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Effects of Packaging Methods on the Shelf Life of Selenium-Supplemented Chicken Meat during Refrigerated Storage

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Abstract Effects of vacuum packaging and modified atmosphere packaging (MAP) on shelf life of selenium-supplemented chicken meat during refrigerated storage were examined. Body weight and feed efficiency were unaffected by dietary selenium level. Dietary treatments and packaging methods had no significant effects on level of microbial growth, lightness, and metmyoglobin content. MAP decreased purge loss (10.9-34.5%) and lipid oxidation (15.2-15.9%) more efficiently than vacuum packaging. Broiler chicks supplemented with α-tocopherol or α-tocopherol + selenium had similar TBA values. Dietary supplementation of 4 and 8 ppm selenium reduced lipid oxidation, and this effect was less significant in MAP breast meat.

Keywords: color stability, lipid oxidation, packaging methods, selenium

Introduction

Lipid oxidation and discoloration are believed to be the major causes of meat quality deterioration during refrigerated storage. Poultry, in particular, is very sensitive to oxidative deterioration due to its higher content of polyunsaturated fatty acids (1). Many studies reported that lipid oxidation in meat products can be effectively controlled using antioxidants (2, 3). Feeding poultry a high level of dietary antioxidants can provide the poultry industry with a simple method for improving the oxidative stability and shelf life of poultry meat. The key dietary factors in the nutritional modulation of oxidant protection are α-tocopherol and selenium (Se) (4). High levels of dietary Se result in higher Se concentrations in the liver, kidneys, plasma, and muscle of poultry (5). Chan and Decker (6) reported that increase in the tissue Se content might not always be accompanied by a corresponding increase in the glutathione peroxidase activity, which suggests that the dietary Se levels can influence the oxidative stability of the skeletal muscle.

The acceptance of vacuum and modified atmosphere packaging (MAP) systems by the meat industry has increased over the past few years (7, 8). Oxygen, nitrogen, and carbon dioxide are the gases generally used in MAP, and the elimination of O_2 from the packaging and the introduction of different CO_2 and N_2 concentrations inhibit the growth of aerobic micro-organisms, proteolytic bacteria, yeast, and fungi (9). The ability of MAP to extend the shelf life has been examined in chickens (10, 11). However, lipid oxidation could limit the shelf life when MAP reduces the other deteriorative mechanisms in meat. Generally, studies on rancidity have been performed using processed meats or antioxidant-supplemented feed (3, 12), whereas only few studies are available on the effects of extended periods of vacuum packaging and MAP on the level of lipid oxidation in poultry. The aim of this study was to determine the effects of vacuum packaging and MAP system on the shelf life of Se-supplemented chicken meat during refrigerated storage.

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Materials and Methods

Birds and diets Two hundred and eighty-eight male broiler chicks (Arbor Acres, 1-day-old) were randomly assigned to 6 pens containing 48 chicks each, and subjected to one of six dietary treatments. This experiment was repeated three times (total 864 chicks, 144 chicks/treatment, 48 birds/pen). Tables 1 and 2 show the ingredients and composition of the starter and basal diets. Up to 3 weeks of age, the chicks were fed the same starter diet. From 3 to 6 weeks, six different treatments were applied to the chicks as follows: control 1 (CON1), basal diet containing 20 IU α-tocopherol/kg of feed and 0.17 ppm Se; control 2 (CON2), basal diet supplemented with 100 IU all-rac-α-tocopheryl acetate/kg of feed; treatments 1 (SE1), 2 (SE2), 3 (SE4), and 4 (SE8), CON2 supplemented with 1, 2, 4, and 8 ppm Na_2SeO_3, respectively. The chicks were maintained on a 24-hr constant lighting schedule and given access to feed and tap water *ad libitum* until slaughter at 42 days of age. The body weight and feed consumption of the individual pens were recorded at 1, 7, 21, and 42 days.

