Dietary Supplementation of Barbatimão (Stryphnodendron Adstringens) and Pacari (Lafoensia Pacari) Extracts on the Oxidative Stability and Quality of Chicken Meat

ABSTRACT

In order to evaluate the antioxidant effects of barbatimão (BAR) or pacari (PAC) on chicken meat oxidative stability and quality, seven dietary treatments containing in three different BAR and PAC concentrations (200, 400 and 600ppm) plus a negative control (CONT) were fed to 350 broilers from 1 to 41 days of age. Ten birds per treatment were slaughtered to collect breast and thigh meat to evaluate pH, color (L*, a*, b*), cooking weight loss (CWL), and shear force (SF) 24 hours postmortem, and TBARS levels in precooked meatballs stored chilled for 8 days. The dietary supplementation with BAR and PAC extracts did not affect pH and color, but reduced (p<0.05) SF in breast meat compared with CONT suggesting improved tenderness. PAC200 increased (p<0.05) L* and protected (p<0.05) yellow pigments (b* values) of thigh meat from degradation compared with the CONT diet. At the end of the chilled storage period, BAR600 and PAC600 significantly reduced (p<0.06) MDA concentrations in breast meatballs compared to the CONT. The dietary supplementation of BAR and PAC improved (p<0.03) oxidative stability of thigh meatballs, except for BAR200. In conclusion, the dietary addition of BAR and PAC extracts may improve meat quality and prevent lipid oxidation in white and dark precooked and chilled chicken meatballs.

INTRODUCTION

Consumer demands for the quality of meat and meat products has changed. Poultry meat consumption has increased due its relatively low fat concentration and high nutrient density (Barroeta, 2007; Pereira & Vicente, 2013). In addition, chicken meat has a higher percentage of monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) compared to other meats, including a beneficial n-6:n-3 PUFA ratio (Grashorn, 2007). Human health studies demonstrated a positive influence of MUFA and PUFA consumption on the prevention and treatment of cardiovascular diseases (Ander et al., 2003; Harris et al., 2009).

On the other hand, high meat PUFA levels in meat and processing techniques, such as grinding, cooking, and salt addition increase the susceptibility of meat to degradation and cause lipid oxidation. Lipid oxidation causes oxidative stress, which is the imbalance between pro-oxidant and antioxidant substances, resulting in meat rancidity (Araújo et al., 2007; Panda & Cherian, 2014).

Lipid oxidation is a chain-reaction process that damage lipids, inducing meat rancid off-flavor and odor, reducing its juiciness and tenderness, in addition of increasing meat spoilage and reducing its shelf life (Adams, 1999; Delles et al., 2014). The dietary supplementation with natural antioxidants may be an alternative to prevent meat deterioration by improving its antioxidant balance and promoting lipid...
stability. Consequently, there is a growing body of research on effective and safely natural antioxidants for poultry meat (Milani et al., 2010).

Brazilian plants naturally occurring in the Cerrado biome, such as barbatimão (Stryphnodendron adstringens) and pacari (Lafoensia pacari), have been used by the local population for decades as medicines due to their empiric effects (Lorenzi, 2000). Recently published studies demonstrated a strong antioxidant capacity of these plants related to the presence of phenolic compounds, including tannins, flavonoids, terpenes, ellagic acid, and others (Solon et al., 2000; Souza et al., 2007; Sampaio & Leão, 2007; Galdino et al., 2009; Oliveira & Vanzeler, 2011). This study aimed at evaluating the antioxidant capacity of the dietary supplementation of barbatimão and pacari extracts on chicken meat quality and lipid oxidation.

**MATERIALS AND METHODS**

**Barbatimão and pacari alcoholic extracts**

Barks from Stryphnodendron adstringens and Lafoensia pacari trees were macerated for 24 hours (80:20 alcohol:water), and distilled. The distilled extracts were reduced using a rotary evaporator cold trap (R-300, Buchi Brazil Ltda, Valinhos, SP) until reaching 20% solid residues with 43.6% (barbatimão) and 35% (pacari) total tannin content.

