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Influence of curry, mint and pawpaw leaves supplemented diets on microbial load of spent layer's muscles and meat at different post mortem ageing period

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Abstract

Microbial contamination causes a major consumers health hazard and economic loss to meat retailers due in terms of food poisoning and meat spoilage. Antibiotics used in animal production to improve meat quality have residual effects of microbial resistance allergy. Therefore, the use of natural substances to enhance meat quality is a promising innovation. This research assessed the microbial load of muscles and meat of spent layer at different ageing period (PMA) fed diets supplemented with curry, mint and pawpaw leaves. A total of 210 spent layers were randomly allocated to seven broiler finisher diets (BFD) containing no supplement, 2 kg pawpaw leaf powder (PLP), 2 kg curry leaf powder (CLP), 2 kg mint leaf powder (MLP), 1 kg CLP + 1 kg PLP, 1 kg MLP + 1 kg PLP and 1 kg CLP +1 kg MLP levels for T_1, T_2, T_3, T_4, T_5, T_6 and T_7 respectively. The hens were exposed to one week adaptation period and two weeks of supplemented feeding trial. Data on microbial loads were subjected to analysis of variance (ANOVA) using factorial design. Results obtained revealed that PLP at 2 kg level of supplementation significantly reduced bacterial loads while PMA did not affect bacterial loads count. Higher frequencies of bacterial loads were observed at 12 hours PMA and different bacteria species were also identify. The study recommended supplement of 2 kg PLP should be used in the diet of spent layers to improve shelf life of spent layer meat.

Keywords: Curry, mint, pawpaw, microbial analysis and spent layer meat

Influence des régimes alimentaires complétés par des feuilles de curry, de menthe et de papaye sur la charge microbienne des muscles et de la viande épuisés de la couche à différentes périodes de vieillissement post mortem

Résumé

La contamination microbienne entraîne un risque majeur pour la santé des consommateurs et une perte économique pour les détaillants de viande en termes d'intoxication alimentaire et de détérioration de la viande. Les antibiotiques utilisés en production animale pour améliorer la qualité de la viande ont des effets résiduels d'allergie à la résistance microbienne. Par conséquent, l'utilisation de substances naturelles pour améliorer la qualité de la viande est une innovation prometteuse. Cette recherche a évalué la charge microbienne des muscles et de la viande de la couche épuisée à différentes périodes de vieillissement (PMA) avec des régimes alimentaires complétés par des feuilles de curry, de menthe et de papaye. Un total de 210 pondeuses épuisées ont été réparties au hasard dans sept régimes alimentaires pour poulets de chair (BFD) ne contenant aucun supplément, 2 kg de poudre de feuilles de papaye (PFP), 2 kg de poudre de feuilles de curry (PFC), 2 kg de poudre de feuilles de menthe (PFM), 1 kg Niveaux de PFC + 1 kg PFC, 1 kg PFM + 1 kg PFP et 1 kg PFC +1 kg PFM pour T1, T2, T3, T4, T5, T6 et T7, respectivement. Les poules ont été exposées à une période d'adaptation d'une semaine et à deux semaines d'essai d'alimentation supplémentée. Les données sur les charges microbiennes ont été soumises à une analyse de variance

(ANOVA) en utilisant un plan factoriel. Les résultats obtenus ont révélé que le PLP à un niveau de supplémentation de 2 kg réduisait significativement les charges bactériennes tandis que le PMA n'affectait pas le nombre de charges bactériennes. Des fréquences plus élevées de charges bactériennes ont été observées à 12 heures de PMA et différentes espèces de bactéries ont également été identifiées. L'étude recommande un supplément de 2 kg de PFP qui doit être utilisé dans le régime alimentaire des pondeuses épuisées afin d'améliorer la durée de conservation de la viande de poule pondeuse épuisée.

Mots-clés : Curry, menthe, papaye, analyse microbienne et viande de couche épuisée

