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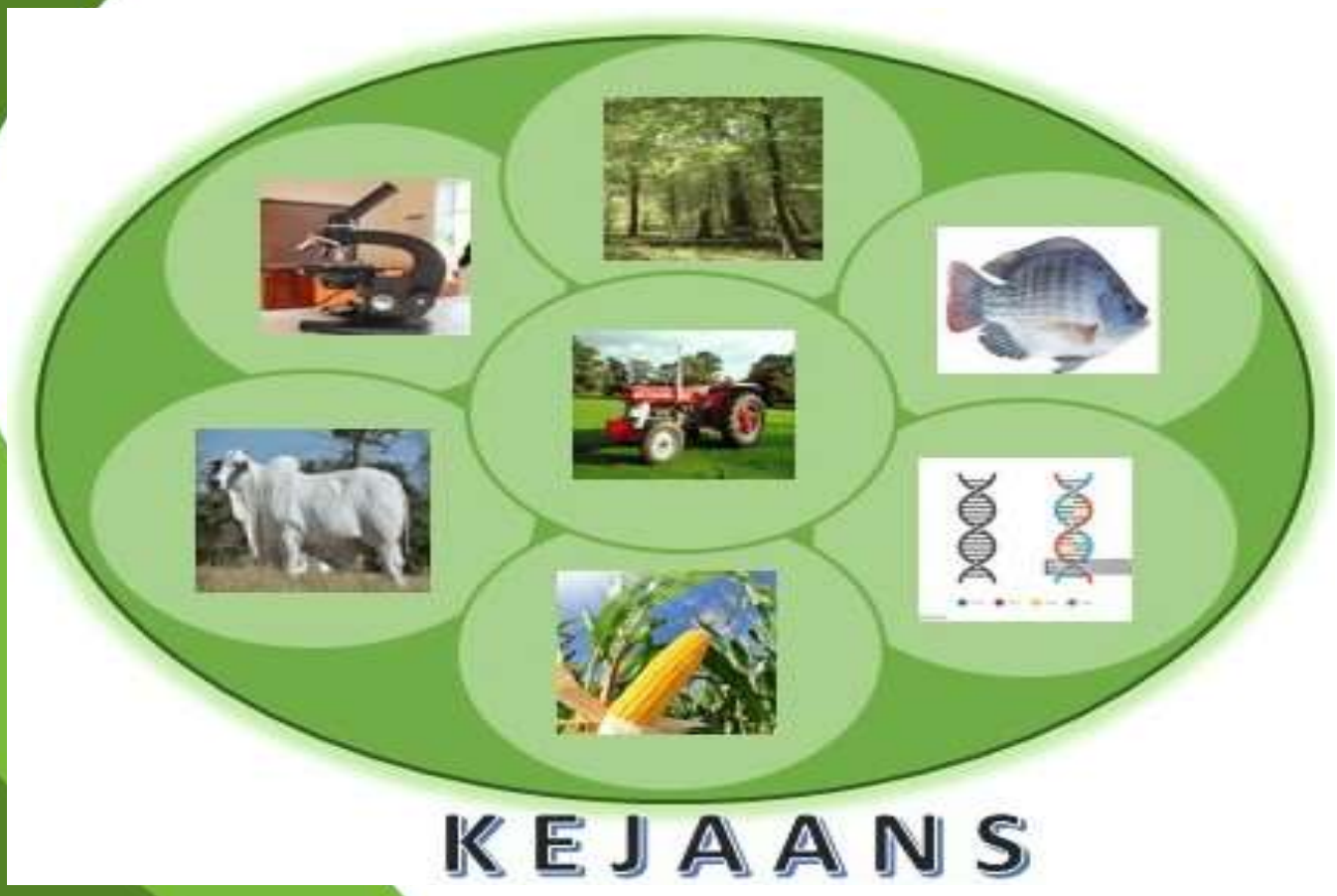

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INFLUENCE OF SELECTED CHEMICAL ADDITIVES AND APPLICATION LEVELS ON BACTERIOLOGICAL SAFETY OF CHICKEN BREAST MEAT