**Birds and diets**

A total of 350 one-d-old male Cobb500® broiler chicks were distributed into seven treatments with five replicates, totaling 35 experimental units with 10 birds each. Birds were housed at the experimental facilities of the Department of Animal Science, Veterinary School, Federal University of Goiás (UFG) in galvanized steel battery cages (0.5 m x 0.4 m x 0.4 m), equipped with trough drinkers and feeders. Water and feed were provided *ad libitum*. A lighting program of 23 hours of light plus one hour of darkness was adopted. Birds were brooded until 14 days of age using 60W lamps, and the room environment was controlled by side plastic curtain management.

The basal diets were fed as mash and were based on corn and soybean meal and formulated to supply the birds’ nutritional requirements during the pre-starter, starter, and grower phases, according to Rostagno et al. (2011) and contained 2,960; 3,050 and 3,150 kcal/kg apparent metabolizable energy (AME); 22.4, 21.2, and 19.8 crude protein (CP), and 1.34, 1.217 and 1.131 digestible lysine, respectively. The composition of the diets is shown in Table 1.

| Ingredients                  | Pre-starter (1-7 days) | Starter (8-21 days) | Grower (22-41 days) |
|-----------------------------|------------------------|---------------------|---------------------|
| Ground corn                 | 54.43                  | 56.86               | 59.82               |
| Soybean meal 45%            | 38.77                  | 35.93               | 32.36               |
| Soybean oil                 | 2.220                  | 3.100               | 4.000               |
| Dicalcium phosphate         | 1.910                  | 1.550               | 1.320               |
| Limestone                   | 0.798                  | 0.843               | 0.803               |
| Salt                        | 0.458                  | 0.437               | 0.416               |
| DL-Methionine 99%           | 0.348                  | 0.294               | 0.274               |
| L-Lysine HCl                | 0.300                  | 0.248               | 0.246               |
| L-threonine 98%             | 0.113                  | 0.078               | 0.069               |
| Starch                      | 0.500                  | 0.500               | 0.500               |
| Vitamin Suplement1          | 0.100                  | 0.100               | 0.100               |
| Mineral Suplement2          | 0.050                  | 0.050               | 0.050               |

| Calculated Values           |                        |                     |                     |
|-----------------------------|------------------------|---------------------|---------------------|
| Metabolizable energy (kcal/kg) | 2,960                  | 3,050               | 3,150               |
| Crude Protein (%)           | 22.40                  | 21.20               | 19.80               |
| Digestible Lysine (%)       | 1.324                  | 1.217               | 1.131               |
| Digestible Arginine (%)     | 1.417                  | 1.337               | 1.235               |
| Digestible Methionine (%)   | 0.658                  | 0.591               | 0.555               |
| Digestible Threonine (%)    | 0.861                  | 0.791               | 0.735               |
| Digestible Tryptophan (%)   | 0.257                  | 0.241               | 0.222               |
| Calcium (%)                 | 0.920                  | 0.841               | 0.758               |
| Available Phosphorus (%)    | 0.470                  | 0.401               | 0.354               |
| Choline (%)                 | 0.309                  | 0.298               | 0.278               |
| Sodium (%)                  | 0.220                  | 0.210               | 0.200               |

1 Amount per kg of supplement: 3,125,000 IU Vitamin A; 550,000 IU Vitamin D3; 3,750 mg Vitamin E; 625 mg Vitamin K3; 250 mg Vitamin B1; 1125 mg Vitamin B2; 250 mg Vitamin B6; 3750mg Vitamin B12; 9,500 mg niacin; 3750 mg calcium pantothenate; 125 mg folic acid; 350,000 mg DL-methionine; 150,000 mg choline chloride 50%; 50 mg selenium.