Packaging and storage After 42 days of feeding, all broilers were slaughtered at a conventional slaughtering plant, and the carcasses were chilled in a spin chiller, weighed, and packed individually using four different packaging methods. MAP 1 and MAP 2 were packed using BB4l pouches with a low gas permeability (7 cc/m^2/24 hr O_2 at 4°C and 80% RH; 150 cc/m^2/24 hr CO_2 at 23 °C and 75% RH; water vapor transmission rate 1.5 g/m^2/24 hr at 38°C and 100% RH) and flushed with 100% N_2, or 80% N_2 and 20% CO_2, respectively. VAC 1 was vacuum-sealed in Cryovac B550T bags and subsequently heat-shrunk in 82°C water following the manufacturer's recom-

Table 1. Ingredients and composition of the starter diets fed up to 3 week of age

Ingredient	%	Composition	Calculated analysis
Corn, yellow	37.51	Moisture	11.44
Wheat	25.00	Crude protein	19.00
Lupine-kernel	5.00	Crude fat	6.61
Soybean meal	20.61	Crude fiber	2.84
Rapeseed meal	1.30	Crude ash	4.64
Fish meal	4.86	Metabolizable energy (cal/g)	3,060
Yellow grease	3.00	Lysine	0.98
Lysine-HCl	0.34	Methionine	0.34
Limestone	0.29	Met + Cys	0.67
Tricalcium phosphate	1.00	Threonine	0.70
NaCl	0.13	Tryptophan	0.21
Wheat bran	0.23	Available lysine	0.90
Methionine	0.21	Available methionine	0.32
Threonine	0.05	Available Met + Cys	0.63
Mineral premix[1)]	0.10	Available threonine	0.65
Vitamin premix[2)]	0.10	Available tryptophan	0.20
Choline chloride	0.12	Calcium	0.80
Clinacox	0.05	Total phosphate	0.61
Virginia-M	0.05	Available phosphate	0.37
Bio-plus	0.05	Sodium	0.14
		Chloride	0.20

[1)]Mineral premix provided per kg of ration with 50 mg Fe, 70 mg Mn, 50 mg Zn, 7 mg Cu, 0.4 mg Co, 0.17 mg Se, and 0.75 mg I.
[2)]Vitamin premix provided per kg of ration with 13,000 IU A, 2,600 IU D_3, 20 IU E, 2.5 mg K_3, 0.02 mg B_{12}, 4.0 mg rivoflavin, 10 mg pantothenic acid, 40 mg niacin, 1.0 mg folic acid, 0.08 mg biotin, and 0.5 mg ethoxyquin.

Table 2. Ingredients and composition of the basal diets fed from 3 to 6 week of age

Ingredient	%	Composition	Calculated analysis
Corn	35.00	Moisture	11.66
Corn gluten meal	1.83	Crude protein	20.00
Wheat	25.00	Crude fat	5.80
Lupine-Kernel	8.00	Crude fiber	2.82
Soybean meal	21.65	Crude ash	4.65
Fish meal	1.35	Metabolizable energy (cal/g)	3,031
Yellow Grease	4.00	Lysine	1.13
Lysine-HCl	0.07	Methionine	0.44
Limestone	0.48	Met + Cys	0.77
Tricalcium phosphate	1.36	Threonine	0.77
NaCl	0.24	Tryptophan	0.23
Wheat bran	0.34	Available lysine	1.04
Methionine	0.12	Available methionine	0.41
Mineral premix[1)]	0.10	Available Met + Cys	0.72
Vitamin premix[2)]	0.09	Available threonine	0.71
Choline chloride	0.12	Available tryptophan	0.21
Salinomycin	0.10	Calcium	0.80
Flavomycin	0.08	Total phosphate	0.64
Bio-plus	0.05	Available phosphate	0.41
		Sodium	0.14
		Chloride	0.20

[1)]Mineral premix provided per kg of ration with 50 mg Fe, 70 mg Mn, 50 mg Zn, 7 mg Cu, 0.4 mg Co, 0.17 mg Se, and 0.75 mg I.
[2)]Vitamin premix provided per kg of ration with 13,000 IU A, 2,600 IU D_3, 20 IU E, 2.5 mg K_3, 0.02 mg B_{12}, 4.0 mg rivoflavin, 10 mg pantothenic acid, 40 mg niacin, 1.0 mg folic acid, 0.08 mg biotin, and 0.5 mg ethoxyquin.

mendation. VAC2 was packed in vacuum bags (Cryovac BB4L; oxygen permeability 30 $cm^3/m^2/24$ hr at 23°C, 0% RH and 1 bar) and sealed with a vacuum packaging machine (GSL-2000; Vac Star, Swiss).

