Evaluation of poultry by-product meal treated with ginger (Zingiber officinale (L) Rosc)

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Abstract
This study was aimed at investigating the effect of ginger as natural antioxidant (NA) treatment in stabilizing poultry by-product meal. Poultry by-products meal (PBPM) were randomly prepared into four treatment groups (T1, T2, T3 and T4) and treated with 0, 750, 1250 and 1750 gram of ginger/100kg respectively and stored for 60 days. Microbial assessment and oxidative properties of PBPM was estimated fortnightly using total aerobic count (TAPC), total coli-form unit count (TCC), peroxide value (POV) and thiobarbituric acid (TBA) test. Poultry by-product meal treated with 1750 gram of ginger/100kg presented the least oxidation for both TBAs (0.064 mg/g) and POV (0.4 mEq/kg) after the 60 days storage period. Both TAPC and TCC for all samples observed in this study are all below satisfactory levels with reference to the standard microbial load specification. It is concluded that ginger rhizome used as a natural antioxidant was effective in stabilizing poultry by-product meal as it presented better microbial and antioxidant status at 1750 g/100kg.

Keywords: Poultry by-product, ginger, antioxidant

Introduction
The cost of feed ingredients is increasing day by day due to their shortage in supply. Among feed ingredients, protein and energy-rich ingredients of poultry feeds are the most expensive. Protein is supplied both from vegetable and animal sources, such as oilseed meals, legumes, abattoir waste, poultry by product meal (PBPM), fish etc. Plants are still the main source of different poultry feed nutrients, but the accelerated pace of livestock growth cannot be maintained at the current crop production volume worldwide. Therefore, exploring and utilizing feed resources that have high protein value for poultry and are not necessarily consumed by humans is important. In this regard, the use of unconventional poultry feed sources may be a solution. Unconventional feed ingredients include synthetic single cell proteins, different animal processing by-products, such as poultry by-product meal (PBPM), and insects (fly larvae). Since 1950, PBPM has been known to be a rich protein and fat source (Kirkland and Fuller, 1971). Poultry by-product meal is also called poultry by-products, poultry offal meal, poultry meal, poultry offal, poultry viscera meal, poultry slaughterhouse waste, chicken offal, chicken by-product meal, hen meal and spent hen meal (Heuze et. al., 2015). It is made by combining the by-products coming from poultry slaughterhouses or poultry processing plants. The AAFCO (USA) defines poultry by-product meal as the ground, rendered, clean parts of the carcass of slaughtered poultry such as necks, heads, feet, undeveloped eggs, gizzards and intestines (provided their content is removed), exclusive of feathers (except in such amounts as might occur unavoidably in good processing practices) (AAFCO cited by Watson, 2006) when subjected to rendering process, i.e., sterilization, dehydration, and re-sizing (Miller, 1996), the material is then dried, fat and protein are
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separated by heating at 140°C (Ockerman and Hansen, 1988), resulting in PBPM after skimming off the fat (AAFCO, 2004). Its inclusion in feeds not only reduces feed cost but also the environment burden of its disposal (FAO, 2011). The inclusion of animal by products in poultry diet affects the bird performance (Caires, et al., 2010; Silva, et al., 2014). The global production of PBPM is expected to double by 2050 (Boushy and Poel, 2000). Therefore, categorizing PBPM as a major feed ingredient (Alex, 2006) may open new dimensions in the animal feed industry in the future. Unfortunately, heat, pressure, mechanical grinding, and mixing during rendering of poultry waste accelerate the process of oxidative degradation of lipids (Wang, 1997). Deterioration due to oxidation of unsaturated fats reduces the quality of PBPM. Therefore, the main obstacle for the use of PBPM in animal feeds is its limited shelf life due to spoilage during storage. Oxidized PBPM not only affects its nutritive value (Pesti, et. al., 2002), but also broiler health (Dibner, 1996), impairing growth performance, reducing hematocrit values and increase intestinal lining sloughing. Oxidized PBPM also produces antigenic stimulation, which result in lymphocyte proliferation. Rancidity reduces PBPM essential fatty acid contents in just six weeks (Kirkland and Fuller, 1971). Antioxidants are the best choice to delay the onset of oxidation by preventing the formation of free radicals. Free radicals oxidize fats, and antioxidants stop the reaction by donating the hydrogen atoms (Sies, 1997). The most commonly applied antioxidants are synthetic phenols, such as, butylated hydroxyl toluene and butylated hydroxyl anisole (BHA). Their safety, however, is doubtful (Imadia, et al., 1983). The initiation of the lipid peroxidation is by the superoxide radical or by hydroxyl radicals. For this reason, antioxidation is an extremely activity, which can be used as a preventive agent against a number of diseases (Basaga, 1990; Halliwell and Chirico, 1993; Aruoma, 1994). Therefore, attention should be focused on natural antioxidants. These antioxidants are polyphenol compounds (Helle and Grete, 1995, as cited by Yen, et al., 2003), which are found in different parts of the plants (tree bark, stalks, leaves, fruits, roots, flowers, pods and seeds) (Aruoma, et al., 1995; Kim and Heo, 1997). Ginger (Zingiber officinale (L.) Rosc) has been used as a spice for over 2000 years (Bartley and Jacobs, 2000). Its rhizome and the obtained extracts contain polyphenol compounds (6- gingerol and its derivatives), which have a high antioxidant activity (Chen, et. al., 1986, Herrmann, 1994). Regardless of the numerous studies dedicated to this area, comprehensive investigations of the antioxidant properties of ginger are not available in the literature. The aim of this study is to assess the effect of ginger in enhancing the oxidative stability of PBPM with regard to the free radical 2, 2-diphenyl-1-picryl hydrazyl, lipid per-oxidation and pro-oxidant activity. The objectives of the study are to compare PBPM treated with graded levels of ginger on spoilage characteristics and also to evaluate the oxidative properties of the treated poultry by-product meal