The dietary treatments consisted of the basal diet with no addition of antioxidants (negative control; CONT), and diets supplemented with 200, 400 or 600 ppm of barbatimão or pacari extracts at the expense of starch. Treatments were applied in a completely randomized experimental design in a 2x3 factorial arrangement (2 plants x 3 concentrations) plus CONT. No synthetic antioxidants were added to the vitamin and mineral premix; only a basal amount of 20 mg of alpha-tocopheryl acetate/kg of diet was supplied to meet the physiological requirements of the birds.

All experimental procedures were previously by the Committee of Ethics on the Use of Animals – CEUA/UFG (protocol O30/2012).

**Meat sampling and analyses**

At 41 days of age, 10 birds per treatment were slaughtered in a commercial processing plant, according to the Brazilian legislation (Brasil, 2000). Raw deboned and skinless breast and thigh meat
samples were stored chilled (4°C) for 24 h, after which meat pH and color (CIELAB System: L*=lightness, a*=redness and b*=yellowness) were recorded in triplicate using a portable pH meter (AG 205, Testo do Brasil®, Campinas, SP) and chroma meter (CR-400, Konica-Minolta Inc., Japan).

Samples were then vacuum packed and stored chilled until meat composition analyses (humidity HU, crude protein CP, total lipid content TLC and ash AS) were performed in quadruplicate. Meat tenderness was evaluated by cooking weight loss (CWL) and shear force (SF) in breast meat samples only. Duplicates of 2.5x2.5x2.5cm meat cubes were collected from the right portion of Pectoralis major muscle, weighed and cooked in electric oven (170°C) until reaching 70°C internal temperature, monitored using a thermocouple (Type K, Testo do Brasil®, Campinas, SP) and chroma meter (CR-400, Konica-Minolta Inc., Japan).

Storage trials

Breast and thigh meat samples were minced separately, 0.5% food-grade salt was added, and shaped into meatballs (30 g ± 0.5 g). Breast and thigh meatballs were vacuum packed and cooked in water bath at 100°C for 10 minutes, according to Racanici et al. (2004). Pre-cooked meatballs were repacked in oxygen-permeable bags and kept chilled at 4°C in the dark for 8 days.

Secondary lipid oxidation products were evaluated on days 0, 2, 4, 6 and 8 of storage by malondialdehyde quantification using TBARS (thiobarbituric acid reactive substances). TBARS was determined in duplicate in two meatballs per treatment, according to Madsen et al. (1998). Absorbance was measured at 532 and 600 nm with spectrophotometer (UV-340G, Gehaka do Brasil, São Paulo, SP) and results were expressed in µmol of malondialdehyde (MDA) per kilogram of meat, using a 1,1,3,3-tetraethoxypropane (TEP) standard curve.

Statistical Analysis

The experiment was analyzed as a completely randomized experimental design in a 2x3 factorial arrangement (2 plants: BAR and PAC; 3 concentrations: 200, 400 and 600 ppm) plus negative control (CONT). Results were analyzed using PROC GLM (meat tenderness) and PROC MIXED procedures (repeated measurements: color, pH, TBARS) of the software SAS® (v.9.3, Statistical Analysis System, NC, USA). Means were compared by Tukey’s test at 5% significance level.

The statistical model used for the analysis of variance was: \[ Y_{ijk} = \mu + P_i + C_j + PxC_{ij} + e_{ijk}, \] where: \( Y_{ijk} \) = dependent variable; \( \mu \) = general mean; \( P_i \) = effect of the ith plant; \( C_j \) = effect of the jth concentration; \( PxC_{ij} \) = interaction between the ith and the jth factors; \( e_{ijk} \) = random residual error.

RESULTS

Meat composition

The results are shown in Table 2. The HU (73.78±0.49 and 73.98±0.52), CP (25.3±0.84 and 20.40±0.44), TLC (1.80±0.23 and 5.79±0.32) and AS (1.52±0.11 and 1.46±0.03) contents determined in the breast and thigh meat samples, respectively, were similar to those found in the Brazilian (NEPA, 2011) and American (USDA, 2012) composition tables and were not affected (p>0.05) by dietary treatments.