Introduction

Poultry meat is highly valued due to its protein content and its relatively low cost, which has resulted to its increased consumption in the world (Del Puerto *et al*., 2016). Among poultry meat products, chicken carcasses, cuts, and processed products are the most consumed, followed by turkey and, to a lesser extent duck (Rouger *et al*., 2017). According to Rouger *et al*. (2017), ensuring the microbial safety of poultry meat products is a vital issue in this perspective of rising production as well as consumption. Microbial proliferation is one of the major cause of quality deterioration in meat and meat products either in fresh, cold or frozen conditions (Mario and Cava, 2004; Akarpat *et al*., 2008). The process of muscle conversion to meats is a series of biochemical events add to the enhancement of many meat value traits (Ouali *et al*., 2006). According to Alvarez-Astorga *et al*. (2002) and Kozacinski *et al*. (2006), during slaughter, processing, storage and market display, the quality attributes of meat also deteriorate faster due to microbial proliferation. Microbial contamination can cause a major consumers health hazards and economic loss to the meat retailers in terms of food poisoning and meat spoilage. Their growth and metabolic activity during shelf life leading to color, odor, taste, or texture defects are responsible for waste and losses of food products and therefore have an important impact on the economy of the poultry meat production sector. As a result, a significant fraction of meat and meat products are spoiled every year (**Rouger *et al*., 2017**). Thus, numerous **studies have been conducted to enhance the quality and shelf life of meat and meat products by creating** unfavorable environment for the proliferation of spoilage organisms using antibiotics (Muhammad *et al.,* 2011). Antibiotics used in animal production to improve performance and meat quality have developed microbial resistance allergy in the consumers (Rolfe, 2000). Despite all the benefits derived from the use of antibiotics, their usage is associated with a lot of risks with regards to human health (Jan, 2007). The misuse of antibiotics in the recent time has resulted in multi-drug resistance (MDR) among bacteria which prompt the search for drugs and dietary supplements effective against such MDR bacteria and to generally reduce microbial load. Different parts of plants, herbs and spices have been used for many years for prevention of infections. This study therefore focuses on evaluation of curry, mint and pawpaw leaves on bacterial load of spent layers' meat at different PMA.

Materials and methods

The trial was conducted at the Poultry Production Unit of Sokoto State Veterinary Centre, located at AliyuJedo Road, in Sokoto Metropolis. Sokoto State is within the savannah agro-ecological zone located between longitude $5^0$14' 51.1872"E and latitude $13^0$0'21.1428" N attain altitude of 305 m above sea level. The rainy season starts in mid-May to early June and reaches peak in August. Dry season starts in mid-October and ends in late May. The hottest periods are March to May while the coldest periods are December to February, characterized by dry harmattan winds (SERC, 2012). Sokoto State Veterinary Centre has a regular annual temperature of 30.26°C with average rainfall of 26.55 inch and mean yearly humidity of 48.54% (SERC, 2012; Ahmed and Egwu, 2014).

Sources of ingredients

The test ingredients (curry, mint and pawpaw leaves) were obtained from the Sokoto main vegetable market within Sokoto metropolis. Other feedstuffs for compounding diet (maize, groundnut cake, blood meal, bone meal, wheat offal, salt, limestone, methionine, lysine and premix) were obtained from the Feed-mill section of the Sokoto Technology Incubation Centre (STIC), Sokoto.

Experimental chickens and their management

The 210 spent layer chickens of ISA Brown strain at 115 weeks old were purchased from the Labana Farms Limited, Aliero, in Aliero Local Government Area of Kebbi State. On arrival, the birds were fed broiler finisher diet (BFD) and presented drinking water containing anti-stress (glucose), to relieving them for journey stress. An adaptation time of seven days was observed for the birds to adjust to their environment. They were afterward dispersed randomly by means of Completely Randomized Design (CRD) to seven treatments and fed for 14 days.

Formulation of experimental diets

Seven dietary treatments were formulated which consisted of a broiler finisher diet (BFD) = T_1, BFD + 2% PLP = T_2, BFD + 2% CLP = T_3, BFD +2% MLP= T_4, BFD + 1%CLP + 1% PLP = T_5, BFD + 1% MLP + 1% PLP = T_6 and BFD + 1% CLP + 1% MLP = T_7. The seven treatment diets used in this experiment were formulated to satisfy 2900 KcalME/kg and 20% CP following NRC (1994) recommendations as presented in Table 1 below.