Materials and Methods

Experimental site
This study was conducted at the Teaching and Research farm, Animal Science Department, Kaduna State University (KASU), Kafanchan campus, Kaduna state. Kafanchan is located at latitude 9°59'N, longitude 8°29'E and it is situated at an elevation of 733m above sea level (World atlas, 2018). The laboratory analysis was carried out at the multipurpose laboratory, Department of Biochemistry
and Public Health Laboratory, Faculty of Veterinary Medicine, ABU Zaria, Nigeria. Zaria, is located on latitude 11°11N and longitude 07°38E. It is situated at an altitude of 686m above sea level (GPS, 2018).

**Experimental layout and sample preparation**

Poultry by-product meal was sourced from various slaughter houses within Kaduna metropolis and its environs. The gathered poultry meals were then homogenized. The experimentally rendered poultry by-products (PBPs) were processed at the Animal Science Laboratory of KASU. The PBPs were divided into four groups and treated with ≈ 0, 750, 1250 and 1750 gram of ginger/100kg, in a completely randomised design. The treatment groups consisted in mixing 50% of the total dose of the natural antioxidant (fresh ginger rhizomes) with the PBPs before cooking and the remaining 50% was added after cooking. The sample mixtures that were prepared are presented in Table 1. During cooking, the PBPs were heated to ≈ 130°C, with only half of the total dose mixed before cooking to prevent the loss of antioxidant. The reason for applying half the dose before cooking is to avoid oxidation of PBP during cooking. This method increases the efficacy of the antioxidant. The cooked PBPs were allowed to drip dry for about 20 min. and mixed with the second dose of ginger (50%) before mincing using pistol and mortar. The minced products were subsequently oven dried on labelled trays for 6-8 h at 70°C to a moisture content of 5-10%. The PBPs were allowed to cool before been resized to powdered form using a hammer mill and then aseptically packaged in labelled plastic containers from were samples were drawn for subsequent analysis. The oxidative stability of the poultry by product meals were evaluated by determining their peroxide values (POV) and thiobarbituric acid values (TBAV) on day 1 and then monthly during the 60-day storage period.

