In this study, we attempted to influence the content of poultry meat fatty acid known to be of detrimental to human health. Breast and leg muscles from broilers fed peas had higher amounts of saturated fatty acids, even if not significant ($P = 0.075$ and 0.061, respectively), and lower monounsaturated fatty acids ($P < 0.05$), but higher amounts of polyunsaturated fatty acids ($P < 0.05$) in the total lipids than did muscles from broilers fed soybean meal. The white (breast) and dark (leg) meats from broilers fed the pea diet had similar amounts of n-6 polyunsaturated fatty acids and higher amounts ($P < 0.05$) of n-3 polyunsaturated fatty acids than the muscles from broilers fed soybean diet. The n-6/n-3 ratio were also higher ($P < 0.05$) in both muscles of broilers fed pea dietary treatment. The unsaturation index (saturated fatty acids/unsaturated fatty acids) of the fatty acids in both muscles was similar in broilers fed control soybean and pea diets (leg muscle: soybean 0.39, pea 0.42; breast muscle: soybean 0.36 and pea 0.37).

The saturated fatty acids and monounsaturated fatty acid are positively correlated with meat quality, since they can improve the characteristics of meat tenderness, juiciness and flavor. Therefore dietary pea inclusion could improve poultry meat quality. However, high levels of polyunsaturated fatty acids in meat are undesirable, especially in poultry, because they adversely affect consistency, storage stability and texture of the processed products. Thus, the fatty acid composition could have a positive and a negative impact on poultry meat quality.

Table 2 Performance of broilers fed pea or soybean meal diets (14–49 days of age)

Item	Diet			
	Soybean	Pea	SEM	*P* value
Body weight gain, g/d	50.4	49.9	0.41	0.521
Feed intake, g/bird/d	105	106	0.69	0.096
Feed conversion ratio, g/g	2.08	2.12	0.02	0.102

Table 3 Total lipids, phospholipids and fatty acid profile (sum of total proportion of SFA, MUFA and PUFA) of dark (leg, $n = 12$) and white (breast, $n = 12$) meat of broilers fed pea or soybean meal diets

Item[a]	Dark meat				White meat			
	Soybean	Pea	SEM	*P* value	Soybean	Pea	SEM	*P* value
Total lipids, %	4.70	3.90	0.41	<0.05	1.95	1.02	0.17	<0.05
Total phospholipids, %	1.05	0.86	0.13	0.077	0.82	0.75	0.11	0.087
Σ SFA[a]	27.94	29.53	1.04	0.061	26.49	27.39	1.05	0.075
Σ MUFA[b]	42.55	39.50	1.07	<0.05	40.97	38.54	1.08	<0.05
Σ PUFA[c]	29.51	30.96	1.06	<0.05	32.54	34.07	1.01	<0.05
n-6 PUFA[d]	27.20	27.99	0.09	0.116	29.55	30.52	0.77	0.085
n-3 PUFA[e]	2.31	2.98	0.61	<0.05	2.99	3.56	0.95	<0.05
n-6/n-3	11.76	9.41	0.84	<0.05	9.88	8.57	0.65	<0.05
SFA/UFA	0.39	0.42	0.19	0.137	0.36	0.37	0.14	0.209

SFA saturated fatty acids, *MUFA* monounsaturated fatty acid, *PUFA* polyunsaturated fatty acid, *SFA*/*UFA* saturated fatty acids/unsaturated fatty acids ratio

[a] SFA = sum of all even chain fatty acid up to 22:0

[b] MUFA = sum of 14:1, 16:1, 18:1, 20:1 and 22:1

[c] PUFA = sum of 18:2, 18:3, 20:2, 20:3, 20:4, 20:5, 22:4, 22:5 and 22:6

[d] n-6 PUFA = sum of 18:2, 18:3 n-6, 20:2, 20:3 n-6, 20:4 and 22:2

[e] n-3 PUFA = sum of 18:3 n-3, 20:3 n-3, 20:5, 22:5 and 22:6

Lipid Oxidation

The leg muscle (dark meat) had a higher thiobarbituric acid-reactive substances value than breast muscle (white meat) in both dietary groups. However, no effect ($P > 0.05$) on lipid oxidation related to the dietary treatment in fresh meat and after 7 days of storage (Figs. 1, 2). These results can be partially explained by the higher total and heme iron contents in poultry leg than in breast muscles [22]. The heme and non-heme iron present in meat can be considered as prooxidants because of their reaction with hydroperoxide to initiate lipid oxidation in meat and meat products [12].

