

Full Length Research Paper

# Effect of dietary mixtures of moringa (*Moringa oleifera*) leaves, broiler finisher and crushed maize on anti-oxidative potential and physico-chemical characteristics of breast meat from broilers

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Accepted 10 October, 2012

This study was carried out to determine the effects of dietary mixtures of moringa (*Moringa oleifera*) leaves and broiler finisher (M-BF) [moringa leaves, broiler finisher and crushed maize (M-BF-CM); broiler finisher and crushed maize (BF-CM); and broiler finisher (BF)] on antioxidative potential and physico-chemical characteristics of breast meat from broilers. Antioxidant activity (AA), ultimate pH (pH<sub>u</sub>), lightness (L\*), redness (a\*), yellowness (b\*), Warner-Bratzler shear force (WBSF) and cooking loss (CL) were determined in breast meat samples from each group. The AA of the extract was evaluated using ferric reducing power and the radical scavenging activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic-acid) (ABTS). Total phenols, flavonoids and proanthocyanidins contents of breast meat were also determined. Similarly, the *in vivo* antioxidant activity of the extract was evaluated in meat by determining the activity of glutathione (GSH), catalase (CAT), superoxide dismutase (SOD) and lipid peroxidation. Moringa fed samples exhibited the highest phenolics (15.5 ± 0.22 mg/g tannic acid equivalent) and flavonoids (29.9 ± 0.32 mg/g tannic acid equivalent) content. There were no significant differences (P > 0.05) observed on the broiler slaughter weight (SLW), pH<sub>u</sub>, CL, a\* and b\* in all the meat samples. The highest carcass weight (CW), and L\* values were observed in breast samples of M-BF-CM and M-BF, respectively. These findings suggest that moringa supplementation could result in free radicals inhibition, thus enhancing the oxidative stability of meat without having effects on the physico-chemical characteristics of meat.

**Key words:** Moringa, broiler meat quality, polyphenols, lipid oxidation, enzymes.

## INTRODUCTION

Poultry meat and its products have a vast consumer market and are making a significant contribution to the supply of quality protein, vitamins and minerals

(Mothershaw et al., 2009). Chicken accounts for more than 90% of the total poultry population of the world (Biswas et al., 2011). The major parameters considered in the assessment of meat quality are appearance, juiciness, tenderness and flavour (Lawrie and Ledward, 2006). The presence of adipose tissue as marbling fat between muscle fibre bundles can weaken the structure so that it is broken down more easily during chewing. Thus, marbling increases juiciness, tenderness, and

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**Table 1.** Composition (g/kg) and calculated chemical composition (g/kg) of the control diet (broiler finisher).

Ingredient	Inclusion (g/kg)
White maize	706
Soyabean meal	174
Sunflower cake	63
Full fat soya	27
Limestone	17.8
Methionine	0.6
Lysine	1.1
Mono calcium phosphate	6.5
Salt	1.5
Premix*	2.5
Total	1000
<b>Calculated chemical composition</b>	
Crude protein	162
Calcium	11
Phosphate (non phytate)	4.6
Metabolizable energy (kcal/kg)	3100.00

\*Vitamin/trace mineral premix/kg diet: Vitamin A ,12 000 IU; vitamin D3 , 1 500 IU; vitamin E, 50 mg; vitamin K3, 5 mg; vitamin B1, 3 mg; vitamin B2, 6 mg; vitamin B6, 5 mg; vitamin B12, 0.03 mg; niacin, 25 mg; Ca-D-pantothenate ,12 mg; folic acid ,1 mg; D-biotin, 0.05 mg; apocarotenoic acid ester, 2.5 mg; choline chloride, 400 mg; Mn, 80 mg; Fe, 60 mg; Zn, 60 mg; Cu, 5 mg; Co, 0.20 mg; I - 1 mg; Se, 0.15 mg.

flavour of the meat (Muchenje et al., 2008a, 2009a,b). At buying point, appearance is the major parameter that influences purchase, selection and initial evaluation of meat quality. These desirable meat parameters tend to be negatively affected by lipid peroxidation.

Besides health concerns to the consumer, lipid peroxidation is also a major cause of meat quality deterioration, affecting colour, flavour, texture and nutritional value (Giannenas et al., 2009; Botsoglou et al., 2010). Antioxidants have been reported to be efficient in diminishing lipid oxidation of meat. However, the use of natural antioxidants to stabilize meat has gained much attention from consumers because they are considered to be safer than synthetic antioxidants (Jung et al., 2010) such as butylated hydroxytoluene (BHT) and tertiary butyl hydroquinone (TBHQ). Natural antioxidants also have the ability to increase the antioxidant capacity of the plasma and reduce the risk of certain diseases such as cancer, stroke and cardiovascular diseases (Chanda and Dave, 2009). It has also been reported that these natural antioxidants, especially of plant source, have greater application potential for consumer's acceptability, palatability, stability and shelf-life of meat products (Jung et al., 2010). One such plant with a potential to be used as an antioxidant is moringa (*Moringa oleifera*).

*M. oleifera* (family: Moringaceae) is native to sub-Himalayan regions of North West India. It is commonly known as horse radish tree, drumstick tree and highly recognised for its nutritional and medicinal properties with some useful minerals, vitamins and amino acids. It is the most economically important species of its kind followed

by *M. stenopetala*. The leaves of the tree have been reported to bear antioxidant activity/contain a higher amount of polyphenols (Sreelatha and Padma, 2009; Verma et al., 2009; Qwele et al., 2011). The polyphenolics content of the leaves are high, comparable to vegetables and fruits of strawberries, hot pepper, carrot and soybean which are high in phenolics, ascorbate, carotene and  $\alpha$ -tocopherol, respectively (Yang et al., 2006).

