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BOOK OF PROCEEDINGS

EFFECT OF KERATINASE PROCESSED FEATHER MEAL-BASED DIETS ON THE MEAT QUALITY CHARACTERISTICS OF BROILER CHICKEN.

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ABSTRACT

Effect of keratinase-processed feather meal-based diets on meat quality of broiler chicken was studied. Keratin- rich soils were collected from feather dumpsites to produce keratinase. White feathers were treated with keratinase and oven-dried to 10 % moisture-content, then milled into Untreated and Treated Feather meals, to formulate 22 % CP and 3,100 Kcal/KgME diets. 1,050 Ross 308 day-old broilers were allotted to seven treatments in a completely randomized design with six replicates per treatment and 25 birds per replicate, in deep litter system for 8 weeks, in a single phase. Treatment 1 (control), Treatments 2, 3 and 4 (8 %, 16 % and 24 %) treated feather meal and 5, 6 and 7 (8 %, 16 % and 24 %) untreated feather meal. Data were analyzed using SPSS, version 2006. Data obtained showed significant differences ($p<0.05$) in meat quality parameters. T1 had the highest cooking yield (71.89 %) while T6 had the least (55.28 %). Cooking loss was highest in T6 (44.72) and lowest in T1 (28.11 %). Highest water holding capacity was in T6 (1.39 %) while the least was in T7 (0.50 %). pH ranged from 6.24 (T3) to 5.96 (T1). Drip loss was highest in T4 (6.51 %) and lowest in T1 (4.08 %). The highest value for appearance (7.56) and flavour (7.64) were in T7 while the least values of 6.76 and 6.88 in T1. The study concluded that keratinase-treated feather meal-based diets improved water holding capacity, appearance and flavor at 24% and juiciness at 16% while untreated enhances pH.

Keywords: *Keratinase, Processed, Feather meal, Meat quality, Broiler chicken*

INTRODUCTION

Feeding has continued to remain the highest contributor to the high cost of inputs needed in poultry production with animal protein ingredients arguably the most expensive in the Nigerian market. In the midst of this trend, high volumes of feathers are produced both locally and globally. Broiler feathers have been reported to contain enormous amounts of crude protein which makes it a potential alternative for the expensive conventional protein concentrate feed (Ajayi and Iyayi, 2015). The over four billion pounds of poultry feathers produced globally, on annual basis, are mostly wasted. Land filling and the continuous burning of this waste by-product of poultry production that has a high carbon footprint, has over the years contributed in the growing emissions of greenhouse gasses responsible for global warming and soil degradation.

Feathers can be degraded to feather meal which can be used in animal feeds and as an organic fertilizer since it contains more than 80 % crude protein and is rich in hydrophobic and essential amino acids (Thyagarajan *et al.*, 2013). The Cur-

rent use of hydrothermal system of production of feather meal with excessive heat, results in the degradation of proteins and their essential amino acids. The product produced is poor in nutrient digestibility, low in biological value and poor in net protein utilization.

The chemical method of hydrolysis using acidic methods could be very hazardous. The use of alkali such as sodium hydroxide, might be too mild to be very effective in breaking the disulphide bonds of the feather. All the methods of treating feather to convert it into a more effective and useful feed ingredient are very expensive. Hence, the need for a less laborious, expensive, protein destructive and more environmentally friendly means of treating feathers. Ajayi and Akoma (2017) reported the beneficial role of enzymes in feather meal. They observed better carcass yield and primal cuts in broilers fed with feather meal with protease supplementation compared to those fed with hydrolyzed feather meal. However, there are no documented reports on the effect of keratinase-processed feather meal based diets on broiler meat quality parameters, the essence of which this research was conducted.

MATERIALS AND METHOD

Study Area

This study was carried out at the Department of Animal Production Teaching and Research Farm of the Federal University of Technology, Minna, Niger State in Nigeria. Minna lies between Latitudes 9°33' and 9°40' North of the Equator and Longitudes 6°29' and 6°35' East of the Greenwich Meridian (Idowu *et al.,* 2020). It falls within the southern guinea savanna agro-ecological zone of Nigeria. The mean rainfall varies from 1100 -1600 mm and mean temperature is between 210c and 350 (FMSN, 2020).

