ANTI-MICROBIAL EFFECTS OF DRIED FERMENTED PAWPAW (*Carica papaya*) SEEDS UNDER DIFFERENT REFRIGERATED STORAGE ON MEAT FROM BROILER CHICKEN.

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ABSTRACT

Poor handling and safety of meat products in most developing countries of the world, occasioned by increase in poultry meat consumption necessitated the use of unconventional feed ingredients with potent pharmaceutical materials in lowering the microbial loads of meat products, hence, the use of fermented dried Pawpaw seed. Pawpaw fruits were locally sourced and the seeds neatly removed, anaerobically fermented for 48 hours before drying for 14 days. The dried fermented pawpaw seeds (FPS) were milled and added to the broiler feed at varying inclusion levels (0%, 5%, 10%, 15%, 20%). One hundred and fifty birds were randomly selected on the basis of weighted average of 1.5kg from a population of 200 birds. The birds were divided into five treatments and each treatment was replicated thrice with 10 birds per replicate. The birds were humanely slaughtered at 8 weeks and a small portion of the thigh muscle was cut with a sterilized Scissors from each treatment. Each sample was stored in a refrigerator for 0, 1, 3 and 6 days. The samples were analyzed in the laboratory using serial dilution method to determine the microbial loads on each treatment in relation to the varying days of storage. The result showed no significant differences (P>0.05) on days 0, 1 and 3 in the bacterial loads across all treatments. However, treatments 4 (15%) and 5 (20%) had pronounced influence (P<0.05) on day 6, indicating that FPS has the tendency to lower microbial loads of stored meat products. The fungal load across all the treatments however did not show any specific effect.

Key Words: Bacteria, Birds, Fungi, Meat safety, Storage

INTRODUCTION

The poultry production in general animal production practices offer the greatest value in terms of quick turnover rate and returns on investment (Sanni and Ogundipe, 2005). Growth production rate in poultry, when compared with ruminants and other monogastric animals, seemed to be the highest according to Braenkaert *et al*. (2000). An independent study carried out by Ojo (2002) revealed that poultry is cheap, available and also the cheapest source of animal protein. Poultry meat consumption is steadily increasing worldwide; the last data available indicated it reached 14.2 kg per capita per year (Rouger *et al.*, 2017). Poultry products, chicken carcasses and, cuts are the most sought after (~75% of total poultry meat), next to it is turkey (~25%), and to a lesser extent, duck (Nieminen *et al.*, 2012).

However, food safety and shelf-life are both important microbial concerns in relation to broiler meat production. To ensure these, vacuum packaging, chilling, or marinades are different practices engaged for guaranteeing microbial quality during the storage of poultry cuts, and that depends on consumers' habits and countries (Rouger *et al.*, 2017). The microbial contamination of carcasses and processed poultry products often emanate from slaughterhouse environment, and the equipment used during and after slaughtering of the birds (Wikipedia, 2022b). Some of these microbial contaminants can develop and stay alive during the processing of food and storage. Food preservatives are food additives often used to preserve food by lowering the pH value and settling the redox potential of the food. Preservatives also help to hinder the development/growth of the microbes in food products. The use of some plant materials with useful phytochemical constituents have assisted in helping to reduce various forms of bacteria loads in meat, especially during storage (Akinduro *et al.*, 2020)

Studies have also shown that, there are some natural agents (plant extracts) possessing both antioxidant and antimicrobial activities that can be used as feed additives or used during processing (Tipu *et al., 2006*) one of which is *Carica papaya* commonly called pawpaw (Aravind *et al.*, 2013). *Carica papaya* seeds have antibacterial properties and are effective against *E. coli*, Salmonella and Staphylococcus infections (Aravind *et al.*, 2013). Pawpaw seeds, irrespective of its fruit maturity stages, have anti-bacteriological effects on gram positive and negative organisms (Aravind *et al.*, 2013). The use of dried pawpaw plant parts as supplements and ingredients in animal feed have been reported by Oloruntola *et al.*, (2018), though there is insufficient information on the possible effect of fermented pawpaw seeds in the diets of broiler chickens on the microbial load on their carcass. Therefore, this study was designed to investigate the effects of fermented dried pawpaw (*Carica papaya*) seeds on the microbial loads of broiler meat under different days of refrigeration storage (0, 1, 3 and 6 days.)