Table 1: Gross and chemical compositions of experimental diets

	Treatment diets						
Ingredients (%)	T_1	T_2	T_3	T_4	T_5	T_6	T_7
Maize	56.00	56.00	56.00	56.00	56.00	56.00	56.00
Wheat offal	22.50	22.50	22.50	22.50	22.50	22.50	22.50
Groundnut cake	15.50	15.50	15.50	15.50	15.50	15.50	15.50
Blood meal	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Bone meal	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Limestone	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Salt	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Premix	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Methionine	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
CLP	0.00	0.00	2.00	0.00	1.00	0.00	1.00
MLP	0.00	0.00	0.00	2.00	0.00	1.00	1.00
PLP	0.00	2.00	0.00	0.00	1.00	1.00	0.00
Calculated Chemical Composition							
ME(Kcal/kg)	2900.00	2920.49	2912.87	2913.75	2916.68	2917.12	2913.31
CP (%)	20.00	20.64	20.50	20.48	20.57	20.56	20.49
CF (%)	4.87	4.93	4.91	4.90	4.92	4.91	4.91
EE (%)	4.10	4.12	4.12	4.11	4.12	4.11	4.12

CLP- Curry leaf powder, MLP- Mint leaf powder, PLP- Pawpaw leaf powder,
ME- Metabolizable energy, CP- Crude protein, CF- crude fibre, EE – ether extract.

Experimental layout

The spent layer birds were divided into seven groups of 30 birds each and assigned to the seven dietary treatments. Each treatment group was replicated into three with 10 birds each. The 21 treatment combinations were laid out in Completely Randomised Design (CRD).

Microbial analysis

Microbial study was carried out at Microbiology Research Laboratory in the Department of Microbiology, UsmanuDanfodiyo University, Sokoto. The microbial analysis was conducted using aseptic technique by serial dilution following the procedure of Adams and Moss (2007); three birds were randomly chosen from each treatment using balloting method for microbial analysis of muscles and meat. The birds were slaughtered, dressed and eviscerated. The dressed carcasses were divided to produce four (4) quarters of approximately equal weight and stored in a chiller at 4°C.

Three quarters of muscles and meat from each treatment were subjected to 0, 6, 12 and 24 hours post-mortem ageing with three replicates per treatment. At completion of each post-mortem ageing, from each replicate, 2.5 gram of muscles and meat samples were cut by sterilized scissors, and placed in 22.5 ml of 0.1% sterilized buffer peptone water and on another separate tube, 2.5 g muscle was homogenized the same way as the muscles and meat using methods Maharjan*et al.* (2019) with slight modification. The homogenate samples were serially diluted in 9 ml of 0.1% sterilized buffer peptone water to achieve a 10-fold dilution. The required dilutions were pour plated on plate count agar made with nutrient agar prepared as described by the manufacturer. Furthermore, 10^{-7} test tubes were used for inoculating media. Colony count was performed by using colony counter as describe by Willey *et al.* (2011) and estimated using the formula below.

Bacterial load=Total number of colonies in a plate x reciprocal of the dilution factor

All plates were inoculated and subsequently incubated at 37°C for period of 24 hours and those with no obvious growth were left for subsequent 24 hours. Culture suspected to be *Staphylococcus aureus* strain was confirmed by result of Gram staining along with biochemical tests; Catalase, Oxidase, and Coagulase while *Escherichiacoli* (E. coli) was confirmed based on the result of series of biochemical tests which include; Methyl Red (MR), Oxidase, Urea Hydrolysis, Catalase, VogesProskauer (VP), Citrate utilization, Triple Sugar Iron agar (TSI), Sulfide Motility and Indole test as recommend by Bailey and Scott's Diagnostic Microbiology (2007).

Data analysis

The data collected for bacterial load were subjected to analysis of variance (ANOVA) using factorial design while data for frequency of occurrence of bacterial species identified were expressed in percentage. Significant difference observed among treatment means were separated by Duncan's multiple range test (DMRT) using SPSS version 21.0 2012, IBM USA.

Results and discussion

Bacterial loads of spent layer muscles and meat at different post mortem ageing

The bacterial loads of muscles and meat of spent layer fed diets supplemented with curry, mint and pawpaw leaves are presented in Table 2, which showed significant difference in bacterial loads observed between treatments for treatment factor. The meat from control birds had significantly ($P<0.05$) higher bacterial loads than the meat obtained in treatment 2, but similar with the bacterial loads of other treatments.