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ABSTRACT

The study was conducted to investigate the effects of selected chemical additives and their application levels on the bacteriological safety of chicken breast meat. A factorial experiment involving four widely used chemical additives (Monosodium glutamate, Sodium nitrate, Monosodium phosphate and Sodium chloride) and three application levels (1, 2 and 3% w/w) was laid in in a completely randomized design (CRD). A total of 15 average weight broiler chickens were slaughtered and cut into primal cuts. Chicken breast meat was collected and each fresh breast was divided into 10 average sized pieces making a total of 150 pieces. The pieces was randomly divided into 12 groups, containing 12 pieces representing the treatments for the study. Each group was administered its treatment specification and grilled using charcoal griller until the meat is cooked. The proximate, sensory and bacteriological data were collected and analyzed using the Analysis of variance (ANOVA) in the statistical packages for social sciences (SPSS) Version 17.0 at 5% using Tukey test. Monosodium glutamate treated samples at 2% had lower microbial load (128×10^5) than all other samples. Total microbial load of chicken breast meat falls within the USDA safety limit for chicken products. The results revealed that despite chemical treatment, 36 bacteria were isolated from nine (9) genera of bacteria. According to prevalence, the bacteria include: Bacillus Cereus (22.22%), Staphylococcus aureus (19.44%), Pseudomonas auregenosa (16.67%), Streptococcus pneumonia (8.33%), Lacto bacillus (Strepto) (8.33%) Bacillus anthricia (8.33%), Wrella Shella pneumonae (5.56%), Bacillus subtilis (5.56%), Bacillus megaterium (2.78%) and Micro coccus (2.78%). The study concluded that Sodium chloride at up to 3% w/w should be used in the grilled beef industries since it ensures lower bacteriological load However, the presence of Streptococcus pneumonia is a human pathogenic bacterium and it is recognized as a major cause of pneumonia and meningitis in children and the elderly which may result to health challenges.

Keywords: Chemical additives, bacteriological load, prevalence, chicken breast meat

Introduction

Chicken breast meat is an excellent source of protein, containing approximately 30-35 grams per 100 grams, chicken breast meat also it is relatively low in fat, with a favorable fatty acid profile USDA (2020). However it is a

good source of various micro nutrients including niacin, vitamin B6, and selenium. Meat is regarded as a valuable food from a nutritional standpoint, and it is one of the main components of the daily diet of a significant percentage of humans. Since the dawn of human civilization. To address these concerns and produce high-quality, sustainable, and safe chicken breast meat products, the poultry industry can benefit from an understanding of consumer preferences and concerns *(Harvey et al., 2017).*

Chicken meat does not contain the trans fats that contribute to coronary heart disease, and can be found in high amounts in beef and lamb. In Canada, values of 2 to 5 percent have been reported for beef and as high as 8 percent for lamb. The World Cancer Research Fund and others (Bingham, 2006) and Acuff (2006) clarified the difference between spoilage organisms and pathogens by stating, "spoilage organisms will not make you sick, as in instigating an infection and creating a real illness." However, spoilage organisms make food undesirable. The meat industry works diligently to prevent, reduce and eliminate both pathogenic and spoilage bacteria before meat are delivered to consumers for purchase.

Decomposition brought on by mold, air, bacteria, or yeast can be slowed by preservatives. Preservatives not only keep food fresh but also assist prevent contamination that can lead to foodborne illnesses like deadly botulism. (Lucy Bell Young, 2021). Of the various methods for food preservation, chemical food preservative is one. Humans have been preserving food since far before civilization was established. Evidence really points to the active usage of sun-drying methods for food preservation as early as 14,000 years ago in the Middle East and the Orient. It has long been practiced to chemically preserve food, especially by using salt to dry meats and fish. (Young Bell, 2021). Other preservation processes, such as cooking, pasteurization, and irradiation which applies heat, high energy, or ionizing radiation are carried out on the food product prior to the addition of chemical preservatives. The food product's shelf life can be increased by destroying germs and fungi by subjecting it to heat or high energy radiation (Lucy Bell Young, 2021).