### Table 1: Levels of ginger (natural anti-oxidant) incorporated in poultry by-product meal

<table>
<thead>
<tr>
<th>Treatment</th>
<th>NAO</th>
<th>Mixing procedure</th>
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<tbody>
<tr>
<td>T1</td>
<td>0</td>
<td>Negative control</td>
</tr>
<tr>
<td>T2</td>
<td>750</td>
<td>375g before cooking + 375g after cooking</td>
</tr>
<tr>
<td>T3</td>
<td>1250</td>
<td>625g before cooking + 625g after cooking</td>
</tr>
<tr>
<td>T4</td>
<td>1750</td>
<td>875g before cooking + 875g after cooking</td>
</tr>
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NAO: natural anti-oxidant

**Laboratory analysis**

The oxidative stability of PBPM batches were analyzed for POV and TBAV on days 1, 30, and 60 of storage according to IUPAC (1992). Peroxides are the primary products of fat and oil oxidation. Peroxide value was calculated according to Horwitz, et al. (2002), and expressed in mEq of peroxide/kg of oil. $POV = S \times M \times 1000/\text{weight of the sample in grams}$, where $S = \text{mL of Na}_2\text{S}_2\text{O}_3$, and $M = 0.01$, the concentration of the Na$_2$S$_2$O$_3$, solution, $POV$ (mEq/kg) = volume of sodium thiosalphte $\times$ molarity of thiosulfate $\times$ 1000/sample weight.

Determination of thiobarbituric acid value involves primarily a reaction of TBA with MDA by heating in the presence of a strong acid to give a red coloration. The TBA analysis was performed directly by heating the sample with TBA, followed by extraction of the coloured complex on a portion of the steam distillate of lipids obtained by aqueous or acid extraction from the samples. The result expressed as TBA
value (mg), which was calculated according to the formula: TBA value = 50 (absorbance of test solution - absorbance of reagent blank)/mass of test portion.

**Microbial analysis**

Microbiological analysis was performed to evaluate coliforms and total viable count in every batch of PBPM using the standard microbial load specification on animal food product (Wilson, *et al*., 1991) as a guide.

### Table 2: Standard microbial load specification on animal food product.

<table>
<thead>
<tr>
<th>Grades</th>
<th>TVC (total viable count)/g at 30°C</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>$\frac{1}{2}$ million</td>
<td>Satisfactory</td>
</tr>
<tr>
<td>II</td>
<td>$\frac{1}{2}$ million to &lt; 10 million</td>
<td>Passable</td>
</tr>
<tr>
<td>III</td>
<td>10 million and more</td>
<td>Unsatisfactory</td>
</tr>
</tbody>
</table>


### Statistical analysis

The study was designed as a completely randomized design (CRD), and data generated was subjected to analysis of variance (ANOVA). The differences among treatment means were compared using Duncan's Multiple Range (DMR) test (Duncan, 1955) using SAS 9.1 (2002-03). The significance level of $p<0.05$ was adopted.