In the literature, the effect of diet on lipid oxidation of meat has been described essentially on beef. The levels of thiobarbituric acid-reactive substances observed in our trial were lower than the value reported by Smet et al. [12] for poultry meat. They found thiobarbituric acid-reactive substances levels ranging from 0.8 to 1.05 μg of MDA/g of muscle of meat stored at 4 °C. Comparison between poultry meat and other meats showed that the thiobarbituric acid-reactive substances values are of the same order [23, 24]. The level of thiobarbituric acid-reactive substances considered as the threshold for perception and acceptability of oxidation by consumer depends on the species, i.e. in beef this threshold ranged from 0.6 to 2 mg MDA/kg meat, while in swine the limit is in average between 0.5 and 1 mg MDA kg/meat [12, 25].

Our results are in good accordance with those of Ponnampalam et al. [26] who demonstrated that lipid oxidation was not affected by the nature of the grass in diet. The concentrations of hydroperoxides in fresh and stored broiler leg and breast muscles are shown in Figs. 3 and 4. The concentrations of lipid hydroperoxides in breast and leg muscle did not differ ($P > 0.05$) among dietary treatments. However, in white meat lipid hydroperoxides were slightly higher compared with dark meat after storage. Moreover, the leg and breast muscles of broilers fed the diet containing peas had lower concentrations of hydroperoxides than broilers fed the control soybean diet.

Protein Oxidation

No effect on protein oxidation ($P > 0.05$) related to the dietary treatment in fresh meat and after 7 days of storage was observed. The carbonyl levels (Figs. 5, 6) tended to be slightly higher in pea group after storage in both leg and breast muscles compared with control soybean diet (1.19 vs. 1.27 and 1.02 vs. 1.09 nmol of dinitrophenylhydrazine/

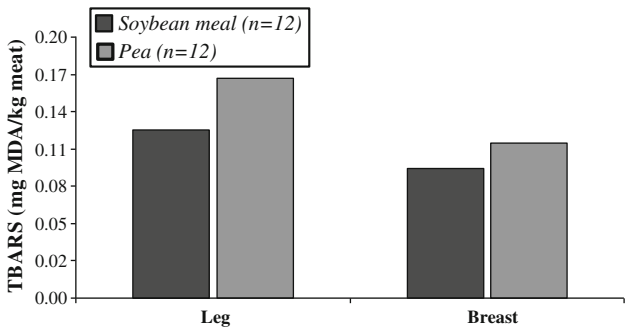

Fig. 1 Thiobarbituric acid-reactive substances levels determined in fresh leg and breast broiler meats

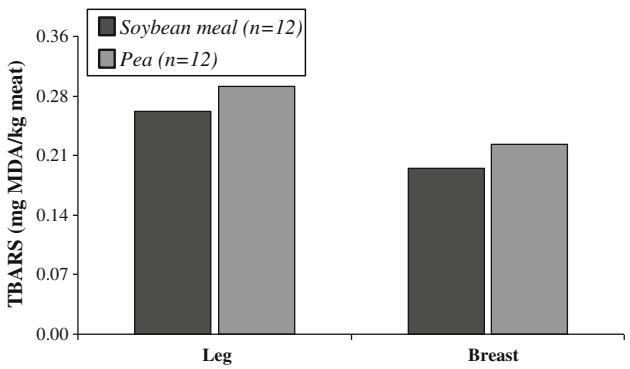

Fig. 2 Thiobarbituric acid-reactive substances levels after 7 days of storage determined in leg and breast broiler meats

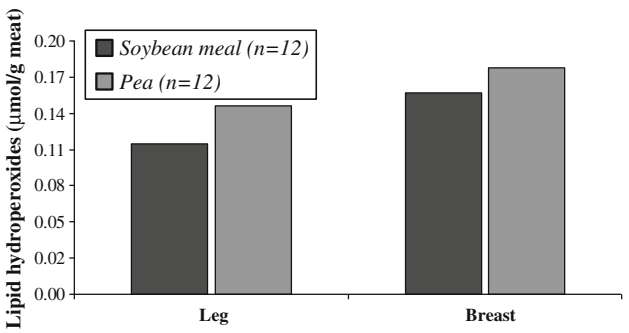

Fig. 3 Lipid hydroperoxides levels determined in fresh leg and breast broiler meats

