

INFLUENCE OF *ACACIA NILOTICA* **ON MEAT QUALITY OF BROILER CHICKENS**

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Abstract

Investigate the impact of A*. nilotica* on meat quality. Conducted at the Poultry Husbandry Department, Sindh Agriculture University Tandojam.300 day-old Hubbard broiler chicks were randomly assigned to five treatment groups, T0 (Control): No A. *nilotica* pods. T3, T6, T9, and T12: Inclusion of 3%, 6%, 9%, and 12% A. *nilotica* pods in the diet. Birds received a corn-soybean based basal diet meeting nutrient requirements. Standard housing, lighting, and vaccination protocols were followed.PH of breast muscles: Influenced by A. nilotica pod levels Highest pH (6.5) in the 12% pod group. Meat color $(L^*, a^*, b^*$ values) Darker meat color in Acacia-fed broilers. L* value highest in control group (60.45), indicating lighter meat color. Water holding capacity: Improved with increasing A. *nilotica* pod levels. Highest capacity (64.71%) in 12% pod group. Antioxidant enzyme activities Superoxide dismutase (SOD) concentration highest in 12% pod group. Malondialdehyde (MDA) concentration (indicator of oxidative stress) lowest in highest pod supplementation group (1.23). The pH of the meat and its water holding capacity improved with Acacia addition, indicating potential improvements in meat texture and juiciness. *A. nilotica* addition, particularly at a 12% level, led to enhanced antioxidant enzyme activity (SOD concentration) and reduced indicators of oxidative stress (MDA concentration), suggesting potential benefits for the oxidative stability of the meat and overall health of the chickens. The use of *A. nilotica* as a supplement in broiler diets could compromise growth performance at higher levels, it has the potential to improve several aspects of health and meat quality. Further studies could focus on optimizing the dosage and form of *A. nilotica* addition to balance growth performance with health and meat quality benefits.

Keywords: Acacia nilotica Meat quality, Treatment groups, Corn-soybean based basal diet PH.

I. Introduction

Poultry meat is the most preferred meat by consumers because it is considered healthier, and cheaper than red meat and consumption of poultry meat is 90% compared to red meat worldwide (Biswas et al. 2010; Hafiz et al., 2015). these statistics displays the importance of chicken production towards feeding the ever- growing human population. The industry ensures the continuous production of nutritious meat products from chickens. However, chicken meat is constrained by its high amount of unsaturated fatty acid Contents, which exposes meat to lipid oxidation, leading to microbial spoilage (Wapi et al., 2013). The lipid oxidation process starts immediately after slaughter when blood circulation stops, and anaerobic metabolism starts. Changes caused by lipid oxidation in meat are seen in sensory attributes (colour, smell), nutritional value (drip loss, rancid) which affects the shell-life, consumer acceptability and price. These parameters including texture are used by consumers when selecting meat quality. Therefore, the control of lipid oxidation in meat is vital to meat quality. (Kolobe et al. 2022; Mthethwa, 2018). Synthetic antioxidants have primarily been used to retard lipid oxidation in chickens, but their continued use is, however, considered harmful to humans as they produce carcinogenic effects (Gheisari et al., 2017; Lorenzo et al., 2018). Because of this, consumers tend to shift their preference to meat produced from natural products without the use of growth hormones and chemicals (Lorenzo et al., 2018; Nikmaram et al., 2018; Cunha et al.,2018). Oxidative stability of poultry meat is influenced not only by bird genotype but also by feeding, rearing practices and the degree of muscle tissue damages during preslaughter, e.g., physical damage, early postmortem conditions, pH and carcass temperature (Kristina et al. 2015). These factors could be manipulated by supplementing the animal diet with

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phytogenic compounds such as di \Box erent essential oils and poly phenols to improve animal productivity and the quality of food derived from those animals (Kristina et al. 2015; Jang et al. 2004).