Poultry raw meat is a perishable and nutritious food and its high moisture content favors bacterial growth and leads to deteriorative changes. Bacterial contamination and lipid oxidation reduce the quality and stability of chicken meat and ultimately result in social health concern. The overuse of antibiotics in poultry production has contributed to the development of antimicrobial resistant bacteria, making it challenging to control microbial growth. (Kondjoyan and Portanguen, 2008). Therefore this study was carried out to assess the effect of chemical additives applied at different levels on microbial safety of chicken breast meat;

Materials and Methods

Study area

The study was conducted in the Animal Science laboratory of the Kebbi State University of Science and Technology Aliero (KSUSTA). Aliero Local Government Area was created in November 1996. Aliero is a town northern Nigeria Kebbi State. Located in the southeast of Kebbi State $12^{0}16'42"N$ $4^{0}27'6"E$ and it is located at 42 kilometers South - East of Birnin - Kebbi and it covers a total of 167 square kilometers with total number of population over 65,973 according to the estimated figure of 2006 census exercise. The federal roads that link Sokoto to Kebbi passes through the area to Jega up to Niger State . This road divided the town into South and North. Aliero shares common boundaries with Sabiyal district in the North, Jega in the west, Tambuwal in the east and it boarded with Gehuru District in the south. The climate

condition has always been on the average temperature of 40°C while the annual average rains rate is 25 inches which normally starts at the middle of the year i.e in the month of May and end mid of September or October the same year. The area is Savannah vegetation, it witness two major seasons dry and rainy season. The rainy season start from May to October and the dry season start from November to January which is characterized by heavy fog and dust as well as extreme cold. The topography of Aliero is at and slightly with compact stony brown soil.(NPC,2024)

Study Plan

The study was conducted to determine the microbial safety of chicken breast meat processed with different chemical additives and their application levels. The objectives were to assess the microbial load, isolation and biochemical characterization to identify bacteria species present in the samples.

Treatments and Experimental Design

The study was a factorial experiment involving four widely used chemical additives (Monosodium glutamate, Sodium nitrate, Monosodium phosphate and Sodium chloride) and three levels of application (1, 2 and 3% w/w) laid in a completely randomized design (CRD) giving 12 treatment combinations. The treatments were replicated 12 times.

Preparation of samples

A total of 15 average weight broiler chickens were used, the birds were slaughtered and cut into primal cuts. Chicken breast meat was collected and each fresh breast was divided into 10 average sized pieces making a total of 150 pieces. The pieces were randomly divided into 12 groups, containing 12 pieces representing the treatments for the study. Each group was administered its treatment specifications and grilled using charcoal griller until the meat is cooked. The grilled meat samples were then kept separately for further analysis.

Experimental Procedures

Determination of Bacteriological Load

For determination of the total viable count, first serial dilutions were prepared from 1 ml of the sample and 9 ml of tryptone water (APHA, 1992). Then, 0.1 ml of each dilution was surface plated onto a previously prepared plate count agar and distributed onto the entire surface of the medium using a plastic spreader. The plates were incubated for 24-48 h at 37 °C. Following incubation, the colonies of bacteria grown on plate count agar were counted using a colony counting chamber. Those plates showed a colony count between 30 and 300 CFU were considered as statistically reliable and accurate for microbial load counting (Eby (2021). The number of distinct colonies on each plate was counted as Colony-forming unit (CFU) per ml of sample volume and was calculated by using the dilution factor of its concentration and converted to log10 CFU/cm^2 values. Mean values of total viable counts in log10 CFU/cm^2 of replicates were determined and reported as mean standard deviation (Swanson *et al*. (1992) of Petri dishes containing samples was done at an appropriate temperature of 37 °C for 24-48 For the purpose of isolation and identification of bacterial species, swab samples from meat contact surfaces of butcher shops were cultured on nutrient agar and blood agar (general media), mac Conkey agar, mannitol salt agar, and brilliant green agar as differential and selective media (ISO/TS (2009). The spread plate method was used for sample inoculation Atlas *et al*. 1995, whereas incubation duration (Ercolini *et al*. (2009). Colony morphology on the plates was observed and colony sub-culturing was done to obtain pure colonies for biochemical testing. Well-isolated colonies were carefully examined microscopically for bacterial

