



Comparative effect of dietary *Morinda lucida* leaf and Butylated hydroxyanisole (BHA) on carcass traits, meat quality, and oxidative stability of broiler chickens

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Abstract This study examined the impact of dietary supplementation of *Morinda lucida* leaf powder (MLLP) and Butylated hydroxyanisole (BHA) on carcass traits, physicochemical properties, and sensory attributes of different muscles in broiler chickens. Two hundred and forty 1-day old Arbor acre chicks were randomly allotted to either a negative control (NC), basal diet without additive; M-0.1, basal diet + 0.1%MLLP; M-0.2, basal diet + 0.2%MLLP; or Positive Control, (PC), basal diet + 0.02%BHA, fed for 42 d, and euthanized. The physicochemical properties and oxidative stability of thigh and breast muscles were assessed over a 5 d *postmortem* chill storage. Diet had no effect ($p > 0.05$) on carcass traits and chemical composition, cook loss, pH and sensory attributes of breast and thigh muscles in broiler chickens. Total phenolic content was higher ($p = 0.032$) in the supplemented meats than in the NC meat. Carbonyl content, TBARS value and drip loss were higher ($p < 0.05$), while redness was lower ($p = 0.021$) in the NC meat compared with the meat of the supplemented birds. Carbonyl content was lower ($p < 0.0001$) in the PC meat compared with the M-0.1 and M-0.2 meats. The M-0.1 and M-0.2 meats had lower ($p < 0.0001$) TBARS value than the PC meat. Chill storage and muscle type influenced ($p < 0.05$) the physicochemical properties and oxidative stability of broiler meat. There were significant interactions between diet, muscle type, and chill storage on the oxidative stability of broiler meat. These results suggest that MLLP exhibited

antioxidant potential that was comparable to that of BHA in the diets of broiler chickens.

Keywords Cook loss · Muscle · Phenolic · Redness · Sensory

Introduction

Broiler meat is rich in polyunsaturated fatty acids, which promote its susceptibility to lipid oxidation (Barroeta 2007). Oxidized lipids, and other pro-oxidants could initiate protein oxidation, which is characterized by breakage of peptide bonds, formation of crosslinks and degradation of amino acid side chains (Bekhit et al. 2013; Soladoye et al. 2015). Lipid and protein oxidation could reduce the nutritional quality and limit the shelf life, and processing possibilities of muscle-foods (Falowo et al. 2014; Min et al. 2008). In addition, consuming products of lipid and protein oxidation could have hazardous effects on consumers' health (Kanner 2007; Soladoye et al. 2015).

Dietary supplementation of antioxidant is a viable strategy for enhancing the oxidative stability of intact muscles, in which the application of exogenous antioxidants may not be feasible (Odhaib et al. 2018; Sierżant et al. 2018; Yusuf et al. 2018). Moreover, dietary strategy ensures that antioxidants are preferentially deposited where they are most needed (Odhaib et al. 2018; Yusuf et al. 2018). Synthetic antioxidants are effective for the prevention of oxidative spoilage in foods (Carocho and Ferreira 2013). Nonetheless, there is potential health risks associated with the consumption of synthetic antioxidants as preservatives in foods (Vandghanooni et al. 2013). Against the backdrop, medicinal plants and their products have been explored for their antioxidant properties in broiler

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meat (Loetscher et al. 2013; Lu et al., 2014; Sierżant et al. 2018).

Various plants with medicinal and antioxidant properties exist in the tropics and one of such is *Morinda lucida* (Brimstone) (Adeyemi et al. 2014; Osuntokun et al. 2016). Various parts of *Morinda lucida* exhibit myriad therapeutic benefits (Lawal et al. 2012; Adeyemi et al. 2014). In spite of the numerous benefits of *Morinda lucida*, its antioxidant potential on muscle-foods has not been explored. Due to the phytochemical contents of *Morinda lucida* leaf (Adeyemi et al. 2014; Osuntokun et al. 2016), it was hypothesized that the dietary supplementation of *Morinda lucida* leaf would affect the oxidative status and the physicochemical properties of broiler meat. The objective of this study was to determine the effects of dietary supplementation of *Morinda lucida* leaf powder in comparison with Butylated hydroxyanisole (BHA), a synthetic antioxidant on carcass traits, physicochemical properties, oxidative stability, and sensory attributes of thigh and breast muscles in broiler chickens.

Materials and methods

Chemicals

Butylated hydroxyanisole, Folin–Ciocalteu reagent and gallic acid were obtained from Sigma-Adrich (St. Louis, MO, USA). Other chemicals were obtained from Integrated Sunaf Nig. Ltd.

Ethical note

The experiment was conducted following the guidelines approved by the University of Ilorin Ethical review committee. All animal procedures were carried out following the animal welfare standards of the Department of Animal Production and Husbandry Services, Federal Ministry of Agriculture and Rural Development, Nigeria.

Source and preparation of *Morinda lucida* leaf powder

Fresh leaves of *Morinda lucida* were harvested in Ikorodu, Lagos State, Nigeria. The identity of the leaves was ascertained at the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria. The leaves were air dried (at ambient temperature (36 ± 1 °C) for approximately 72 h), ground into powder, and stored in an airtight container until analysis and usage.