Table 2*:* Bacterial load according to treatments and post mortem ageing

Factor		Bacteria load
Treatment		
	1	6.67×10^{6a}
	2	1.50×10^{6b}
	3	3.30×10^{6ab}
	4	2.81×10^{6ab}
	5	3.93×10^{6ab}
	6	2.17×10^{6ab}
	7	3.32×10^{6ab}
SEM		1.51×10^{6}
PMA		
	0	2.43×10^{6}
	6	3.28×10^{6}
	12	4.08×10^{6}
	24	3.76×10^{6}
SEM		1.14×10^{6}
Interaction		*

SEM = Standard error of mean,
PMA = Post-mortem ageing
a, b = Means bearing different superscripts along the column within a subset differ ($P<0.05$

The higher bacteria load of 6.67×10^6 in the control treatment (T_1) may be as a result of absence of supplements, while the lower bacteria count in treatment 2 (T_2) may be attributed to the presence of PLP 2% supplement. Similar result was reported by Peter *et al.* (2014) who proved that pawpaw leaf has antimicrobial potential as shown to have acted against some bacteria species such as *Pseudomonas* and *Listeria monocytogens*. Therefore, the lower bacteria load obtained for meat of birds in treatment 2 is due to anti-bacterial effect of the test ingredients pawpaw fed to the birds. This is explained in terms of bacteriostatic effects of the secondary metabolites present in the pawpaw leaves known to reduce bacteria. Anibijuwon and Udeze (2009) and Okunola *et al*. (2012) reported pawpaw leaf have shown antimicrobial activity against both gram-negative and gram-positive bacteria which indicates the plant is a potential source for production of drugs with a broad spectrum of activity. The low bacterial load of 1.50×10^6obtained in this study is lesser to the values of 2.9 (log10 CFU/g) obtained by Najeeb *et al*. (2015). The researcher assessed the efficacy of leaves (drumstick, mint and curry leaves) powder as natural preservatives in restructured chicken block. Bacterial loads frequency of occurrence different post mortem ageing. The frequency of occurrence at different PMA are presented in Table 3. The frequency of occurrence of bacterial loads obtained on muscles and meat indicated high occurrence at 12 hour PMAand low occurrence at 0 hour PMA.

Table 3: Bacterial loads frequency of occurrence at different post mortem ageing (PMA)

S/N	PMA	Bacterial loads frequency of occurrence (%)
1	0	8.13
2	6	16.56
3	12	39.62
4	24	35.69

S/N = Serial Number
PMA = Post Mortem Ageing

The PMA of 12 hours showed the highest bacteria load frequency of occurrence which can be attributed to as the log phase of this research, where the activities of the initial bacterial load increases in cell division within the short term ageing.The period is considered metabolically active and availability of enough surface area on the meat permit division even though there will be competition for food among varying species.Furthermore, such growth is considered mixed population referred to as poly microbial proliferation. However, at 24hours the population decreases.The findings of this study agreed with Lázaro *et al.* (2015), who emphasized the effect of temperature on microbial proliferation of meat which was recorded at 4^0C storage in their research as same condition of storage of this work.

Bacteria isolate identification on muscles and meat

Bacteria isolate identify on muscles and meat of spent layer are presented in Table 4. The following bacteria, *Escherichia coli, Streptococcus morbillorum* and *Streptococcus zooepidemicus* are gram positive while *Enterobacteriaceae, Lactic acid bacteria* (LAB), *Staphylococcus aureus* and *Salmonella typhi* are gram negative bacterial species

Table 4*: Bacterial isolate identification on muscles and meat*

S/N	Gram Stain	Bacterial Isolate
1	+ve	*Escherichia coli*
2	-ve	*Enterobacteriaceae*
3	-ve	*Lactic acid bacteria (LAB)*
4	-ve	*Staphylococcus aureus*
5	-ve	*Salmonella typhi*
6	+ve	*Streptococcus morbillorum*
7	+ve	*Streptococcus zooepidemicus*

S/N = Serial number,
+ve = Positive,
-ve = Negative

The bacterial isolates identified in this studywhich include E. coli as a common contaminant of meat agreed with the report of Rouger *et al.* (2017) on some meat contaminating bacteria. Some other bacteria such as Lactic acid bacteria (LAB) further participate in the spoilage of meat in long term storage and were isolated as participants in meat spoilage by Rouger *et al.* (2017). The presence of *Salmonella typhi* and *S. aureus* in this study is an indication of health hazard if not thoroughly cooked for consumers. The *salmonella typhi* can be present in the intestinal tract of birds but has to be watched in the course of processing their meat to avoid human infection. *Staph.aureus* which was also identified remain a problem because of its opportunity to cause infection in human. It is an opportunistic pathogen causing a variety of diseases, therefore remain a burden when perceived in food (Thippeswamy *et al.,*2011).

Conclusion

The result of the study indicates that PLP can be encouraged to be added as supplement to spent layers diet in order to significantly reduced bacterial load because the record here showed a drastic static nature in the rate of bacteria growth as compared to the initial and final PMA when analyzed.

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