EFFECT OF *Vernonia amygdalina* EXTRACTS AND MEAL ON COLOUR, PHYSICOCHEMICAL PROPERTIES AND MICROBIAL LOAD OF BROILER MEAT

Adenike Bilikis Hameed[1] and Azeez Kehinde Tella[1]*

[1]Department of Animal Science Department, University of Ibadan, Ibadan.

ABSTRACT

Aims: This study was carried out to investigate the meat colour, physicochemical properties and microbial load of broiler meat fed *Vernonia amygdalina* extracts and meal.

Study Design: The experiment employed a complete randomized design; all data generated were subjected to analysis of variance, P=0.05.

Place and Duration of Study: The feeding trial of the study was carried out at the Teaching and Research Farm, University of Ibadan, Ibadan, Nigeria while meat quality attributes were evaluated at Animal Products and Processing Laboratory, Department of Animal Science of the same institution between June and September, 2016

Methodology: Twelve 8 weeks old broiler chickens with an average weight of 2.5±0.2kg used in this study were obtained from an experimental unit where they were assigned to four treatments. Treatment1 and treatment 2 were offered *Vernonia amygdalina* aqueous extract and ethanolic extract in drinking water respectively and leaf meal was added to the feed of treatment 3 in powdered form. Treatment 4 was offered water and feed without *Vernonia amygdalina* leaf meal or extracts. Meat produced from the carcass was refrigerated before being analyzed

Results: The result revealed that supplementation of VA extract for T1 and T2 gave better meat physicochemical parameter compared to control. However, pH ranged from 5.51-5.87 and cooking loss (29.84 -37.19) were not significantly (P>0.05) different among the treatments. T2 (ethanolic extract) had the highest extract release volume. T1 (aqueous extract) had the highest meat swelling capacity (227.62) and water holding capacity (70.33). T3 (leaf meal) showed a significantly (P<0.05) lower lightness (L*) and yellowness b* while T1 (Aqueous extract) had higher redness (a*). Total viable count (TVC) of treatment 2 was significantly lower compared to others.

E-mail address: tellakehinde96@gmail.com

Conclusion: The result of these findings showed that the use of the ethanolic extract of *Vernonia amygdalina* in water was able to inhibit microbial load and improve physicochemical properties of fresh meat compared to aqueous extract and VA leaf meal.

Keywords: *Vernonia amygdalina*, microbial load, physicochemical, plant extract

E-mail address: tellakehinde96@gmail.com

1. INTRODUCTION

Diet composition and feed play an important role in meat quality of broiler chicken. This can affect the chemical composition of meat to a greater or lesser extent. Materials added to diets for reason other than to supply nutrient are feed additives. For example antibiotics added at sub-therapeutic level in order to improve feed utilization by lowering the population of some unwanted microbes can be considered as feed additives (1). Economic benefit of feed additives is typically lower production cost as a result of an improvement in production efficiency. A feed additive is typically used in small quantities and is classified into both organic and synthetic in poultry production. The organic feed additives are the product derived from plants which are used in feeding animals to improve their performance (2), (3). In order to improve the utilization of feed and to reduce the use of synthetic products which have toxicological effects, there is an increase in the search for alternatives plant growth promoters such as *Moringa oleifera, Vernonia amygdalina.*etc.

Vernonia amygdalina(VA) is a shrub or small tree that grows throughout tropical Africa. It is popularly called bitter leaf because of its abundant bitter properties (4). The findings by (5) reported that the young leaves often preferred for human consumption, contain high cyanide (60.1mg 100^{-1}g DM) and tannin content (40.6 100^{-1}g) than older ones. Several research works have been documented on the use of *V. amygdalina* as a treatment for coccidiosis and bacterial infections in poultry among which is the use of *V. amygdalina* leaf extract to treat coccidiosis *(*6)(7), the extract from the leaf used to treat *bacilliary* white diarrhea and *bronchitis* (8). Furthermore, *VA* meal has also been fed to broilers, where it was able to replace 300g kg^{-1} of maize-based diet without negative effect on feed intake, body weight gain and feed efficiency (9). The use *o*f *VA* in poultry production as feed/ diet replacement and treatment of various diseases have been documented**.** However, research on quality evaluation of meat produced from its usage is still undermined. This study was conducted to assess the effect of *VA* leaf meal and extract on colour, physicochemical properties and microbial load on broiler meat.