The search for feed manipulation has since been placed on protein-rich legumes which are highly abundant in the tropics (D'Mello, 1995; Abdulrazak et al., 2000). Nitrogen-rich leguminous leaf meals are a good source of protein and contain natural antioxidants with high levels of polyphenolic compounds (Dube et al, 2001; Ndou et al., 2015). Leguminous leaf meals contain a range of different compounds which possess antioxidant properties including vitamin E, flavonoids, phenolics, carotenoids and ascorbic acid (Makkar et al, 2003; Muhaisen et al., 2002; Camo et al., 2008). These properties of compounds are present in considerable amounts in *A. nilotica*. The use of leguminous trees reduces the use of conventional feed sources and feed costs in poultry industry. Legume meals such as *Acacia* species are high in protein, contain natural antioxidants and are believed to be safer than synthetic antioxidants (Dube et al., 2001; Moyo et al., 2011). Moreover, *Acacia* pods meals possess antimicrobial and anti-inflammatory effects (Alajmi, et al., 2017). *A. nilotica* pod is a rich protein source, with high contents of polyphenolic compounds. Polyphenolic compounds reduce protein and amino acid digestibility in chickens (Hlatini et al, 2016), however, they possess a wide range of positive biological effects. Using plant extracts that display the natural antioxidant properties in broiler feeds reduces microbial spoilage of meat and improves its quality, aiding in extended shelf life (Djenane et al., 2002).

A. nilotica contains bioactive compounds that may impart distinct flavors or odors to the meat. The sensory attributes of broiler meat, such as taste, texture, and aroma, could potentially be influenced by the inclusion of *A. nilotica* in the diet. However, the specific impact on sensory attributes requires further investigation. *A. nilotica* contains various nutrients, including vitamins, minerals, and dietary fiber (Beya et al., 2021). The nutritional composition of broiler meat can be influenced by the nutrients present in the diet. Therefore, the inclusion of *A. nilotica* in the broiler diet may have implications for the nutritional profile of the meat. *A. nilotica* contains bioactive compounds, such as tannins and flavonoids, which may have an impact on meat quality parameters. For example, these compounds can affect the water-holding capacity, tenderness, and color stability of the meat. However, the specific influence of *A. nilotica* on these physicochemical properties requires further investigation. *A. nilotica* contains antioxidant compounds, such as polyphenols, which can help reduce oxidative stress and protect against lipid oxidation in meat. By reducing oxidative damage, *A. nilotica* may potentially contribute to improved meat quality attributes such as colour stability and shelf life (Hassan et al., 2017).

To date, little is known about the optimum inclusion levels of *A. nilotica* pods and its effect on carcass yield and meat quality of broiler chicken. Increased consumer awareness of meat quality is also a key driver in efforts to determine the effect of the leaf meal on meat quality characteristics. This would ensure satisfaction of consumer expectations (Grunert et al., 2004). Therefore, recommendations on the use of *A. nilotica* pods meal in broiler diets should consider its effect on major meat quality parameters. This would help reduce the chances of product rejection in the market. Recommendations on the use of the pods meal in broiler diets are silent on its effect on meat quality parameters (Ncube, 2018). Therefore, there is a need to determine the appropriate level of including in broiler diets for optimum growth and meat quality characteristics. The objective of this study was therefore to determine the appropriate levels of including *A. nilotica* pods meal in broilers diets for optimum performance and meat quality.

II. Materials and Methods

A. Feeding management

A total of three hundred day old Hubbard broiler chicks were randomly allocated to five treatment groups, each with three replicates. The dietary treatments included the following levels of Acacia nilotica pods: T3 (3%), T6 (6%), T9 (9%), and T12 (12%). The control group (T0) received a basal diet without any Acacia nilotica pod supplementation. The basal diet was formulated based on the recommendations of the National Research Council (NRC, 1994) to meet the nutrient requirements of broiler chickens. During the study, the birds were provided with starter feed from day 1 to 14 and finisher feed from day 15 to 40. All experimental procedures were approved by the Institutional Animal Care and Ethics Committee of Sindh Agriculture University Tandojam.

B. Sample collection

At 40 day of age, blood samples were taken from randomly selected bird per replicate; plasma was separated by centrifugation at 300 × g for 10 min at 4°C and stored at -20° C for further analysis. After blood collection, all birds were slaughtered and defeathering, the colour and pH of the meat was measured after 45 minutes. The meat samples were cut into 4×3×1-inch cubes and all of the visible fat and connective tissues were removed. The antioxidant parameters were determined three hrs after slaughtering.