The carcasses were stored in a 4°C room for 6, 12, 18 or 24 days. After storage, individual carcasses were reweighed, with the level of purge loss reported as a percentage. Subsequently, individual carcasses were trimmed to obtain *pectoralis major* muscles.

Shelf life evaluation Total mesophilic count, discoloration, and lipid oxidation of the samples were analyzed on days 6, 12, 18, and 24 days storage at 4°C. For total mesophilic count, a 10×10 cm area of the skin was swabbed with a sterile cotton swab (Fisher Scientific, Pittsburgh, PA, USA), and serially diluted (10^{-1} to 10^{-7}) with 0.2% sterile peptone water. After 10-fold dilutions, 1 mL each diluent was transferred into petri dishes and pour-plated with Plate Count Agar (Difco Laboratories, Detroit, MI, USA) in duplicates. After thorough mixing, the plates were incubated at 32°C for 48 hr. The total mesophilic counts were then enumerated and reported as colony forming units (CFU)/cm^2 meat sample. The muscle color (CIE L*, a, and b) was measured using a Minolta chromameter (CR-300; Minolta Camera Co., Osaka, Japan). The percentage of metmyoglobin accumulation on the muscle surface was determined as described by Krzywicki (13). The lipid oxidation level was determined using the thiobarbituric acid (TBA) procedure reported by Salih *et al.* (14) and modified by Ahn *et al.* (15). The results are expressed in milligrams of malondialdehyde (MDA)/kg of tissue.

Statistical analyses Using the GLM procedure in the SAS PC software (SAS Inst., Inc., Cary, NC, USA), the data were subjected to the ANOVA procedures, appropriate for a randomized complete block design. The model included the effects of the dietary treatment, packaging method, and their interactions. When significant differences ($p<0.05$) were detected, the mean values were separated using the probability difference (PDIFF) option

Table 3. Effect of dietary selenium concentration on body weight and feed efficiency in broiler chicks

Variables	Treatment[1)]					
	CON1	CON2	SE1	SE2	SE4	SE8
Body weight (g)						
1 day	45 (0.8)[2)]	45 (0.8)	46 (0.8)	45 (0.8)	47 (0.8)	46 (0.8)
7 day	169 (12.8)	167 (12.8)	179 (14.5)	168 (12.8)	168 (14.5)	187 (14.5)
21 day	682 (20.5)	645 (20.5)	711 (28.4)	656 (18.1)	639 (28.4)	640 (28.4)
42 day	1903 (65.8)	1882 (65.8)	1922 (83.2)	1841 (58.1)	1856 (83.1)	1837 (83.1)
Feed efficiency (gain/feed)						
1-7 day	0.64 (0.05)	0.65 (0.05)	0.80 (0.11)	0.66 (0.05)	0.75 (0.11)	0.80 (0.11)
7-21 day	0.40 (0.05)	0.35 (0.05)	0.40 (0.08)	0.39 (0.04)	0.40 (0.09)	0.35 (0.09)
21-42 day	0.35 (0.02)	0.34 (0.02)	0.40 (0.04)	0.37 (0.02)	0.35 (0.04)	0.35 (0.04)

[1)]CON1, basal diet (α-tocopherol at a rate of 20 IU/kg feed and 0.17 ppm selenium); CON2, basal diet + 100 IU of all-rac-α-tocopheryl acetate/kg feed; SE1, CON2 + 1 ppm of sodium selenite. SE2, CON2 + 2 ppm of sodium selenite; SE4, CON2 + 4 ppm of sodium selenite; SE8, CON2 + 8 ppm of sodium selenite.
[2)]Standard error of least square means.

at a predetermined probability rate of 5%. The results are presented as the least square means (LSM) for each treatment group along with the standard error.

Results and Discussion

Performance of broiler chicks The body weight and feed efficiency were unaffected by the dietary Se when the broilers were fed 1, 2, 4, and 8 ppm Se in the form of Na_2SeO_3 in their diets from 3 to 6 weeks of age (Table 3). Moksnes and Norheim (16) observed that similar concentrations of Se fed to broilers as Na_2SeO_3 also caused no differences in body weight. However, Echevarria *et al.* (5) reported that the feed intake and weight gain were lower in birds fed 9 mg Se/kg, suggesting the development of Se toxicities. In addition 100 IU α-tocopherol (CON2) had no beneficial effect on the growth performance. This is in agreement with Guo *et al.* (17), who showed that the performance of the broiler chicks was not influenced by vitamin E supplementation at 50 and 100 mg/kg.

