ENHANCING QUALITY AND SAFETY OF INDIGENOUS CHICKEN MEAT PROCESSING IN KENYA

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A Thesis Submitted to the Graduate School in Partial Fulfilment of the Requirements for the Doctor of Philosophy Degree in Food Science of Egerton University

EGERTON UNIVERSITY

JULY, 2021

DECLARATION AND RECOMMENDATION

Declaration

I declare that this thesis is my original work and has not been presented in this university or any other for the award of a degree.

Signature…  …….. Date …..3rd June, 2021…….

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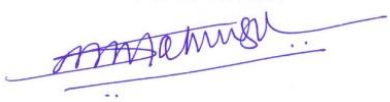
Signature……  Date……4th June, 2021…….

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DEDICATION

To the Almighty God whose Grace has been awesome in my life.

In loving memory of my late dad Mr. Dick Oloo Omolo, my late step mum Pamela Osoo Oloo, my loving mum Joyce A. Oloo and my siblings (Peter, the late-Caroline, James, George, Sabin, David, Moureen, Willis, Stephen, Victor, Dorothy, Fredrick, and Quinter).

Last but not least is to my wife and love of my life Sheillah and our wonderful daughters Tehilah Amor and Annabel Gweth.

ACKNOWLEDGEMENTS

My heartfelt gratitude goes to God Almighty for his grace to come this far. In deed He is Ebenezer. I also want to acknowledge Egerton University, the Department of Dairy and Food Science and Technology for the opportunity to undertake my studies. I am greatly indebted to the sponsors of the ABCF fellowship programme at the ILRI BeCA hub, the ILRI research community especially the Capacity Development, team headed by Dr. Wellington Ekaya, my research supervisor Dr. Roger Pelle and my research Associate, Ms. Eunice Machuka and the rest of the ABCF-2017/18 fellows. The development of the HACCP programme was supported by Nakuru County through the County Veterinary office in its bid to ensure a supply of high quality and safe indigenous chicken products to its dwellers. Thank you for the support during this study. I am also most grateful to my supervisors, Professor Symon Mahungu for unwavering support in my academic journey but more so in making this study a success. Professor, thank you for your selflessness, timely inputs, and an inspiring acumen. To Dr. Hon. Lillian Gogo, for her inspirational support through the different stages of this work, particularly with her keenness on making every presentation unforgettable. In equal measure I send my out pouring gratitude to Professor Alexander Kahi for the amazing support in my academic, professional, and financial through the InCIP project and through personal finances when the InCIP project wound up before the end of my studies. Very special thanks go to Professor Eric Amonsou for being an amazing help and for hosting me for 3 months of research at Durban University of Technology, South Africa. I also want to take this opportunity to thank the fellow graduate students in the Department of Dairy and Food Science and Technology of Egerton University including Ms. Linet Mwangi, Ms. Milkah Wambua, Mr. Johnson Mwove and Mr. Nobert Wafula for their support. I am appreciative to Mr. Kamau, David and Belindah of JKUAT for fatty acid profile analysis support. Last but not least, I take this opportunity to thank my family members from my dear wife Sheillah, my mum Joyce, my siblings and my fellow church members at GOC Egerton University. Your kind of support is exactly what it took to see me through this phase. Finally, I had the chance to work with many farmers, agricultural officers, data collection assistants, slaughter assistants among many other stakeholders. Though space does not allow me to mention all of you by name, your help will forever be appreciated. May God bless you all abundantly.

ABSTRACT

Poultry meat is a low acid food that is associated with the presence of foodborne pathogens such as *Campylobacter*, *Escherichia coli*, *Salmonella enteritidis*, and *Staphylococcus aureus*. The study developed a Hazard Analysis and Critical Control Point (HACCP) plan for IC processing at Nakuru Slaughter house.It also evaluated metagenomics of microbial ecology of indigenous chicken along the value chain with main emphasis on the microbial characterisation based 16S rRNA for typing of pathogenic strains. The results from IC ecotypes were contrasted to that of the broiler. The sensory quality of IC under intensive system was evaluated based on Quantitative Descriptive Analysis (QDA) and Just About Right (JAR) scale by a trained panel of 13. The nutritional quality of indigenous chicken was evaluated by proximate analysis based on AOAC official methods, while fatty acid profile was determined using a Gas Chromatograph interfaced with Flame Ionisation Detector, GC-FID. Amino acid profile was determined based on PiCO Tag, by derivatization in Reverse Phase High Performance Liquid Chromatography, RP-HPLC attached to RF2000 Fluorescence detector. One dimensional SDS-PAGE analysis was used to evaluate the molecular weight profile of sarcoplasmic and myofibrillar proteins. Protein isolates' functionality was studied through, digestibility, solubility, emulsification and foaming capacities. Nakuru top market slaughter house demonstrated a process out of control hence high prevalence of pathogenic microorganisms such as *Salmonella*(16%), *Staphylococcus Aureus* (15.7%). Four Critical Control Points (CCPs)to deal with the identified hazards were reported. The farm had CCP1 to eliminate antibiotic residues, CCP2-was at the final rinse to eliminate microbial contamination (*E. coli* and *Listeria*), CCP3 at packaging to eliminate any chemical toxins from packaging material and CCP4 at storage to eliminate *E. coli* proliferation. There was significant effect $P<0.05$ of IC on a 9 point QDA scale for aroma (5.17 and 4.39), flavour (5.52 and 4.3), and brown colour intensity (3.89 and 6.94) for breast and thigh respectively. The amino acid profile, registered the highest value for Glutamic acid (14.3 g/100g) which correlates well to the taste of chicken. The ratios of omega -6: omega -3 was above the generally recommended ratio of 4:1. The Kakamega ecotype had the lowest ratio (9:1), suggesting better source of nutrition. Distinct protein band pattern was reported between sarcoplasmic (12) and myofibrillar maximum of 10. IC microbiome hadwider diversity and relative abundance of novel bacteria associated with improved heath, feed intake, and utilisation of vegetable-based diets. Thestudy recommends complete implementation of designed HACCP to guarantee safety and exploitation of knowledge of IC protein functionality and microbiome composition to improve IC's processing quality and safety.

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LIST OF ABBREVIATIONS AND ACRONYMS

ABCF	Africa Biosciences Challenge Fund
AOAC	Association of Analytical Chemists
BeCA	Biosciences Eastern and Central Africa
BRC	British Retail Consortium
CAC	Codex Alimentarius Commission
CCP	Critical Control Point
CDC	Centre for Disease Control
DMS	Degree/Minutes/Seconds
DNA	Deoxy-ribonucleic Acid
EU	European Union
FAO	Food and Agricultural Organisation of the United Nations
FID	Flame Ionisation Detector
GC	Gas Chromatography
GIT	Gastro Intestinal Tract
GPS	Global Positioning System
HACCP	Hazard Analysis and Critical Control Point
HPLC	High Performance Liquid Chromatography
HPvR	Hypervariable Regions
HS	High Sensitivity
HT-NGS	High Throughput-Next Generation Sequencing
IC	Indigenous Chicken
ILRI	International Livestock Research Institute
InCIP	Indigenous Chicken Improvement Programme
ISO	International Organisation for Standardization
JAR	Just About Right
KEBS	Kenya Bureau of Standards
KNBS	Kenya National Bureau of Statistics
LAB	Lactic Acid Bacteria
MCH	Histocompatibility Complex
MUFA	Mono Unsaturated Fatty Acids
NACMCF	National Advisory Committee on Microbiological Criteria for Foods
NAFIS	National Farmers Information Services