### Results and discussion

Thiobarbituric acid value of poultry by-products treated with 0, 750, 1250 and 1750 gram of ginger/100 kg (T1, T2, T3 and T4) is shown in Figure 1. Treatment 1 had TBA values of 0.119, 0.081 and 0.067mg/g for day 1, day 30 and day 60, respectively. The highest initial value was recorded in Treatment 2 which had values of 0.141, 0.068 and 0.066mg/g for day 1, 30 and 60 respectively. The lowest value was recorded in Treatment 3; 0.116, 0.062 and 0.060 for the 60 days storage period. Treatment 4 had 0.122 in day 1, 0.065 and 0.060 for day 30 and 60 respectively. The results obtained from all samples shows a general decline in the TBARS values with increase in days of storage. The thiobarbituric acid value indicated that the samples (treated and untreated) were not rancid, as the fat oxidation are reported to be higher with elevated temperatures although can be reduced by addition of antioxidants (Pereira, *et al.* 1975), this could account for the higher values recorded across the treatments in day 1. The result of the present study does not agree with report by El-
Although the results obtained were not statistically (P>0.05) different, treatment 1 had the higher value (0.067mg/g) after 60 days storage. It is important to note that all values recorded in the current study were all below the acceptable limit of >1 mg MDA/Kg as reported by ES (2005). This oxidation parameter is equally far below levels associated with oil rancidity, and below TBAVs level (1 mg of MDA/kg of sample) associated with meat rancidity (Witte, et al., 1970; Ripoll, et al., 2011). This could also be because of the nature and proportion of the by-product (head, legs and emptied gastro intestinal track). Poultry by-product meal treated with ginger at 1250 and 1750g/kg had the lowest TBA (0.06mg/g) value.

Peroxide value (Figure 2) indicated that the control sample (T1), with no antioxidant inclusion had lower rancidity than samples treated with ginger as a natural antioxidant at day 1. The observed result is this study does not support report by Ahmad, et al., (2017) who reported significant decrease in POV of PBPM with increase in antioxidant application. This could be due to the method of processing employed in this study and/or the application of synthetic antioxidant which offered immediate free radicals to control/reduces the rancidity from the first day. Wang (1997), stated that; unfortunately, heat, pressure, mechanical grinding, and mixing during rendering of poultry waste accelerate the process of oxidative degradation of lipids. The natural antioxidant (ginger) used in this study is assumed to released its antioxidant properties (gingerols, shogaols, paradols etc.) over time as it was observed in this study with sample treated with 1750 gram of ginger/100kg (Treatment 4) having a remarkable drop in peroxide value over time (60 days).

The Total plate count for aerobic bacteria and coli-form unit count in poultry by-product meal is shown in table 3. All samples of PBPM were free from coli-forms at 1- and 30-days post-storage, the highest count (18*10³Cfu/g) was recorded in PBPM treated with 750NA g/kg. The total viable microbial count showed no relationship with the ginger treatment levels. This agrees with report by Ahmad, et al. (2016) who used a commercial antioxidant to stabilize animal by products. Both TAPC and TCC observed in this study are all below satisfactory levels with reference to the standard microbial load specification (Wilson, et al., 1991).
Table 3: Total aerobic plate count (TAPC) $10^6$cfu/g and total coli-form count (TCC) $10^6$cfu/g of untreated and treated PBPM

<table>
<thead>
<tr>
<th>Treatment</th>
<th>NAO (g/kg)</th>
<th>Analytical test</th>
<th>Storage Period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 1</td>
<td>Day 30</td>
</tr>
<tr>
<td>T1</td>
<td>0</td>
<td>TAPC 0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TCC 0</td>
<td>0</td>
</tr>
<tr>
<td>T2</td>
<td>750</td>
<td>TAPC 3</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TCC 0</td>
<td>3</td>
</tr>
<tr>
<td>T3</td>
<td>1250</td>
<td>TAPC 3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TCC 0</td>
<td>0</td>
</tr>
<tr>
<td>T4</td>
<td>1750</td>
<td>TAPC 1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TCC 0</td>
<td>0</td>
</tr>
</tbody>
</table>

NAO: national antioxidant, PBPM: poultry by-product meal

**Conclusion**
Based on the findings under the conditions of this study, poultry by-product Meal treated with 1750g/100kg of ginger rhizome as an antioxidant source presented minimum oxidative status. Therefore, ginger rhizome at 1750g/100kg can be used to stabilize poultry by-product meal as it presented better oxidative status at 60 days of storage.

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