Qualitative and quantitative phytochemical analysis of *Morinda lucida* leaf powder

Morinda lucida leaf powder (MLLP) was subjected to qualitative and quantitative phytochemical screening following the method described by Osuntokun et al. (2016). The MLLP contained saponin (0.92 mg/100 g), tannin (0.37 mg/100 g), phenol (0.93 mg/100 g), flavonoids (24.44 mg/100 g), steroids (0.10 mg/100 g), coumarins (0.02 mg/100 g), terpenoids (0.48 mg/100 g), glycosides (8.65 mg/100 g), and alkaloids (0.46 mg/100 g).

Experimental birds, diets, and management

Two hundred and forty 1-day old Arbor acre chicks were obtained from a reputable hatchery. The birds were individually wing banded, weighed and randomly allotted to either a negative control (NC), basal diet without additive; M-0.1, basal diet + 0.1% MLLP; M-0.2, basal diet + 0.2% MLLP; or a positive control (PC), basal diet + 0.02% BHA and fed for 42 d. Each treatment group was replicated six times with ten chicks per replicate. The experimental diets were formulated to meet the nutrient requirement of broiler chickens following the National Research Council (NRC 1994) guidelines. The starter diet was fed from d 1 to d 21 while the finisher diet was fed from d 22 to d 42. Birds were raised in a deep litter facility. The bedding material was wood shavings spread at a depth of 5 cm. The birds had ad libitum access to water and feed throughout the feeding trial. Routine management procedures followed commercial recommendation. The chemical composition of the diets was determined according to the procedure of AOAC (2000) and presented in Table 1.

Slaughter and carcass analysis

On d 42, the birds were fasted overnight with ad libitum access to water. Five birds per replicate (30 birds per dietary group) were randomly selected and euthanized by a neck cut with an exquisitely sharp knife. Thereafter, the slaughtered birds were defeathered and eviscerated. Weights of abdominal fat and prime cuts were measured. Dressing percentage and the relative weights of carcass cuts and their components were calculated. The thigh and breast muscles obtained from the carcasses were trimmed free of skin and external fat and used for meat quality analyses.

Chemical composition and total phenolic content of meat

The chemical composition of the meat samples was determined by the AOAC (2000) method. The total

Table 1 Ingredients and chemical composition of dietary treatments

Ingredient (g/kg as fed)	Dietary treatment ¹							
	Starter diet				Finisher diet			
	NC	M-0.1	M-0.2	PC	NC	M-0.1	M-0.2	PC
Maize	500.00	500.00	500.00	500.00	540.00	540.00	540.00	540.00
Soybean meal	300.00	300.00	300.00	300.00	240.00	240.00	240.00	240.00
Maize offal	90.00	90.00	90.00	90.00	100.00	100.00	100.00	100.00
Groundnut cake	30.00	30.00	30.00	30.00	50.00	50.00	50.00	50.00
Fish meal	20.00	20.00	20.00	20.00	10.00	10.00	10.00	10.00
Bone meal	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Oyster shell	16.50	16.50	16.50	16.50	10.50	10.50	10.50	10.50
Dicalcium phosphate	14.00	14.00	14.00	14.00	20.00	20.00	20.00	20.00
Methionine	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Lysine	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Salt	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Vitamin-mineral Premix ²	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Enzyme ³	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Toxin binder ⁴	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
<i>Morinda lucida</i> leaf powder	0.00	1.00	2.00	0.00	0.00	1.00	2.00	0.00
Butylated hydroxyanisole	0.00	0.00	0.00	0.20	0.00	0.00	0.00	0.20
<i>Analyzed Composition</i>								
Dry matter (%)	93.41	92.90	93.23	92.89	93.00	93.10	92.40	92.70
Crude protein (%)	22.76	22.56	22.50	22.60	20.11	20.10	20.10	20.22
Ether extract (%)	4.01	4.05	4.00	4.04	3.68	3.65	3.66	3.68
Crude fibre (%)	3.22	3.54	3.50	3.53	4.21	4.18	4.20	4.18
Ash (%)	3.64	3.70	3.70	3.72	3.95	3.95	3.95	3.96
Total polyphenols (%)	0.10	0.19	0.27	0.24	0.11	0.19	0.28	0.23
<i>Calculated analysis</i>								
Metabolizable energy (kcal/kg)	2948.00	2948.00	2948.00	2948.00	3113.00	3113.00	3113.00	3113.00
Calcium (%)	1.06	1.06	1.06	1.06	1.10	1.10	1.10	1.10
Phosphorus (%)	0.65	0.65	0.65	0.65	0.60	0.60	0.60	0.60
Methionine (%)	0.73	0.73	0.73	0.73	0.70	0.70	0.70	0.70
Lysine (%)	1.20	1.20	1.20	1.20	1.18	1.18	1.18	1.18

¹NC, negative control, basal diet without additive; M-0.1, basal diet + 0.1% *Morinda lucida* leaf powder; M-0.2, basal diet + 0.2% *Morinda lucida* leaf powder; PC, positive control, basal diet + 0.02% Butylated hydroxyanisole