2. MATERIAL AND METHODS

2.1 Experimental site

This study was carried out at the Teaching and Research Farm University of Ibadan, Ibadan and lasted for eight weeks.

2.2 Preparation of *Vernonia amygdalina* Samples

2.2.1 Preparation of *Vernonia amygdalina* leave meal

Fresh leaves of *V. amygdalina* were collected from a farm at Moniya area of Ibadan, Oyo State, Nigeria. The leaves collected per time were rinsed with distilled water and were air-dried for 14 days. The dried *V.*

E-mail address: tellakehinde96@gmail.com

amygdalina leaves were pulverized using a hammer mill and stored in an air-tight plastic container until required for usage.

2.2.2 Preparation of *Vernonia amygdalina* ethanolic extract

Ten kilograms of pulverized *V. amygdalina* was poured into a container, 2.5 litres absolute ethanol and 2.5 litres distilled water was added to make 50% ethanol and stirred properly using a glass rod to ensure proper mixing after which it was left for 72 hours with intermittent stirring every 12 hours. After 72 hours, the solution was sieved with a muslin cloth after which it was concentrated using a rotary evaporator.

2.2.3 Preparation of *Vernonia amygdalina* aqueous extract

Ten kilogram of pulverized *V. amygdalina* was poured into a container, five litres of distilled water was added and stirred properly using a glass rod and left for 72 hours with intermittent stirring every 12 hours. The mixture was then sieved with a muslin cloth after which it was concentrated using a rotary evaporator.

2.3 Preparation of Experimental chicken

Twenty-four broiler chickens of about 2.5± 0.2kg average live weight and 8 wks of age with six replicate per treatment were used in this study. The chickens were slaughtered using a sharp knife. Birds were bled for about 10 minutes then the chickens were defeathered, eviscerated, cleaned with water and chilled for 30 minutes before breast portion was removed and evaluated.

2.4 Physico-chemical parameters

2.4.1 pH

Meats from each sample (10g) were homogenized in 90 mL distilled water .The pH of homogenized samples was measured using a glass pH.

2.4.2 Extract released volume (ERV)

This was determined according to the method described by Jay (10). Twenty grams of sample was weighed and homogenized with 100mL of distilled water for 2 minutes using a blender (Mixer/grinder, India). The homogenate was poured directly into a funnel lined with Whatman N0 1 filter paper, which was folded thrice so as to make eight sections. The homogenate was allowed to seep between the folds and extract was collected in a 100mL graduated cylinder for 15minutes.

Interpretation of the reading

ERV (mL)	Meat Quality
>25mL	Good Quality
>20mL	incipient spoilage

E-mail address: tellakehinde96@gmail.com

<20mL spoiled meat

2.4.3 Meat swelling capacity (MSC)

This was determined according to Wierbicky *et al.*(11). Twenty five grams of sample was blended (VTLC Mixer/Grinder, India) with 100mL distilled water for 2 minutes. 35mL of the homogenate was taken and centrifuge at 2000rpm for 15 minutes (Bosch, UK). The volume of the supernatant (S) was measured using a graduated cylinder. Meat swelling capacity was determined using the formula below.

$$\%\text{meat swelling} = \frac{(35 - S - 7) \times 100}{7}$$

2.4.4 Water holding capacity (WHC)

The water-holding capacity (WHC) was determined by the method of Zayas *et al.*(12) which was calculated as follows:

$$WHC = 1 - \frac{\text{(Meat film area)}}{\text{Area of spread juice}}$$

An intact sample was pressed between 2 filter papers with a plexi glass for over 1 minute using a table device. The amount of juice released from the sample was measured indirectly by measuring the area of the filter paper wetted relative to the area of pressed sample.

2.4.5 Cooking loss

This was determined according to the method described by Mahendrakar and Dam (13). Broiler meat sample was weighed and placed in boiling water for 20mins. Samples were allowed to cool before weighing.

$$\text{Cooking loss\%} = \frac{\text{weight of sample before cooking} - \text{weight of sample after cooking}}{\text{Weight of sample before cooking}} \times 100$$

2.4.6 Colour evaluation

Colour of the chicken meat samples was evaluated using Chromameter Minolta CR-100 Tristimulus Colour Analyzer, which gave CIELAB colour evaluation in the form of lightness (L*), redness (a*) and yellowness (b*). Three random measurements per sample were taken. The colorimeter was calibrated by using a standard white ceramic plate prior to colour measurement.