NASA	National Aeronautics and Space Administration
NGS	Next Generation Sequencing
PCA	Principal Component Analysis
PCoA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PUFA	Poly Unsaturated Saturated Fatty Acids
QDA	Quantitative Descriptive Analysis
QIIME	Quantitative Insights into Microbial Ecology
QRA	Quantitative Risk Assessment
RP	Reverse Phase
rRNA	ribosomal Ribonucleic Acid
SDS PAGE	Sodium Dodecyl Acrylamide Gel Electrophoresis
SFA	Saturated Fatty acids
TCC	Total Coliform Counts
TQM	Total Quality Management
TVC	Total Viable Counts
USAID	United States Agency for International Development
WHC	Water Holding Capacity
WHO	World Health Organisation

CHAPTER ONE
INTRODUCTION

1. 1 Background Information

According to the Food and Agricultural Organisation of the United Nations (FAO), the most important challenge facing the world today is food insecurity (FAO, 2014). In response, the sub-Saharan countries made a commitment to invest in improved poultry production as an area of focus in provision of dietary protein. In Kenya, the government responded by the revitalisation of Kenya National Poultry Improvement & Programme ((KNPIP)(2010) within the Ministry of Livestock to enhance competitiveness of indigenous chicken (IC) in Kenya. This has elicited renewed interest in indigenous chicken in the past few years with evidence suggesting that rearing of IC can improve the livelihood of most households in Kenya (Ahlers *et al.,* 2009). This is because; poultry in most rural households are managed by women and children. The poultry products are also expected to contribute to the world's demand for animal proteins cheaply, quickly, and safely (FAO, 2009). The indigenous chicken has several advantages over their exotic counterparts. First, they are adaptable scavengers who are very resistant to most parasites and diseases in the tropical regions. Secondly, they are very good feed converters; converting various locally available foods into high proteineous meat (Kingori *et al*., 2010). According to Kingori *et al*. (2010), quality attributes of the IC meat are concordant with consumer demands for its unique taste, texture, and nutritious leaner meat. Okello *et al*. (2010) reported that, the demand for indigenous poultry is high as some consumers prefer IC meat to meat from broilers and layers. This is because, they argue, it tastes better and it is more nutritious. In addition, the cost of producing indigenous chicken is considerably lower due to its higher resistance to pests and diseases (Bebe & Owuor, 2008; Ngeno, 2014). Bett *et al*. (2013) estimated the consumers' responsiveness to a premium price and their willingness to pay for the indigenous chicken products in Kenya. Their finding revealed that; consumers were willing to pay about 23% per kg more for indigenous chicken meat and about 42 % more for a single IC egg. This supports the report of the (United States Agency for International Development, [USAID], 2010).

In spite of these marked advantages, there is not yet a documented systematic study to validate this perception and provide impetus responsible for the desirable qualities of indigenous chicken over the exotic broilers. This is especially so from the point of view of nutrition, protein functionality, and microbiome composition. Furthermore, not much has been reported in terms of the evaluation of the microbial, hygienic, functionality, and sensory quality of IC in Kenya. The consumer demand for IC in Kenya and most parts of the world is on the

rise (Bett *et al*., 2013). Consumers on the other hand however must be assured of the quality of IC meat if they are to enjoy a continuous supply. In the interest of the worldwide breakout of avian flu in 2005, the question of biosecurity of indigenous chicken has solicited keen interest (Aila *et al*., 2012). This however, is a challenge to most farmers who are not capable of processing their chicken in a manner that guarantees food safety. The farmers' challenges are multi prong. A steady supply of high-quality IC meat is not yet realised due to lack of adequate processing facilities that take care of the food safety challenges in the chicken industry; such as the HACCP processing system. In spite of the well documented evidence of the benefits of dressing and selling IC chicken to a wider market, a simple and reliable processing method based on science that guarantees high quality IC meat product remains a hurdle to a majority of farmers in Kenya (Aila *et al*., 2012). Chicken meat being highly nutritious provides a good medium for the thriving of microorganisms some of which may be pathogenic. The most common pathogenic microorganisms associated with chicken meat are *Listeria spp*., *E.coli*, *Salmonella spp*., *Staphylococcus Spp*. and *Campylobacter spp*. One of the ways to address the safety concerns in IC processing is to develop a Hazard Analysis and Critical Control Point (HACCP) system for the IC processing. The methods or systems and facilities currently used for processing IC in the country raises several and serious safety concerns for the dressed carcass.

The use of Hazard Analysis and Critical Control Point (HACCP) system improves product safety by systematically anticipating and preventing health hazards before they occur (Schlosser *et al*., 2000). The HACCP system is a reliable way to guarantee safety and quality of meat products and promote regular supply of high-quality IC meat to the market. It can sufficiently supplement packaging, labelling, and supply of dressed IC chicken meat to supermarkets.Indigenous chicken dressed this way and supplied to supermarkets have reported about 50% price increase for suppliers (farmers) (Ondwasy *et al.,* 2006). Nakuru top market slaughter point is the oldest chicken slaughter house in the country. It has experienced various challenges post-independence including several closures along the way, due to public health concerns. Design of a model for the application and operationalisation of HACCP system was undertaken at the Nakuru top market slaughter house toguarantee consumers enjoy safe products of indigenous chicken meat from the slaughter house.This work set out to designan on-farm processing method of IC meat, based on HACCP principles and then evaluating the hygienic, nutritional, and sensory characteristics of IC meat from the different ecotype clusters. To effectively do so, the situational parameters of the slaughter house including the prevalence of pathogenic microorganisms were established through a baseline survey. There was no report

of any similar study ever having been conducted at the slaughter house. A gap analysis report based on Codex Alimentarius Code of hygienic practice for meat and meat products and BRC hygienic principles was employed to collate the necessary data on hygienic quality of the premise. A comprehensive study on the possible determinants of indigenous chicken in Kenya's sensory quality attributes is yet to be conducted. Given that indigenous chicken is clustered genetically based on the different ecotypes, this may have an impact. Up to the point of commissioning of this study, there were very limited reports of sensory quality of IC meat based on this ecotype cluster demarcation. To reduce on variability of the determinants of indigenous chicken's sensory attributes, sensory evaluation was undertaken for indigenous chicken grown under intensive system at Indigenous chicken Improvement Programme (InCIP) at Egerton University, Njoro Campus. The chickens were all reared under same agricultural practices and slaughtered at the same age. This chapter established the effect of the IC rearing method on the quality and safety of IC meat processed under HACCP. By comparing the results against broilers, the study points out the quality characteristics of indigenous chicken breeds that are responsible for the preferences experienced by consumers.

Though only scanty reports exist in Kenya about the specific causes of food borne illnesses, the available data already points to a serious food borne disease burden due to the contamination of foods by these pathogens (Ombui *et al.,* 2001). It also suggests that different nodes of the indigenous chicken value chain present different conditions for the thriving of these micro-organisms. A systematic study of ICs' microbial quality at the different nodes of the value chain has not yet been reported. While the conventional method of pathogen detection in Kenya and worldwide remains the classic culturing techniques, evidence suggest that only about 10% of all microorganism can be reproduced through culturing techniques (Amman, 1995; Hanson & Henson, 1996; Kieft *et al*., 1998).