²Supplied per kg diet: Vitamin A 11 494 IU; vitamin B₁ 1.43 mg; vitamin B₂ 3.44 mg; vitamin B₃ 40.17 mg; vitamin B₅ 6.46 mg; vitamin B₆ 2.29 mg; vitamin B₇ 0.05 mg; vitamin B₉ 0.56 mg; vitamin B₁₂ 0.05 mg; vitamin D 1 725 IU; vitamin E 40 IU; vitamin K₃ 2.29 mg; Fe 120 mg; Zn 120 mg; Cu 15 mg; Mn 150 mg; Co 0.4 mg; Se 0.3 mg; I 1.5 mg;

³Maxigrain®; contains Xylanase 10,000 IU, Beta glucanase 200 IU, Cellulase 10,000 IU, Phytase 2500 FTU

⁴AnfatoxPro®; contains Mannan-Oligosaccharides, Bacillus, subtilis, Benzoic acid, sorbic acid, activated charcoal, liver extract, silicon dioxide, Hydrated Sodium Calcium Aluminosilicate

phenolic content of the meat sample was determined using Folin–Ciocalteu reagent (Sigma-Aldrich) according to the method of Jang et al. (2008) with slight modifications. Five gram of each meat sample was homogenized with 15 mL of distilled water at 4000 × g for 90 s. Thereafter, 10 mL of chloroform was added to the homogenate, and the mixture was vortexed for 30 s. An aliquot (1 mL) of the

diluted sample was added to 500 µL of Folin–Ciocalteu reagent, followed by the addition of 1 mL of sodium carbonate solution. The mixture was vortexed and incubated in the dark at 35 °C for 30 min and the absorbance was read at 700 nm with spectronic 21D digital spectrophotometer (Milton Roy, NY, USA). Total phenolic content was quantified based on the standard curve generated with

gallic acid (Sigma-Adrich). The results were expressed as gallic acid equivalent/kg of meat.

Measurement of muscle pH

Approximately 5 g of meat sample was homogenized with 20 mL of distilled water. Thereafter, the pH of the resultant homogenate was measured with a pre-calibrated pH meter (Mettler Toledo, China).

Determination of meat color coordinates

The meat color was measured with a hand held colorimeter (WR-10, Shenzhen, China) based on CIELAB values with D₆₅ illuminant and 10° standard observer. For each sample, three spectral readings were obtained at different locations and averaged. The device was calibrated before use.

Determination of drip loss of muscle samples

Muscle samples were weighed and the weight was recorded as initial weight (W_a). The weighed samples were placed in transparent vacuum bags, vacuum sealed and stored in a refrigerator (Haier HR-170 T) at 5 ± 1 °C. After 1, 3, and 5 d postmortem, the samples were removed from the vacuum bags, blotted dry and weighed and the weight was recorded as final weight (W_b). Drip loss was estimated using the formula below:

$$\text{Drip loss (\%)} = [(W_a - W_b) \div W_a] \times 100$$

Determination of cooking loss of muscle samples

Muscle samples were weighed and the weight was recorded as initial weight (W_a). The samples were placed in a vacuum bags, vacuum sealed and cooked in pre-heated water bath at 80 °C until the internal temperature of the samples reached 78 °C as monitored by a stabbing temperature probe, which was inserted into the center of the meat sample. The cooked meat samples were cooled with running tap water for 15 min, removed from the vacuum bags, blotted dry without squeezing, and reweighed (W_b). Cooking loss was calculated using the equation below:

$$\text{Cooking loss (\%)} = [(W_a - W_b) \div W_a] \times 100$$

Determination of meat oxidative stability

Lipid oxidation was assayed by measuring the formation of thiobarbituric acid reactive substances (TBARS) following the protocol of Varshney and Kale (1990). Results were presented as nmol malondialdehyde (MDA) per mg protein of sample. The protein carbonyl content was determined

according to the protocol of Levine et al. (1990). Results were presented as μmol carbonyl per mg protein of sample.

Sensory analysis The sensory attributes of meat samples were determined following the protocols described by Meilgaard et al. (2007) and AMSA (1995). Sensory assessment was carried out on meat samples (from 29 birds per dietary group) that were used for the determination of cook loss on 1 d postmortem. Meat samples were sectioned into blocks (2.5 cm length × 1.5 cm width × 1.5 cm height), wrapped in a foil paper and placed in a plastic container with a lid. The plastic containing the meat sample was assigned a three-digit random number and kept in an oven at 50 °C until analysis (the holding time was 15 min). A consumer type sensory assessment (Meilgaard et al. 2007) was conducted on the meat samples using a 9-point hedonic scale (9 = like extremely; 8 = like very much; 7 = like moderately; 6 = like slightly; 5 = neither like nor dislike; 4 = dislike slightly; 3 = dislike moderately, 2 = dislike very much; 1 = dislike extremely). Twenty-nine assessors (12 males and 17 females; Age 17–45 years) participated in the sensory analysis. The assessors comprised of staff and students of University of Ilorin and were recruited through verbal communication. The sensory variables (flavour, juiciness, appearance, tenderness, and overall acceptability) and other sensory protocols were explained to the assessors prior to the sensory analysis. Two sessions of assessment were held in one day with a 30 min break between sessions. Breast samples were assessed in the first session while thigh samples were assessed in the second session. Each panelist assessed four samples in each section, and the samples assessed always included one sample from each dietary group. The assessors were provided water and unsalted crackers to rinse their palate after assessing each sample.