C. pH of breast muscles

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The pH of breast meat was determined using pH meter (CRISON pH, 2000) calibrated before each measurement at pH 4 and pH 9 standard solution. Each pH reading was taken by immersing the electrode into the mixture. Each sample on each treatment was weighed as 10g and homogenized in 50mL of deionized water for a minute. The pH meter electrode was rinsed using distilled water between each measurement to avoid treatment contamination. The measurements of pH were done at room temperature.

D.Colour of breast muscles

Colour of thigh and breast muscles was observed using colour spectrophotometer in colorimetric parameters CIE Labs. The measurements taken were lightness (L*), redness (a*) and yellowness (b*) (Commission Internationale de l'Eclairage, 1976). Before taking colour readings, the meat samples were left to bloom for 30 min. A 30 g portion of meat was measured in triplicate per sample.

E. Water holding capacity

The water holding capacity of the meat was measured according to the method of Omana et al. (2010). Raw meat (approximately 2 g) was placed between a pre-weighed Whatman (No. 1) filter paper and between two glass plates. Using the texture profile analyser (TA-XT Express, Stable micro systems, Ltd., Surrey, England) in adhesive test mode, the samples were tested with a target force of 111 kg for 5 min. After the test, the filter paper with the absorbed water was immediately weighed. Expressible moisture was measured as the quantity of water released per gram of meat and expressed as a percentage.

Expressible water (%) = (wet paper – dry paper/meat weight) × 100

F. Drip loss

After slaughter, 2.5 g of samples were placed in plastics and sealed with rope. After, they were kept at 4°C for 24 hours. Drip loss was calculated using the following equation:

% Drip $\log s = [(Weight before - Weight after) / (Weight before)] \times 100$

G. Cooking loss

For the determination of cooking loss breast muscles were weighed using analytical balance, placed in polyethylene bags. For cooking of meat, water bath was used and set at 80°C for an hour. After cooking, samples were removed in the water bath including excess water and final weight was recorded. The calculations were done using the following equation:

% Cooking loss = $[(Weight before - Weight after) / (Weight before)] \times 100$

H. Texture analysis

Soon after determination of cooking loss, the cooked samples were used for texture measurements. For texture measurements, breasts muscles were made identical. For breasts, a cylindrical of 14mm was used to cut similar pieces. The texture of meat samples was determined using the Warner-Bratzler shear force (Honikel, 1998).

I. Antioxidant activities

The antioxidant activities were measured with enzymatic methods and the corresponding diagnostic kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) for TAOC, SOD and MDA according to the instructions of the manufacturer. The MDA Concentrations, which reflect thiobarbituric acid reactive substance (TBARS), were determined according to the procedure described by Zhang et al. (2009). The methodology used in the kit was the nitrite method for SOD determination, as described by Oyanagui, (1984).

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J. Thiobarbituric Acid Reactive Substances (TBARS)

Determination of TBARs of the meat samples, the procedure of (Berry and Leddy 1984) was followed. TBA 2.88g was dissolved in slightly warm double distilled water and then add 150g TCA. Again add double distilled water to make the final volume 1 liter. Make a 10 percent stock solution by dissolving BHT in ethanol. Lipid oxidation of the samples was determined using a 2-TBA reactive substances (TBARS) method. Two grams of each sample were weighed through an analytical weighing balance and pour into a 50ml falcon tube. 50 μ L of butylated hydroxytoluene (7.2%) was added into the tube along with 15 mL of double distilled water and then homogenize using a Polytron homogenizer (type PT 10/35, Brinkman Instruments Inc.) for 20-30 seconds at max speed. 1ml of meat sample homogenate was transferred to a disposable test tube $(13 \times 100 \text{ mm})$ along with the 2ml solution mixture of TBA/TCA.

