INFLUENCE OF DIETS SUPPLEMENTED WITH CURRY, MINT AND PAWPAW LEAVES ON MICROBIAL ANALYSIS OF SPENT LAYER'S MUSCLES AND MEAT AT DIFFERENT POST MORTEM AGEING PERIOD

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ABSTARCT
A study conducted on microbial analysis of spent layer muscles and meat at different ageing period fed diets supplemented with curry, mint and pawpaw leaves. A total of 210 spent layers were randomly allocated to seven broiler finisher diets in Completely Randomized Design (CRD) manner: (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 T1, T2, T3, T4, T5, T6 and T7 respectively. The research was based on one week adaptation period and two weeks feeding exposure to the supplement compositions. 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 post-mortem Ageing (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.

INTRODUCTION
Poultry meat is highly valued due to its protein content and it relatively low in cost, this has resulted to its increase in 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), storage process of muscle conversion to meat, 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). Different parts of plants, herbs and spices have been used for many years for prevention of infections. This study would focus on evaluation of curry, mint and pawpaw leaves on bacterial load of spent layers' meat at different PMA.

MATERIAL AND METHODS
The trial was conducted at the Poultry Production Unit of Sokoto State Veterinary Centre, located at Aliyu Jedo 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^{o}C with average rainfall of 26.55 mm and mean yearly humidity of 48.54% (SERC, 2012; Ahmed and Egwu, 2014).

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. The treatments consisted of single BFD without additive as T1, BFD + 2 kg pawpaw leaf powder (PLP) as T2, BFD + 2 kg curry leaf powder (CLP) as T3, BFD + 2 kg mint leaf powder (MLP) as T4, BFD + 1 kg CLP + 1 kg PLP as T5, BFD + 1 kg MLP + 1 kg PLP as T6 and BFD + 1 kg CLP + 1 kg MLP as T7.

Experimental Layout

Two hundred and ten (210) spent layer birds were separated into seven groups of 30 birds each and apportioned to the seven dietary treatments. Each treatment group was divided into three replicate with 10 birds per replicate. The 7 x 3 = 21 treatment combinations were laid out in Completely Randomized Design (CRD).

Microbial Analysis

Microbial study was carried out at Microbiology Research Laboratory in the Department of Microbiology, Usmanu Danfodiyo 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 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^{o}C. The twelve (12) quarters of muscle and meat samples for each treatment were subjected to 0, 6, 12 and 24 hours PMA with three (3) replicate per treatment. At completion of each PMA, muscles and meat from each replicate were collected for microbial load count according to treatments and then recorded.

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 ($P<0.05$).

Bacterial load of 6.67×10^{6} in the control Treatment (T1) may be as a result of absence of supplements while the lower bacterial count in treatment (T2) may be attributed to the presence of PLP 2 kg supplement. 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 leaves 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.

Bacterial Loads Frequency of Occurrence Different Post Mortem Ageing

The frequencies of occurrence at different PMA are presented in Table 4. 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.

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 because they are 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 because such growth is considered mixed population referred to as poly microbial proliferation whereas at 24hours the population decreases and this agrees to the research of Rodríguez-Lázaro*et al.* (2015) who emphasized the effect of temperature on microbial proliferation of meat which was recorded at 4^{0}C storage in their research as same condition of storage of this work.

CONCLUSION AND RECOMMENDATION

Pawpaw plant is a popular plant known for usage as tradition medicine. The leaf can be encouraged to be added as supplement to spent layers feed 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. Thus, supplementation of 2 kg PLP should be used in the diet of spent layers.

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