Statistical analysis

Data were checked for normality using the PROC UNIVARIATE procedure of Statistical Analysis System (SAS) (SAS Institute Inc., Cary, NC, USA) and were found to be normally distributed. The growth and carcass data were subjected to analysis of variance model suitable for a completely randomized design with six replicates. For the growth performance, the experimental unit was a pen of 10 birds and each dietary group had six pens. For the carcass attributes, the experimental unit was a pen of five birds and each dietary group had six pens. The data were analyzed using the Generalized Linear Model procedure of SAS. Means were separated with Tukey HSD test. Level of significance was set at $p < 0.05$.

Meat chemical composition data was analyzed using the PROC-MIXED procedure of SAS, in which diet, muscle

type and their interaction were fitted as fixed effects. Least-square means were separated by using the PDIF option of SAS. The experimental unit was the individual muscle within birds in a pen. The data obtained for meat physicochemical properties and oxidative stability were analyzed using the PROC MIXED procedure of SAS with repeated measures. The experimental unit was the individual muscle within birds in a pen. Diet, muscle type and chill storage and their second order interactions were fitted as fixed effects. Least-square means were separated by using the PDIF option of SAS. The sensory data were analyzed using PROC MIXED procedure of SAS. Diet, muscle type and diet-muscle type interaction were treated as fixed effects. The experimental unit was the individual muscle in a bird nested within a panelist. Least square means were generated, and PDIF was assessed for significance.

Results and discussion

Carcass traits and meat chemical composition

In this study, we examined the antioxidant potential of *Morinda lucida* leaf in comparison with that of BHA in the diet of broiler chickens. Dietary treatments had no effect ($p > 0.05$) on growth traits, carcass weight, dressing percentage, and abdominal fat in broiler chickens (Table 2). The percentage weight of prime cuts was not significantly different ($p > 0.05$) among the treatments. The percentage composition of lean, skin and bone in the prime cuts was not influenced ($p > 0.05$) by diets. These observations may be due to the homogenous dietary energy and protein contents and the similar management conditions employed during the trial. These findings are akin to that of Nobakht et al. (2015), who reported that the supplementation of *Tanacetum balsamita* powder and extract had no effect on the carcass attributes of broiler chickens.

The chemical composition and total phenolic content of different muscles in broiler chickens fed diet supplemented with different sources of antioxidants are presented in Table 3. There was no significant interaction ($p > 0.05$) between diet and muscle type on the chemical composition of broiler meat. Diet did not affect ($p > 0.05$) the percentage of moisture, protein, ash and ether extract in broiler meat. These findings may be due to the similar slaughter weight of the birds. Consistently, dietary supplementation of mix of medicinal herb extract did not affect breast meat composition in broiler chickens (Jang et al. 2008). Muscle type did not affect ($p > 0.05$) the moisture, crude protein, and ash content of broiler meat. The thigh muscle had higher ($p = 0.047$) ether extract than did the breast muscle. This may be because oxidative muscles need higher intramuscular fat to meet the need for cellular metabolism

by fat oxidation (Adeyemi et al. 2016). Further, muscles with high glycolytic activity have reduced intramuscular fat (Hocquette et al. 2010). The proximate composition of broiler meat found in this study is consistent with those reported by previous workers (Jang et al. 2008; Okarini et al. 2013; Adeyemi et al. 2020). The total phenolic content of the meat reflected dietary polyphenol, and was higher ($p = 0.032$) in the meat of the supplemented birds than did that of the NC birds. This finding is in accord to that of Jang et al. (2008) who reported that the supplementation of medicinal herb extract mix enhanced the total phenol content of breast muscle in broiler chickens. Total phenolic content did not differ ($p > 0.05$) between the thigh and breast meat in broiler chickens.

3.2. Sensory profile of broiler meat

Dietary supplementation of antioxidants did not affect ($p > 0.05$) the flavor, appearance, tenderness, juiciness and overall acceptability of broiler meat (Table 4). The similar chemical composition of meat in the treatments may account for this observation. The thigh meat had higher tenderness ($p = 0.020$), juiciness ($p = 0.042$), flavor ($p = 0.031$) and overall acceptability ($p = 0.014$) than did the breast meat. This finding may be due to the higher water holding capacity and fat content of the thigh meat. Improved water holding capacity (a lower drip and cook losses) may likely improve meat tenderness because a given cross-sectional area of meat sample will contain more water and less structural components (Adeyemi et al. 2016). Moreover, intramuscular fat in the perimysium can cause a remodeling of intramuscular connective tissues (IMCT), which can lower the mechanical strength of IMCT thereby improving meat tenderness (Nishimura 2010). There was no significant interaction between dietary antioxidants and muscle type on the sensory attributes of broiler meat.