Shelf life evaluation The dietary treatments and packaging methods had no significant effects on total mesophilic count (Table 4); similar levels of microbial growth were observed among the four packaging methods. However, total mesophilic counts increased with storage time. After 18 days storage, total viable counts reached high numbers ($p<0.05$). Despite the high microbial populations, the overall count remained acceptable throughout the storage period. Nitrogen delays the oxidative rancidity and inhibits the growth of aerobic microorganisms by displacing oxygen in the package (18). Because carbon dioxide acts as an antimicrobial agent, it inhibits the growth of microorganisms during the logarithmic phase and extends the lag phase (18). In this study, no significant difference was observed in microbial growth between the vacuum and MAP samples.

No differences attributable to diet were observed in the purge loss (data not shown). This is consistent with the results reported by Cannon *et al.* (19), in which no beneficial effect of vitamin E supplementation was observed on the water-holding capacity. However, the effects of packaging methods on the purge loss at 6 and 12 days approached significance (Table 5, $p<0.001$). Samples packed with N_2 or $N_2 + CO_2$ showed similar purge losses during the storage period. However, vacuum packaging increased the level of purge loss by 12 days of storage, and remained similar up to 24 days, indicating MAP decreased the level of purge loss more efficiently than the vacuum packaging by almost 2 weeks. Bentley *et al.* (20) also reported that nitrogen atmosphere packages had the lowest level of purge loss, while those packaged with no gas backflush showed the highest levels.

The surface meat lightness (L*) of the broiler chicks was unaffected by the different packaging methods (Fig. 1A) and dietary Se supplementation (Fig. 1C). The lightness

Table 4. Total mesophilic counts (CFU/cm^2) in broiler chicks during storage

Packaging methods[1)]	Days of storage				Level of significance[3)]
	6	12	18	24	
MAP1	1.2×10^{4a} (0.7×10^4)[2)]	2.7×10^{4a} (1.2×10^4)	1.5×10^{5b} (2.2×10^4)	1.4×10^{6c} (2.2×10^5)	*
MAP2	3.7×10^{3a} (1.8×10^3)	2.6×10^{4ab} (1.8×10^4)	1.1×10^{5b} (1.8×10^4)	1.4×10^{6c} (1.8×10^5)	*
VAC1	5.5×10^{3a} (1.6×10^3)	2.7×10^{4a} (1.3×10^4)	1.6×10^{5b} (1.9×10^4)	1.4×10^{6c} (1.9×10^5)	*
VAC2	2.6×10^{4a} (1.9×10^4)	2.8×10^{4ab} (1.9×10^4)	1.3×10^{5b} (3.9×10^4)	2.0×10^{6c} (3.9×10^5)	*

[a-c]Least square means within row with no common superscripts differ significantly ($p<0.05$).
[1)]MAP1, modified atmosphere package (N_2 100%); MAP2, modified atmosphere package (N_2 : CO_2 = 80 : 20); VAC1, heat-shrink vacuum package; VAC2, vacuum package.
[2)]Standard error of least-square means.
[3)]Level of significance: *$p<0.05$.

Table 5. Effect of packaging methods on purge loss (%) in broiler chicks during storage

Days of storage	Packaging methods[1)]				Level of significance[3)]
	MAP1	MAP2	VAC1	VAC2	
6	3.83[a] (0.25)[2)]	4.43[a] (0.25)	5.15[b] (0.25)	5.85[c] (0.25)	***
12	4.66[a] (0.24)	5.00[ab] (0.24)	5.61[b] (0.24)	6.56[c] (0.24)	***
18	5.07 (0.27)	5.52 (0.27)	5.72 (0.27)	5.75 (0.27)	NS
24	5.27 (0.38)	5.33 (0.38)	5.83 (0.38)	5.82 (0.38)	NS

[a-c]Least square means within row with no common superscripts differ significantly ($p<0.05$).
[1)]MAP1, modified atmosphere package (N_2 100%); MAP2, modified atmosphere package (N_2 : CO_2 = 80 : 20); VAC1, heat-shrink vacuum package; VAC2, vacuum package.
[2)]Standard error of least-square means.
[3)]Level of significance: NS = not significant; *** $p<0.001$.