K. TBARs Analysis

The mixture was mixed thoroughly using a vortex. After this tube was incubated in a hot water bath operate at 90°C for 15 min to develop color. In an ice water bath, the samples were allowed to cooldown the temperature for 10 min. Then test tubes were vortex and then centrifuged for 20 min at 2000 rpm at 4 °C. The 1ml of the resultant supernatant solution was separated and absorbance was determined with blank references (which contain 1ml of deionized distilled water and 2 mL of TBA/TCA solution) at 531 nm in a spectrophotometer. The quantity of TBARS was expressed as milligrams of malondialdehyde (MDA) / kilogram of meat. A standard curve was constructed and TBARS values of the samples were calculated using that curve. For Calculation mentioned formula was used. TBARS value (mg MDA/kg of meat) = $4 *$ absorbance at 531nm

L.Statistical analysis

Data obtained from all experiments were summarized using Excel software (Microsoft Co., Redmond, WA, USA), entered into SPSS 20 software (International Business Machines Co., Armonk, NY, USA) and subsequently analysed using one-way ANOVA. Tukey's multiple comparison test was used to test the homogeneity of variance. The results were expressed as arithmetic means and SD, and a difference was considered statistically significant when the P-value was ≤ 0.05 .

III. RESULTS

A. pH of breast muscles

The results regarding muscles pH are presented in Table-1 which shows the pH response on various concentration of *A. nilotica* pods meal. The results shows that pH of breast muscle was higher (6.5) in T12 (12% *A. nilotica* Pod) group followed by T9 (9% *A. nilotica* Pod) (6.29), T6 (6% *A. nilotica* Pod) (6.13), T3 (3% *A. nilotica* Pod) (6.03) and T0 (control) (6.00). The pH values ranged within a range of $(6.0 - 6.5)$ in all treatments. The inclusion level of 12% had higher pH, while control group broiler breast meat had lower pH and the effect of *A. nilotica* pods was significant $(P<0.038)$.

^{abc} Mean values in the same row that do not share a common letter differ significantly ($P < 0.05$)

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B. Colour of breast muscles

Highest L* value (lightness) was observed in the control group (T0) with a value of 60.45. Acacia-fed broilers (T9, T3, T12, and T6) exhibited slightly lower L* values.The breast meat color was comparatively darker in Acacia-fed broilers than in the control group $(P < 0.0233)$.

The highest a* value (redness) occurred in the T12 group with 16.55. T9 and T6 groups followed with values of 15.41 and 13.88, respectively Redness was not significantly affected by dietary treatment ($\overline{P} = 0.1149$).

T9 group had the highest b* value (yellowness) at 14.39. T12, T6, T0, and T3 groups followed with slightly lower values. Yellowness of breast muscle meat was lower in broilers fed A. nilotica compared to the control group (P < 0.2315).

 L^* = Lightness; a^* = Redness; b^* = Yellowness

abc Mean values in the same row that do not share a common letter differ significantly $(P < 0.05)$

C. Water Holding Capacity

The results regarding water holding capacity are presented in Table 3. The results show that water holding capacity of breast muscle was higher (64.71) in T12 (12% *A. nilotica* Pod) group followed by T9 (9% *A. nilotica* Pod) (62.01), T6 (6% *A. nilotica* Pod) (61.62), T3 (3% *A. nilotica* Pod) (61.56) and T0 (control) (61.40). The inclusion level of 12% *A. nilotica* pods had higher water holding capacity compared to control group and the effect of *A. nilotica* pods was significant (P<0.0374).

Table 3 Effect of dietary addition of *Acacia nilotica* **on water holding capacity of breast muscle of broiler.**

Treatments	$Mean \pm SEp$ DH
T ₀ (control)	61.40 ± 1.34^b
T3 $(3\% A. \nnilotica Pod)$	61.56 ± 1.50 ^{ab}
$T6 (6\% A. \nnilotica Pod)$	61.62 ± 1.80 ^{ab}
T9 (9% Λ . nilotica Pod)	62.01 ± 1.60 ^{ab}
T12 (12% A. nilotica Pod)	64.71 ± 1.25^a
P-value= 0.0374	

abc Mean values in the same row that do not share a common letter differ significantly $(P < 0.05)$