A study conducted by Kakengi et al. (2007) revealed high pepsin and total soluble protein in *M. oleifera* leaf meal (MOLM). The high pepsin and total soluble protein makes MOLM more suitable to monogastric animals such as poultry. On the other hand, broiler finisher and crushed maize have been used for many years both commercially and communally to supply essential nutrients for growth and to reach maximum weight gain with lowest feed conversion ratio. According to Moyo et al. (2011, 2012b) moringa leaves have nutritional and antimicrobial properties; there is a need to investigate alternative feed. However, there are no reports on the effect and antioxidative potential of dietary mixtures of moringa leaves with feeds such as broiler finisher and crushed maize on the quality of meat from broilers. Therefore, the current study was designed to determine the effects of dietary mixtures of moringa leaves, broiler finisher and crushed maize on antioxidative potential and the physico-chemical characteristics of breast meat from broilers.

## MATERIALS AND METHODS

### Feeding management of broilers

Thirty-two (32) day-old commercial male broilers were purchased from an agricultural Co-operation located in Berlin, South Africa. The birds were randomly assigned to four groups of dietary supplementation with each group having six birds. All the groups were fed the commercial broiler starter mesh for the first 28 days. However, after 28 days, the diets were gradually changed. The diets were formulated to be isonitrogenous. The first group of the birds were 100% fed broiler finisher (control); the second group, 80% broiler finisher and 20% crushed maize; the third group, 5% moringa and 95% broiler finisher and the birds from the fourth group were supplemented with 5% moringa, 80% broiler finisher and 15% crushed maize. Tables 1 and 2 present the composition and calculated chemical composition of the control diet (broiler finisher); and the percentage composition of broiler finisher, crushed maize and moringa leaves, respectively. All groups of birds were housed in a rearing house under the same management and environmental conditions. The birds were supplied *ad libitum* feed and water for four weeks (until slaughter).

Slaughter was done by decapitating the bird's heads from the neck using a sharp knife. The slaughter and carcass weights were measured using a digital scale. After slaughter, fresh breast samples were used. Meat samples were stored at 4°C for 24 h before assays for antioxidant activity were performed. 10 g of each meat sample was homogenized. A volume of 10% w/v homogenate was prepared in 0.05 M phosphate buffer (pH 7) and centrifuged at 12,000 × g for 60 min at 4°C. The supernatant obtained was used for the estimation of total polyphenols, and Broiler physico-chemical meat quality characteristics were also determined.

**Table 2.** Composition (g/kg) of broiler finisher, crushed maize and *Moringa oleifera* leaves diet.

Treatment	Broiler finisher	Crushed maize	<i>Moringa oleifera</i>	Total
M-BF-CM	800	150	50	1000
BF-CM	800	200	00	1000
M-BF	950	00	50	1000
BF	1000	0	0	1000

BF-CM = Broiler finisher and crushed maize; M-BF = Moringa and broiler finisher; M-BF-CM = Moringa + broiler finisher and crushed maize.

### Phenol content determination

The total phenol content was determined by the Folin-Ciocalteu method (Subramanian et al., 1965). To 0.2 ml of the Folin-Ciocalteu reagent, 0.1 ml of the supernatant was added, followed by an addition of 3 ml sodium carbonate solution (5%). Total phenolics were determined after 1 h of incubation of the reaction mixture at 23°C. Absorbance was measured with a spectrophotometer (Hewlett Packard, UV/visible light) at 765 nm. The quantification of phenolics was based on the standard curve generated with the use of gallic acid and expressed as gallic acid equivalent.

### Total flavonoids

The method of Ordonez et al. (2006) was used to determine the total flavonoid contents in meat. A volume of 0.5 ml of 2% AlCl<sub>3</sub> ethanol solution was added to 0.5 ml of the supernatant. After 1 h of incubation at the room temperature, the absorbance was measured at 420 nm using UV-VIS spectrophotometer. A yellow colour indicated the presence of flavonoids. All determinations were done in triplicate and the total flavonoids content was calculated as quercetin (mg/g) using the following equation based on the calibration curve:  $Y = 0.0255x$ ,  $R^2 = 0.9812$ , where  $x$  was the absorbance and  $Y$  was the quercetin equivalent (mg/g).

### Total proanthocyanidins

Total proanthocyanidins was determined based on the procedure of Sun et al. (1998). A volume of 0.5 ml of 0.1 mg/ml of the supernatant was mixed with 3 ml of 4% vanillin-methanol solution and 1.5 ml of non diluted hydrochloric acid then the mixture was vortexed. The absorbance of resulting mixture was measured at 500 nm after 15 min at room temperature. Total proanthocyanidin content was expressed as catechin equivalents (mg/g) using the following equation from the calibration curve:

$$Y = 0.5825x, R^2 = 0.9277,$$

Where  $x$  was the absorbance and  $Y$  the catechin equivalent (mg/g).

### DPPH radical scavenging activity

DPPH radical scavenging activity was estimated according to the method of Blois (1958) with slight modifications. A 200  $\mu$ L of the supernatant was added to 800  $\mu$ L distilled water and 1 mL methanolic DPPH solution (0.2 mM). The mixture was vortexed and left to stand at room temperature (20 to 22°C) for 30 min. A tube containing 1 mL of distilled water and 1 mL of methanolic DPPH

solution (0.2 mM) was served as the control. The absorbance of the solution was measured at 517 nm using a spectrophotometer (Hewlett Packard, UV/visible light). The percentage inhibition of DPPH radical was obtained from the following equation:

$$\% \text{DPPH scavenging activity} = [1 - (\text{absorbance of sample} / \text{absorbance of control})] \times 100$$

### ABTS<sup>+</sup> reducing activity

The free radical scavenging activity was determined by ABTS radical cation decolorization assay described by Erel (2004). ABTS was dissolved in distilled water to a 7 mM concentration. The working solution was prepared by mixing two stock solutions of 7 mM ABTS and 2.4 mM potassium persulphate in equal amounts and allowed to react for 12 to 16 h at room temperature in the dark to allow the completion of radical generation. This solution was then diluted with ethanol so that its absorbance was adjusted to  $0.70 \pm 0.02$  at 734 nm. The diluted ABTS<sup>+</sup> solution (3 mL) were added to 20  $\mu$ L aqueous supernatant and the absorbance was measured by a spectrophotometer (Hewlett Packard, UV/visible light) at 734 nm, using ethanol as a blank. The percentage inhibition was calculated by the following equation:

$$\text{ABTS}^+ \text{ Scavenging activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