Limited work has been done if any to establish microbial quality based on DNA sequencing of these micro-organisms at different nodes of the value chain and from the different ecotype clusters of indigenous chicken in Kenya (Hoffman & Baral, 2019). Application of DNA and Next Generation Sequencing (NGS) techniques to microbial studies in poultry and meat show great promise (Weinroth *et al*., 2019). DNA based techniques are robust in determining potential hazards and offers opportunity for faster and more reliable detection of beneficial and pathogenic residents of IC chicken gastrointestinal (GIT) track. Information is very scanty in literature supporting this kind of work in Kenya. A literature search on the nutritional quality of indigenous chicken reveals a gap particularly of the amino acid and fatty acid profiles of indigenous chicken. Whereas studies have focused on fatty acid

profile especially of broilers, the amino acid profile studies of IC in Kenya remain most limited (Chepkemoi *et al*., 2017). Furthermore, to the best of my knowledge no reports are available in literature with regard to the results of functionality of the proteins isolated from Kenyan indigenous chicken. Yet, such information is vital to the development of innovative products from indigenous chicken for the ever changing consumer needs landscape. The results of such a study will also form the basis for acquisition of functional ingredients from chicken.

Technological quality of chicken meat is an important parameter in ensuring better understanding of the functionality of indigenous chicken. Apart from information regarding proximate analysis which is crucial to laying the basis of nutritional quality of a food product, analysis of fatty acid profiles and amino acid profiles is crucial to conferring nutritional benefits or lack thereof of consuming certain foods. Such information on fatty acid profile and amino acid profile, is increasingly becoming important in the face of the worlds' increasing cases of lifestyle diseases. Not only are the results of nutritional profiles important to address the sources of lifestyle diseases, they are equally vital to understanding of parameters that could be connected to genetic studies for enhanced expression of those characteristics in indigenous chicken. Though this work did not explore the genetic expression of the desirable sensory and quality characteristics, the results can be used in creating markers for enhancing indigenous chicken's genetic production and improvement. Meat quality characteristic such as colour, texture, cooking loss and moisture retention are crucial to determining of consumer preference (Chepkemoi *et al*., 2017). The characterisation of IC protein quality based on SDS PAGE provides clues that can be correlated to the protein functionality. Functionality of IC protein can be studied based on parameters such as protein solubility, protein digestibility, protein foaming capacity, and emulsifying capacity. All these results for the 3 IC ecotypes in Kenya are presented in this study.

In summary, the aim of the current work was to conduct a baseline survey of quality and safety of Kenyan indigenous chicken slaughter houses represented by Nakuru top market slaughter house and then design a HACCP processing system plan for improved safety and quality of IC meat in the country.This work set out to develop an on-farm processing method of IC meat, based on the HACCP principles and then evaluating the hygienic, nutritional, and sensory characteristics of IC meat from the different ecotype clusters. It has also realised the effect of the IC rearing method on the quality and safety of IC meat. By comparing the results against broilers, the study points out the quality characteristics of indigenous chicken breeds that are responsible for the preferences expressed by consumers of these breeds.It has gone beyond establishing the nutritional and proximate quality of indigenous chicken to present

results of nutritional profile including amino acid and fatty acid profiles. It has also determined the functionality of protein isolates from the three ecotype clusters.This presents a complete picture that connects the observed quality and functionality characteristics to the genetic variability among these three Kenyan IC ecotypes.

To address the gap with regard to application of DNA metagenomics to the characterisation and identification of microbial communities along the indigenous chicken value chain, this study evaluated microbial ecology of indigenous chicken along the value chain based on 16S rRNA marker gene. Three different parts of chicken were sampled (breast, thigh and caecum). These samples were justified on the basis that the thigh and breast are most commercially important chicken parts as far as consumers are concerned while, caecum has been demonstrated to contain the widest abundance of microorganisms from chicken Gastro Intestinal Tract (GIT). The main emphasis was on molecular characterisation and DNA typing of pathogenic strains, their relative abundance, and diversity from different ecotype and parts of chicken. The study also evaluated metagenomics of IC at different nodes of the indigenous chicken meat value chain, and compared to that of the broilers.

1.2 Statement of the Problem

Indigenous chicken meat products are generally considered to be of high quality by consumers. This has resulted in increased demand with consumers showing a willingness to pay premium prices for IC meat products. However, obtaining a regular supply of high-quality IC meat to the market at an affordable price has not been realised. There is no reliable on farm science-based processing system in preparing their chicken for the market, leading to the low supply and restricted market. Though HACCP studies have been developed in many areas of food business, the existing models cannot be adopted without due consideration of the important factors surrounding chicken production. Of note is that, HACCP systems are premise, location specific and product line specific. Furthermore, the quality control measures for IC meat and meat products are quite underdeveloped in Kenya. To improve the safety of the IC meat products, the meat must be healthy and free from disease causing pathogens that are a risk to human health (Hoffmann & Baral, 2019). In fact, several slaughter houses in the country including the Nakuru municipal council's (Top Market poultry slaughter point) have been closed due to public health concerns. Public health records indicate that the foodborne disease burden due to eating of contaminated foods is on the rise. Chicken meat is a major cause of such contamination. This in turn leads to the low supply and restricted market. Furthermore, the traders have resorted to illegal slaughtering of IC without even the involvement of the meat inspectors from the veterinary department. This further puts the safety

of IC meat consumers at risk. The high global and local food borne disease burden, rampant misdiagnosis, and mounting numbers of unreported cases present a challenge to food safety practice in the world and specifically in the developing countries. Most of the food borne illness have been attributed to non-typhoidal diarrhoealeading to more than half of death in children. Poultry meat and meat products have been a culprit. In many cases, there are unreported illnesses and where reports are done, the tedious culture techniques sometimes lead to misdiagnosis or are too slow. In cases of outbreaks, culturing techniques may not identify the offending strains from others in circulation (Hoshide, 2019). Furthermore, 90 % of bacteria in natural environments fail to grow under artificial conditions (Amman, 1995; Hanson & Henson, 1996; Kieft *et al*., 1998). Molecular tools and DNA sequencing can contribute significantly to address these challenges and even ascribe unique functionalities to the microbial communities in poultry. However, High Throughput Next Generation Sequencing (HT-NGS) techniques are yet to be substantially employed. This information can improve indigenous chicken safety and aid public health departments in curbing foodborne illnesses.

1.3 Objectives

1.3.1 General Objective

The main objective of this research was to contribute to safety and quality of IC meat processing by developing a HACCP plan, describing the sensory nutritional, protein functionality and, metagenomic analyses of ICecotypes and body parts for improvedproductivity, profitability, and enhancedlivelihoods of IC stakeholders.

1.3.2 Specific Objectives

The specific objectives were:

i. To conduct a baseline study of the current microbial quality and safety status of the IC processing facility at the Nakuru Top market slaughter house.
ii. To design a Hazard Analysis and Critical Control Point (HACCP) system for Kenyan IC meat processingat Nakuru top market slaughter house.
iii. To determine the effect of indigenous chicken ecotype, and body part on quantity and functionality of protein isolates from IC chicken meat.
iv. To determine the descriptive quantitative sensory quality of Kenyan indigenous chicken from the different ecotype clusters.
v. To evaluate the nutritional quality of indigenous chickens' breasts and drum sticksfrom different IC cluster ecotypes.

vi. To determine the metagenomics of microbial communities at different nodes of the value chain of indigenous chicken ecotype clusters in Kenya based on 16S rRNA molecular techniques.

1.4 Hypotheses

i. The current state of quality and safety of the Nakuru top market slaughter house is out of sync with processing technologies that ensure safety.

ii. A design of HACCP systemthat guarantees safety of IC at Nakuru top market is possible

iii. The indigenous chicken ecotype, and body part does not have a significant effect on quantity and functionality of protein isolates from IC chicken meat.

iv. Descriptive Quantitative sensory quality of Kenyan indigenous chicken from the different ecotype clusters is neither affected by sex nor ecotype cluster.

v. The nutritional quality of indigenous chickens' breasts and drum sticks is not different for the different IC cluster ecotypes.

vi. The metagenomics of Microbial communities of IC is not affected by different nodes of the value chain and Indigenous chicken ecotype clusters in Kenya based on 16S rRNA molecular techniques.