Sample collection

Keratin- rich soil samples were collected from abattoir, hair, horn dumpsite and poultry feather dumpsite in Bosso, Niger State. The samples were then placed in sample bottles, properly labeled and preserved at 4^0C. Chicken feathers were also collected and treated by washing and drying at 50^0C in a forced drought oven. The feathers were then grinded into fine fractions and stored at room temperature until needed for further use.

Isolation and identification of Keratinase-producing micro-organisms

For isolation, an enriched media was produced according to the procedure described by Paul *et al.* (2013). This was followed by the addition of nutrient agar for preparation of bacteria culture and potato dextrose agar for fungi culture preparation. Serial dilutions were then carried out and dilution factors of 10^{-6} were plated for both bacteria and fungi cultures using pour plate techniques. The bacteria plates were then incubated at 37^0C for 24 hours while the fungal plates were incubated at room temperature for 5 days.

Identification of bacteria and fungi

The isolation of bacteria was done based on cellular morphology, grams staining and biochemical tests (Raju *et al.,* 2013). Fungal isolates were defined and identified based on their macroscopic and microscopic characteristics. The identified pure cultures were then inoculated into slants for preservation and stored at 4^0C until required for use.

Enzyme production

The production of enzymes was done by introducing 1ml of each isolate into 250ml Erlenmeyer flask containing 100ml broth enriched with the following mineral salts (gram/100ml):

NH_4Cl – 0.05, NaCl – 0.05, K_2HPO_4 – 0.03, KH_2PO_4 – 0.04, $MgCl_2$ – 0.024, Yeast extract – 0.1, Raw feather – 1, pH -7.5. The flasks was then continuously shaken at 150rpm and incubated at 37^0C for 5 days. The 5-day culture was then centrifuged at 1000rpm for 10minutes at 45^0C. The supernatant was collected to determine the keratinase activity using spectrophotometer by reading the optical density at 280nm of uv-vis spectrophotometer against no hydrolysis mixture as a blank as described by Mazotto *et al.* (2011). An increase of 0.01 in the absorbance was considered as equivalent to 1 unit of enzyme activity per ml (Kumar *et al.,* 2014).

Preparation of Feather meal

Preparation of Untreated Feather meal

White feathers from broiler chickens were collected from the poultry slaughterhouse at the Kure International Market, Minna, Niger State. The feathers were then washed to remove contaminants like blood, sand and other visible dirt.

Thereafter, they were subjected to sun-drying until completely dried. A certain quantity was milled using a simple hammer mill and an attrition mill. The hammer mill was used for pre-crushing the feather and the attrition meal was used for grinding into a fine form. The product obtained was designated as Untreated Feather Meal (UTFM). The untreated feathermeal obtained was then subjected to proximate analysis at the Department of Animal Production Laboratory, Federal University of Technology, Minna. The analysis was carried out in line with the procedure of A.O.A.C. (2000).

Preparation of Enzyme-treated Feather meal

5kg of the broiler chickens white feather that has been washed and cleaned of visible contaminants was poured into a pH and temperature dependent bioreactor containing 50 litres of water. The mixture was pretreated with 50g sodium hydroxide and the bioreactor was agitated for 10minutes at a temperature of 40^0C to allow for even mixing. The feather was then rinsed with water until a steady pH of 7 was achieved. Thereafter, 5 litres of Keratinase enzyme was added to the mixture. The mixture was agitated again in the bioreactor for 30 – 40 minutes in order to achieve complete treatment of the feather. After this duration of treatment was attained, the bioreactor was then drained of any liquid and the feather left behind was rinsed again with water until a steady pH of 7 (neutral) was achieved. The feather was then oven-dried at a temperature of 80^0 C until a moisture content of 10 % was attained. The dried chicken feather was then milled by means of a simple hammer mill and an attrition mill. The hammer mill served to pre-crush the feather while the attrition mill was used for grinding the feather into a fine form. The product obtained was designated as Treated Feather Meal (TFM). The treated feather meal obtained was then subjected to proximate analysis at the Department of Animal Production Laboratory, Federal University of Technology, Minna. The analysis was carried out in line with procedure of A.O.A.C. (2000).