### Reducing power

The reducing power of the tissue was evaluated according to the method of Oyaizu (1986). A 200  $\mu$ L supernatant was mixed with 500  $\mu$ L sodium phosphate buffer (0.2 M, pH 6.6) and 500  $\mu$ L potassium ferricyanide (1%), and the resultant mixture was incubated at 50°C for 20 min. After addition of 2.5 mL trichloroacetic acid [TCA, (10%)], the mixture was centrifuged (Hanil) at 2200  $\times$  g for 10 min. The upper layer (500  $\mu$ L) was mixed with 500  $\mu$ L distilled water and 100  $\mu$ L ferric chloride (0.1%), and the absorbance was measured at 700 nm using UV/visible spectrophotometer. Increased absorbance of the reaction mixture indicated higher reducing power of the tissue extract.

### Lipid peroxidation assay

Lipid oxidation was measured as increases in thiobarbituric acid-reactive substances (TBARS). Briefly, an egg-yolk homogenate was used as an egg rich media. A volume of 0.5 mL of egg yolk prepared in distilled water (10% v/v) and 0.1 mL of extract were mixed in a test tube and the volume was made up to 1 mL, by

adding distilled water. Then, 0.05 ml of FeSO<sub>4</sub> (0.07 M) was added to the above mixture and incubated for 30 min to induce lipid peroxidation. Finally, 1.5 mL of 20% acetic acid (pH adjusted to 3.5 with NaOH) and 1.5 mL of 0.8% TBA (w/v) (prepared in 1.1% sodium dodecyl sulphate) and 0.05 mL 20% TCA was added. Tubes were heated (90°C) in a boiling water bath for 60 min then cooled. After cooling, 5.0 mL of 1-butanol was added to each tube and then centrifuged at 3000 × g for 10 min. Absorbance of the supernatant was measured at 532 nm with a spectrophotometer (Hewlett Packard, UV/visible light). Distilled water (0.1 mL) was used in place of the extract for the blank.

#### Glutathione (GSH) assay

The method of Akerboom and Sies (1981) was used to determine reduced Glutathione (GSH) in meat. Preparation of glutathione standard solutions was done by serial dilutions of the 50 μM glutathione solution according to the manufacture instructions. The fresh meat was first deproteinized with the 5% 5-sulfosalicylic acid solution, centrifuged to remove the precipitated protein. The first 2 wells contained 10 μL of the 5% 5-sulfosalicylic acid solution as a reagent blank, followed by adding 10 μL duplicate samples of the prepared glutathione standard solutions. Later, varying volumes of the unknown sample were added into separate wells up to 10 μL sample to desire concentrations (2, 4, 6, 8 and 10 μL). A 150 μL of the working mixture was added, which comprises of 8 mL of 1× assay buffer added to 228 μL of the diluted enzyme solution (6 unit/mL) and 228 μL of DTNB stock solution (1.5 mg/mL) to each well and resuspended. After incubation for 5 min at room temperature, 50 μL of the diluted NADPH solution was added and then mixed to generate yellow colour. The value reagent blank was subtracted from measurement. The values of the glutathione standard solutions were used to determine the standard curve and the ΔA412/min equivalent was calculated to 1 nmole of reduced glutathione per well. The nmoles of GSH in the unknown sample was calculated as follows:

$$\text{nmoles GSH per ml of sample} = \frac{\Delta A_{412}/\text{min} (\text{sample}) \times \text{dil}}{\Delta A_{412}/\text{min} (1 \text{ nmole}) \times \text{vol}}$$

Where, ΔA412/min (sample) = slope generated by sample (after subtracting the values generated by the blank reaction), ΔA412/min (1 nmole) = slope calculated from standard curve for 1 nmole of GSH, dil = dilution factor of original sample, vol = volume of sample in the reaction in mL.

#### Catalase (CAT) activity

The method of Deisseroth and Dounce (1970) was used to determine the catalase (CAT) activity in meat. For the hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) standard curve, the reaction was initiated by 0, 125, 250, 500, and 750 μL of 10 mM H<sub>2</sub>O<sub>2</sub> solution into micro-centrifuge tubes. To this reaction was added 1× assay buffer to a final volume of 1.0 mL and mixed by inversion. The assay system consisted of 10 μL aliquot of each solution being transferred to a second tube and 1 mL of the colour reagent was added to each tubes. After 15 mins, the absorbance was read at 520 nm. A standard curve of the absorbance was plotted at 520 nm versus the final amount of H<sub>2</sub>O<sub>2</sub> in the reaction mixture. For catalase colorimetric enzymatic reaction, the assay reaction was performed at room temperature (25°C). The 1× assay buffer, colorimetric assay substrate solution (200 mM H<sub>2</sub>O<sub>2</sub>), and colour reagent were allowed to equilibrate to room temperature. A volume of 20 μL of the aliquot and 55 μL of 1× assay buffer was added to the micro-centrifuge tube. The reaction was started by addition of 25 mL of the colorimetric assay substrate solution, mixed by inversion and

the colorimetric assay substrate solution, mixed by inversion and then incubated for 5 mins followed by adding 0.9 mL of the stop solution and the tube was then inverted. A 10 mL aliquot of the catalase enzymatic reaction mixture was added to another micro-centrifuge tube and 1 mL of the colour reagent was added. The mixture was left for 15 mins at room temperature for colour development and the absorbance was measured at 520 nm.