3.3. Physicochemical properties of broiler meat

The physicochemical properties of thigh and breast muscles of broiler chickens fed diets supplemented with different antioxidants are presented in Table 5. The interaction between diet, muscle and chill storage was not significant ($p > 0.05$). Dietary antioxidants did not affect ($p = 0.700$) muscle pH in broilers. This may indicate the homogenous dietary energy and the management conditions employed during the trial. Nonetheless, the pH of the meat samples was within the normal pH range for broiler chickens (Jang et al. 2008; Salwani et al. 2016). Breast muscle had lower ($p = 0.040$) pH than did the thigh muscle. Breast muscle consists majorly of type IIB fiber that has higher glycolytic potential, and thus the potential to

Table 2 Growth and carcass traits of broiler chickens fed diet supplemented with different antioxidants from 1 to 42 days of age

Item	Dietary treatment ¹				SEM	P value
	NC	M-0.1	M-0.2	PC		
<i>Growth traits</i> [†]						
Initial body weight (g/bird)	43.00	43.00	42.50	43.00	1.13	0.214
Final body weight (g/bird)	1900.00	1833.33	2116.67	1883.33	113.65	0.364
Body weight gain (g/bird/day)	44.21	42.62	49.37	43.81	6.44	0.090
Feed intake (g/bird/day)	81.79	77.14	85.91	78.43	12.10	0.109
Feed conversion ratio	1.85	1.81	1.74	1.79	0.12	0.142
<i>Carcass traits</i> [‡]						
Live weight (g/bird)	1937.00	1898.55	2063.20	1924.67	100.21	0.122
Carcass weight (g/bird)	1374.88	1313.60	1464.11	13,331.60	70.71	0.195
Dressing percentage	70.98	69.21	70.97	69.21	4.52	0.880
Abdominal fat (% body weight)	0.77	0.87	0.72	0.83	0.21	0.181
Carcass cuts (% Carcass weight)						
Breast	31.52	31.50	30.76	30.71	4.18	0.105
Thigh	16.62	16.48	15.98	16.48	1.03	0.771
Drumstick	16.07	16.69	16.96	17.01	0.65	0.092
Wing	12.64	12.19	12.86	12.47	0.77	0.163
Back	23.29	22.92	23.31	23.33	0.97	0.559
Composition of prime cuts (%)						
Breast						
Lean	74.72	75.01	75.00	75.21	2.45	0.071
Skin	9.80	9.83	9.49	9.50	0.81	0.056
Bone	15.24	15.04	15.88	15.29	0.90	0.092
Thigh						
Lean	75.45	74.21	75.00	74.23	2.45	0.123
Skin	9.27	8.89	10.02	9.45	0.52	0.213
Bone	14.95	15.67	14.89	15.04	0.65	0.093
Drumstick						
Lean	67.89	69.21	68.24	68.78	4.23	0.218
Skin	4.54	5.00	4.78	5.13	0.76	0.341
Bone	26.97	24.24	25.21	24.89	1.90	0.125
Wing						
Lean	35.32	36.21	36.01	36.00	2.41	0.170
Skin	27.21	26.26	27.01	26.54	1.98	0.146
Bone	39.07	38.24	37.98	38.32	3.08	0.186

¹basal diet without additive; M-0.1, basal diet + 0.1% *Morinda lucida* leaf powder; M-0.2, basal diet + 0.2% *Morinda lucida* leaf powder; basal diet + 0.02% Butylated hydroxyanisole

[†]Each dietary group was replicated six times and each replicate consisted of 10 birds

[‡]Each dietary group was replicated six times and each replicate consisted of five birds

SEM, standard error of mean, NC, negative control, PC, positive control

produce lactic acid, which causes a lower pH (Barbut et al. 2008; Ismail and Joo 2017). Muscle pH on day 0 was higher ($p < 0.0001$) than that on days 1, 3, and 5 post-mortem. The finding may be attributed to postmortem glycolysis characterized by the conversion of muscle glycogen to lactic acid, which lowers muscle pH (Sabow et al. 2016). The stability of pH on days 1, 3, 5 postmortem

may indicate that postmortem glycolysis was completed within the first 24 h postmortem.

The ability to retain moisture is one of the most important meat quality traits (Huff-Lonergan and Lonergan 2005). The meat of the NC birds had higher ($p = 0.027$) drip loss than the meat of birds fed other diets (Table 5). However, cook loss was not affected ($p = 0.273$) by diets. The lower drip loss in the meat of the supplemented birds

Table 3 Chemical composition and total phenolic content of different muscles in broiler chickens fed different antioxidants from 1 to 42 days of age

Factor	Item [†]	Item [†]				
		moisture (%)	Crude protein (%)	Ether extract (%)	Ash (%)	Total phenols (mg gallic acid equivalent/kg)
Diet ¹	NC	75.22	20.46	3.09	1.53	51.25 ^c
	M-0.1	74.45	21.09	3.12	1.34	78.27 ^b
	M-0.2	74.78	20.55	3.15	1.52	94.21 ^a
	PC	75.05	20.78	3.00	1.17	86.21 ^{ab}
	SEM	6.45	2.33	0.21	0.18	8.79
	P value	0.180	0.154	0.081	0.118	0.032
Muscle	Breast	74.24	21.23	3.21 ^b	1.32	78.94
	Thigh	74.20	20.04	4.09 ^a	1.48	75.54
	SEM	5.60	2.49	0.15	0.19	6.00
	P value	0.127	0.110	0.047	0.105	0.211
Diet*muscle	P value	0.111	0.125	0.065	0.219	0.094