Management of experimental birds

The experimental birds were managed on a deep litter housing system. Before the arrival of the birds at the farm, the pens were washed and disinfected against parasite using Vinkokill® at 150 ml for each 20 litres of water used. Thereafter, the pens were allowed to completely dry after which sufficient quantity of wood shavings were evenly spread on the floor to a height of 30 cm to serve as bedding material for the birds. Drinkers and feeders were also thoroughly washed and made ready for use.

Upon arrival of the chicks, they were weighed individually to obtain their initial weights after which they were randomly allotted to the treatment groups. The birds were also given anti-stress (Vitalyte®) through their drinking water according to the manufacturer's instructions. Gumboro vaccine was also administered twice at the 7^{th} and 21^{st} days while Lasota vaccine was given on the 14^{th} and 28^{th} day. Two thousand doses of these vaccines were diluted in 20 litres of water into which a sachet of powdered peak milk has been added. The powdered milk served to neutralise the presence of any trace of chlorine in the water. The vaccine was then administered to the birds after the birds had been starved of water for 6 hours.

Experimental design and Procedure

A total of 1,050 Ross 308 day old broilers were purchased from a hatchery in Ibadan, Oyo State, Nigeria. The birds were acclimatized on a commercial starter diet for one week before being given the experimental diets. At the start of the experiment, the birds were weighed and randomly allocated into seven dietary treatments in a completely randomized design (CRD). Each of the treatments was replicated six times with twenty five birds per replicate. Treatment 1 served as the control with 0 % feather meal (FM), Treatments 2, 3 and 4 contained 8%, 16% and 24% treated Feather meal (TFM) while treatments 5, 6 and 7 contained 8%, 16% and 24% untreated feather meal (UTFM)

Experimental Diet

The diets used for the experiment were formulated in accordance with the recommendations of the NRC (1994). A single phase feeding regime was used for the experiment. The composition of the experimental diet is as shown on Table 1.

Meat Quality Evaluation

Determination of pH

pH of the cold carcass was determined using a pH meter. 10 g of meat samples was homogenized for two minutes with 90 mls of distilled water using a laboratory blender (plate 5mm) model 242, Nakal Japan. The meat suspension was filtered and the pH of the meat was then measured using a digital pH meter (model H18424 micro-computer, Hanna Instruments Romania)

Water holding capacity

The water holding capacity was measured according to the procedures described by Kauffman *et al.* (1992). A section of meat from breast muscles was cut, weighed and kept in a plastic container. Water holding capacity was determined by cutting a portion of meat (20 g) from the breast portion. The sample was then pressed using a screw jack until all the free water was expelled. The meat sample was then removed, unwrapped and re-weighed. The difference in weight of meat sample represented the weight of expelled fluid. This was then expressed as percentage of the initial sample weight and recorded as water holding capacity of the meat.

Cooking yield/loss

The meat from breast portions were cut and frozen at -4^0C. The meat was then thawed and the thawed weights were recorded before broiling. Broiling was done in an open gas oven. The racks were covered with perforated aluminum foils for ease of drainage; the oven was then preheated for 5 minutes, before loading in the samples which was then broiled to a temperature of 72^0C as measured with a skewer meat thermometer. The samples were then allowed to cool to room temperature, excess fluid were mopped using paper serviette and the weights were taken and recorded. The difference between the precooked weight and post-cooked weight was the cooking loss. While the cooking yield percentage was calculated as: Cooking yield % = (Cooked weight/ Thawed weight) × 100

Organoleptic quality of meat

Boiled meat from the breast portions were used for taste panel evaluation. A 30-member panel of semi-trained tasters drawn from the University Community was used for this evaluation. 10 g of meat boiled with a pinch of salt was served to the panelists. This evaluation was done according to the method described by Grunert *et al.* (2004) using a 9- point Hedonic scale ranked thus: 9= like extremely, 8= like very much, 7= like moderately, 6= like slightly, 5= neither like nor dislike, 4= dislike slightly, 3= dislike moderately, 2= dislike very much, 1= dislike extremely. The meat samples given to the panelists were evaluated for their organoleptic properties such as appearance, juiciness, taste, tenderness, flavour, aroma, texture and general acceptability. Bottled water was given to each panelist to rinse their mouth after tasting each sample in order to reduce carryover effects of taste from previous samples.