#### Superoxide dismutase (SOD) assay

The assay mixture contained 1 mL of working solution (WST) diluted with 19 mL of buffer solution, centrifuged enzyme solution tube for 5 s/10,000 × g, then mixed by pipeting and 15 μL of enzyme solution was diluted with 2.5 mL of dilution buffer, SOD solution, where SOD is diluted with dilution buffer to prepare SOD standard solution were 200, 100, 50, 20, 10, 5, 1, 0.1, 0.05, 0.01, and 0.001 U/mL, respectively. To each sample and blank 2, was added 20 μL of ddH<sub>2</sub>O (double distilled water) as well as blank 1 and blank 3. A volume of 200 mL of WST was added to each well and mixed. To blank 2 and 3 was added 20 mL of dilution buffer. Then, 20 mL of enzyme working solution was added to each sample and blank 1 well, and then mixed thoroughly. After incubation at 37°C for 20 min, the absorbance was read at 450 nm using a microplate reader. The SOD activity (inhibition rate %) was calculated using the following equation:

$$\text{SOD activity (inhibition rate \%)} = \frac{\{(A_{\text{blank 1}} - A_{\text{blank 3}}) - (A_{\text{sample}} - A_{\text{blank 2}})\}}{(A_{\text{blank 1}} - A_{\text{blank 3}})} \times 100$$

#### Physico-chemical meat quality characteristics

##### Ultimate pH of breast meat

The *post-mortem* ultimate pH (pH<sub>u</sub>) was determined on the breast muscle of each bird using a digital pH meter with a piercing electrode. It was measured 24 h *post mortem*. The measurement was carried out using a portable pH meter (CRISON pH25, CRISON Instruments SA, Spain). The pH meter was calibrated using pH 4, 7 and 9 standard solutions (CRISON Instruments, SA, Spain) before each day's measurement.

##### Colour determination

The colour of meat (L\* = Lightness, a\* = Redness and b\* = Yellowness) was determined on the breast 24 h after slaughter using a colour-guide 45/0 BYK-Gardener GmbH machine, with a 20 mm diameter measurement area and illuminant D65-day light, 10° standard observer. Three readings were taken by rotating the Colour Guide 90° between each measurement, in order to obtain a representative average value of the colour. The guide was calibrated before each day's measurements using the green standard.

##### Warner Bratzler shear force (WBSF)

Triplicate 10 mm thick sub samples were cut from the centre of each breast muscle after measurement of cooking loss. The samples were sheared perpendicular to the fibre direction rather than across using a Warner Bratzler (WB) shear device mounted on an Instron (Model 3344) Universal testing apparatus (cross head speed at 400 mm/min, one shear in the centre of each core).

##### Cooking loss

Cooking loss (CL) was determined using breast meat measuring 5

**Table 3.** Total polyphenolic contents of the leaf extracts of *M. oleifera*.

Solvent extract	Phenolic (mg TE/g)	Flavonoid (mg QE/g)	Flavonol (mg QE/g)	Proanthocyanidin (mg CE/g)
Acetone <i>M. oleifera</i> leaves	120.33±0.76 <sup>b</sup>	295.01±1.99 <sup>b</sup>	132.74±0.83 <sup>b</sup>	32.59±0.50 <sup>b</sup>
Aqueous <i>M. oleifera</i> leaves	40.27±0.99 <sup>a</sup>	45.1±0.47 <sup>a</sup>	18.10±0.18 <sup>a</sup>	16.91±0.87 <sup>a</sup>

Values are standard errors of mean (n=6). <sup>a,b</sup>Means superscript with different letters in the same column differ significantly (P < 0.05). QE = quercetin equivalent; TE = tannic acid equivalent; CE = catechin equivalent.

**Table 4.** Polyphenol contents (mg gallic acid equivalent/g meat ± standard error) of breast meat from broilers fed with broiler finisher, mixture of broiler finisher and crushed maize, *Moringa oleifera* leaves and broiler finisher, mixture of moringa leaves, broiler finisher and crushed maize.

Treatment	Phenol	Flavonoid	Proanthocyanidin
BF (n=6)	12.0±0.12 <sup>a</sup>	23.9±0.28 <sup>a</sup>	0.5±0.01 <sup>b</sup>
BF-CM (n=6)	11.1±0.15 <sup>a</sup>	23.7±0.12 <sup>a</sup>	0.4±0.02 <sup>a</sup>
M-BF (n=6)	14.1±0.36 <sup>b</sup>	26.7±0.38 <sup>b</sup>	0.5±0.02 <sup>b</sup>
M-BF-CM (n=6)	15.5±0.22 <sup>c</sup>	29.9±0.32 <sup>c</sup>	0.4±0.01 <sup>a</sup>

Values are standard errors of mean (n=6). <sup>abc</sup>Means with different letters within the same column significantly differ at p < 0.05. Phenols are expressed as mg tannic acid/g of meat. Flavonoids are expressed as mg quercetin/g of meat. Proanthocyanidins are expressed as mg quercetin/g of meat. BF = broiler finisher; BF-CM = broiler finisher and crushed maize; M-BF = moringa and broiler finisher; M-BF-CM = moringa + broiler finisher and crushed maize.

× 4 cm long. The samples weighing 170 g on average were placed inside plastic bags in a water bath and cooked at 70°C for 90 min. Samples were cooled at room temperature and reweighed. Cooking loss (%) was calculated as follows:

$$\text{Cooking loss (\%)} = \frac{\text{Weight before cooked} - \text{weight after cooked} \times 100}{\text{Weight before cooked}}$$

### Statistical analysis

The PROC GLM procedure of SAS (2003) was used to analyse *in vivo* and *in vitro* antioxidant (AA) parameters, the effect of diet on slaughter weight, carcass weight, pH<sub>u</sub>, L\*, a\*, b\*, WBSF values and cooking loss. Comparisons of means were analyzed using the Turkey's HSD procedure in SAS (2003). The model used was:

$$Y_{ij} = \mu + \alpha_i + e_{ij}$$

Where, Y<sub>ij</sub> = AA variable, slaughter weight, carcass weight, ultimate, pH, L\*, a\*, b\*, WBSF values and cooking loss, μ is the overall mean, α<sub>i</sub> is the effect of dietary supplementation, e<sub>ij</sub> is the random error.

In the model, it was assumed that the data was normally distributed, with sum of errors equal to zero (0) and variances were identical (Iσ<sup>2</sup>).