1.5 Justification of the Study

Indigenous chicken by FAO have been singled out as an important strategy toward improved livelihoods for small scale farmers and rural households while providing an affordable source of high-quality protein. Global forbornedisease burden estimations show significant costs. Chicken meat has also been considered among top foods leading to foodborne illnesses. In Kenya, stakeholders established the indigenous chicken improvement programme as a policy directive toward improved productivity, quality, and safety of indigenous chicken. As farmers embrace indigenous chicken farming for increased income and food security in Africa, it is important that a processing system that ensures consumer safety is established and adhered to. This will enable farmers to access more high value markets while ensuring consumer safety and reduced disease burden. The IC have been classified in recent years based on microsatellite alleles into different ecotypes. The significance of this classification can be best utilised by determining the relationship between the differentecotypes and sensorial, technological, nutritional, and protein functionality. It is also important that the physical, compositional, and functional quality of the indigenous chickens' meat is evaluated and documented. This would ensure a regular supply of high-quality meat to the market at

minimum costs. Furthermore, it wouldbetter inform the pricing and value of the produce to ensure farmers or producers get a price value commensurate with the quality of meat they supply. Development of HACCP system for IC meat processing and evaluating the quality of IC meat will provide an opportunity for extending the market for indigenous chicken meat products far beyond the East African region to the global markets. But to achieve this, 16S rRNA metagenomics as a next generation sequencing tool was useful in availing scientific data for action of the public health departments in curbing foodborne illnesses. To improve the safety of the IC meat products, the meat must be healthy and free from disease causing pathogens that are a risk to human health. HACCP studies are used because of its robustness in dealing with control of hazards based on the 'farm to fork' approach. A baseline of microbial ecology of indigenous chicken in Kenya was studied, using a rapid, reliable, next generation sequencing technology based on 16 sRNA for bacterial identification to the species level. The classical method for microbial detection in food and related matter has been use of culture techniques. There are however some limitations to the reliability on culture techniques for microbial detection and enumeration. To succeed with culture techniques, one must be able to mimic the growth characteristics of the medium being used for microbial growth in the laboratory conditions. Even though most media have been designed to take care of this purpose, there are always limitations. It is either very difficult or in some cases not possible and impractical with current technology to mimic the complex anaerobic environments within the GIT. In such cases, culture-independent methods HT-NGS techniques become critical. In addition to simple microbial identification, NGS techniques can be harnessed to predict nutritional and ecological roles of GIT microbes.The 16s rRNA metagenomics was chosen over short gun metagenomics because of its application of PCR to amplify a specific region of DNA while ignoring the host DNA. This ensured none microbe reads (chicken DNA) did not obscure the readings. The shotgun on the other hand would amplify all the DNA including host DNA from chicken which would have obscured the microbiome DNA. The current study fits well into the indigenous chicken improvement programmes and the country's climate smart agricultural development programmes being supported by the World Bank. The right to safe food is entrenched into the Kenyan constitution. This work fits well into Kenyan Government's Big 4 agenda under the nodeof Food Security and Nutrition. The chicken improvement programme fits in to the Nakuru County Integrated Development Plan (CIDP).

1.6 Scope and Limitations

The covered indigenous chicken ecotypes from three different counties which represent the 3 ecotypes in Kenya. It also designed a HACCP plan for Nakuru Top market slaughter house. As a result, it is was limited to the sample size and time.

CHAPTER TWO
LITERATURE REVIEW

2.1 Introduction to the Poultry Industry in Kenya

Most developing countries are experiencing rapid population growth rate which has placed an increasingly high demand on land for agriculture. This has led to a decrease in arable land, hence the need to focus on enterprises that will give rise to readily acceptable products and other enterprises with less land requirements. Poultry and other enterprises that will give rise to readily acceptable products to the consumers will be key in alleviating the arable land shortage (Kiptarus, 2005). The Kenyan livestock sub sector has poultry as one of its main components.Of the Kenyan poultry population, 76% are indigenous chicken (IC), with a population of 42 million birds (FAO, 2014; KNBS, 2016). This figure has increased to 46 million as of a report by Vajana, Lozano-Jaramillo and ILRI (2019). The IC in Kenya contribute to the economic, social, and cultural welfare of the populace (Kimani *et al*., 2006; Kitalyi, 1998). They are characterised by a unique set of feeds which is different from that used for conventional broilers in commercial farms. The indigenous chickens remain an important part of the rural households in Kenya (Bebora *et al*., 2005). Poultry contribute to employment, family nutrition, and income from sale of eggs and the birds (Maina, 2005).

The poultry production in Kenya has been characterised by a rapid growth and expansion. There has been a gradual increase of poultry in Kenya. The birds' population increased from 21 million in 1993 to 29 million in 2001 and in 5 years' time the population had reached 34 million in 2006. It is estimated was the current population of birds in Kenya was about 42.4 million in 2016 and 46 million in 2019 just after 3 years. Before 2000 the IC population represented 80% but the proportion of indigenous chicken is currently estimated at over 76% (NAFIS-National Farmers Information Services, 2018). Indigenous chicken in the rural areas are associated with free range management system or backyard flocks in the urban and peri-urban areas of most developing countries (Zephania, 2009). After the promulgation of Kenya's new constitution in 2010, counties have spearheaded the construction of slaughter houses to ensure high quality chicken meat and the safety of consumers. This move is yet to bear fruits because of very slow and inadequate support from County governments (Nairobi City County Assembly, 2014).

In order to link farmers to high value markets, policy makers, stakeholders, and Government need to improve the whole IC value chain. This can be done through proper production management , conservation ofgenetic resources, and sustainable utilisation (Bett *et al*., 2013). The other key enabler is the promotion of infrastructural developments and

strengthening poultry producers' organisations to champion their marketing needs.However, there are several constraints to the expansion and improvement of the poultry farming in Kenya. Some of these constrains include the targeting of the indigenous poultry for decimation by bacterial and viraldiseases such as the New Castle disease, *Escherichia coli*, and *Ecto-endo-haemoparasites* (Bett *et al*., 2013).

2.1.1 Indigenous Chicken Production System and Demand

Poultry sector contributes to 30% of agricultural GDP which in itself accounts for 25% of overall Kenyan GDP. It has been estimated that it contributes to 3.7% of per capita annual animal protein consumption. Chicken is an absolutely important protein source among poor households.The poultry sector is estimated to employ more than 3 million people and it has been known to play a great Socio-cultural role among various communities for various reasons. The average smallholder IC farmers have about 20 chickens per household and this number fluctuates based on the time of the year as the chicken are traded for other food sources or simply converted to money to serve other means.

Indigenous poultry –are mostly reared in rural areas, but this trend is changing as peri-urban dwellers adopt the rearing of IC or improved breeds of IC particularly for meet or to boost their economic situations. The commercial rearing or exotic chicken predominates in the – peri-urban & urban areas. The poultry industry in Kenya may be subdivided into 4 main sectors. Each sector shows certain distinctcharacteristics but there are also very many overlaps. The first sector consists of the industrial integrated system where the mode of processing is Commercial and there is use of huge amounts of inputs. A good example here includes KenChic Ltd. The production system is in large-scale commercial farms carrying several thousand birds. There is clear demonstration of upstreamintegration day old chicks'production withdownstream –integration made possible by use of contract farmers with 3- 12,000 birds. They also have poultry meat outlets and they operate high bio-security measures at all points of production. For most, their slogan is– "From farm to fork".The second sector refers to commercial sector. Most of the players in this sector also carry out processing at a commercial scale. The biosecurity measures practiced by farmers in this sector range from moderate to high. This sector also consists of hatcheries. They typically rear 10,000-12,000-layer breeders and approximately 10,000-18,000 broiler breeders per annum. There is presence of high bio-security measures in place in this sector. They are characterised by use of moderate to high levels of inputs.