Data Analysis

Data collected on all parameters were analyzed using one-way analysis of variance (SPSS, 2006). Where there were significant differences ($p<0.05$), Duncan test for multiple comparisons was used to separate the treatment means.

RESULTS

pH, water holding capacity, cooking yield, cooking loss as affected by diets containing enzyme-treated and untreated feather meal

Table 2 shows the effect of enzyme-treated and untreated feather meal on pH, water holding capacity (WHC), cooking yield and cooking loss of broiler chicken meat. The result showed that the experimental diet significantly influenced ($p< 0.05$) these parameters across the treatments. T1 had the highest cooking yield (71.89 %). This was closely followed by T3 with a yield of 61.31 %. The least cooking yield of 55.28 % was recorded in the T6. Cooking loss was highest in T6 (44.72) and this was significantly different ($P<0.05$) from the control T1 (28.11 %). The best value for water holding capacity (WHC) was recorded in T6 (1.39 %) while the least value was recorded in T7 (0.50 %). pH was highest in T3 (6.24) while the least value was obtained from the control (5.96). Drip loss was highest at T4 (6.51 %) and lowest in the control (4.08 %).

Effects of enzyme-treated and untreated feather meal on the organoleptic properties of broiler chicken meat.

The results of the organoleptic properties of broiler chickens fed enzyme-treated and untreat-

Table 1: Ingredient Composition of the Experimental Diets

Ingredients	Control	Untreated feather meal			Treated feather meal		
	T1	T2	T3	T4	T5	T6	T7
Maize	58.00	57.20	59.55	60.00	57.20	59.55	60.00
Groundnut cake	20.40	11.30	17.30	17.40	11.30	17.30	17.40
Full fat Soybean meal	10.30	18.50	08.25	03.00	18.50	08.25	03. 00
Fish meal	03.00	02.80	02.00	02.00	02.80	02.00	02.00
Feather meal	-	02.20	04.40	06.60	02.20	04.40	06.60
Wheat offal	04.00	04.00	04.50	07.00	04.00	04.50	07.00
Bone meal	02.00	02.00	02.00	02.00	02.00	02.00	02.00
$CaC0_3$	01.00	01.00	01.00	01.00	01.00	01.00	01.00
Lysine	00.25	00.25	00.25	00.25	00.25	00.25	00.25
Methionine	00.25	00.25	00.25	00.25	00.25	00.25	00.25
Salt	00.25	00.25	00.25	00.25	00.25	00.25	00.25
Premix	00.25	00.25	00.25	00.25	00.25	00.25	00.25
Total	100	100	100	100	100	100	100
Calculated values							
Crude Protein (%)	22.01	22.01	22.01	22.02	22.01	22.01	22.02
Energy(Kcal/KgME)	3100.00	3108.60	3103.62	3118.68	3108.60	3103.62	3118.68
Crude fibre	03.50	03.42	03.34	03.34	03.42	03.34	03.34
Ether Extract	05.73	06.80	05.57	04.98	06.80	05.57	04.98
Calcium	01.11	01.40	01.63	01.93	01.40	01.63	01.93
Phosphorus	00.51	00.70	00.87	01.08	00.70	00.87	01.08
Lysine	01.10	01.18	01.05	00.98	01.18	01.05	00.98
Methionine	00.86	00.93	00.97	01.03	00.93	00.97	01.03

**Each 2.5kg contain vitamin. A – 10,000,000iu:, vitamin D-2,000,000iv, vitamin E- 20,000iv, vitamin k-2,250mg; thiamine – 17omg; Riboflavin – 5,000mg; Pyridoxine – 2,750mg; Niacin- 27,500mg; vit B12 -15mg; Pantothenic acid – 7,500mg; folic Acid – 7,500mg; Biotin – 50mg; manganese – 80g; zinc – 50g; copper – 5g; iodine 1.5g; selenium – 200mg and cobalt – 200mg*