## RESULTS AND DISCUSSION

### Total polyphenols

Table 3 presents the polyphenolic contents of the leaf

extracts of *M. oleifera*. The total phenolic contents in breast meat from broilers fed with BF, BF-CM, M-BF and M-BF-CM are presented in Table 4. The phenols compound were highest (P < 0.05) in breast meat of broilers fed on M-BF-CM followed by those fed on M-BF, BF and BF-CM. The flavonoids content was highest in breast meat of broilers fed on M-BF-CM followed by those fed on M-BF, BF and BF-CM, respectively. The breast meat from broilers fed on M-BF had the highest concentration of proanthocyanidins followed by those fed on BF, BF-CM and M-BF-CM. Findings in this study corroborated with other reports on the leaves of moringa, which suggest that moringa can be used as an antioxidant to scavenge free radicals (Qwele et al., 2011) in order to prevent chronic, arterial and cardiovascular diseases. Sreelatha and Padma (2009) also observed higher levels of total phenolics and total flavonoids in antioxidant activity of *M. oleifera* leaves in two stages of maturity. Similarly, higher amount of phenols were observed in antioxidant properties of different fractions of *M. oleifera* leaves (Verma et al., 2009).

Among phytochemicals, flavonoids and phenols have been demonstrated to have significant antioxidant activity (Sreelatha and Padma, 2009). Phenols are well known to have anti-inflammatory activities (Ezeamuzle et al., 1996; Jyotsna Mishra et al., 2007) and prevent oxidative damage caused by reactive oxygen species (ROS) in tissues, DNA (Chanda and Dave, 2009) RNA, enzymes and proteins (Jyotsna Mishra et al., 2007). Flavonoids have also been reported to scavenge free radicals and

**Table 5.** Antioxidative potential of breast meat from broilers fed with broiler finisher, mixture of broiler finisher and crushed maize, *Moringa oleifera* leaves and broiler finisher, mixture of moringa leaves, broiler finisher and crushed maize.

Treatment	DPPH (%)	ABTS (%)	Reducing power	% of LOI (mg/ml)
BF (n=6)	66.4±0.45 <sup>c</sup>	43.9±0.67 <sup>b</sup>	0.6±0.01 <sup>a</sup>	0.3±0.01 <sup>c</sup>
BF-CM (n=6)	68.3±0.45 <sup>d</sup>	27.9±0.67 <sup>a</sup>	0.6±0.01 <sup>a</sup>	0.2±0.01 <sup>b</sup>
M-BF (n=6)	57.2±0.45 <sup>a</sup>	68.6±0.67 <sup>d</sup>	0.7±0.01 <sup>b</sup>	0.1±0.01 <sup>a</sup>
M-BF-CM (n=6)	62.3±0.45 <sup>b</sup>	52.4±0.67 <sup>c</sup>	0.7±0.01 <sup>b</sup>	0.1±0.01 <sup>a</sup>

Values are standard errors of mean (n=6). <sup>abcd</sup>Means within the same column with different superscripts differ significantly at P < 0.05. BF = broiler finisher; BF-CM = broiler finisher and crushed maize; M-BF = moringa and broiler finisher; M-BF-CM = moringa + broiler finisher and crushed maize. DPPH, 1, 1 diphenylpicrylhydrazyl; ABTS, 2,2-azino-bis (3-ethylbenzothiazoline-6-sulphonic diammonium salt; LOI, lipid oxidation inhibition.

combat pathological disorders generated by ROS (Jyotsna Mishra et al., 2007).

### Antioxidative activity

The DPPH results are presented in Table 5. The DPPH was highest in BF-CM fed broilers, followed by BF then M-BF-CM and M-BF. Even though breast samples that were supplemented with moringa leaves showed the lowest DPPH free radical scavenging activity, it did show proton-donating ability and could serve as free radical inhibitors or scavengers that can be used as antioxidants. The efficacies of antioxidants are often associated with their ability to scavenge stable free radicals of DPPH by donating electron to the unpaired valence electron at one atom of nitrogen bridge (Krishnaraju et al., 2009; Sharma and Bhat, 2008). The stable radical DPPH has been used widely for the determination of antioxidant activity. When it is mixed with antioxidants, it donates hydrogen atom to form a stable DPPH-H (2,2-diphenyl-1-picrylhydrazine). The degree of discoloration indicates the scavenging potential of the antioxidant extract, which is due to the hydrogen donating or radical scavenging ability (Adedapo et al., 2008).

All supplements showed effective scavenging activity against ABTS radical as shown in Table 5. Meat from the M-BF-fed broilers produced the highest percentage inhibition followed by M-BF-CM then BF and BF-CM, respectively. The high inhibitory concentration in breast samples of broilers supplemented with M-BF and M-BF-CM could be attributed to the presence of polyphenolics compounds in moringa leaves (Sreelatha and Padma, 2009). Even though DPPH and ABTS<sup>+</sup> are both radical scavenging assays, percentage inhibition will vary due to different methods of preparation. The DPPH radical is stable at formation whereas the ABTS<sup>+</sup> assay allows formation of a radical which remains stable for several days due to the optimised pH (Cano et al., 1998), hence lower antioxidant scavenging activity. Wootton-Beard et al. (2011) reported a weak correlation between DPPH and ABTS<sup>+</sup> assays.

The reducing power of meat from M-BF-CM and M-BF-fed broilers was significantly (P < 0.05) higher than those fed on BF and BF-CM (Table 5). The presence of antioxidants in meat causes the reduction of Fe<sup>3+</sup>/Ferric cyanide complex to the ferrous form (Fe<sup>2+</sup>) (Chung et al., 2002). Higher absorbance exhibited in meat from M-BF-CM and M-BF-fed broilers is due to the high polyphenolic content in moringa leaves. Results of scavenging activity also suggest the ability of moringa supplement to minimize oxidative damage to some vital tissues in the body (Kojic et al., 1998; Weighand et al., 1999). Additionally, it has been reported that the reducing power of bioactive compounds is directly related to its antioxidant activity (Iqbal and Bhanger, 2006).