The third sector refers to the semi-commercial farms where they sell live birds. These practiceslow to minimal levels of biosecurity. It is dominated by small-scale producers and accounted forby 23,661 broilers & 11,311-layer farms. These farmers derive about 73% of income from poultry. Some are contract farmers with KenChic Ltd. They more often obtain day old chicks from hatcheries. Those who practice contract farming get day old chicks, from vet care& market from KenChic Ltd. They often practice minimal to low biosecurity except KenChic farmers and their level of biosecurity is dependent on level of income& level of awareness. The final sector 4 is the village/ backyard farming system. This sector is characterised by verylittle marketing; with product being live bird. They practice minimal biosecurity measures with littleif any investmenton input is supplies.There are estimated 1.5 million households – with over 31.4 million birds in (2006). Theestimated average number of chickensbeing 20.8 chickens per household. Their focus is subsistence-oriented withvery low-input – most of it diverted from other sources. Because of this, they often experience low-output-of about 85% hatchability; with 55% chick survival rate. They practice very low bio-security; as chickens share houses with humans. The chicken is transported by foot, bicycle or public vehicles to reach various market destinations.Poultry consumption in Kenya is estimated to be about: 1.1 kg per capita p.a. of poultry meat. The consumption of eggs is estimated to be at 37.5 eggs per capita p.a. The major exports of day-old chicks go to the countries of: Uganda, Tanzania, Ethiopia, and Somalia. Whereas poultry meat from Kenya is exported to– Tanzania, Sudan, Ethiopia, Ghana, and Rwanda. Major imports for hatchingeggs come from – USA, and United Kingdom.

2.1.2 Geneticdiversity and Histocompatibility Complex (MHC) Region Variability

Ngeno (2014), investigated genetic diversity and major histocompatibility complex (MHC) region in indigenous chicken (IC) ecotypes in Kenya. They collected blood samples from eight counties of Kenya, namely; Kakamega and Siaya in the Western region, West Pokot and Turkana in North Rift, Bomet and Narok in South Rift, Lamu and Taita-Taveta in coastal region. There after the blood samples were genotyped with twelve microsatellite markers. In total, 140 alleles were detected from their study. Their findingwas that the chicken MHC marker LEI0258 revealed 46 alleles and thus indicated that IC host many and highly diverse alleles which are associated with disease and parasite tolerance (Ngeno, 2014). They also conducted a clustering analysis that indicated a clear ecotype subdivision into three genetically distinct groups. Their results revealed two main population clusters indicated by ad hoc statistic ΔK, posterior probability (Ln P (D)) of the data and PCoA were revealed as Lamu (one cluster),

Taita-Taveta (second cluster) and populations from Kakamega, West Pokot, Turkana, Bomet, Narok and Siaya as a third cluster. Chicken meat is a widely consumed product among most communities of the world (Chepkemoi *et al*., 2017). Globalisation and industrialisation have provided more market opportunities for these products to a great extent. There are five main characteristics that contribute to the overall eating quality of meat and meat products. These are: taste, texture, juiciness, appearance and aroma. Among these characteristics, texture is probably considered the most important attribute by the average consumer.Mechanical factors (tenderness) and juiciness (succulence) contribute to the difference in meat texture (Wattanachant, 2008). The tenderness of meat is the sum total of the mechanical strength of skeletal muscle tissue and it's weakening during the post-mortem aging of meat. The former depends on species, breed, age, sex and individual skeletal muscle tissue of animals and fowls (Takahashi, 1996). Meat tenderness originates in structural and biochemical properties of skeletal muscle fibres, especially myofibrils and intermediate filaments. It is also contributed to by intramuscular connective tissue, (the endomysium and perimysium), which are composed of collagen fibrils and fibres (Takahashi, 1996).

2.1.3 Demand and Trends of Indigenous Chicken

According to the Food and Agricultural Organisation of the United Nations (FAO), food insecurity is among the most important challenges facing the world (FAO, 2014). As a result, the sub-Saharan Africa countries took necessary steps to invest in poultry breeding. The aim was to utilisepoultry in provision of dietary protein, and improve livelihoods. This has raised interest in indigenous chicken (*Gallus domestica*) in the past few years with evidence suggesting that rearing of IC (*Gallus domestica*) can improve the livelihood of most households in Kenya (Ahlers *et al.*, 2007). In most rural households, women and children manage poultry keeping. To this extent, poultry raring extends financial benefits to the most deserving populace. The poultry products are also expected to contribute to the world's increasing demand for animal products cheaply, quickly, and safely (FAO, 2014).

Consumer demand for IC in Kenya has been on an upward trajectory. Studies show that Kenyan indigenous chickens (IC) (*Gallus domestica*) are generally reported to be most preferred by consumers due to several desirable characteristics; (Bett *et al.*, 2011; Islam & Nishibori, 2009; Kingori *et al.*, 2010). According to Kingori *et al*. (2010) quality attributes of the IC meat are concordant with consumer preferences for its unique taste, texture, and nutritious leaner meat. Okello *et al*. (2010) reported that, the demand for indigenous poultry is high as some consumers prefer IC meat to meat from broilers and layers (Okello *et al*., 2010).

This is because, they report that IC tastes better and it is more nutritious. Current trends in Kenya and in most developing countriesis to move away from the traditional free range scavenging rearing methods to semi-intensive and intensive systems of chicken rearing (Kamau *et al.,* 2018).

2.2 Chicken Meat Quality Determinants

Many factors such as breeds or genotypes, rearing systems, feed, age, muscle pH, chemical compositions, microstructure of muscle, post-mortem aging, and processing methods influence the quality of indigenous chicken meat (Jaturasitha *et al*., 2008a). Breeds or genotypes have more impact on colour and appearance of indigenous chicken meat but less influence on chemical compositions. This is most noticeable when determined comparatively at the same age of slaughter. The quality of the indigenous chicken meat also relates to its composition especially the muscle proteins, intramuscular collagen, and intramuscular fat content. These attributes are strongly affected by the breed, rearing system, and age of the chicken (Baracho *et al*., 2006; Shen *et al*., 2005). Quality description is often incomplete unless the poultry meat is free of hazards including: microbial, chemical, and biological that renders the meat unsafe for human consumption.

According to the ISO standard (ISO 8402-94), quality is defined as the set of characteristics of an entity that give that entity the ability to satisfy the expressed and implicit needs of its user or consumer (International Organisation for Standardization, ISO, 2015; Tougan *et al*., 2013). From this definition, the complex nature of the concept of quality is realised. Quality evaluation for indigenous chicken therefore entails the detailed analysis of a combination of factors that contribute to the consumers' perception about the product. The meat quality concept is used to add to the overall meat characteristics including its physical, chemical, morphological, biochemical, microbial, sensory, technological, hygienic, nutritional, and culinary properties (Jassim *et al*., 2011). Many consumers judge meat quality on the basis of appearance, texture, juiciness, water holding capacity, firmness, tenderness, odour, and flavour (Jassim *et al.,* 2011). These are the most important parameters of judgment across all cultures. They are also the most perceptible qualities that affect the initial and final judgment of the consumers. Quality of meat includes: quantifiable properties of meat such as water holding capacity (WHC), shear force, drip loss, cooking loss, pH, shelf life.Other important quality determinants include; collagen content, protein solubility, cohesiveness, and fat binding capacity. These attributes are indispensable for processors involved in the manufacture of value-added meat products (Allen *et al*., 1998).