MATERIALS AND METHOD

Experimental Site: The experiment was conducted at the Poultry Unit of the University Teaching and Research Farm, College of Agriculture, Osun State University, Osogbo, Ejigbo Campus, Ejigbo, Osun State. The site is located on latitude 7º54`N and longitude 4º18`E at an altitude 426 m above sea level. Ejigbo is located at the centre of the state, about 35 km north-east of Iwo, 30 km from Ogbomoso in the north, and 24 km from Ede in the south-east. It is about 40 km north-west of Osogbo, the capital of Osun State and about 95 km north-east of Ibadan. The mean annual rainfall in Ejigbo is 52.35 mm and there are variations from the mean value from year to year (Wikipedia, 2022a).

Plant sample collection

Source of Pawpaw Seeds and the Fermentation Process

Matured Pawpaw fruits (*carica papaya*) (variety: yellow pawpaw) were obtained from various towns within Osun state. The fruits were identified and authenticated at the Department of Agronomy, Osun State University, Osogbo, Nigeria. The fruits were cut open to access the seeds**.** The seeds were neatly removed, kept in air tight polythene bags for a period of 48 hours for fermentation to take place after which the fermented seeds were completely air dried in a room away from direct sunlight, in order to keep the phytochemical components intact. The dried seeds were grinded (pulverized) using a hammer mill after which it was incorporated into the feed at various inclusion levels.

Experimental birds

Preparation before arrival

Disinfection, screening and fumigation of the brooding pen was done before the broiler chicks were brought in, as this served as a biosecurity measure. The nylon screening was removed after 48hrs of fumigation, so as to allow the escape of any residual gas (fumigants).

Arrival of Birds

On arrival, the chicks were graded, i.e., unboxed and counted while weak ones were isolation from active ones which were kept in the already prepared pen. Also, the birds were given a solution of glucose and vitamins as anti-stress.

Experimental procedure

The experiment was conducted using one-hundred-and-fifty-day-old broiler chicks. These birds were obtained from a reputable farm in Osogbo, Osun State. The chicks were brooded for fourteen (14) days on a deep litter system using 200 wats electricity bulbs as source of heat. From 0-4 weeks, the chicks were group-fed for acclimatization and the development of the GIT (gastro intestinal tract) prior to inclusion of the test ingredient at the finisher phase. Feeding trial was between day 28-56. The chicks, which had already been randomly selected and assigned to five treatments, were replicated three times, ten (10) birds were allocated to each replicate making a total of 30 birds per treatment. Vaccination and medication programs were administered based on the standard procedure laid down in the University Teaching and Research Farm. Both routine and occasional management practices were thoroughly carried out with strict hygiene measures. The feed composition at both starter and finisher phases are shown in Tables 1 below. Water was supplied *ad-libitum* throughout the experiment and dried fermented pawpaw seed at varying inclusion levels (0%, 5%, 10%, 15%, and 20%) were added to their diet at the finisher phase respectively.

Experimental Diet

Five (5) experimental diets which consisted of:

Treatment 1 (Control): 0% fermented pawpaw seed inclusion

Treatment 2: 5% fermented pawpaw seed inclusion

Treatment 3: 10% fermented pawpaw seed inclusion

Treatment 4: 15% fermented pawpaw seed inclusion

Treatment 5: 20% fermented pawpaw seed inclusion

The inclusions were done on w/w basis.

TABLE 1: FORMULATED EXPERIMENTAL DIETS CONTAINING THE TEST INGREDIENTS IN VARYING PROPORTION

Ingredients	0%	5%	10%	15%	20%	STARTER
Maize	50.00	45.00	45.00	43.00	38.00	50.00
Wheat Offal	10.50	12.00	10.50	10.00	14.00	3.90
BDG	10.00	10.00	10.00	12.50	12.00	5.00
GNC	12.00	11.00	10.00	8.00	7.00	10.00
SBM	13.00	12.00	10.00	7.00	4.50	24.50
Fish Meal	0.00	0.00	0.00	0.00	0.00	3.00
Oyster Shell	0.20	0.20	0.20	0.20	0.20	0.20
Bone Meal	3.00	3.00	3.00	3.00	3.00	2.50
Methionine	0.35	0.35	0.35	0.35	0.35	0.25
Lysine	0.30	0.30	0.30	0.30	0.30	0.00
Broiler Premix	0.30	0.30	0.30	0.30	0.30	0.25
Salt	0.40	0.40	0.40	0.40	0.40	0.40
FDPS	0.00	5.00	10.00	15.00	20.00	0.00
Total	100.00	100.00	100.00	100.00	100.00	100.00