KEY

T1= Formulated diet without feather meal, T2= diet containing 8% untreated feather meal, T3= diet containing 16% untreated feather meal, T4= diet containing 24% untreated feather meal, T5= diet containing 8% treated feather meal, T6= diet containing 16% treated feather meal, T7= diet containing 24% treated feather meal

ed feather meal is presented on Table 3. From the results, it was observed that the experimental diet had no significant influence ($P>0.05$) on the colour, juiciness, aroma, tenderness and overall acceptability of the broiler chicken meat. However, there were significant differences ($P<0.05$) in the appearance and flavor of the meat samples tasted. The highest values for appearance (7.56) and flavor (7.64) were recorded in birds fed 24 % enzyme-treated feather meal (T7) while the least values of 6.76 and 6.88 for appearance and flavour respectively were recorded in the control group (T1).

DISCUSSION

pH, water holding capacity, cooking yield, cooking loss, and drip loss of broiler chickens fed enzyme-treated and untreated feather meal

The cooking yield obtained from the current study agrees with the 65% cooking yield for poultry reported by Hertanto *et al.* (2017). Losses during cooking of meat samples have been linked with temperature-induced changes in meat proteins (Bruggemann *et al.*, 2010). The slightly higher cooking losses recorded in the meat from birds fed enzyme-treated feather meal diets might be as a result of the cooking temperatures of the meat.

Low WHC and pH have been linked with high degrees of post-mortem muscle protein denaturation and reduced protein solubility (Yin *et al.*, 2014). The influence of diet on WHC in chickens have also been reported (Jiang, 2009). The improved WHC recorded in T6 may have been as a result of reduced muscle protein denaturation in birds fed enzyme-treated feather meal. The enzyme-treated feather meal diets fed to the birds may have influenced the higher WHC values recorded in T6. Under the current study, higher cooking loss was greatly related to higher water holding capacity. The higher WHC and higher pH in T6 is in agreement with the finding of Mir *et al.* (2017) who reported that broiler breast meat with higher WHC tend to have a higher pH. Meat pH is an important indicator of meat quality which is closely linked with tenderness, water holding capacity, colour, juiciness and shelf life. Usually, when an animal is slaughtered, muscle cells undergo glycolysis anaerobically to obtain energy for other post mortem metabolic processes (Yin *et al.*, 2014). Consequently, glycogen reserves are broken down into lactic acid resulting in a drop of the pH values. The pH values obtained in the current study are within the normal pH range of 5.81-6.3 reported by Hertanto *et al.* (2017) for chickens. Drip loss shows the extent of loss of myofibers, water, iron and proteins as muscle changes to meat (Ponsuksili *et al.*, 2008). The drip loss values of meat samples in this study were slightly higher than those reported by Abdulla *et al.* (2017).

Organoleptic properties of broiler chickens fed enzyme-treated and untreated feather meal

Consumers' acceptability of meat products is based on organoleptic (sensory) qualities such as taste, juiciness, colour, juiciness and aroma (Schivazappa and Virgili, 2020). Consumer's first physical assessment of meat is on the basis of its appearance. This assessment basically depends on how attractive or otherwise the colour of the meat appears on sighting (Wideman *et al.*, 2016). The decision of the panelist to adjudge T7 as the meat sample with the best appearance might be due to the fact that the meat appeared fresher than other samples tasted. This agrees with the report by Kralik *et al.* (2017) that consumers associate the appearance of meat with its freshness and this promotes their desirability or otherwise for such product. The type of feed given to broiler chickens during the rearing period have been suggested to contribute to the ap-

Table 2: Effects of enzyme-treated and untreated feather meal based diets on pH, water holding capacity, cooking yield and cooking loss of broiler Chickens