Meat processing requires a transformation in the physical, chemical and biochemical characteristics of meat. The ability of meat to undergo these sets of transformation, preservation, and packing during an industrial or artisanal process is what constitutes the technological quality (Boutten*et al*., 2003). The meat's technological quality is best appreciated through colour, WHC, and texture of raw meat or during industrial transformation (Gigaud *et al*., 2006).

The quality of poultry meat, in terms of textural characteristic, muscle microstructure, and the chemical composition of muscle protein are very important for comparing meat from different breeds. Main factors that influence the chemical composition, properties, and structure of the indigenous chicken muscle should be studied since they all relate to its meat quality. The information gained from the understanding of these factors, should be beneficial for controlling the fundamental quality factors on further production, breeding, and processing of indigenous chicken to improve the live weight, growth rate, chicken meat quality, and safety. The genotype (the breed and strain) of chickens plays a major role in carcass fatness and meat quality (Jaturasitha *et al*., 2008a).

With regard to nutrition, there are specific nutrient attributes of chicken that result in desired quality. Nutritional quality relates to capability of meat to nourish consumers with major food nutrients, as well as many other essential compounds (vitamins, minerals, and trace elements) (Tougan *et al*., 2013). The content and nature of existence of these nutrients, determine the contribution of a given meat product to nutrition. Together with provision of nutrients, nutritious food should result in positive health outcome for the consumer. Much of the value of a protein food is based on its amino acid profile whereby the high nutritional value is related to a high presence of essential amino acids. Amino acids represent over 90% of the crude protein in the body of poultry (Hunton, 1995).

Providing different feeds to indigenous chickens also has effects on the colour of chicken meat especially skin colour. Meat pH is affected by type of breed, muscle types, and post-mortem aging of the indigenous chicken.It is additionally vitalto determining the textural properties and colour of the indigenous chicken meat. The information on proteolytic enzymes relating to post-mortem changes during aging of the indigenous chicken muscle is still limited. Microstructure of muscle; especially of intramuscular connective tissue highly influence the texture of the indigenous chicken meat which is related to the breed and age of the chicken.

Fat content and fatty acid profile in muscle are also strongly related to meat quality, especially in terms of flavour, juiciness, and tenderness. Chicken supply fatty acids including the essential fatty acids and the Mono-unsaturated (MUFA) and Poly Unsaturated Fatty acids (PUFA) to

the human diet. Hunton (1995) noted that high unsaturated fat intakes may be preferable for humans; however, unsaturated fatty acids are more prone to oxidation. Wattanachant and others (2004) reported that indigenous chicken muscle contained a higher percentage of saturated fatty acids ($P < 0.05$) and a lower percentage of polyunsaturated fatty acids ($P< 0.05$) as compared with broiler chicken muscle. The current study focused on development of a HACP plan based on the HACCP principles. The aim was adoption of HACCP plan applicable to an-on farm processing of IC to replace traditional inspection and introduce safety to areas that had no inspection at all so as to avert occurrence of hazards in the final product.

Poultry farming provides a food security component to the rural communities particularly in the growing economies. For a long time, indigenous chicken has been a ready source of affordable excellent source of proteins for rural households. The muscle portion used for food represent up to 35-60% in some cases of the poultry carcass by weight. Poultry is also one of the best nutritional sources with regards to proteins which result in higher digestibility and a high concentration of essential amino acids (Listrat *et al*., 2016). Several factors including the combination of the contractile, and metabolic types, sizes and number of muscle fibres determine consumer preferences. The content, composition, distribution of the connective tissue, and lipid composition of the intramuscular fat play a role in the determination of flesh appearance, colour, tenderness, juiciness flavour and technological (processing) value of chicken (Listrat *et al*., 2016). These factors are most important determinants of consumer choices and preference in the purchase of poultry and other meat products (Mudalal *et al*., 2014).

The most important quality aspect of meat for average consumers is the sensory quality. This is the perception of the meat as discerned by consumers. Sensory quality regroups the aspect of colour, taste, flavour, odour, and the aroma. It also includes consistency and the texture of a food (Gigaud *et al*., 2006). Sensorial quality is a key determinant of consumer preference to chicken products. Sensory quality consists of qualitative, quantitative, or hedonic quality measurement. For chicken, most important quality attributes by the estimation of consumers are: colour, tenderness, juiciness and flavour (Santé *et al*., 2001).The firm texture of the indigenous chicken meat after cooking is a dominant quality that influences consumer acceptance. This textural characteristic of the indigenous chicken meat can result in resistance of the IC meat to more severe processing techniques. However, high cooking loss during heating process of IC chicken meat may result in a lower yield of the chicken products after processing as compared to that of broiler meat. Therefore, the development of breeds to improve muscle protein quality of this chicken meat needs to be studied.According to the ISO

standard (ISO 8402-94), quality is the set of characteristics of an entity that give that entity the ability to satisfy the expressed and implicit needs of its user or consumer(ISO, 2015). From this definition, the complex nature of the concept of quality is realised. Quality evaluation for indigenous chicken therefore entails the detailed analysis of a combination of factors that contribute to the consumers' perception about the product. The meat quality concept is used to add to the overall meat characteristics including its physical, chemical, morphological, biochemical, microbial, sensory, technological, hygienic, nutritional, and culinary properties (Jassim *et al.*, 2011).

Even when meat is of acceptable sensory quality, it still has to be safe to be of any value to a consumer. Poultry meat can be contaminated with a variety of microorganisms including spoilage ones during chill storage and even food borne pathogens. Handling of raw meat, undercooking or even mishandling of cooked products may lead to human illness due to *Salmonella* and *Campylobacter* species; *E.coli* 0157 strain, *Listeria*, *Vibrio* and *Yersinia* which are of the greatest global concern (World Health Organization [WHO], 2015a; Mead, 2006). The *Aerobacter* and the Helicobacter species and the *VerotoxigenicE. Coli* are also a growing threat. There is equally the growing problem of the antimicrobial resistance among poultry associated pathogens. As a result, there is a need for Universal systematic and applicable approach to food safety which has led to the increased introduction of the HACCP concept to poultry industry (Mead, 2006). With this dominance of HACCP has come Quantitative Risk Assessment (QRA) which is also being applied to microbial hazards according to FAO/WHO had completed studies on QRA application to broilers (Crotta *et al.*, 2017). The role of HACCP in poultry processing is multi-prong. First, it addresses zoonotic agents that are not detectable by conventional meat inspection procedures. At the same time, it also controls contamination of carcass with spoilage organisms. It has benefits in optimising plant hygiene that ensures compliance with legislations and is an evidence of 'Due diligence' on the part of the processors.

