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

5 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.

8 Study Design: The experiment employed a complete randomized design; all data generated were
 9 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 was-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 <u>analysedanalyzed</u>

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 (aqeousaqueous extract) had the highest meat swelling capacity (227.62) and Water water holding capacity (70.33). T3 (leaf meal) showed a significantly (P<0.05) lower lightness (L*) and yellowness b* while T1 (AqueesAqueous extract) had higher redness (a*). Total viable count (TVC) of treatment 2 was significantly lower compared to others.

Conclusion: The result of these findings showed that the use of <u>the</u> ethanolic extract of *Vernonia amygdalina* in water was able to inhibit microbial load and improve physico-chemical properties of fresh
 meat compared to aqueusagueous extract and VA leaf meal.

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

31 1. INTRODUCTION

32 Diet composition and feed plays an important role in meat quality of broiler chicken. This can affect the 33 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-sub-therapeutic level in order 34 35 to improve feed utilization by lowering the population of some unwanted microbes can be considered as 36 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 37 into both organic and synthetic in poultry production. The organic feed additives are the product derived 38 from plants which are used in feeding animals to improve their performance (2), (3). In order to improve 39 the utilization of feed and to reduce the use of synthetic products which have toxicological effects, there is 40 41 an increase in the search for alternatives plant growth promoters such as Moringa oleifera, Vernonia 42 amygdalina., etc.

Vernonia amygdalina (VA) is a shrub or small tree that grows throughout tropical Africa. It is popularly 43 called bitter leaf because of its abundant bitter properties (4). The findings by (5) reported that the young 44 leaves often preferred for human consumption, contain high cyanide (60.1mg 100⁻¹g DM) and tannin 45 content (40.6 100^{-1} g) than older ones. Several research works have been documented on the use of V. 46 47 amygdalina as a treatment for coccidiosis and bacterial infections in poultry among which are is the use of 48 V. amygdalina leaf extract to treat coccidiosis (6), the extract from the leaf used to treat bacilliary white diarrhea and brochitis bronchitis (7). Furthermore, Vernonia amygdalina meal has also been fed to 49 broilers, where it was able to replace 300g kg¹ of maize-based diet without negative effect on feed intake, 50 body weight gain and feed efficiency (8). The use of Vernonia amygdalina in poultry production as feed/ 51 52 diet replacement and treatment of various diseases have been documented. However, research on 53 quality evaluation of meat produced from its usage is still undermined. This study was conducted to 54 assess the effect of VA leaf meal and extract on colour, physicochemical properties, and microbial load 55 on broiler meat.

57 2. MATERIAL AND METHODS

58 2.1 Experimental site

59 This study was carried out at the Teaching and Research Farm University of Ibadan, Ibadan and lasted 60 for eight weeks.

61 2.2 Preparation of Vernonia amygdalina Samples

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63 2.2.1 Preparation of Vernonia amygdalina leave meal

64 Fresh leaves of *V. amygdalina* were collected from a farm at Moniya area of Ibadan, Oyo State, Nigeria.

65 The leaves collected per time were rinsed with distilled water and were air-dried for 14 days. The dried V.

Comment [DM1]: Once the full scientific name is written, please write the abbreviated form thereafter 66 amygdalina leaves were pulverized using a hammer mill and stored in an air-tight plastic container until

67 required for <u>usedusage</u>.

68 2.2.2 Preparation of Vernonia amygdalina ethanolic extract

69 Ten kilograms of pulverized V. amygdalina was poured into a container, 2.5 litres absolute ethanol and

70 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.

73 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

77 evaporator.

78

79 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 <u>a</u>_sharp knife. Birds were bled for about 10 minutes then the chickens were de_feathered, eviscerated, cleaned with water, and chilled for 30 minutes before breast portion was removed and evaluated.

84

85 2.4 Physico-chemical parameters

86 <u>2.4.1 pH</u>

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

89

90 2.4.2 Extract released volume (ERV)

This was determined according to the method described by (9). Twenty grams of sample was weighed and homogenized with 100mL of distilled water for 2 minutes using a blender (Mixer/grinder, India). The

93 homogenate was poured directly into a funnel lined with whatman-Whatman No-No. 1 filter paper, which

94 was folded thrice so as to make eight sections. The homogenate was allowed to seep between the folds

Meat Quality

Good Quality

incipient spoilage

- 95 and extract was collected in a 100mL graduated cylinder for 15minutes.
- 96 Interpretation of the reading
- 97 ERV (mL)
- 98 >25mL
- 99 >20mL
- 100 <20mL spoiled meat

Comment [DM2]: Please write the surname of first author followed by et al. In italics instead of simply writing method (8) or (9)

101 2.4.3 Meat swelling capacity (MSC)

