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Fatty acid composition and oxidative stability of breast meat from broiler chickens supplemented with *Moringa oleifera* leaf meal over a period of refrigeration

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ABSTRACT

Effects of diets supplemented with or without *Moringa oleifera* leaf meal (MOLM) on fatty acid (FA) composition and oxidative stability of broiler breast meat during refrigerated storage was determined. Dietary treatments (T) were as follows: T1, positive control, 668 g/ton Salinomycin and 500 g/ton Albac; T2, T3 and T4 contained graded levels of MOLM at 1%, 3% and 5% of dry matter (DM) intake, respectively; and T5, a negative control (0% additives). Oxidative stability was evaluated by thiobarbituric acid reactive substances (TBARS) on day (D) 1–8 of storage at 4 °C; and FA analysis was done on samples obtained on D1 and D8. Significant effects on TBARS were noted on day (D) 1, 3, 4 and 7; increased with increasing storage time, and with increase in MOLM supplementation. Highest ($P < 0.05$) C18:0 and C15:0 levels were noted on D1 in T2; C20:0 in T4 on D8; C20:2, C20:3n6 and C22:6n3 in T2; C18:3n6 and P/S ratio in T4 on D1; and *n*-3 in T3. Thus, despite the high SFA content, additive supplementation of *M. oleifera* leaf meal up to 5% of the bird's DMI improved the FA profile and reduced lipid oxidation in broiler breast meat.

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1. Introduction

Poultry meat has many desirable nutritional characteristics, such as low lipid content and relatively high concentrations of polyunsaturated fatty acids. The objectives in modern breeding strategies, particularly in pigs and meat-type chickens, have been to cost effectively achieve maximum lean muscle tissue (Maltin, Balcerzak, Tilley, & Delday, 2003). However, although bird genotype strongly influences meat functional properties as well as nutritional characteristics (Sirri et al., 2011); the feeding and conditions under which the animals are produced and slaughtered may influence the oxidative stability of the meat (Jung et al., 2010). Currently, there is an increasing trend towards replacement of saturated with unsaturated fats in poultry products through feeding, which raises concerns over long term stability of meat quality (Bou, Guardiola, Tres, Barroeta, & Codony, 2004; Leeson, 1999). One reason for the success of the broiler meat industry has been the consumer perception of a healthy product that contains less fat, most predominantly unsaturated fatty acids (especially polyunsaturated fatty acids) as comparable to beef or pork products (Bonoli, Caboni, Rodriguez-Estrada, & Lercker, 2007; Leeson, 1999).

Lipid oxidation is a major cause of meat quality deterioration, resulting in rancidity and the formation of undesirable odors and flavours, which lowers the functional, sensory and nutritive values of meat products; and therefore, consumer acceptability (Bou et al., 2004). Three main factors define the susceptibility of lipids to peroxidation in tissue, that is, the proportion of PUFA in lipid bilayers, the amount of reactive oxygen species produced and the level of endogenous or nutritional antioxidants (Brenes et al., 2008). In practise, the addition of antioxidants, which are organic molecules of either synthetic or natural origin capable of scavenging the active forms of oxygen involved in the initiation step or progression of oxidation is the major preventive measure against lipid oxidation in meat and meat products (Valenzuela, 1995). Antioxidants get incorporated within cell membranes and protect tissues against oxidation from reactive oxygen species, thus maintaining the overall quality of meat (Descalzo & Sancho, 2008). In the same manner, phenolics or flavonoids in plants have the affinity to bind to biological polymers and heavy metal ions, terminating free radical chain reactions (Botsoglou, Christaki, Fletouris, Florou-Paneri, & Spais, 2002; Milos, Mastelic, & Jerkovic, 2000; Valenzuela, 1995). As a result, research on antioxidants has focused on naturally occurring

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molecules and numerous medicinal herbs currently being suggested to eliminate consumers' concerns about the safety and toxicity of their synthetic counterparts (Botsoglou et al., 2002).

Moringa oleifera leaves act as a good source of natural antioxidant due to the presence of various types of antioxidant compounds such as ascorbic acid, flavonoids, phenolics and carotenoids (Anwar, Latif, Ashraf, & Gilani, 2007; Makkar & Becker, 1996; Moyo, Oyedemi, Masika, & Muchenje, 2012). These leaves can be eaten fresh, cooked, or as ground dried powder that can be stored for many months without refrigeration, without possibly loss of nutritional value (Fahey, 2005). A high content of γ-tocopherol has been found in practically the whole Moringa plant, ranging from 5.7 μg/g (adult leaves) to 27.8 μg/g (6 month-old leaves) of dry mass. Even though, variations have been encountered in α-tocopherol values ranging from 95.9 μg/g (green seeds) to 744.5 μg/g (adult plant leaves); variation between the age of the plants and their varied parts is the probably explanation (Makkar & Becker, 1996). The presence of β-carotene, vitamins A, C and E in *M. oleifera* leaves further explains their reducing potential albeit the mode of action is yet to be elucidated (Anwar et al., 2007). Vitamin C is known to act as a scavenger of free radicals, while it indirectly regenerates vitamin E. In South Africa, ground *M. oleifera* leaves are already being propagated and market as health supplements for humans. The objective of the present study was therefore to evaluate the effect of *M. oleifera* leaf meal (MOLM) on the fatty acid profile and lipid stability of broiler breast meat quality.