In the present investigation, levels of lipid peroxides in breast meat of broilers supplemented with BF were significantly high, followed by those supplemented with BF-CM then M-BF and M-BF-CM (Table 5). The decrease in lipid peroxidation level in breast meat indicates the role of *M. oleifera* leaves as an antioxidant, where meat from M-BF and M-BF-CM-fed showed higher inhibition than BF and BF-CM (Sreelatha and Padma, 2009). This is in agreement with the study conducted by Kumar and Pari (2003), who observed that *M. oleifera* inhibited lipid peroxidation against anti-tubercular drugs induced lipid peroxidation in rats. Previous studies have shown that the negative outcome of lipid oxidation in chicken meat and eggs was diminished by the use of diets containing antioxidants such as medicinal herb mix (Jung et al., 2010).

### Glutathione and enzymatic antioxidants

The levels of tissue glutathione in breast were significantly higher (P < 0.05) in breast meat from broilers supplemented with M-BF-CM followed by M-BF, BF-CM and BF (Table 6). Thus, moringa have demonstrated to have a protective role against oxidative damage and can be used as an antioxidant to inhibit tissue injury. Glutathione is a non-protein in thiol cells in a living organism which is responsible for cellular oxygen defence

**Table 6.** Activities of glutathione and enzymatic antioxidants of breast meat from broiler fed with broiler finisher, mixture of broiler finisher and crushed maize, *Moringa oleifera* leaves and broiler finisher, mixture of *Moringa* leaves, broiler finisher and crushed maize.

Treatment	GSH (nm/ml)	CAT ( $\mu\text{m}/\text{min}/\text{ml}$ )	SOD (%)
BF (n=6)	97.0 $\pm$ 0.77 <sup>a</sup>	0.07 $\pm$ 0.01 <sup>a</sup>	70.3 $\pm$ 0.55 <sup>a</sup>
BF-CM (n=6)	99.0 $\pm$ 0.77 <sup>b</sup>	0.07 $\pm$ 0.01 <sup>a</sup>	73.0 $\pm$ 0.55 <sup>b</sup>
M-BF (n=6)	110.0 $\pm$ 0.77 <sup>c</sup>	0.08 $\pm$ 0.01 <sup>ab</sup>	75.0 $\pm$ 0.55 <sup>c</sup>
M-BF-CM (n=6)	121.0 $\pm$ 0.77 <sup>d</sup>	0.09 $\pm$ 0.01 <sup>b</sup>	79.0 $\pm$ 0.55 <sup>d</sup>

Values are standard errors of mean (n=6). <sup>abcd</sup>Means within the same column with different superscripts differ significantly at  $P < 0.05$ . BF = broiler finisher; BF-CM = broiler finisher and crushed maize; M-BF = moringa and broiler finisher; M-BF-CM = moringa + broiler finisher and crushed maize; GSH, glutathione; CAT, catalase; SOD, superoxide dismutase.

**Table 7.** Meat quality characteristics, slaughter weight and carcass weight of broilers fed with broiler finisher, mixture of broiler finisher and crushed maize, *Moringa oleifera* leaves and broiler finisher, mixture of moringa leaves, broiler finisher and crushed maize.

Parameter	BF	BF-CM	M-BF	M-BF-CM	SEM
SLW (kg)	4.0 <sup>a</sup>	4.7 <sup>a</sup>	4.7 <sup>a</sup>	4.8 <sup>a</sup>	0.31
CW (kg)	2.9 <sup>a</sup>	3.5 <sup>b</sup>	3.12 <sup>b</sup>	3.6 <sup>b</sup>	0.38
CL (%)	15.4 <sup>b</sup>	14.3 <sup>b</sup>	12.9 <sup>a</sup>	12.3 <sup>a</sup>	2.21
L <sub>24</sub> *	49.4 <sup>ab</sup>	48.9 <sup>ab</sup>	47.5 <sup>a</sup>	51.6 <sup>b</sup>	5.93
a <sub>24</sub> *	4.2 <sup>a</sup>	3.8 <sup>a</sup>	4.01 <sup>a</sup>	3.8 <sup>a</sup>	1.58
b <sub>24</sub> *	18.3 <sup>a</sup>	17.6 <sup>a</sup>	16.5 <sup>a</sup>	17.8 <sup>a</sup>	6.12
WSBF (N)	14.9 <sup>a</sup>	11.7 <sup>a</sup>	13.3 <sup>a</sup>	16.8 <sup>a</sup>	3.03
pH <sub>24</sub>	5.7 <sup>a</sup>	5.8 <sup>a</sup>	5.9 <sup>a</sup>	5.9 <sup>a</sup>	1.44

Values are standard errors of mean (n=6). <sup>ab</sup>Means in the same row with different superscript are significantly different ( $P < 0.05$ ). BF = broiler finisher; BF-CM = broiler finisher and crushed maize; M-BF = moringa and broiler finisher; M-BF-CM = moringa + broiler finisher and crushed maize; SLW = slaughter weight; CW = carcass weight; CL = cooking loss; L\* = lightness; a\* = redness; b\* = yellowness; WBF = Warner Bratzler shear force value.

(Jyotsna et al., 2007). Administration of thiol compounds such as glutathione, cysteine and methionine have been shown to protect against oxidative stress in humans and animals (Krishnaraju et al., 2009).

Higher activity of catalase was observed in moringa supplemented broiler meat samples compared to BF and BF-CM (Table 6). These results indicate that M-BF and M-BF-CM is able to protect meat against lipid peroxidation and ROS due to antioxidant property of moringa. Catalase is an enzymatic antioxidant located in the mitochondria and cytosol of a living organism which is responsible for the removal of hydrogen peroxides (Jyotsna Mishra et al., 2007).

Table 6 also presents the effect of BF, BF-CM, M-BF and M-BF-CM on the activities of superoxide dismutase. Higher percentage inhibition of superoxide anion in meat from moringa supplemented broilers was observed, which implies an efficient protective mechanism of the plant. Superoxide anion is one of the main reactive oxygen species in the cell (Curtis and Mortiz, 1972), thus SOD would play a key antioxidant role. Although the fatty acid profiles and antioxidant activities of the moringa mixtures could not be determined in this study, addition of

moringa leaves in broiler diets increases antioxidant activities in broiler meat with the potential to improve its shelf life.