Sample Collection and Laboratory Analysis

Two birds from each replicate were humanely slaughtered with special consideration to animal ethical conducts. Small portion of the thigh from each replicate (i.e., a total of six samples per treatment) were collected and were cut with a sterilized scissors.

The samples were then divided into three:

- the first portion was kept in the freezer (< 0^0C) to inhibit the growth of microbes (Day 0)
- the second portion was kept in the refrigerator (4 – 8^0C) for twenty-four hours (Day 1)
- the third portion was kept in the refrigerator for three (3) days and
- the fourth portion was kept in the refrigerator for six (6) days

After that, the samples were taken to the laboratory for analysis using Serial Dilution Method.

Experimental Design

The experimental design was Completely Randomized Design (CRD).

Statistical Analysis

All the data collected were analyzed using analysis of variance (ANOVA) with the procedure of SAS (2008). Statistically significant observed means were compared using LSD of the same package at 5% level of probability.

RESULTS AND DISCUSSION

Bacterial Load on Day 1

Table 2 showed the presence/absence of *Enterobacter aerogenes*, *Bacillus subtilis*, *Pseudomonas fluorescens*, *Serratia marcescens*, *Staphylococcus aureus*, and *Lactobacillus plantarum* as well as the overall bacterial load of broiler meat fed the experimental diet and refrigerated at 1 day. *Enterobacter aerogenes* and *Lactobacillus plantarum* were present across all treatments. *Bacillus subtilis* and *Pseudomonas fluorescens* were absent across all treatments.

For *Serratia marcescens*, it was absent in T_2 but was present in all other treatments. *Staphylococcus aureus* was absent in T_5 but present in all other treatments. There was no significant difference (P>0.05) in treatments 1(0%) which is the control and treatment 5(20%) which is the highest inclusion level of the test ingredient, though higher microbial loads were noticed in treatments 2, 3 and 4, this implied that further increase in the percentage inclusion level could help reduce the microbial loads of meat from broiler chicken fed fermented pawpaw seed in their diets. Reduced microbial loads seen in treatment 5(20% inclusion level) (7.300 ± 1.007), was close the findings of Anihouvi *et al*. (2013) on roasted meat, skewers, and seasoned wrapped meat stored for 24 hours (6.26 ± 0.24 to 6.97± 0.21).

Bacterial Load on Day 3

Table 3 showed the presence/absence of *Enterobacter aerogenes*, *Bacillus subtilis, Pseudomonas fluorescens, Serratia marcescens*, *Staphylococcus aureus*, and *Lactobacillus plantarum* as well as the overall bacterial load of broiler meat fed the experimental diet and refrigerated for 3 days. *Enterobacter aerogenes* was absent in T_1 but present in other treatments. *Bacillus subtilis* was absent in all treatments. *Pseudomonas fluorescens* was present in T_1 but absent in other treatments. *Serratia marcescens* was present in T_4 and T_5 but absent in the other three treatments. *Staphylococcus aureus* was absent in T_4 and T_5 but present in the other three treatments**.** *Lactobacillus plantarum* was absent in T_5 but present in the other treatments. For bacterial load, T_3 (7.900 ± 0.289) and T_4 (7.700 ± 0.493) were significantly different (P<0.05) highest, while T_1 (5.267 ± 0.484) was significantly (P<0.05) lowest. Alabi *et al*. (2012) carried out a comparative study on antimicrobial and anti-fungal property of extracts of fresh and dried leaves of *Carica papaya*. The study was repeated by various concentration of extract using the disc diffusion method. *C. papaya* leaves showed better antibacterial activity than antifungal activity, indicating that the effects of *C. papaya* could be felt more on anti-microbial activities and not necessarily on anti-fungal activities since some of the phytochemicals in the seeds are also known to be present in the leaves, though with varying concentrations.