102 This was determined according to (10). Twenty five grams of sample was blended (VTLC Mixer/Grinder, 103 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 104 105 graduated cylinder. Meat swelling capacity was determined using the formula below. 106 %meat swelling = (35 – S-7) x 100 107 7 108 2.4.4 Water holding capacity (WHC) 109 The water-holding capacity (WHC) was determined by the method of (11) which was calculated as 110 follows: 111 WHC = 1 -(Meat film area) 112 Area of spread juice 113 An intact sample was pressed between 2 filter papers with a plexi glass for over 1 minute using a table 114 device. The amount of juice released from the sample was measured indirectly by measuring the area of 115 the filter paper wetted relative to the area of pressed sample. 116 2.4.5 Cooking loss This was determined according to the method described by (12). Broiler meat sample were was weighed 117 118 and placed in boiling water for 20mins. Samples were allowed to cool before weighing. 119 120 Cooking loss%= weight of sample before cooking – weight of sample after cooking x 100 121 Weight of sample before cooking 122 2.4.6 Colour evaluation 123 124 Colour of the chicken meat samples was evaluated using Chromameter Minolta CR-100 Tristimulus 125 Colour Analyzer, which gave CIELAB colour evaluation in the form of lightness (L*), redness (a*) and 126 yellowness (b*). Three random measurements per sample were taken. The colorimeter was calibrated by 127 using a standard white ceramic plate prior to colour measurement. 128 2.5 Microbial Analysis 129 Culture media: Potato Dextrose Agar (PDA), Total Plate Count and EMB for fungi, mould, and yeast, total 130 131 viable and Coliform count, respectively were determined as recommended by the America public health 132 association for food-food-stuff examination (13). 133 3. **RESULTS AND DISCUSSION** 134

Comment [DM3]: A section (2.6) mentioning the statistical function must be added n the Materials and Methods section depicting the applied statistical method

135 3.1 Colour of broiler meat-meat-fed Vernonia amygdalina leaf meal and extract

136 Table 1 showed the effect of Vernonia amygdalina leaf meal and extract on the colour of the meat. Colour 137 is an important quality attribute that influences consumer acceptance of many food products, including poultry meat. Meat-The meat of broilers fed control diet was significantly lighter than treatment 1, 2, and 3 138 with treatment 3 having the lowest mean value (58.19). This contradicts the result obtained by (14) that 139 140 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 141 a weaker light scattering and higher transmittance into its depth and across individual muscle fibers and 142 appeared darker. Treatment 3 with leaf meal was significantly lower compared to other treatments. 143

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

	T1	T2	Т3	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.

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

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

149 3.2 Physicochemical properties of broiler meat-meat-fed Vernonia amygdalina leaf 150 meal and extract

151 Extract Release Volume (ERV) determination is helpful in detecting the incipient spoilage of meat. It 152 refers to the aqueous release in meat homogenate when it is filtered through filter paper over a period of 153 time. Fresh meat of good organoleptic quality with a relatively low bacteria number releases large 154 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 155 treatments 1, 3, and 4. This might be due to the use of ethanol for the extraction which contains higher 156 157 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 (15) where the results of the 158 159 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 Vernonia amygdalina which in turn affect meat quality.
 However, there is a linear correlation between meat swelling capacity and pH which indicate the

163 increasing rate of deterioration of the meat. The result of this study is in accordance with the findings of (16). 164

Cooking loss (Table 2) showed no significant differences amidst treatment with the mean value ranging 165 166 from 29.84 to 37.19. These results contradict the findings of (15) where the broiler diet were-was 167 supplemented with onion and garlic.

168 Table 2: The effect of Vernonia amygdalina leaf meal and extract on physicochemical 169 quality of broiler meat

Parameters	T1	T2	T3	T4	SEM	Comment [DM4]: What is SE Please mention in the footnot
pH	5.80	5.57	5.51	5.70	0.22	
Extract	34.87 ^b	61.11 ^a	40.67 ^{ab}	46.89 ^{ab}	9.16	
release				\sim		
volume (mL)						
Meat swelling	227.62 ^a	210.47 ^{ab}	180.79 ^c	194.82 ^{bc}	6.58	
capacity (%)						
Cooking loss	30.90	29.84	30.84	37.19	1.54	
(%)						
Water holding	70.33 ^a	57.67 ^b	47.33 ^b	55.33 ^b	2.86	
capacity (%)						

EM in Table 2? e of Table

170 ^{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 171

172 Water holding capacity: The water holding capacity is the capacity of muscle and meat products to keep 173 the water-water-bound under specific processing conditions (17). The reduction in WHC of Vernonia 174 amagnalina samples might be due to lower pH and this drop in pH may be responsible for an overall 175 reduction in reactive groups of proteins available for water-holding (18). The extent and rate of pH fall 176 post mortem affect the water holding capacity (18), with a positive correlation between these attributes 177 registered in several studies (19); (20). Water holding capacity data obtained ranged between 47.33-178 70.33.

179 3.3 Microbial analysis of broiler meat fed Vernonia amygdalina leaf meal and extract

Comment [DM5]: No information regarding Minimum Inhibitory Concentration (MIC) is given in this study

180 Microbial growth could deteriorate the meat and meat product quality. Figure 1 showed the mean value 181 obtained for the total viable count; it was within a range of low level 0.1-7.3 log CFU/g in stored fresh 182 meat. Total viable count of treatment 2 is significantly lower (p≤0.0.5) compared to other treatments. The 183 low microbial -levels could be due to the fact that microbial growth is inhibited at low activity water (21), (



184 22) which is also revealed by treatment 2 high extract release volume. Extract from ethanol also has 185 higher



Coliform

total viable count

photochemical yield compared to <u>aquousaqueous</u> 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 contradicts
microbial status findings of (23).

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186

194 4.0 CONCLUSION

0.50

Fungi /mould

The result of these findings showed that the use of ethanolic extract of *Vernonia amygdalina* in drinking water of broiler chicken was able to inhibit microbial load and improve physico-chemical properties of fresh meat compared to aquous extract and VA leaf meal.

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199 5. RECOMMENDATION

200 It could therefore be recommended that supplementing broiler chicken diets with VA could improve201 acceptability of

202 203

Comment [DM6]: What is Y-Axis in this

Figure

Comment [DM7]: The conclusion must be comprehensive and elaborative with more focus on future perspectives

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