The quality of meat products must meet strict standards with regard to safety. Meat product safety includes microbial, chemical and physical. The hygienic quality demands that it should not contain any toxic chemical residues or growth of harmful bacteria. Hygienic quality of chicken meat is well documented especially with regard to microbial quality and toxic residues including antibiotic. Good quality chicken meat product that does not consist of any hazard is a primary right of the consumer (Tougan *et al.*, 2013). Poultry products are desirable, delicious, palatable, highly digestible and nutritious for all ages. Compared to beef or mutton products, the chicken meat products are low priced and affordable to a majority of consumers (AL-Dughaym & Altabari, 2010). Good quality meat products must be wholesome, safe, and

well. Further processing of poultry products into reconstructed cuts, or breaded forms lead to improvement in shelf life, juiciness, and the water holding capacity. Because of their nutritious nature, favourable pH, and lightly salted medium, the poultry meat products are an ideal ground for growth and proliferation of harmful microorganisms (AL-Dughaym & Altabari, 2010).

2.3 Role of HACCP in Determining Hygienic Quality of IC

The processing of indigenous chicken in a large scale in Kenya was begun by colonists in the 1960s. The Nakuru top market slaughter house became the very first one of its kind in the country. The municipal market has been slaughtering, de-feathering, and eviscerating the chicken for their set of customers since its establishment by white settlers in Rift Valley in late 1960. The chickens are usually held in cages next to the slaughter place in an open entrance to the main vegetables market in Nakuru. The chickens are often slaughtered at the small slaughter room besides the gate. There is no test on the quality of water, the microbial quality of the knives and working surfaces and the process does not follow a given quality system. Meat quality inspectors in the premise have not been consistently present. This leaves a gap in the management of quality and safety of chicken and could be a source contamination to consumers of the products.

Hazards are any chemical, biological, or physical substance or property that could render food unsafe for consumption (The National Advisory Committee on Microbiological Criteria for Foods, 2006). Hazard analysis refers to the process of the evaluation of all the potential hazards and deciding on which ones are significant as to be controlled by a HACCP plan. Hazard analysis consists of two main steps. Step one involves the identification of the potential threats to human health based on the production conditions of the premise and including raw materials and ingredients (ISO, 2018). The threats included those which may be introduced into the chicken meat product during processing or those associated with the raw material, upcoming ingredients used in processing, and dressing of poultry at the premise (Khaliduzzaman, 2017). The second step involves identification of a critical step at which a specific control must be applied to prevent, eliminate or reduce the occurrence of the specific hazard to an acceptable level (FAO, 2013).

Processed and none processed poultry products may harbour spoilage microorganisms as well as food borne pathogens. These microorganismswhere present, often lead to contamination of poultry meat. However, poultry products can also harbour food borne pathogens, such as *Salmonella* stereotypes, *C. jejuni*, *L. monocytogens*, *C. perfringens* and S. *aureus.* Poultry and poultry products rank very highly among foods linked tofood borne

diseases in most of the countries all over the world. For example, in the USA it ranked third of the reported food-borne disease outbreaks in the 1980s and 1990s (Waldroup, 1996). Currently they are the leading of cause of outbreaks associated with foodborne illnesses according to the CDC 2018 report entitled 'Surveillance for Foodborne Disease Outbreaks — United States, 2009–2015" (Centre for Disease Control and Prevention (CDC), 2018).

The HACCP plan is aimed at ensuring production, storage and distribution of safe foods. It therefore provides for precise process control measures for each and every step of the entire manufacturing process (Khairuzzaman *et al*., 2014). The HACCP system is not new in the food manufacturing industry. It was developed through a joint team of Food scientists and Engineers, from the Pillsbury, company the Natick Research Laboratories and NASA. It was designed to create a built-in quality into the product to ensure food safety for the manned space programme(Surak, 2008). From then on, the early results of its applications proved so great that it's become synonymous to food safety management systems. The HACCP system has been referred to as generic meaning they are applicable to processing plant of whatever size and whatever products. It takes into account only the actual conditions in the plant. The spread of diseases from unsafe poultry products due to the *Listeria monocytogenes*, *Escherichia col*i, and even food adulterants and chemical residues have been reported (Odwar *et al*., 2014b). This calls for the need to pay attention to the potential contaminants in chicken production. It is therefore very important to design prerequisite programmes, Hazard Analysis and Critical Control points. This exercise should be basedonscrupulous analysisof the production premise and processes.

The Codex Alimentarius Commission issued the first HACCP standard in 1993, which provided the first International definition of HACCP. As of the year 2000, several private, industry and National food safety standards existed.This existence of numerous standardsresulted in problems during third party audits. This led to steps by national organisations to harmonise these many standards leading to the formation of ISO family of standards. The ISO 22000 is a Food safety management system based on HACCP. HACCP is a scientific, systematic method for process control. It has been used for long in food production to prevent problems by applying controls at points in a food production process where the hazards could be controlled, reduced to acceptable levels or eliminated (Consumer Federation of America, n.d.).It involves an extensive evaluation and control over entire food production processes for the purposes of reducing potential for food related health risks to the consumer (Khaliduzzaman, 2017). HACCPsystem maintains the safety and freshness of the meat and poultry products because it anticipates potential hazards that may occur during rearing,

processing, and distribution. It then evaluates the hazards and either controls, or prevents their occurrence.

The nature of the HACCP system is product or commodity and premise specific.This implies that even where a company produces several products on the same production line, each product should have its own HACCP plan (Northcutt & Russell, 2010). In Kenya, indigenous chicken meat processing by HACCP technology is relatively new as a field of study, research, and even business. However, the increased consumer preference and demand for IC meat products has led to an expansion of products and business in this area, making HACCP application most timely.Poultry meat is a low acid food and is therefore associated with presence of food borne pathogens. The most dominant pathogens include;*Listeria monocytogens, Salmonella enteridis, Staphyloccocus aureus, Escherichia coli*, and others. The traditional quality control practicefocuses on testing and inspection services.This method is employed by regulatory bodies such as KEBs and is applied once the food safety problem has presented itself. In many parts of the country including at Nakuru market slaughter house, no such testing is even done. At the same time, it is difficult to maintain the product inspection, due to limited personnel, difficulty in sample handling and preservation. In addition,there is lack of awareness of the magnitude of the health concerns involved.HACCP is a scientific system that can be used to ensure that food safety hazards are controlled to prevent unsafe foods from reaching the target consumers (Bardic, 2001;IFST, 1998; Mortimore & Wallace, 1998). To ensure safety, the design of the HACCP is very essential and has a great importance in IC chicken processing safety for wider market access in Kenya and beyond.

It is evidenced that increasing consumer demand for safety and high-quality food with a prolonged shelf life is the desire of most consumers. One of the products with increased demands in this regard is the IC. Many safety and quality management systems such as ISO 9000, TQM, Lean Manufacturing or Sigma 6 and HACCP were developed to address this challenge. Since its invention HACCP has been widely applied and recognised as an effective and rational means of assuring food safety from production to consumption in a ‘farm to fork or table’ approach (Hofi & Ismail, 2008). When Pillsbury Company and NASA developed HACCP in 1963, the focus was on microbial safety system to ensure food safety for the astronauts. At the time, the rest of the food safety systems were based on end use testing which ultimately led to huge losses due to waste of finished product. These systems were never preventative at all but only reactive. As a result, the search for a preventive system was initiated that would give a high level of food safety assurance (Bardic, 2001; Bennet & Steed, 1999; Mortimore & Wallace, 1998).