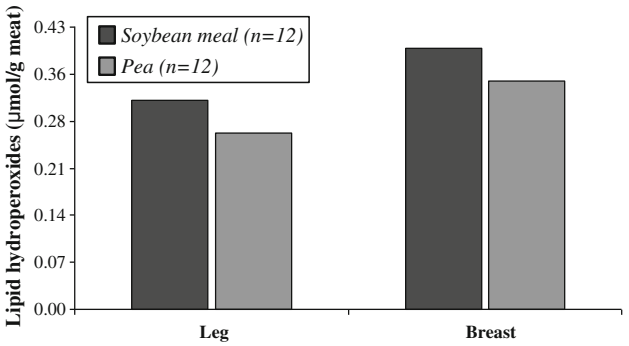

Fig. 4 Lipid hydroperoxides levels after 7 days of storage determined in leg and breast broiler meats

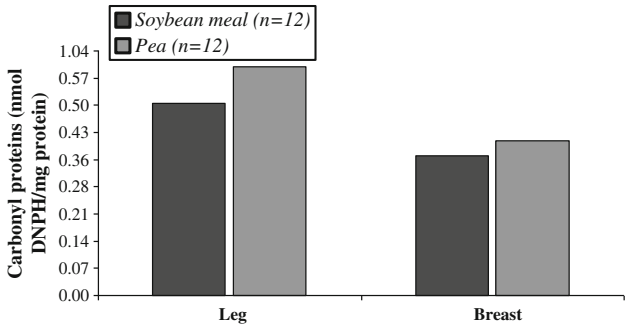

Fig. 5 Carbonyl protein levels determined in fresh leg and breast broiler meats

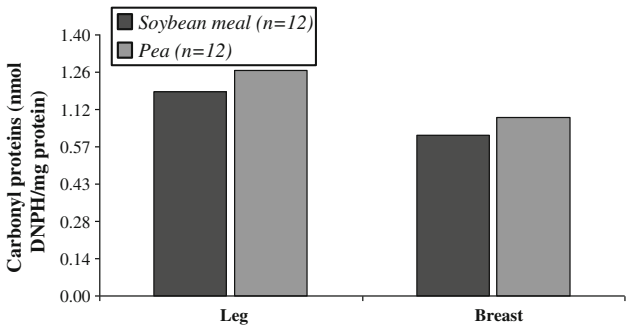

Fig. 6 Carbonyl protein levels after 7 days of storage determined in leg and breast broiler meats

mg protein, respectively). Our results disagree with those of Petron et al. [27] who observed an effect of the diet (i.e. different pastures) on protein oxidation without any effect on lipid oxidation in lamb meat, but are in line with findings of Smet et al. [12] in poultry fed ingredients containing natural antioxidants.

The protein carbonyl content was used as a measure of the extent of oxidative reactions affecting muscle proteins during storage of meat patties. The onset of lipid oxidation in refrigerated broiler meats and the impact of these reactions on poultry meat quality have been extensively studied by Ganhão et al. [10], whereas research on the onset and extent of protein oxidation in poultry meat products is limited. The increase of protein carbonyls show that muscle proteins in fresh patties are susceptible to oxidative reactions leading to carbonyl gain.

Carbonyl compounds are formed as a result of the oxidative degradation of side chains of lysine, proline, arginine and histidine residues [8]. The level of protein carbonyls in the present study indicates optimal oxidative reactions than described in previous studies on raw pork, lamb and poultry subjected to storage [6, 12, 24]. By inhibiting protein carbonyl formation, pea meal does not seem to alter the nutritional value of poultry dark and white meats. Besides the loss of essential amino acids, the formation of carbonyl compounds from oxidizing protein might have additional repercussions on particular quality traits. Protein oxidation seems to be influenced by the level of lipid oxidation in meat [22]. The levels of thiobarbituric acid-reactive substances observed in the present study were correlated with carbonyl proteins levels.

Conclusion

The results obtained confirm the suitability of peas as a dietary protein source. The lipid and protein oxidation processes for leg and breast meat from poultry fed a pea-based diet were similar to those poultry fed a soybean meal-based diet.