D. Drip loss, cooking loss, Tenderness

The results regarding drip loss, cooking loss and tenderness are presented in Table 4. The results showed that drip was higher in T9 group followed by T12, T6, T3 and T0 (control) group. While, cooking loss was higher in T12 group followed by T6, T0, T3 and T9 groups. On the other hands, Tenderness was significantly higher in T9 group followed by T6, T0, T3 and T12 group. The inclusion level of 9% *A. nilotica* pods had higher tenderness compared to T12 group and the effect of *A. nilotica* pods was significant (P<0.0392). The present study showed that the dietary inclusion of *A. nilotica* pods at lower levels decreased drip loss and cooking loss and increased meat tenderness as compared to control group

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E. Antioxidant Enzyme

The results regarding antioxidant enzymes activity are presented in Table.5. The results show that SOD concentration was higher in T12 (91.60) group followed by T9 (82.26), T6 (70.92), T0 (70.29) and T3 (69.48). On the contrary, the MDA concentration was higher (1.99) in T0 group followed by T3 (1.90), T6 (1.65), T9 (1.40) and T12 (1.23). The inclusion level of 12% *A. nilotica* pods had lower MDA compared to T0 group and the effect of *A. nilotica* pods was significant (P<0.0103). The present study showed that the dietary inclusion of *A. nilotica* pods at various levels increased SOD activity and decreased MDA levels as compared to T0 group. These results indicate that natural antioxidants enhanced the activity of antioxidant enzymes in broilers.

Table 5 Effect of dietary addition of *Acacia nilotica* **on Antioxidant enzymes of breast muscle of broiler chicken**

Parameter	GROUPS					P. value
	T0 (control)	$T3 \t(3\% \tA)$	T6 $(6\%$ A_{\cdot}	T9 (9% \boldsymbol{A} .	\Box T12 (12% A.	
		<i>nilotica</i> Pod)	<i>nilotica</i> Pod)	<i>nilotica</i> Pod)	<i>nilotica</i> Pod)	
MDA	1.99 ± 0.37 ^a	1.90 ± 0.24 ^a	1.65 ± 0.27 ^{ab}	1.40 ± 0.25 ^{ab}	1.23 ± 0.31^b	0.0103
SOD	70.29 ± 8.58 ^{ab}	$69.48 \pm 8.61^{\rm b}$	70.92 ± 9.11 ^{ab}	82.26 ± 11.40 ^{ab}	91.60 \pm 11.08 ^a	0.0226
$abc + c$	$\mathbf{1} \cdot \mathbf{0}$ \cdot \cdot \sim \cdot \cdot \sqrt{D} \wedge \wedge \wedge $\mathbf{1}$ $\mathbf{1}$ $\mathbf{1}$ $\mathbf{1}$ \blacksquare					

abc Mean values in the same row that do not share a common letter differ significantly ($P < 0.05$)

IV Discussions

Meat colour is considered the most important factor for consumers when assessing meat quality in the market. In our study the breast meat pigmentation analysis showed that the L^* value (lightness) of breast meat was significantly lower in broilers fed *A. nilotica* pods meal than T0 control group broilers (P<0.05). The decreased L* value (lightness) of the meat of supplemented broilers might be due to the lower intramuscular fat Content of this meat (Priolo et al., 2001). Our results also showed that inclusion of *A. nilotica* pods meal decreased fat and cholesterol in broiler chicken. The a* values (redness) and b* values (yellowness) of breast meat were non-significantly affected by inclusion of *A. nilotica* pods meal. However, the redness was numerically higher in breast meat of broiler fed 12% *A. nilotica* pods meal diet, which may be due to higher levels of haemoglobin in the tissue as found in present study. A decreased a* value, on the other hand, may be linked to decreased mineral availability in muscle tissue (Mancini & Hunt, 2005) and natural antioxidants like Acacia can attenuate these effects (Anosa & Okoro, 2011).

Lipid oxidation is believed to stimulate changes in protein structures and thus alter the water-holding capacity of the muscle (Stadtman & Berlett, 1997); loss of water can also lead to the loss of protein. Most of the water in meat is trapped between the myofibrils and the cell membrane, within the myofibrils and between the myofibrils. Proteins, especially the myofibrillar protein that holds and stores water, play a vital role in promoting the water-holding capacity of muscle; pH and protein oxidation also have a direct effect on the water-holding capacity of the muscle tissue (Huff-Lonergan & Steven, 2005). In the present study, the water holding capacity of breast muscle tissue was higher in broiler chickens fed *A. nilotica* pods meal and lower in the broiler chicken of T0 (control) group. The lower waterholding capacity might be due to the significantly higher oxidation and relatively lower pH of control chicken breast muscle. In addition, the variation in the water-holding capacity is to some extent due to differences in proteolysis and cell shrinkage and to the mobilisation of water to the extra cellular space (Huff-Lonergan & Steven, 2005).