Table 2 – Average pH, color (L*, a*, b*), cooking weight loss (CWL, %), shear force (SF, KgF) values obtained in breast meat samples.

| Treatment* | pH   | L*   | a*   | b*   | CWL | SF   |
|------------|------|------|------|------|-----|------|
| CONT       | 5.97 | 47.49| 3.02 | 8.33 | 15.88| 2.85 |
| BAR200     | 5.87 | 47.73| 3.48 | 8.72 | 14.04| 2.17 |
| BAR400     | 5.92 | 46.58| 3.52 | 7.59 | 10.27| 1.40 |
| BAR600     | 5.83 | 48.19| 3.10 | 7.99 | 13.71| 1.71 |
| PAC200     | 5.86 | 49.64| 3.30 | 9.71 | 20.70| 1.63 |
| PAC400     | 5.89 | 49.13| 3.22 | 8.73 | 15.62| 1.82 |
| PAC600     | 5.87 | 47.45| 2.98 | 8.35 | 12.47| 1.65 |
| Stand. Dev.| 0.50 | 0.69 | 0.31 | 0.60 | 1.09 | 0.41 |

*Means with different letters in the same row are statistically different (p<0.05).

Meat pH and color

The addition of BAR and PAC did not affect breast meat pH or a* color (Table 2). The diets containing BAR400 promoted the lowest (46.58 and 7.59) and PAC200 the highest (49.64 and 9.71) L* and b* values, respectively. Likewise, the pH of the thigh meat samples (Table 3) were not different when BAC and PAC were compared with the CONT treatment. Thigh meat a* values were not different among treatments; however, the birds fed the PAC200 diet presented higher thigh meat (p<0.05) L* (48.86) and b* (11.27) values compared with those fed the CONT diet.
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Table 3 – Average pH and color (L*, a*, b*) values obtained in thigh meat samples.

| Treatment* | pH  | L*      | a*  | b*    |
|------------|-----|---------|-----|-------|
| CONT       | 6.11ab | 46.68b  | 13.81 | 9.54ab |
| BAR200     | 6.12ab | 47.56ab  | 15.49 | 10.71ab |
| BAR400     | 6.17a  | 47.31ab  | 15.01 | 10.33ab |
| BAR600     | 5.98b  | 47.48ab  | 15.03 | 10.06ab |
| PAC200     | 6.06ab | 48.86a  | 13.59 | 11.27a |
| PAC400     | 6.08ab | 47.64ab  | 14.45 | 10.67ab |
| PAC600     | 6.02ab | 46.64b  | 14.78 | 10.35ab |
| Stand.Dev. | 0.06  | 0.74    | 0.97 | 0.59 |

a,b,c Means with different letters in the same row are statistically different (p<0.05).
*Treatments: negative control diet with no antioxidants (CONT) and diets supplemented with of 200, 400, or 600 ppm of barbatimão (BAR) or pacari (PAC) alcoholic extracts.

Meat tenderness

CWL and SF values were significantly affected (p<0.05) by the dietary treatments (Table 2). The meat of the birds fed the BAR400 diets presented the lowest CWL value (10.27%; p<0.05) whereas the opposite was observed in the meat of PAC200-fed birds (20.70%). The inclusion of the evaluated plant extracts to broiler diets significantly reduced (p<0.05) breast meat SF relative to the CONT diet, except for BAR200.

Meat oxidation

Statistical differences (p<0.07) among treatments regarding malondialdehyde (MDA) accumulation in precooked breast meat were detected during the entire storage period (Figure 1). On day zero, broilers fed the diets with BAR and PAC inclusion presented similar oxidation levels compared with the CONT diet, except for BAR200, which pro-oxidant effect was detected immediately after cooking. From day 2 to 6 of chilled storage, dietary PAC400 inclusion effectively (p<0.06) prevented lipid peroxidation compared with the CONT diet, while the oxidation levels determined with the other dietary BAR and PAC levels was similar to that of the CONT diet. Up to day 8, the dietary addition of BAR600 and PAC600 significantly reduced (p<0.06) MDA levels in cooked breast meatballs, efficiently delaying oxidation compared with the CONT diet.