^{a,b,c} means with different superscripts along the same column differ significantly ($p < 0.05$)

¹NC, negative control, basal diet without additive; M-0.1, basal diet + 0.1% *Morinda lucida* leaf powder; M-0.2, basal diet + 0.2% *Morinda lucida* leaf powder; basal diet + 0.02% Butylated hydroxyanisole

[†]Each dietary group was replicated six times and each replicate consisted of five birds

SEM, standard error of mean, NC, negative control, PC, positive control

Table 4 Sensory attributes of different muscles in broiler chickens fed different antioxidants from 1 to 42 days of age

Factor		Sensory indices [†]				
		Flavor	Appearance	Juiciness	Tenderness	Overall acceptability
Diet ¹	NC	5.90	6.00	5.80	5.90	6.21
	M-0.1	5.86	6.02	5.90	5.90	6.20
	M-0.2	6.00	6.00	5.85	5.95	6.42
	PC	5.90	6.00	5.85	5.90	6.40
	SEM	0.52	0.57	0.43	0.24	0.40
	P value	0.110	0.210	0.176	0.091	0.093
Muscle	Breast	5.70 ^b	6.04	5.70 ^b	5.60 ^b	5.84 ^b
	Thigh	6.10 ^a	6.12	6.40 ^a	6.00 ^a	6.20 ^a
	SEM	0.23	0.10	0.17	0.21	0.18
	P value	0.031	0.092	0.042	0.020	0.014
Diet*muscle	P value	0.167	0.219	0.119	0.218	0.148

^{a, b}, means with different superscripts along the same column differ significantly ($p < 0.05$)

¹basal diet without additive; M-0.1, basal diet + 0.1% *Morinda lucida* leaf powder; M-0.2, basal diet + 0.2% *Morinda lucida* leaf powder; basal diet + 0.02% Butylated hydroxyanisole

[†]Meat from each dietary group was assessed by 29 panelists with each panelist assessed a sample of each muscle from one bird from each group using a 9 point hedonic scale (9 = like extremely; 8 = like very much; 7 = like moderately; 6 = like slightly; 5 = neither like nor dislike; 4 = dislike slightly; 3 = dislike moderately, 2 = dislike very much; 1 = dislike extremely)

SEM, standard error of mean, NC, negative control, PC, positive control

may reflect the higher polyphenol contents, which reduced oxidative-induced conformational alterations and fragmentation of myofibrillar proteins that could modify their functions, particularly the water holding capacity. Likewise, a reduction in drip loss was observed in the breast

muscle of broiler chickens fed antioxidant blends (Lu et al. 2014). The PC meat had lower ($p = 0.027$) drip loss than did the M-0.1 and M-0.2 meats. This finding reflects the lower carbonyl content in the PC meat. Breast muscle had higher drip loss ($p = 0.007$) and cook loss ($p = 0.001$) than

Table 5 Physicochemical properties and oxidative stability of different muscles in broiler chickens fed diet supplemented with different antioxidants from 1 to 42 days of age

Factor	Item [†]	L*		a*		b*		Drip loss (%)	Cook loss (%)	pH	TBARS (nmol MDA/mg protein)	Carbonyl (μmol/mg protein)
		L*	a*	b*	a*	b*						
Diet ¹	NC	46.09	4.46 ^b	9.06	6.80 ^a	13.96	5.86	0.72 ^a	0.53 ^a			
	M-0.1	47.42	5.60 ^a	8.72	6.05 ^b	16.88	5.86	0.38 ^c	0.39 ^b			
	M-0.2	44.15	5.64 ^a	8.89	5.88 ^b	14.51	5.88	0.26 ^d	0.40 ^b			
	PC	45.18	5.27 ^a	8.46	4.24 ^c	14.19	5.90	0.50 ^b	0.30 ^c			
	SEM	1.26	0.48	0.42	0.59	1.19	0.02	0.02	0.02			
	P value	0.317	0.021	0.067	0.027	0.273	0.70	< .0001	< .0001			
Muscle	Breast	46.51	3.63 ^a	9.42	6.59 ^a	16.94 ^a	5.70 ^b	0.12 ^b	0.47 ^a			
	Thigh	44.92	6.75 ^b	9.24	4.92 ^b	12.84 ^b	5.87 ^a	0.42 ^a	0.15 ^b			
	SEM	0.89	0.34	0.30	0.42	0.83	0.02	0.02	0.02			
	P value	0.215	< .0001	0.671	0.007	0.001	0.04	< .0001	< .0001			
Day	0						6.12 ^a	0.12 ^c	0.10 ^c			
	1	46.29	6.12 ^a	8.93	6.84 ^a	14.82 ^{ab}	5.75 ^b					
	3	46.10	4.96 ^b	9.46	5.76 ^{ab}	17.07 ^a	5.76 ^b	0.22 ^b	0.16 ^b			
	5	44.74	4.50 ^b	9.61	4.66 ^b	12.78 ^b	5.75 ^b	0.36 ^a	0.35 ^a			
	SEM	1.09	0.42	0.36	0.51	1.01	0.02	0.02	0.02			
	P value	0.548	0.026	0.391	0.016	0.116	< .0001	< .0001	< .0001			
Diet*muscle	P value	0.253	0.077	0.064	0.225	0.547	0.145	< .0001	0.001			
Diet*day	P value	0.491	0.748	0.428	0.932	0.109	0.971	< .0001	0.004			
Muscle*day	P value	0.639	0.057	0.918	0.245	0.136	0.015	< .0001	< .0001			
Diet*muscle*day	P value	0.749	0.789	0.422	0.451	0.203	0.985	0.017	0.005			