characteristics such as shape and color. Gram staining, as well as an appropriate biochemical test such as lysine, Triple Sugar-Iron, catalase, motility, indole production, and citrate utilization, were performed following the standard protocol Oyeleke and Magna, 2008. The isolates were identified by comparing their morphological and biochemical characteristics with standard reference organisms of known taxa, as described by Bergy's manual for determinative bacteriology Vos *et al*. 2009.

Data Analysis

The data collected for bacteriological analysis were analyzed using the Analysis of variance (ANOVA) in the statistical packages for social sciences (SPSS) Version 17.0. Significant means were separated at 5% using the Tukey test. Bacteriological results were expressed as CFU/g.

Results and Discussion

Total Viable bacterial count from Chicken Breast Meat Cured with Different Levels of Chemical Additives

The result in Table 1 shows the total viable bacterial count indicate that all microbiological test carried out indicated that there is significant difference ($p<0.05$) in the chicken breast meat. However, monosodium glutamate was found to have higher log ($128x10^{-5}$) followed by monosodium phosphate ($124x10^{-5}$) and sodium nitrate has the least with ($15x10^{-5}$). However all the microbial load of chicken breast meat falls within the USDA safety limit for chicken breast meat. However, Asmara *et al.* (1994) who reported TVC in raw chicken meat in the range of 6.55 -7.15.log10cfu/g. The recommended microbiological standard for raw meat was in the range of 10^{-5} – 10^{-7} log10cfu/g. The results of the study are in agreement with Chaiba *et al*. (2007) who reported the presence of Staphylococcus spp. and Salmonella spp. in raw chicken meat. The presence of such high microbial counts can be attributed to improper handling of raw chicken products and inadequate storage conditions (Jay *et al, 2005).*

Table 1 Total Viable Bacterial Count from Chicken Breast Meat Cured with Different Chemical Additives

Meat samples	Tvc/g 10^{-4}	Tvc/g 10^{-5}	Total	Mean	Cfu/g	USDA
MSG 1%	NA	$95X10^{-5}$	95	95	95.0×10^5	Acceptable
MSG 2%	NA	$128X10^{-5}$	128	128	128-× 10^5	Acceptable
MSG 3%	$200X10^4$	$100X10^{-5}$	300	150	150-× 10^5	Acceptable
SNT 1%	NA	$63X10^{-5}$	63	63	630×10^5	Acceptable
SNT 2%	NA	$21X10^{-5}$	21	21	21.0×10^5	Acceptable
SNT 3%	$175X10^4$	$15X10^{-5}$	190	95	95.0×10^5	Acceptable
MSP 1%	NA	$31X10^{-5}$	31	31	31.0×10^5	Acceptable
MSP 2%	NA	$48X10^{-5}$	48	48	48.0×10^5	Acceptable
MSP 3%	$87X10^{-4}$	$124X10^5$	211	105.5	105.5×10^5	Acceptable
SCL 1%	NA	$68X10^{-5}$	68	68	68×010^5	Acceptable
SCL 2%	NA	$55X10^{-5}$	55	55	55.0- × 10^5	Acceptable
SCL 3%	$203X10^4$	$76X10^{-5}$	279	139.5	139.5- × 10^5	Acceptable

MSG = Monosodium glutamate MSP = Monosodium phosphate
SCL = Sodium chloride SNT = Sodium Nitrite

Prevalence of Bacterial Isolates Identified from Chicken Breast Meat Cured with Different levels of Chemical Additives

Table 2 shows the result for prevalence of bacterial isolates identified from chicken breast meat treated with different levels of chemical additives. Results shows that a total of 36 bacteria were isolated from nine (9) genera of bacteria including *Bacillus Cereus* (22.22%), *Staphylococcus aureus* (19.44%), *Pseudomonas auregenosa* (16.67%), *Streptococcus pneumonia* (8.33%), *Lacto bacillus (Strepto)* (8.33%) *Bacillus anthricia* (8.33%), *Wrella Shella pneumonae* (5.56%), *Bacillus subtilis* (5.56%), *Bacillus megaterium* (2.78%) and *Micro coccus* (2.78%).