In thigh meatballs (Figure 2), the dietary inclusion of BAR400 and PAC200 was able to protect lipids from oxidation (p<0.02) during cooking (day zero) compared with the CONT diet, whereas the treatment PAC600 increased (p<0.0002) TBARS levels. Between days 2 and 6 of storage, the dietary supplementation of BAR and PAC was not effective to prevent the formation of TBARS compared with the CONT diet. However, at the end of storage (day 8), the dietary inclusion of BAR and PAC showed (p<0.03) significant antioxidant activity, preventing lipid oxidation relative to the CONT diet, except for BAR200.

DISCUSSION

Meat pH and color

Average breast meat pH values within the range expected for this type of meat 24 hours post mortem, according to Lesiow et al. (2009) and Glamoclija et al. (2015). As expected, higher pH values were determined in thigh meat (dark meat) than in breast meat (white meat) due to the different types of their
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**Meat tenderness**

In general, the average CWL values observed in this study (10.27-20.70%) were lower than those reported by Almeida et al. (2002) and Shafey et al. (2014) in normal breast meat (23.0-31.69%). On the other hand, Barbut et al. (2005) considered normal meat when CWL average values were close to 11.25%. Shear force results (1.40-2.85) were within the expected range for chicken breast meat (Souza et al., 2011; An et al., 2015).

Cooking loss and shear force are related with meat tenderness and water holding capacity, i.e., with the capacity of retaining water associated with the intramuscular fibers (Müller et al., 2012). The results of the present study showed that the breast meat fed 400 ppm of barbatimão was capable of retaining water inside the muscle fibers and, therefore, being more tender than the meat of those fed the control diet. These results are in agreement with other studies evaluating plant extract supplementation in broiler diets (Lahucky et al., 2010; Luna et al., 2010).

**Lipid oxidation**

The inclusion of plant extracts in broiler diets, such as BAR and PAC, led to the incorporation of antioxidant compounds in the cell membrane and muscle tissue (breast meat, Figure 1 and thigh meat, Figure 2) after these compounds were metabolized by the birds. The uniform distribution of the antioxidant substances derived from the plant extracts is directly correlated with their antioxidant efficiency after the muscle is converted into meat, because these compounds are available close to the damaged sites (Sies & Stahl, 1995; Cui & Decker, 2016).

Although the dietary inclusion of pacari at 400 ppm delayed breast meat lipid oxidation up to day 6 of storage, only the highest dose (600 ppm) effectively prevented lipid oxidation on day 8 (Figure 1). This result probably is related to the depletion of most of the antioxidant compounds accumulated in the muscle at lower supplementation levels, as the supplementation of both evaluated plant extracts at 600 ppm was able to maintain higher antioxidant levels available to prevent lipid oxidation until the end of the storage period.

On the other hand, the dietary inclusion of BAR and PAC at any level delayed thigh meat lipid oxidation until the day 6 (except on day zero) (Figure 2). Nevertheless, on day 8, the dietary inclusion of all levels of BAR and PAC, with except for BAR200, significantly (p<0.03) delayed thigh meat oxidation compared with the CONT diet.

 muscle fibers. The dark color of thigh meat is given by its high amount of type I muscle fibers, which are aerobic, and therefore, have low glycolytic potential. The metabolism of muscle I fibers results in low glycogen and lactic acid production, which are involved in the transformation of muscle into meat (Dransfield & Sosnicki, 1999; Joo et al., 2013).