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References

1. Igbasan FA, Guenter W (1996) The enhancement of the nutritive value of peas for broiler chickens: an evaluation of micronization and dehulling processes. Poult Sci 75:1243–1252
2. Meng X, Slominski BA (2005) Nutritive values of corn, soybean meal, canola meal, and peas for broiler chickens as affected by a multicarbohydrase preparation of cell wall degrading enzymes. Poult Sci 84:1242–1251
3. Laudadio V, Tufarelli V (2010) Growth performance and carcass and meat quality of broiler chickens fed diets containing micronized-dehulled peas (*Pisum sativum* cv. Spirale) as a substitute of soybean meal. Poult Sci 89:1537–1543
4. Yoshida H, Tomiyama Y, Saiki M, Mizushina Y (2007) Tocopherol content and fatty acid distribution of peas (*Pisum sativum* L.). J Am Oil Chem Soc 84:1031–1038
5. Bou R, Codony R, Tres A, Decker EA, Guardiola F (2009) Dietary strategies to improve nutritional value, oxidative stability, and sensory properties of poultry products. Crit Rev Food Sci Nutr 49:800–822
6. Lòpez-Bote CJ, Gray JI, Gomaa EA, Flegal CJ (1998) Effect of dietary administration of oil extracts from rosemary and sage on lipid oxidation in broiler meat. Br Poult Sci 39:235–240
7. Ganhão R, Morcuende D, Estévez M (2010) Protein oxidation in emulsified cooked burger patties with added fruit extracts: influence on colour and texture deterioration during chill storage. Meat Sci 85:402–409
8. Stadtman ER, Levine RL (2003) Free radical-mediated oxidation of free amino acids and amino acid residues in proteins. Amino Acids 25:207–218
9. Abdel-Kader ZM (1996) Lipid oxidation in chicken as affected by cooking and frozen storage. Nahrung 40:21–24
10. Terevinto A, Ramos A, Castroman G, Cabrera MC, Saadoun A (2010) Oxidative status, in vitro iron-induced lipid oxidation and superoxide dismutase, catalase and glutathione peroxidase activities in rhea meat. Meat Sci 84:706–710
11. Crepon K (2006) Nutritional value of legumes (pea and faba bean) and economics of their use. In: Garnsworthy PC, Wiseman J (eds) Recent advances in animal nutrition. Nottingham University Press, Nottingham, pp 332–366

12. Smet K, Raes K, Huyghebaert G, Haak L, Arnouts S, De Smet S (2008) Lipid and protein oxidation of broiler meat as influenced by dietary natural antioxidant supplementation. Poult Sci 87:1682–1688
13. NRC, National Research Council (1994) Nutrient requirements of poultry. 9th edn. National Academic Press, Washington Revised
14. AOAC (2000) Official methods of analysis, 17th edn. Association of Official Analytical Chemists, Gaithersburg
15. Folch J, Lees M, Sloane-Stanley GHA (1957) A simple methods for the isolation and purification of total lipids from animal tissues. J Biol Chem 226:497–507
16. Bartlett GR (1959) Phosphorus assay in column chromatography. J Biol Chem 234:466–468
17. Sukhija PS, Palmquist DL (1988) Rapid method for determination of total fatty acid content and composition of feedstuffs and feces. J Agric Food Chem 36:1202–1206
18. McDonald RE, Hultin HO (1987) Some characteristics of the enzymic lipid peroxidation system in the microsomal fraction of flounder skeletal muscle. J Food Sci 52:15–27
19. Shantha NC, Decker EA (1994) Rapid, sensitive, iron-based spectrophotometric methods for determination of peroxide values of food lipids. J AOAC Int 77:421–424
20. Oliver CN, Ahn BW, Moerman EJ, Goldstein S, Stadtman ER (1987) Age related changes in oxidized proteins. J Biol Chem 262:5488–5491
21. SAS Institute (2000) SAS/STAT user's guide. Release 8.1. Statistical analysis system. SAS Institute Inc. Cary, NC
22. Mercier Y, Gatellier P, Viau M, Remignon H, Renerre M (1998) Effect of dietary fat and vitamin E on colour stability and on lipid and protein oxidation in turkey meat during storage. Meat Sci 48:301–318
23. Castellini C, Dal Bosco A, Mugnai C, Pedrazoli M (2006) Comparison of two chicken genotypes organically reared: oxidative stability and other qualitative traits of the meat. Ital J Anim Sci 5:355–363
24. Santé-Lhoutellier V, Engel E, Gatellier Ph (2008) Assessment of the influence of diet on lamb meat oxidation. Food Chem 109:573–579
25. Campo MM, Nute GR, Hughes SI, Enser M, Wood JD, Richardson RI (2006) Flavour perception of oxidation in beef. Meat Sci 72:303–311
26. Ponnampalam EN, Trout GR, Sinclair AJ, Egan AR, Leury BJ (2001) Comparison of the color stability and lipid oxidative stability of fresh and vacuum packaged lamb muscle containing elevated omega-3 and omega-6 fatty acid levels from dietary manipulation. Meat Sci 58:151–161
27. Petron MJ, Raes K, Claeys E, Lourenco M et al (2007) Effect of grazing pastures of different botanical composition on antioxidant enzyme activities and oxidative stability of lamb meat. Meat Sci 75:737–745