Table 2 Prevalence of Bacterial Isolates Identified from Chicken Breast Meat Treated with Different Chemical Additives and their Concentration

Bacteria Isolates	Prevalence	
	Frequency	Percentage (%)
Bacillus Cereus	8	22.22
Staphylococcus aureus	7	19.44
Pseudomonas auregenosa	6	16.67
Streptococcus pneumonia	3	8.33
Lacto bacillus (Strepto)	3	8.33
Bacillus anthricia	3	8.33
Wrella Shella pneumonae	2	5.56
Bacillus subtilis	2	5.56
Bacillus megaterium	1	2.78
Micro coccus	1	2.78
Total	36	100

The presence of Bacteria spp in chicken breast meat identified from all chicken breast meat sample were 36 bacteria which Bacillus cereus 22.44% with frequency of 8, Staphylococcus aureus 19.44% with frequency of 7, *Pseudomonas auregenosa* 16.67% with frequency of 6, Streptococcus pneumonia 8.33% with frequency of 3, *Lacto bacillus* (strepto) 8.33% with frequency of 3, Bacillus anthricia 8.33% with frequency of 3, Wrella shella pneumonia 5.56% with frequency of 2, *Bacillus subtilis* 5.56% with frequency of 2, *Bacillus megaterium* 2.78% with frequency of 1 and lastly Micro coccus 2.28% with frequency of 1. Ten (10) bacteria isolates identified from chicken breast meat cured with different chemical additives and twelve samples were contaminated with a total of ten (10) bacteria spp. From the result obtained from the experiment Bacillus cereus was found to have the highest prevalence of bacteria accounting for (22.22%) of the bacterial population isolated. This has indicated a higher prevalence of bacterial contamination from the study area. Followed by Staphylococcus aureus (19.44%), *Pseudomonas auregenosa* (16.67%), and Streptococcus pneumonia (8.33%) each. However, Beli, *et al.* (2001) have revealed a low prevalence of Salmonella in turkey meat in Albania (8.2%). In Ireland, Jordan, *et al*. (2006) have found (3.1%).which the report show a significance differences with the current studies.

Conclusion

The results revealed that Monosodium glutamate treated samples at 2% had lower microbial load (128×10^5) than all other

samples Total microbial load of chicken breast meat falls within the USDA safety limit for chicken breast meat, which indicated it was acceptable with in the consumer. However, despite chemical treatment, 36 bacteria were isolated from nine (9) genera of bacteria. According to prevalence, the bacteria include: *Bacillus Cereus* (22.22%), *Staphylococcus aureus* (19.44%), *Pseudomonas auregenosa* (16.67%), *Streptococcus pneumonia* (8.33%), *Lacto bacillus (Strepto)* (8.33%) *Bacillus anthricia* (8.33%), *Wrella Shella pneumonae* (5.56%), *Bacillus subtilis* (5.56%), *Bacillus megaterium* (2.78%) and *Micro coccus* (2.78%). The presence of *Streptococcus pneumonia* is a human pathogenic bacterium and it is recognized as a major cause of pneumonia and meningitis in children and the elderly which may result to health challenges. The research finding revealed *Bacillus cereus* was found to be more prevalent in all the total chicken breast meat samples collected and also the presence of *Streptococcus pneumonia* is a human pathogenic bacterium and it is recognized as a major cause of pneumonia and meningitis in children and the elderly which may result to health challenges, Bacillus cereus was found to be more prevalent in all the total chicken breast meat samples collected. There should be enlightenment and awareness to the public as regarding the quantity of all the chemical additives that we use in our foods and meat so that adequate and right proportion of the chemicals should be used in order not to exceed the recommended standard set by World Health Organization (WHO) and United State Department of Agriculture (USDA) for guarantee safety of both food and meats for consumptions.

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