According to Beraquet (2000), normal chicken meat pH ranges between 5.8-6.2. In this experiment, the meat pH values obtained with all treatments were within this range. However, there was no effect of the tested antioxidants on pH, as observed by Lee et al. (2012), who detected an increase in meat pH values in the meat of broilers fed garlic and linoleic acid with those fed a control diet. Those authors concluded that the dietary supplementation of those natural antioxidants was able to slow down pH decline post-mortem, possibly due to the antioxidant effect of the phenolic compounds deposited in the meat. Lima et al. (2015) concluded that the inclusion of 500ppm of the Cerrado plants copaiba (Stryphnodendron adstringens) and sucupira (Lafoensia pacari) oil resins in broiler diets delayed thigh meat lipid oxidation compared with a control diet, but did not detect any pH differences.

Barbut et al. (1997) classified chicken breast meat as normal, DFD (dark, firm and dry), or PSE (pale, soft and exudative) according to the pH and L* (luminosity) values evaluated 24 hours post-mortem in chilled pectoralis major muscle. DFD meat is characterized by L* values lower than 46 and pH values higher than 6.1, whereas PSE meat presents L* values higher than 53 and pH lower than 5.7. According to these values, all treatments applied in the present study produced breast meat that can be classified as normal. Breast meat color was not affected by natural extracts supplementation when compared with the CONT treatment, as previously reported by Leonel et al. (2007) evaluating different levels of vitamin E.

Overall, the higher redness (a*) value detected in thigh meat compared with breast meat is related to the tissue concentrations of hemoglobin, and specially, of myoglobin (Hedrick et al., 1994; Muhlisin et al., 2016). These heme proteins are responsible for meat pigmentation and their concentration in meat is related to several factors, such as tissue muscular activity, blood supply, oxygen availability, and age (Kranen et al., 1999; Min et al., 2008). As observed in other studies with natural antioxidants (Chouliara et al., 2007; Simitzis et al., 2008), the supplementation of PAC200 was capable of delaying deterioration of yellowness (b*) in thigh meat, suggesting improvement in myoglobin stability.
Therefore, the results observed in this study are consistent with those reported by Botogloou et al. (2002), who detected antioxidant activity of the dietary supplementation of oregano essential oil (50 and 100 ppm) on both breast and thigh meat compared with a control diet, obtaining the best results with the highest dosage. Moreover, Narciso-Gaytán et al. (2011) found that DL-α-tocopheryl acetate supplemented at 200 ppm in broiler diets was effective in preventing both breast and thigh meat oxidation. However, Mariutti et al. (2011) obtained different results when evaluating 0.1% inclusion of dried garlic directly in minced breast meat before cooking. The authors did not detect antioxidant activity of garlic when compared to a control without the use of antioxidants.

The antioxidant efficacy of BAR and PAC natural extracts, as well as of other plant extracts, is influenced by several plant-related factors, including the geological region where the plants are grown, harvesting season, climate, and part of the plant used (Fernandez-Panchon et al., 2008). Most part of the essential oils compounds present in plant extracts is rapidly absorbed in the gut after oral administration, metabolized, and excreted by the kidneys. Only a small amount of these compounds is deposited in the body, especially on cellular membranes (Mitumoto, 2000; Cui & Decker, 2016). However, the balance between the amount of compounds stored and excreted can vary according to the composition of these essential oils (Igmi et al., 1974; Lee, 2004).

The in-vitro antioxidant potential of barbatimão extract was previously reported by Lopes et al. (2005), who demonstrated the antioxidant activity of this plant by DPPH (1,1-diphenyl-2-picryl-hydrazyl) radical scavenging. Solon et al. (2000) also showed that pacari extract has antioxidant activity in vitro. Therefore, the results of the present study confirm those previous findings, as shown by the effective lipid oxidation control at the end of the storage period of the breast and thigh meat of broilers fed BAR and PAC compared with the CONT diet.

CONCLUSIONS

The dietary supplementation of broilers with alcoholic extracts of barbatimão and pacari seems to improve breast meat quality and preserve yellowness in thigh meat. Low dietary supplementation levels of these extract maybe used to prevent early lipid oxidation in chicken breast meatballs; however, higher levels are needed to protect breast meat lipids for longer periods, whereas the antioxidant effect of barbatimão and pacari in chicken thigh meatballs was detected at the end of chilled storage period.

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