Parameters	control	untreated feather meal			enzyme-treated feather meal			SEM	P-VALUE
	T1	T2	T3	T4	T5	T6	T7		
pH	5.96^{e}	6.08^{c}	6.24^{a}	6.08^{c}	6.04^{cd}	6.13^{b}	6.00^{de}	0.017	0.001
WHC (%)	0.56^{d}	0.73^{c}	0.53^{de}	1.21^{b}	0.75^{c}	1.39^{a}	0.50^{e}	0.063	0.001
Cooking yield (%)	71.89^{a}	60.08^{e}	65.31^{b}	56.39^{d}	60.25^{c}	55.28^{d}	55.58^{d}	1.141	0.003
Cooking Loss (%)	28.11^{g}	39.90^{c}	34.69^{f}	43.61^{b}	39.75^{d}	44.72^{a}	39.42^{e}	1.007	0.001
Drip Loss	4.08^{f}	5.12^{d}	5.13^{d}	6.51^{a}	5.03^{e}	5.79^{c}	5.91^{b}	0.139	0.002

abcdef Means in the same row with different superscripts are significantly different (P<0.05), SEM = Standard Error of Means,
WHC = Water Holding Capacity, T1 = 0% Feather meal, T2 = 8% Untreated feather meal, T3 = 16% Untreated feather meal, T4 = 24% Untreated feather meal, T5 = 8% Enzyme-treated feather meal, T6 = 16% Enzyme-treated feather meal, T7 = 24% Enzyme-treated feather meal

Table 3: Effects of enzyme-treated and untreated feather meal on the organoleptic properties of broiler chickens meat

Parameters	control	untreated feather meal			enzyme-treated feather meal			SEM	P-VALUE
	T1	T2	T3	T4	T5	T6	T7		
Colour	7.24	7.23	7.32	7.08	7.36	7.16	7.20	0.062	0.92
Juiciness	6.92	7.28	7.08	6.96	7.36	7.40	7.35	0.081	0.49
Appearance	6.76^{c}	6.96^{bc}	7.40^{ab}	7.24^{abc}	7.23^{abc}	7.28^{abc}	7.56^{a}	0.070	0.047
Flavour	6.88^{c}	7.52^{ab}	7.56^{ab}	7.00^{bc}	7.36^{abc}	7.56^{ab}	7.64^{a}	0.071	0.01
Aroma	7.24	7.38	7.60	7.28	7.32	7.44	7.28	0.071	0.86
Tenderness	7.60	7.64	7.63	7.40	7.56	7.48	7.64	0.072	0.97
Overall Acceptability	7.56	7.62	7.50	7.32	7.64	7.76	7.72	0.069	0.69

abc Means in the same row with different superscripts are significantly different (P<0.05), SEM = Standard Error of Means
T1 = 0% Feather meal, T2 = 8% Untreated feather meal, T3 = 16% Untreated feather meal, T4 = 24% Untreated feather meal
T5 = 8% Enzyme-treated feather meal, T6 = 16% Enzyme-treated feather meal, T7 = 24% Enzyme-treated feather meal

pearance of the meat from such animals which eventually influences consumer's preference for the product (Kim *et al.*, 2014). Enzyme treated feather meal fed to broiler chickens under the current study could have promoted better appearance of the meat and hence the decision made by the panel of tasters. Similarly, meat flavor has been regarded as a major determinant of consumer's meat-purchasing preference (Jayasena *et al.*, 2013). Meat samples are prone to the development of undesirable flavours through lipid oxidation and this reduces consumers' preference for such meat products. The quality of free amino acid present in animal feed have been suggested to contribute greatly to the flavor and taste of meat from animals (Ma *et al.*, 2019). The impressive flavor of meat obtained from this study could be attributed to the presence of free amino acids and their derivatives in the feather meal-based diets offered to the broiler chickens.

CONCLUSION

Based on the results obtained from this study, it is concluded that:

- enzyme-treated feather meal based diets enhanced broiler meat water holding capacity while untreated feather meal based diets increased the pH of broiler chickens meat.
- the organoleptic properties of appearance and flavour were enhanced by enzyme-treated feather meal diets at 24% level of inclusion while meat juiciness was improved at 16% inclusion level of enzyme-treated feather meal.

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