^{a,b}, means with different superscripts along the same column differ significantly ($p < 0.05$)

¹basal diet without additive; M-0.1, basal diet + 0.1% *Morinda lucida* leaf powder; M-0.2, basal diet + 0.2% *Morinda lucida* leaf powder; basal diet + 0.02% Butylated hydroxyanisole

[†]Each dietary group was replicated six times and each replicate consisted of five birds

SEM, standard error of mean, PC, positive control

did the thigh muscle. This finding may be due to the differences in pH between the muscles. The reduction in muscle pH resulting from postmortem glycolysis decreased the electrostatic forces that aid the maintenance of myofibril spacing, which reduces the space between the thick and thin filaments thereby causing loss of water (Huff-Lonergan and Lonergan 2005). Drip loss on day 1 was higher ($p = 0.016$) than that on day 5. However, drip loss on day 3 was not different from that of day 1 and day 5. The changes in muscle pH during chill storage may explain this observation.

The color of meat affects meat-purchasing decisions by consumers and it is thus a useful criterion for meat quality

assessments (Sabow et al. 2016). Meat from the supplemented birds had higher ($p = 0.021$) redness than did the meat of the NC birds (Table 5). Diets had no effect on the lightness ($p = 0.317$) and yellowness ($p = 0.067$) of broiler meat. The higher redness in the meat of the supplemented birds could be due to the higher phenolic contents in the meat, which prevented the oxidation of myoglobin. Moreover, the improved meat redness could be attributed to the lower drip loss because myoglobin is soluble in water and could be lost during drip loss or purge (Barbut et al. 2008). This observation is consistent with the finding of Abbood et al. (2017), who reported that the supplementation of *Borreria latifolia* enhanced meat redness in

Table 6 Details of interaction between dietary antioxidants, muscle type and chill storage on the oxidative stability of broiler meat

Factor			Item [†]	
Diet ¹	Muscle type	Chill storage (d)	TBARS (nmol MDA/ mg protein)	Carbonyl (μmol/mg protein)
NC	Breast	0	0.05 ^{hi}	0.41 ^{cdefg}
NC	Breast	3	0.05 ^{hi}	0.54 ^{cde}
NC	Breast	5	0.46 ^{fg}	1.53 ^a
NC	Thigh	0	0.66 ^{def}	0.18 ^{fghi}
NC	Thigh	3	1.21 ^{bc}	0.23 ^{fghi}
NC	Thigh	5	1.97 ^a	0.33 ^{defghi}
M-0.1	Breast	0	0.02 ⁱ	0.37 ^{cdefgh}
M-0.1	Breast	3	0.04 ⁱ	0.66 ^c
M-0.1	Breast	5	0.18 ^{ghi}	1.00 ^b
M-0.1	Thigh	0	0.38 ^{fgh}	0.05 ⁱ
M-0.1	Thigh	3	0.70 ^{def}	0.09 ^{hi}
M-0.1	Thigh	5	0.97 ^{bcd}	0.17 ^{fghi}
M-0.2	Breast	0	0.02 ⁱ	0.23 ^{fghi}
M-0.2	Breast	3	0.07 ^{hi}	0.23 ^{fghi}
M-0.2	Breast	5	0.20 ^{ghi}	0.97 ^b
M-0.2	Thigh	0	0.12 ^{hi}	0.04 ⁱ
M-0.2	Thigh	3	0.46 ^{fg}	0.11 ^{hi}
M-0.2	Thigh	5	0.70 ^{def}	0.18 ^{fghi}
PC	Breast	0	0.02 ⁱ	0.43 ^{cdef}
PC	Breast	3	0.04 ⁱ	0.56 ^{cd}
PC	Breast	5	0.22 ^{ghi}	1.20 ^b
PC	Thigh	0	0.57 ^{ef}	0.04 ⁱ
PC	Thigh	3	0.89 ^{cde}	0.15 ^{fghi}
PC	Thigh	5	1.26 ^b	0.22 ^{fghi}

a, b, c, d, e, f, g, h, i means with different superscripts along the same column differ significantly ($p < 0.05$)

¹basal diet without additive; M-0.1, basal diet + 0.1% *Morinda lucida* leaf powder; M-0.2, basal diet + 0.2% *Morinda lucida* leaf powder; basal diet + 0.02% Butylated hydroxyanisole

[†]Each dietary group was replicated six times and each replicate consisted of five birds

SEM standard error of mean, NC negative control, PC positive control

chickens. Conversely, dietary herb extract mix had no effect on meat redness in broiler chickens (Jang et al. 2008). The thigh muscle had higher ($p < 0.0001$) redness than did the breast muscle. Thigh muscles consist majorly of type I fiber type, which is rich in myoglobin and whose metabolism is primarily oxidative (Barbut et al. 2008; Ismail and Joo 2017). Neither muscle type nor chill storage affected ($p > 0.05$) the lightness and yellowness of meat. However, the value of redness on d 0 (5 h postmortem) was higher ($p = 0.026$) than that of days 3 and 5 postmortem. The decrease in meat redness over storage suggests an increase in myoglobin oxidation or an increase in the concentration of metmyoglobin. The increase in drip loss over storage could also be responsible for the loss of water-soluble myoglobin and other pigments.