2.5 Microbial Analysis

Culture media: Potato Dextrose Agar (PDA), Total Plate Count and EMB for fungi, mould and yeast, total viable and Coliform count respectively were determined as recommended by the America public health association for food-stuff examination (14).

E-mail address: tellakehinde96@gmail.com

2.6 Experimental Design

Complete randomized design was employed. The data collected were subjected to analysis of variance (ANOVA) using SAS v. 9.3 (2011) package where significant differences were found at 5% level of significance. The means were compared using Duncan Multiple Range Test of the same software [15].

3. RESULTS AND DISCUSSION

3.1 Colour of broiler meat fed V*ernonia amygdalina* leaf meal and extract

Table 1 showed the effect of V*ernonia amygdalina* leaf meal and extract on the colour of the meat. Colour is an important quality attribute that influences consumer acceptance of many food products, including poultry meat. The mMeat of broilers fed control diet was significantly lighter than treatment 1, 2 and 3 with treatment 3 having the lowest mean value (58.19). This contradicts the result obtained by (16) that chicken breast meat, as well as pork, with low pH had higher reflectance and light scattering and appeared lighter. However, treatment 1 appears darker than treatment 4 because meat with high pH had a weaker light scattering and higher transmittance into its depth and across individual muscle fibres and appeared darker. Treatment 3 with leaf meal was significantly lower compared to other treatments.

Table 1: Effect of *Vernonia amygdalina* leaf meal and extract on the colour of the broiler breast meat

	T1	T2	T3	T4	SEM
L*	60.28^{c}	64.28^{b}	58.19^{d}	67.09^{a}	1.05
a*	18.94^{a}	7.78^{d}	11.20^{b}	8.82^{c}	1.31
b*	10.09^{b}	7.76^{c}	10.38^{a}	10.44^{a}	0.33

^{a,b,c,d} Means along the same row with superscripts are significantly ($P<0.05$)different.

T1: Aqueous extract, T2: Ethanolic extract, T3: Leaf meal, T4: Control

L*: lightness, a*: redness, b*: yellowness

3.2 Physicochemical properties of broiler meat fed V*ernonia amygdalina* leaf meal and extract

E-mail address: tellakehinde96@gmail.com

Extract Release Volume (ERV) determination is helpful in detecting the incipient spoilage of meat. It refers to the aqueous release in meat homogenate when it is filtered through filter paper over a period of time. Fresh meat of good organoleptic quality with a relatively low bacteria number releases large volumes of extract. The mean value ranged from 34.97 to 61.11 mL which were above 25mL required for good quality meat. However, the mean value of treatment 2 (61.11mL) was significantly higher than treatments 1, 3 and 4. This might be due to the use of ethanol for the extraction which contains higher phytochemical constituents after the extraction. It can be deduced that the result of this study is inversely related to the microbial load. This was not in agreement with the findings of (17) where the results of the extract release volume observed were not significant.

Meat Swelling Capacity: Mean value ranged between 180 and 227. This could be as a result of a reduction in bioactive ingredient present in V*ernonia amygdalina* which in turn affect meat quality. However, there is a linear correlation between meat swelling capacity and pH which indicate the increasing rate of deterioration of the meat. The result of this study is in accordance with the findings of (18).

Cooking loss (Table 2) showed no significant differences amidst treatment with the mean value ranging from 29.84 to 37.19. These results contradict the findings of (17) where the broiler diet was supplemented with onion and garlic.

Table 2: The effect of *Vernonia amygdalina* leaf meal and extract on physicochemical quality of broiler meat

Parameters	T1	T2	T3	T4	SEM
pH	5.80	5.57	5.51	5.70	0.22
Extract release volume (mL)	34.87^{b}	61.11^{a}	40.67^{ab}	46.89^{ab}	9.16
Meat swelling capacity (%)	227.62^{a}	210.47^{ab}	180.79^{c}	194.82^{bc}	6.58
Cooking loss (%)	30.90	29.84	30.84	37.19	1.54
Water holding capacity (%)	70.33^{a}	57.67^{b}	47.33^{b}	55.33^{b}	2.86

^{a,b,c,d} Means along the same row and column with superscripts are significantly ($P<0.05$)different.

T1: Aqueous extract, T2: Ethanolic extract, T3: Leaf meal, T4: Control

E-mail address: tellakehinde96@gmail.com

Water holding capacity: The water holding capacity is the capacity of muscle and meat products to keep the water-bound under specific processing conditions (19). The reduction in WHC of *Vernonia amagnalina* samples might be due to lower pH and this drop in pH may be responsible for an overall reduction in reactive groups of proteins available for water-holding (20). The extent and rate of pH fall post mortem affect the water holding capacity (19), with a positive correlation between these attributes registered in several studies (21); (22). Water holding capacity data obtained ranged between 47.33 - 70.33.