### Carcass characteristics

The effect of diet on SLW and CW are shown in Table 7. No significant differences ( $P > 0.05$ ) were observed in SLW from broilers, although there was a different ( $P < 0.05$ ) CW. The CW of broilers fed BF was significantly lower ( $P < 0.05$ ) than broilers fed BF-CM, M-BF and M-BF-CM. High CW from broilers supplemented with M-BF and M-BF-CM could be attributed to high amount of nutrients and antioxidants contained in moringa (Yang et al., 2006).

### Physico-chemical quality characteristics

The pH<sub>u</sub> values of breast samples are presented in Table 7. Dietary supplementation had no effect on pH<sub>u</sub>. Similar results were reported by Perlo et al. (2010) in broiler

breast filets. When animals are slaughtered, glycogen is broken down to glucose and glucose undergoes glycolysis. In the absence of oxygen, lactic acid is produced which is responsible for the drop of muscle pH such as drop aid in the conversion of muscle to meat (Muchenje et al., 2009c). Meat pH is a vital characteristic that influences the acceptability of meat. Higher meat pH results in lower L\* (lightness), implying that high meat pH is darker than normal meat pH (Zhang et al., 2005; Muchenje et al., 2008b).

Meat samples from broilers supplemented with M-BF-CM had the highest L\* values while no significant differences ( $P > 0.05$ ) were observed for a\*, b\*, WBSF and cooking loss in all meat samples (Table 7). High L\* values in meat are preferable because the lightness of broiler meat is more attractive and acceptable by consumers. Meat colour is usually associated with factors such as breed (Ekiz et al., 2010; Santos et al., 2007; Muchenje et al., 2008b, 2009a, c), slaughter weight (Martínez-Cerezo et al., 2005) production system and pH<sub>u</sub> (Ekiz et al., 2010). Colour is an important quality attribute that influences consumer acceptance of poultry meat. Perlo et al. (2010) found that meat discoloration was caused by oxidation processes and enzymatic reducing systems. Several studies have reported a significant relationship between colour and pH (Perlo et al., 2010; Mothershaw et al., 2009). Contrary, Muchenje et al. (2008a) reported poor correlations between L\* and pH<sub>u</sub>.

## Conclusion

Dietary supplementation of moringa formulated diets for broilers was effective in enhancing the oxidative stability of chicken meat, but did not result in differences in the physico-chemical characteristics of meat.

## REFERENCES

- Adedapo AA, Jimoh FO, Afolayan AJ, Masika PJ (2008). Antioxidant activities and phenolic contents of the methanol extracts of the stems of *Acokanthera oppositifolia* and *Adenia gummifera*. *BMC Comp. Alt. Med.* 8:54-59.
- Akerboom TP, Sies H (1981). Assay of glutathione, glutathione disulfide, and glutathione mixed disulfides in biological samples. *Methods Enzymol.* 77:373-382.
- Biswas A, Ahmed M, Bharti VK, Singh SB (2011). Effect of antioxidants on physio-biochemical and hematological parameters in broiler chicken at high altitude. *Asian-Aust. J. Anim. Sci.* 24:246-249.
- Blois MS (1958). Antioxidant determination by the use of a stable free radical. *Nature* 181:1199-1200.
- Botsoglou E, Govarisi A, Moulas A, Botsoglou N (2010). Oxidative stability and microbial growth of turkey breast filets during refrigerated storage as influenced by feed supplementation with olive leaves, oregano and/or  $\alpha$ -tocopheryl acetate. *Br. Poult. Sci.* 51:760-768.
- Cano A, Hernandez-Ruiz J, Garcia-Canovas F, Acosta M, Arnao MB (1998). An end-point method for estimation of the total antioxidant activity in plant material. *Phytochem. Anal.* 9:196-202.
- Chanda S, Dave R (2009). *In vitro* models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: An overview. *Afr. J. Microb. Res.* 3:981-996.
- Chung YC, Chang CT, Chao WW, Lin CF, Chou ST (2002). Antioxidative activity and safety of the 50% ethanolic extract from red bean fermented by *Bacillus subtilis* IMR-NK1. *J. Agric. Food Chem.* 50:2454-2458.
- Curtis JJ, Mortiz M (1972). Serum enzymes derived from liver cell fraction and response to carbon tetrachloride intoxication in rats. *Gastroenterology* 62:84-92.
- Deisseroth A, Dounce AL (1970). Catalase: Physical and chemical properties, mechanism of catalysis, and physiological role. *Phys. Rev.* 50:319-375.
- Ekiz B, Ozcan M, Yilmaz A, Tölü C, Savas T (2010). Carcass measurements and meat quality characteristics of dairy suckling kids compared to an indigenous genotype. *Meat Sci.* 85:245-249.
- Erel O (2004). A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. *Clin. Biochem.* 37:277-285.
- Ezeamuzie IC, Ambadederomo AW, Shode FO, Ekwebelem SC (1996). Anti-inflammatory effects of *Moringa oleifera* root extract. *Int. J. Pharm.* 34:207-212.
- Giannenas I, Pappas IIS, Mavridis S, Kontopidis G, Skoufos J, Kyriazakis I (2009). Performance and antioxidant status of broiler chickens supplemented with dried mushrooms (*Agaricus bisporus*) in their diet. *Poult. Sci.* 89:303-311.
- Iqbal S, Bhangar MI (2006). Effect of season and production location on antioxidant activity of *Moringa oleifera* leaves grown in Pakistan. *J. Food Comp. Anal.* 19:544-551.
- Jung S, Choe J, Kim B, Yun H, Kruk ZA, Jo C (2010). Effect of dietary mixture of gallic acid and linoleic acid on antioxidative potential and quality of breast meat from broilers. *Meat Sci.* 86:520-526.
- Jyotsna Mishra RK, Srivastava SV, Shukla Raghav CS (2007). Antioxidants in aromatic and medicinal plants. *Science Tech Entrepreneur.* pp.1-16. Fragrance and Flavour Development Centre. Kammuaaj.
- Kakengi AMV, Kaijage JT, Sarwatt SV, Mutayoba SK, Shem MN, Fujihara T (2007). Effect of *Moringa oleifera* leaf meal as a substitute for sunflower seed meal on performance of laying hens in Tanzania. *Livest. Res. Rural Dev.* 19:120.
- Kojic G, Vlahonic P, Ravloure D (1998). The possible importance of the cation binding site for the oxidative modification of liver nucleotidase. *Arch. Phys. Biochem.* 106:91-99.
- Krishnaraju AV, Rao CV, Rao TVN, Reddy KN, Trimurtulu G (2009). *In vitro* and *In vivo* antioxidant activity of *Aphanamixis polystachya* bark. *Am. J. Inf. Dis.* 5:60-67.
- Kumar NA, Pari L (2003). Antioxidant action of *Moringa oleifera* Lam. (Drumstick) against antitubercular drugs induced lipid peroxidation in rats. *J. Med. Food.* 6:255-259.
- Lawrie RA, Ledward DA (2006). *Lawrie's meat science* (7th ed.). Cambridge, England: Woodhead Publishing.
- Martínez-Cerezo S, Sañudo C, Panea B, Medel I, Delfa R, Sierra I (2005). Breed, slaughter weight and ageing time effects on physico-chemical characteristics of lamb meat. *Meat Sci.* 69:325-333.
- Mothershaw AS, Gaffer T, Kadim I, Guizani N, Al-Amri I, Mahgoub O, Al-Bahry S (2009). Quality characteristics of broiler chicken meat on salt at different temperatures. *Int. J. Food Prop.* 12:681-690.
- Muchenje V, Dzama K, Chimonyo M, Raats JG, Hugo A, Strydom PE (2008a). Sensory evaluation and its relationship to physical meat quality attributes of beef from Nguni and Bonsmara steers raised on natural pasture. *Animal* 2:1700-1706.
- Muchenje V, Dzama K, Chimonyo M, Raats JG, Strydom PE (2008b). Meat quality of Nguni, Bonsmara and Angus steers raised on natural pasture in the Eastern Cape, South Africa. *Meat Sci.* 79:20-28.
- Muchenje V, Dzama K, Chimonyo M, Strydom PE, Hugo A, Raats JG (2009a). Some biochemical aspects pertaining to beef eating quality and consumer health: A review. *Food Chem.* 112:279-289.
- Muchenje V, Dzama K, Chimonyo M, Strydom PE, Raats JG (2009c). Relationship between stress responsiveness and meat quality in three cattle breeds. *Meat Sci.* 81:653-657.
- Muchenje V, Hugo A, Dzama K, Chimonyo M, Raats JG, Strydom PE (2009b). Cholesterol levels and fatty acid profiles of beef from three cattle breeds raised on natural pasture. *J. Food Comp. Anal.*