2. Materials and methods

2.1. Preparation of M. oleifera leaf meal and dietary treatments

Fresh, green and undamaged mature *M. oleifera* leaves were collected during the month of June from a number of trees from the same village to avoid variations in soil micronutrient content. The leaves were air-dried during the day with no direct sunlight exposure, with constant turning over to avert fungal growth. After 5 days of drying the leaves were grinded to a fine powder to pass through a 0.15-mm sieve. The leaf meal was tightly packaged in polythene plastic bags, sealed and kept at room temperature until required.

2.2. Dietary treatments

The feeding programme consisted of starter (0–21 d), grower (22–28 d), and finisher (29–35 d) basal diets that were formulated to meet the bird's dietary nutrient requirements (NRC, 1994). Each basal feed was split into 5 treatment (T) groups, which were prepared as follows: T_1: positive control, basal diet supplemented with 668 g/ton Salinomycin and 500 g/ton Albac; T_2: basal diet supplemented with 1% MOLM; T_3: basal diet supplemented with 3% MOLM; T_4, basal diet supplemented with 5% MOLM; and T_5: a negative control, basal diet with no supplementation (Table 1). All diets from starter to finisher were pelleted. Proximate analysis for moisture (Table 1), crude protein, ash and ether extract; and mineral composition was performed on all experimental diets and on MOLM samples according to methods of the Association of Official Analytical Chemists (AOAC, 2000). The techniques described by Van Soest, Robertson, and Lewis (1991) were used to determine neutral detergent fibre (NDF) and acid detergent fibre (ADF) concentrations.

2.3. Bird management and experimental design

A total 2400 day-old unsexed Cobb 500 broiler chicks were purchased from a commercial hatchery, weighted and allocated to 30 floor pens containing fresh wood shavings to the depth of 10 cm in an environmentally controlled house. The experiment was a completely randomized design divided into 5 dietary treatments with 6 replicate groups of 80 birds per pen (5 diets × 6 replicates). House temperature was set and maintained at 34 °C during the first week and then reduced by 3 °C per week until 22 °C was reached and maintained at this level until the end of the experiment. Birds were vaccinated for Marek's disease and infectious bursal disease at the hatchery, but no vaccines were administered during rearing. Chicks were inspected daily and dead birds removed following recording of mortality (pen, date and body weight). Feed and fresh water were offered *ad libitum* throughout the 35-day rearing period. Care and management of birds were in accordance with principles of animal care in experimentation (NRC, 1994). The experiment was also subjected to an assessment for its ethical acceptability and approved by the Ethics Committees of the University of Fort Hare (Animal Ethics No. NKU01–1SWAP01).

2.4. Slaughter procedure and sampling

At five weeks of age two broilers from each replicate pen, 12 birds per treatment were electrically stunned at 70 V and killed by cervical dislocation. After 24 h of chilling (4 °C), carcasses were trimmed for breast meat by removing skin, bones, and connective tissue. The left breasts from the respective treatments were sliced longitudinally into two equal parts, equating to 24 breast samples per treatment. Each sample was weighed, placed on a Styrofoam tray, wrapped and vacuum sealed in transparent oxygen-permeable polyvinyl chloride film then refrigerated at 4 °C. For eight consecutive days, ten grams from each fillet were sampled from three trays per treatment, wrapped in tinfoil, vacuum sealed and stored at −18 °C for lipid oxidation studies on a later date. On day one and eight, respectively, three additional samples per treatment were wrapped in tinfoil, vacuum sealed and stored at −18 °C until required for fatty acid analysis.

2.5. Determination of fatty acid composition

After thawing at 4 °C over night, total lipids of each 2 g meat sample were extracted using chloroform–methanol (2:1, v/v) according to the procedure of Folch, Lees, and Stanley (1957). Samples were then homogenised using a polytron mixer (Wiggen Hauser, D-500 Homogenizer) with the extraction solvent containing 0.01% butylated hydroxytoluene (BHT) as an antioxidant. Individual fatty acids were quantified using Heptadecanoic acid (C17:0) as an internal standard (catalogue number H3500, Sigma–Aldrich Inc., 3050 Spruce Street, St. Louis, MO 63103, USA). A sub-sample of the extracted lipids was trans-methylated for 2 h at 70 °C using methanol–sulphuric acid (19:1; v/v) solution. After cooling at room temperature, the resulting fatty acid methyl esters (FAMEs) were extracted with water and hexane, and the top phase was transferred to a spotting tube and dried with liquid nitrogen.

The fatty acid methyl esters were separated on a Thermo Focus GC equipped with a flame ionised detector using a BPX70 capillary column (60 m × 0.25 mm internal diameter, 0.25 μm film, SGE [SGE International Pty Ltd, 7 Argent Place, Ringwood, Victoria 3134] Australia). Gas flow rates were 30 ml/min for the hydrogen gas. Helium was the carrier gas at constant flow of 0.7 ml/min. The initial spectrometer temperature reading was programmed at 60 °C, and at 160 °C for the final reading. Injector and detector temperatures were set at 220 °C and 260 °C, respectively. The FAMEs were identified by comparing the retention times to those of a standard FAME mixture (Supelco™ 37 Component FAME Mix, 10 mg/ml in CH_2Cl_2, Catalogue Number 47885-U. Supelco, North Harrison Road, Bellefonte, PA 16823–0048, USA). Ratios

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