Bacterial Load on Day 6

Results from table 4 showed the presence/absence of *Enterobacter aerogenes*, *Bacillus subtilis*, *Pseudomonas fluorescens*, *Serratia marcescens*, *Staphylococcus aureus*, and *Lactobacillus plantarum* as well as the overall bacterial load of broiler meat fed the experimental diet and refrigerated for 6 days. *Enterobacter aerogenes* was absent in T_3 and T_5 but present in the other three treatments. *Bacillus subtilis*, *Pseudomonas fluorescens*, and *Serratia marcescens* were absent across all treatments. *Staphylococcus aureus* was present in all treatments. *Lactobacillus plantarum* was absent in T_3 but present in all other treatments. There was no significant difference (P>0.05) in the bacterial load across all the treatments. Although, treatments 3, 4 and 5 had the least bacterial loads, which implied that higher inclusion of fermented pawpaw seed could help reduce bacterial load on meat. Further increase in the percentage level could help reduce the bacterial loads to a level of significance. A similar study carried out by (Okoye, 2011;

Adesola *et al*., 2019) revealed the characteristic potentials of *Carica papaya* seed as a phytogenic substance that has the tendency to lower the bacterial loads on meat. This was revealed in his study where the antibacterial and antifungal activity of crude ethanolic and aqueous extracts of seeds from *Carica papaya* was used against four different bacteria and fungi.

Fungal Load on Day 1

The presence/absence of *Aspergillus flavus, Penicillium chrysogenum, Rhizopus stolonifer, Saccharomycos cerevisiae, Alternaria tenuis,* and *Penicillium citrinum* were shown in table 5 as well as the overall fungal load of broiler meat fed the experimental diet and refrigerated for 1 day. *Aspergillus flavus* was absent in T_2 but present in the other treatments. *Penicillium chrysogenum, Alternaria tenuis,* and *Penicillium citrinum* were absent across all treatments. *Rhizopus stolonifer* was present in T_3 and T_5 but absent in the other treatments. *Saccharomycos cerevisiae* was absent in T_4 but present in the other four treatments. After day 1 of refrigeration, there was no significant difference (P>0.05) in the fungal load across all treatments indicating that the test ingredients did not exhibit any potentials of lowering the fungal loads of stored meat products when included in their diets, contradicting the report by Okoye (2011) in which a test on antifungal activity of crude ethanolic and aqueous extracts of seeds of *Carica papaya* against four fungi (*Aspergillusniger*, *Penicilliumnotatum*, *Fusariumsolani* and *Candida albicans*) were tested, with relative reduction in fungal loads.

Fungal Loads on days 3 & 6

There were similarities in the results obtained on days 3 and 6 on the fungal load. Table 7 below showed the presence/absence of *Aspergillus flavus*, *Penicillium chrysogenum*, *Rhizopus stolonifer, Saccharomycos cerevisiae*, *Alternaria tenuis,* and *Penicillium citrinum* as well as the overall fungal load of broiler meat fed the experimental diet and refrigerated at 6 days. *Aspergillus flavus* was absent across all treatments except T_4. *Penicillium chrysogenum*, *Rhizopus stolonifer*, *Alternaria tenuis,* and *Penicillium citrinum* were absent across all dietary treatments. *Saccharomycos cerevisiae* was present across all treatments except T_4. On day 6 of refrigeration storage, there was no significant difference (P>0.05) in the fungal load across all treatments indicating that the fermented dried pawpaw seed had no effects on the meat samples, this contradicted the report made by Adesola *et al*. (2019) wherein an ethanolic extraction was made on pawpaw seed using petroleum ether and the extracts topically applied on the meat samples, the results showed a decline in microbial loads on the meat samples.

TABLE 2: BACTERIAL LOAD ON DAY 1

Treatment	*Enterobacter aerogenes*	*Bacillus subtilis*	*Pseudomonas fluorescens*	*Serratia marcescens*	*Staphylococcus aureus*	*Lactobacillus plantarum*	Bacterial Load (x10cfu/ml)
1	+	-	-	+	+	+	7.133 ± 0.371^{b}
2	+	-	-	-	+	+	9.933 ± 0.384^{a}
3	+	-	-	+	+	+	10.900 ± 0.907^{a}
4	+	-	-	+	+	+	9.200 ± 0.872^{ab}
5	+	-	-	+	-	+	7.300 ± 1.007^{b}