The superoxide anions are responsible to initiate or to activate the free radicals which can react with biological molecule systems and bring tissue damage in meat (Pardini 1995). So these Superoxide anions are less in the amount in treated breast fillets that are scavenged by *Moringa oleifera* leaves extract (Sreelatha & Padma, 2009). The same result was found when *Moringa oleifera* was applied on packed raw beef and showed a non-significant result (Ahmad et al. 2015). Hazra et al. (2012) described that significantly reduce the cooking loss% in ground meat of buffalo when it is treated with 1.5% and 2% crude extract of *Moringa oleifera* leaves.

The antioxidant defence mechanism consists of both natural antioxidant enzymes present in the biological system and synthetic antioxidants (Sies, 1991). T-SOD is a well-known antioxidant enzyme McCord, (1979). In the present study, dietary supplementation with the phytochemicals curcumin and lutein enhanced T-SOD and TAOC activity. Lipid oxidation in meat associated with deterioration of flavor, color, odor, quality and nutritive value (Luciano et al., 2009). The extreme oxidation of lipids caused by high levels of free radicals is the main cause of oxidative stress; this oxidation, in turn, enhances the levels of malondialdehyde (MDA), which is the end product of lipid per-oxidation (Jung et al., 2012). In our study, lipid oxidation (as measured by MDA and SOD level) was significantly lower in the breast meat of broiler chicken fed *A. nilotica* pods. In addition, lipid oxidation tends to increase with longer frozen storage times and higher meat fat contents (Wood & Enser, 1997). Lipid and pigment oxidation in meat are closely associated, and supplementation with antioxidants can increase the color stability of the meat (Faustman $\&$ Cassens,

1990). The increased levels of lipid and protein oxidation might be due to the secretion of pro-oxidant and oxidative enzymes from various ruptured cellular organelles (Xia et al., 2009). Such ruptured organelles may reduce the function of muscle proteins and promote water losses in muscle tissue (Xiong, 2000). Lipid oxidation is determined by TBARS values (the analysis of reactive substances with thiobarbituric acid. A few numbers of research have been conducted to find out the antioxidant effect of *Moringa oleifera* leaves in diet, raw beef meat, beef burgers patties, pre-cooked patties. Feihrmann et al. (2017) reported that TBARS value is less (0.10 to 0.11mg/kg) in beef cubes immersed in *Moringa oleifera* water extract of different concentrations on the 7th day of vacuum-packed storage at 4^oC and in control group are high (0.14 mg/kg). In chicken meat patties peroxide value decreased as the concentration *Moringa oleifera* leaf powder increased and significantly delayed the process of oxidation when it is compared with control during storage. TBARS values were found to be less from 0.35 to 0.75 mg malonaldehyde (MDA) in mechanically debone breast meat dipped in pomegranate fruit juice phenolic and higher (0.51 to 1.07 mg) in without treated pomegranate fruit juice phenolic butthere was little difference in different level of treatment during chilling storage temperature at 4°C (Vaithiyanathan et al. 2011). Naveena et al. (2008) have testified that pomegranate juice (natural fruit antioxidant) and BHT (synthetic) have no significant difference in TBARS values but pomegranate rindpowder values were significantly different in chicken cooked patties. Bazargani-Gilani et al. (2015) also found similar results for pomegranate juice dipping and chitosan coating in chicken meat during refrigerated storage. Dietary inclusion of ground *Moringa oleifera* leaf meal also improves the shelf life of pork (Mukumbo, 2013) and incorporation of *Moringa oleifera* leaves extract to reduce lipid oxidation in uncooked meat and cooked meat patties to a much greater extent during chilled storage temperature (Muthukumar et al., 2014).

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