- 22:354-358.
- Ordonez AAL, Gomez JD, Vattuone MA, Isla MI (2006). Antioxidant activities of *Sechium edule* (Jacq). Food Chem. 97:452-458.
- Oyaizu M (1986). Studies on products of browning reaction: Antioxidant activities of products of browning reaction prepared from glucosamine. Jpn. J. Nutr. 44:307-315.
- Perlo F, Bonato P, Fabre R, Teira G, Tisocco O (2010). Meat quality evaluation of broiler breast fillets affected by aging time and marination. Int. J. Poult. Sci. 9:1063-1068.
- Qwele K, Muchenje V, Oyedemi S, Moyo B, Masika PJ (2011). Antioxidant activity of meat from goats supplemented with *Moringa oleifera* leaves. Submitted to Meat Science.
- Santos VAC, Silva AO, Cardoso JVF, Silvestre AJD, Silva SR, Martins C, Azevedo JMT (2007). Genotype and sex effects on carcass and meat quality of suckling kids protected by the PGI "Cabrito de Barroso". Meat Sci. 75:725-736.
- Sharma OP, Bhat TK (2008). DPPH antioxidant assay revisited. Food Chem. 113:1202-1205.
- Sreelatha S, Padma PR (2009). Antioxidant activity and total phenolic content of *Moringa oleifera* leaves in two stages of maturity. Plant Foods Hum. Nutr. 64:303-311.
- Statistical Analysis Systems [SAS] (2003). Statistical analysis system user's guide (5<sup>th</sup> Edition), Version 6, (SAS Institute Inc., Raleigh, North Carolina, USA).
- Subramanian KN, Padmanaban G, Sarma PS (1965). Folin-Ciocalteu reagent for the estimation of siderochromes. Anal. Biochem. 12:106-112.
- Sun JS, Tsuang YW, Chen JJ, Huang WC, Hang YS, Lu FJ (1998). An ultra-weak chemiluminescence study on oxidative stress in rabbits following acute thermal injury. Burns 24:225-231.
- Verma AR, Vijayakumar M, Mathela CS, Rao CV (2009). *In vitro* and *in vivo* antioxidant properties of different fractions of *Moringa oleifera* leaves. Food Chem. Toxicol. 47:2196-2201.
- Weighand MA, Laipple A, Plascke K (1999). Concentration changes of malondialdehyde across the cerebral vascular bed and shedding of L selectin during carotid endarterectomy. Stroke 30:306-311.
- Wootton-Beard PC, Moran A, Ryan L (2011). Stability of the total antioxidant capacity and total polyphenol content of 23 commercially available vegetable juices before and after *in vitro* digestion measured by FRAP, DPPH, ABTS and Folin-Ciocalteu methods. Food Res. Int. 44:217-224.
- Yang R, Chang L, Hsu J, Weng BBC, Palada MC, Chadha ML, Levasseur V (2006). Nutritional and functional properties of *Moringa* leaves - From Germplasm, to Plant, to Food, to Health, American Chemical Society, pp. 1-17.
- Zhang SX, Farouk MM, Young OA, Wieliczko KJ, Podmore C (2005). Functional stability of frozen normal and high pH beef. Meat Sci. 69:765-772.