- means not present

+ means present

cfu: coliform unit/ml

TABLE 3: BACTERIAL LOAD ON DAY 3

Treatment	*Enterobacter aerogenes*	*Bacillus subtilis*	*Pseudomonas fluorescens*	*Serratia marcescens*	*Staphylococcus aureus*	*Lactobacillus plantarum*	Bacterial Load (x10cfu/ml)
1	-	-	+	-	+	+	5.267 ± 0.484^{b}
2	+	-	-	-	+	+	7.367 ± 1.084^{ab}
3	+	-	-	-	+	+	7.900 ± 0.289^{a}
4	+	-	-	+	-	+	7.700 ± 0.493^{a}
5	+	-	-	+	-	-	6.433 ± 0.754^{ab}

$^{a, b, c, d}$*: Means within the same row with different superscript are significantly (P<0.05) different*

- means not present

+ means present

cfu: coliform unit/ml

TABLE 4: BACTERIAL LOAD ON DAY 6

Treatment	*Enterobacter aerogenes*	*Bacillus subtilis*	*Pseudomonas fluorescens*	*Serratia marcescens*	*Staphylococcus aureus*	*Lactobacillus plantarum*	Bacterial Load (x10cfu/ml)
1	+	-	-	-	+	+	7.033 ± 2.585
2	+	-	-	-	+	+	7.367 ± 1.267
3	-	-	-	-	+	-	6.400 ± 1.582
4	+	-	-	-	+	+	5.133 ± 0.825
5	-	-	-	-	+	+	6.100 ± 1.716

[a, b, c, d]: *Means within the same row with different superscript are significantly (p<0.05) different*

- means not present

+ means present

cfu: coliform unit/ml

TABLE 5: FUNGAL LOAD ON DAY 1

Treatment	*Aspergillus flavus*	*Penicillium chrysogenum*	*Rhizopus stolonifer*	*Saccharomycos cerevisiae*	*Alternaria tenuis*	*Penicillium citrinum*	Fungal Load (x10cfu/ml)
1	+	-	-	+	-	-	2.000 ± 0.100
2	-	-	-	+	-	-	2.133 ± 0.088
3	+	-	+	+	-	-	1.900 ± 0.289
4	+	-	-	-	-	-	1.933 ± 0.667
5	+	-	+	+	-	-	2.367 ± 0.067

- means not present

+ means present

cfu: coliform unit/ml

TABLE 7: FUNGAL LOAD ON DAY 6

Treatment	***Aspergillus flavus***	***Penicillium chrysogenum***	***Rhizopus stolonifer***	***Saccharomycos cerevisiae***	***Alternaria tenuis***	***Penicillium citrinum***	**Fungal Load (x10cfu/ml)**
1	-	-	-	+	-	-	1.367 ± 0.088
2	-	-	-	+	-	-	1.567 ± 0.176
3	-	-	-	+	-	-	1.533 ± 0.773
4	+	-	-	-	-	-	1.467 ± 0.145
5	-	-	-	+	-	-	1.567 ± 0.296

- means not present

+ means present

cfu: coliform unit/ml

CONCLUSION

This study showed pawpaw seeds have some useful pharmaceutical ingredients that could impede the growth of bacteria, known to be the major source of contamination during meat processing and storage. The inclusion levels at 15 and 20% in treatments 4 and 5 respectively, at day 6 (Highest length of days) of refrigeration storage recorded the lowest bacterial loads. This means that the fermented dried pawpaw seed used as the test ingredient has antimicrobial properties which could assist in reducing the microbial loads in meat and thereby improving the quality characteristics of the meat products, hence, enhance consumer's appeal. The fungal load as revealed from the result of the experiment showed that there was no significant effect (P>0.05) of the fermented dried pawpaw seed on the cooked meat samples across all the treatments especially when incorporated in the diet, this goes contrary to the findings of (Okoye, 2011; Adesola *et al*., 2019) where different tests were carried out on antibacterial and antifungal activity of crude ethanolic and aqueous extracts of seeds of *Carica papaya* on different bacteria and fungi types, there was decline in the microbial loads on meat samples examined. Their results could have been possible because the extracts were applied to the meat samples directly and not to the feed given to the broiler chickens, which was the focus of this experiment.

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