3.3 Microbial analysis of broiler meat fed V*ernonia amygdalina* leaf meal and extract

Microbial growth could deteriorate the meat and meat product quality. Figure 1 showed the mean value obtained for the total viable count; it was within a range of low level 0.1-7.3 log CFU/g in stored fresh meat. Total viable count of treatment 2 is significantly lower (p≤0.0.5) compared to other treatments. The low microbial levels could be due to the fact that microbial growth is inhibited at low activity water (23),(24) which is also revealed by treatment 2 high extract release volume. Extract from ethanol also has higher

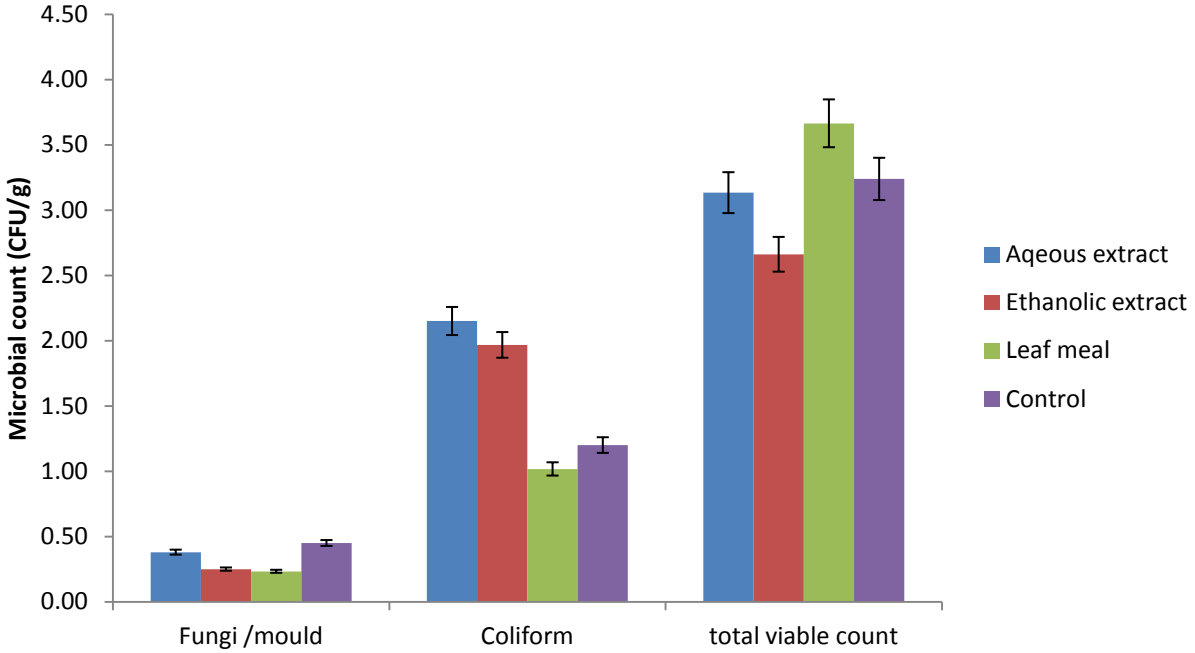

Figure 1: Effect of *Vernonia amygdalina* leaf meal and extract on microbial analysis of broiler breast meat

photochemical yield compared to aqueous extraction which may also influence microbial inhibition. Though *Vernonia amygdalina* leaf meal and extracts contain antibacterial and antifungal properties that are capable of reducing or inhibit microbial growth, the result of this finding contradict microbial status findings of (25).

E-mail address: tellakehinde96@gmail.com

4.0 CONCLUSION

In conclusion, the result of the present study showed that VA extract could inhibit microbial load and improve meat physicochemical properties compared with leaf meal. However, ethanolic extract of VA in drinking water of broiler chickens proved to be more effective compared to aqueous extract. The total viable count of bacteria which is critical to spoilage of meat was significantly lower in broiler meat with ethanolic extract compared with aqueous extract.

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E-mail address: tellakehinde96@gmail.com

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E-mail address: tellakehinde96@gmail.com

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E-mail address: tellakehinde96@gmail.com