of the breast muscle showed large variations during storage in the broiler chicks supplemented with different Se levels. However, no significant differences in this parameter were observed in the samples in both vacuum and modified atmosphere packages. The level of surface metmyoglobin accumulation was also unaffected by the different packaging methods (Fig. 1B) and dietary Se supplements (Fig. 1D), showing a similar discoloration rate. On day 6, the breast muscle samples were highly discolored and contained a larger percentage of surface metmyoglobin. The metmyoglobin content increased in all samples during storage up to 24 days, with the four different packaging methods showing similar levels. The differences in the metmyoglobin content during the storage period were related to the intensity of the oxidation process that occurs during storage. These results showed no clear advantage of the modified atmospheres (100% N_2 and 20% CO_2 + 80% N_2) over the vacuum packaging of broiler chicks in terms of meat color and discoloration.

Table 6 shows the effect of dietary Se and the packaging methods on the level of lipid oxidation in broiler chicks stored at 4°C for 6, 12, 18, and 24 days. In breast muscles, the interaction between the dietary Se level and the packaging method was significant for the TBA values at all storage times ($p<0.05$). The TBA values were highest in the vacuum packaged meats from the control diet and lowest in the MAP breast meat from SE4 and SE8 diets.

The TBA value increased with all treatments during

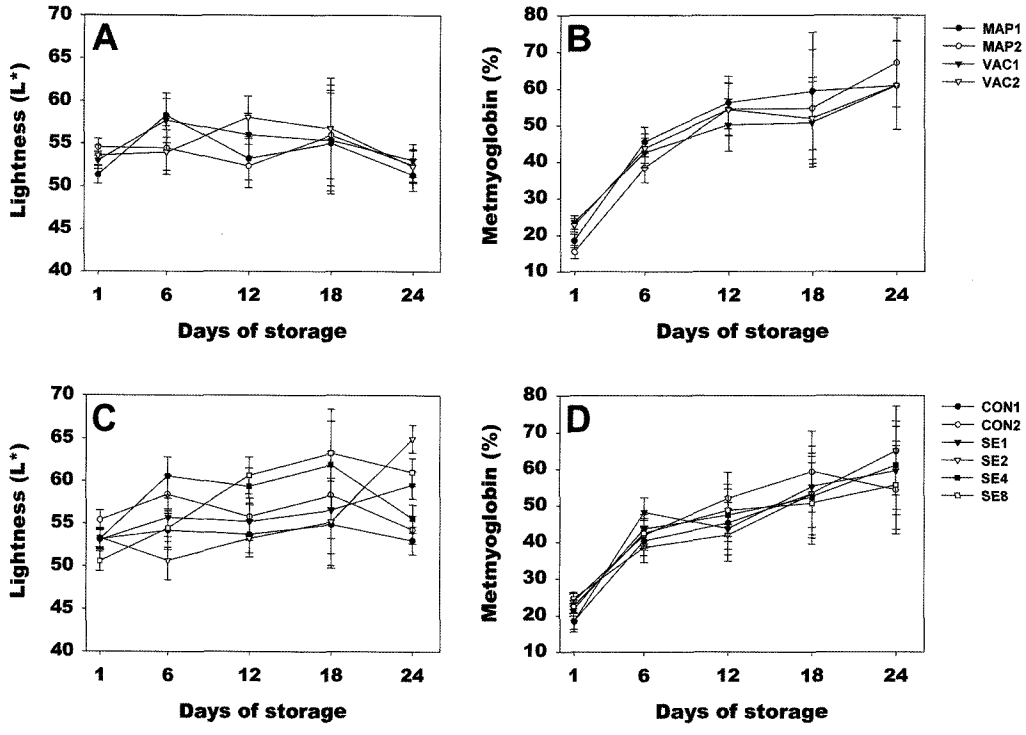

Fig. 1. Effects of packaging methods (A, B) and dietary selenium levels (C, D) for lightness and metmyoglobin content in breast muscle of broiler chicks during storage.