3.4. Oxidative stability of broiler meat

The indicators of oxidative status in different muscles of broiler chickens fed diet supplemented with different antioxidants are presented in Table 5. Carbonyl content and TBARS values were higher ($p < 0.0001$) in the meat of the NC birds compared with the meat of birds fed antioxidants. The higher total phenolic contents in the meats of the supplemented birds may be responsible for the lower carbonyl content and TBARS values. *Morinda lucida* leaf contains tannin, flavonoids, phenol, and other secondary metabolites whose antioxidant properties have been espoused (Adeyemi et al. 2014; Osuntokun et al. 2016). Phenolic compounds in medicinal plants exert their antioxidant properties by scavenging free radicals, chelating metal ions, and reacting with other antioxidants (Moran

et al. 1997; van Acker et al. 1996). The current observation agrees with the findings of Loetscher et al. (2013), who reported a lower TBARS value in breast meat of broiler chickens fed diet supplemented with *Rosa canina*, *Rosmarinus officinalis*, *Urtica dioica*, and *Aronia melanocarpa*. Moreover, dietary supplementation of *Ribes nigrum* extract reduced TBARS value in broiler meat (Sierżant et al. 2018). The pattern of antioxidant potential of MLLP and BHA differs significantly. The MLLP meat had lower TBARS value than did the BHA meat. Conversely, the BHA meat had lower carbonyl content than did the MLLP meat. The discrepancies in the antioxidant potential of the supplements may be indicative of the differences in the amounts and nature of their phytochemical contents.

The thigh muscle had higher ($p < 0.0001$) TBARS value than did the breast muscles. This observation may be due to the higher fat and myoglobin contents of the thigh muscle. The heme pigment in myoglobin produces H_2O_2 during the autoxidation of oxymyoglobin (Min et al. 2008). The H_2O_2 is capable of reacting with metmyoglobin to form ferric myoglobin, which can initiate lipid oxidation (Min et al. 2008). The higher carbonyl content in the breast muscle could be attributed to the low pH-induced degradation of myofibrillar proteins during ageing. The increase in carbonyl content and TBARS over ageing is consistent with the reports of previous findings in broiler meat (Lu et al. 2014).

There was a significant interaction between diet, muscle type and chill storage on the carbonyl content ($p = 0.005$) and TBARS value ($p = 0.017$) of broiler meat and the details are presented in Table 6. On day 0, the TBARS value of the thigh and breast muscles did not differ ($p > 0.05$) among the treatments. On day 3, the TBARS value of the breast muscle was not significantly different among the treatments. On day 5, the TBARS value of the M-0.2 breast meat was lower ($p = 0.017$) than that of the NC breast meat. On days 3 and 5, the TBARS value of the thigh muscle was higher ($p = 0.017$) than that of the breast muscle for all the dietary treatments. On day 3, the TBARS value of the thigh muscle of the NC and PC birds was not different but was higher ($p = 0.017$) than that of M-0.2 and M-0.1 birds. The TBARS value of the thigh muscle of the NC birds was higher ($p = 0.017$) than that of the supplemented birds on day 5 postmortem. On days 0 and 3, the carbonyl content of the breast muscle was not significantly different among the diets. However, the NC breast meat had higher ($p = 0.005$) carbonyl content than the breast meat of the supplemented birds on day 5 postmortem. In all dietary treatments, the carbonyl content of the thigh muscle did not change over the 5 d chill storage. These results suggest that the antioxidant potential of MLLP and BHA was muscle dependent. The ability of the antioxidant supplements to reduce lipid oxidation was more potent in

the breast muscle than in the thigh muscle. Contrarily, the potential of the antioxidant treatments to reduce protein oxidation was more pronounced in the thigh muscle than in the breast muscle.

Conclusion

The antioxidant effect of *Morinda lucida* leaf powder in the diet of broiler chickens was similar to that of BHA in enhancing meat redness and decreasing drip loss. The antioxidant potential of MLLP and BHA was muscle-dependent. The breast muscle was readily susceptible to protein oxidation than did the thigh muscle. Conversely, the thigh muscle was more liable to lipid oxidation than did the breast muscle. The supplementation of 0.2% MLLP seems to be a potential alternative for BHA in the diet of broiler chickens. Further research to examine the antioxidant potential of MLLP in birds fed oxidized diet is suggested. The mechanisms by which MLLP reduced oxidative spoilage in muscle-foods deserve further investigation.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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