Table 6. Two-way interaction between selenium supplementation and packaging methods on TBA values (mg MDA/kg) in broiler chicks during storage

Days	Packaging methods	Dietary treatments						
		CON1	CON2	SE1	SE2	SE4	SE8	SE
6	MAP1	0.361^{abc}	0.340^{abcd}	0.293^{cd}	0.242^{d}	0.324^{bcd}	0.352^{abcd}	0.048
	MAP2	0.371^{abc}	0.377^{abc}	0.392^{abc}	0.331^{bcd}	0.434^{ab}	0.293^{cd}	
	VAC1	0.324^{bcd}	0.291^{cd}	0.358^{abc}	0.308^{cd}	0.406^{abc}	0.306^{cd}	
	VAC2*	0.446^{a}	0.448^{a}	0.297^{cd}	0.445^{a}	0.352^{abcd}	0.309^{bcd}	
12	MAP1	0.606^{cd}	0.769^{bcd}	0.493^{d}	0.621^{cd}	0.837^{abcd}	0.772^{bcd}	0.163
	MAP2	0.768^{bcd}	0.746^{bcd}	0.841^{abcd}	1.258^{a}	1.093^{ab}	0.902^{abcd}	
	VAC1	0.660^{cd}	0.931^{abcd}	1.011^{abc}	1.222^{a}	0.555^{d}	0.748^{bcd}	
	VAC2	0.843^{abcd}	1.262^{a}	0.525^{d}	0.692^{cd}	0.659^{cd}	1.238^{a}	
18	MAP1	2.072^{gh}	2.770^{bcdef}	2.016^{h}	2.710^{cdef}	2.404^{efgh}	2.782^{bcdef}	0.232
	MAP2	3.174^{abc}	3.755^{a}	2.488^{efgh}	2.889^{bcde}	1.804^{h}	1.989^{h}	
	VAC1	2.851^{bcdef}	2.940^{bcde}	2.277^{fgh}	3.156^{bcd}	2.573^{defgh}	2.678^{cdefg}	
	VAC2	3.344^{ab}	2.037^{gh}	2.385^{efgh}	2.527^{efgh}	2.316^{efgh}	3.266^{abc}	
24	MAP1	2.655^{fgh}	2.771^{efgh}	2.515^{gh}	3.059^{def}	2.848^{efg}	2.480^{gh}	0.173
	MAP2	3.644^{ab}	3.660^{ab}	2.723^{fgh}	2.937^{efg}	2.277^{h}	2.355^{h}	
	VAC1	3.603^{ab}	3.382^{bcd}	2.944^{defg}	3.476^{bc}	3.068^{cdef}	3.099^{cdef}	
	VAC2	3.998^{a}	3.009^{def}	2.719^{fgh}	2.852^{efg}	2.941^{defg}	3.225^{bcde}	

[a-h]Least square means with different superscripts significantly differ ($p<0.05$).

storage for up to 24 days, with some differences ($p<0.05$) among treatments; the TBA value was highest in the CON1 group (3.475 mg MDA/kg). Until 24 days of refrigerated storage, the breast meats from the chicks supplemented with Se (2.275 to 3.082 mg MDA/kg) had lower TBA values than those of the CON2 group (3.206 mg MDA/kg). However, the broiler chicks supplemented with either α-tocopherol or α-tocopherol + selenium showed no statistical differences in the TBA values. Avanzo *et al.* (2) reported that α-tocopherol affected the peroxidation of the mitochondrial membranes by one order of magnitude higher than Se, which also suggested that α-tocopherol, and not Se, is the factor that markedly affects the course of lipid peroxidation (21, 22).

Significant differences were observed in the TBA values, showing 24 days values of 3.293, 3.086, 2.934, and 2.769 mg MDA/kg for the VAC1, VAC2, MAP1, and MAP2 groups, respectively, showing that MAP was more effective in reducing the level of lipid oxidation than vacuum packaging. This is consistent with the results of Wang *et al.* (23), who reported that the TBA and peroxide values were lower under the MAP than the vacuum conditions.

In summary, broiler chicks stored in vacuum and modified atmospheres (100% N_2 and 20% CO_2 + 80% N_2) showed similar color and microbiological stabilities. However, MAP was more effective in reducing the levels of purge loss and lipid oxidation than vacuum packaging. Dietary supplementation with 4 or 8 ppm Se had an added effect in reducing the level of lipid oxidation, which was limited in the modified atmosphere packaged breast meat.

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