The HACCP system is preventative in that it assesses the potential hazards, estimates the risks, and establishes specific control measures.It therefore emphasises prevention rather than reliance on end product testing (Ropkins & Beck, 2000). The main potential hazards in poultry products are microbial in nature. The chicken or poultry industry has been enhanced through HACCP processing worldwide but more needs to be done to streamline this process to the IC chicken in Kenya. Strict adherence to quality systems of processing Indigenous chicken meat should be paramount. The HACCP system identifies, assesses the hazards, and risks associated with manufacturing, storage, and distribution of a product. It then institutes appropriate control measures aimed at eliminating or reducing the hazards to acceptable level at a specific identified point of the production line (Zhao, 2003). The traditional inspection and testing, was only applicable once the problem had presented itself.Besides, getting 100% product inspection is difficult due to inherent human errorand impracticality where destructive sampling is necessary. In addition, there exist difficulty in getting sufficient samples. From the beginning HACCP, was invented as a means to a 'zero defect' programme and has almost become synonymous with food safety. The present work focused on the flow diagram based on the production line of Indigenous chicken meat.It presents a HACCP design based on analysis of the hazards and the institution of CCPs at the Nakuru, Municipal Market.

In the world two paradigms exist with regard to control of microbes in chicken meat processing facilities. First is the USA where carcass contamination is clearly reduced as carcass passes through processing. The carcass is finally chilled in super chlorinated water or a chemical rinse treatment for the reduction of microbial contamination (Mead, 2006). In the second scenario, the European Union processors are not allowed use of super chlorinated water.In this case, water chilling only has an important washing effect.Instead, they are confined to ensure carcass is frozen. The European Union had equally abolished any chemical decontamination until the year 2006 (Mead, 2006). For the freshly chilled carcass there is no marked reduction in carcass contamination and no Critical Control point at which a significant reduction in pathogen contamination can be guaranteed. Overall, effective control of microorganisms is best realised through a 'farm to fork' approach at all the stages of the supply chain. It must therefore be realised that the microbial quality and safety of poultry products are important to producers, retailers, and consumers.This is because both involve microbial contaminants on the final product (Mead, 2006). Generally, two classes of microorganisms are involved in the deterioration of quality and safety of poultry meat. These are spoilage organisms or the pathogenic ones.

2. 4 Pathogenic and Spoilage Microorganisms of Poultry Meat

The spoilage microorganisms (mostly the harmless and psychotropic) have the capacity to multiply in the chilled storage resulting in off flavours. The product shelf life is normally determined by both the number of spoilage microorganisms present initially and the temperature history of the product at all the stages of production, subsequent storage, and handling. Currently, accelerated shelf-life studies and computer models are effective tools being used to effectively predict shelf lives within a given level of accuracy and precision. Food contamination by foodborne pathogens remains an important Public health concern (WHO, 2015b). It leads to illness and this leads to suffering and loss of productivity. This also leads to high cost of production and increased cost in healthcare. These pathogens may be a cause of mortality particularly in developing regions due to compromised health status of many people.

According to a report by Havelaar and colleagues, the EU is most affected by *Campylobacter*and *Salmonella*species (Havelaar *et al*., 2015). But since these diseases are under reported the figures are likely to be higher (Hoffmann & Baral, 2019). Even though chickens are not the only source of these microbes, they are a majorculprit due to the following reasons. There is symptomless carriage in the birds, the intensive rearing, and the high processing rates. This puts carcass in close proximity throughout processing and can escalate the problem. These conditions may favour the spread of any pathogens that could gain access to the flock.The antimicrobial use of (prophylactic, therapeutic or performance enhancers) contribute to resistance in pathogens.This can lead to serious consequences for treatment of human illness from these organisms. For example, resistance of *S. Paralymphi B.* variant Java increased in poultry in the Netherlands from less than 2% in 1996 to over 60% by 2003 (Wannet *et al*., 2003). They indicated that resistance to *Campylobacter*to antimicrobials was also rising. In Kenya, a study by KEMRI, 2015 demonstrated that the existing microbial communities in chicken from selected outlets in Nairobi had microorganisms which were resistant to the commonly used antimicrobials or antibiotics (Odwar *et al*., 2014b).

Status and variety of microbial organisms in poultry are greatly diverse.Bacterial load has been reported to vary even from cuts stored under the same conditions. Microbial ecology reported so far in poultry meat are mostly based on culturing techniques. However, this is biased since relative selectivity is often experienced with culture media. Assessment of spoilage has been conducted via various methods. The challenges encountered include non- and/or poorly selective media to target LABS, or *enterobacteriacea*leading to incorrect characterisation of bacterial species present in a given food matrix(Mead, 2004).

Currently,three approaches are employed to the study of bacterial species in poultry matrix (Diaz-Sanchez *et al*., 2013). The first approach involves targeting few species (pathogens) with little attention paid to microbiota.This is usually because of low contamination level of pathogens in relation to total counts. The second one focuses on a wide range of microbes; meaning the microbiota is assessed with techniques which include bias in the identification of that genera list because of the media used.The third, approach involves the study of complex environments by non-cultural methods in poultry.This is found to be advantageous because it offers best description of the bacterial species present. This is irrespective of the pathogens which are often present in very small quantities. Though the GIT of birds and slaughtering facilities have been identified as main reservoir of poultry meat contaminants, knowledge of flux of microbiota along the production value chain from animals to end product is very limited. This is thereason why High Through put sequencing approaches, combined with highly selective cultural methods through the production value chain are necessary to assess the sources of meat contaminants, their identification, and dynamic on storage. Meta-transcriptomics may help determine the metabolic functions expressed by bacterial contaminants during meat process and storage (Diaz-Sanchez *et al*., 2013). Combined with metabolomics, the results enhance knowledge on the complex behaviour of poultry meat contaminants along the value chain. Such results can be harnessed to better manage meat the ecosystem and improve the microbial quality and safety of food (Diaz-Sanchez *et al*., 2013).

Detection and enumeration of microbes in foods is an important aspect of quality and safety control. The number of microbes or even their species define safety or acceptability of food for human consumption. The culturing technique and sample growing in the media including isolation are responsible for the period of analysis. This is then followed by biochemical/serological and subspecies characterisation. Current methods such as DNA and antibody-based tests or at least the modification of the conventional methods can improve on the period limitations.They are faster, have more sensitivity, and present a huge convenience. Currently, almost all the assays used to detect microorganisms are qualitative and this lacks sensitivity for direct testing and require growth in an enrichment media.One of the challenges to fullexploitation the new methods is the difficulty of sample preparation. By combining several methods, this challenge can be overcome and better diagnostics of microbes in poultry meat can be achieved.

2.5 The Next Generation Sequencing: Genome Based Approaches in IC Pathogenic Detections

The start of the era of molecular biology and automation of Sanger chain-terminator, heralded an era that has resulted in advanced diagnostic and discovery in poultry. The last 2 decades were dominated by the Sanger sequencing technology (Sanger & Coulson, 1975; Sanger *et al.*, 1977). This has given way to significant technological advancements in DNA sequencing and analysis. The high through-put next generation sequencing technologies were invented purposefully to undo any limitations of the first-generation technologies (Brockhurst et *al.,* 2011; Pareek *et al*., 2011). Some of the advantages that next generation High Throughput (HT-NGS) bring to the table includes: faster speeds, less tedious hence less labour cost and lower overall sequencing cost.Many platforms evolved as a result of these advances including sequencing by synthesis under 454 Lifesciences, Illumina technologies, SOLiD sequencing, and the Ion Torrent semiconductor sequencing technologies that apply different principles of detection. This progress has already lead to early advances and progress toward 3rd generation sequencing technologies including real time PCR, monitoring by fluorescent resonant energy transfer, and the Nanopore Sequencing (Diaz-Sanchez *et al*., 2013).

These technologies haveseveral advantages including: ease of use, scalability, better DNA polymerase performance and yields, reduced error and at lower cost. The fact that they aim ultimately to real time results is even most critical. Culture techniques in poultry safety has limitations that can be overcome by embracing the Next Generation Technologies in enhancing production, safety and quality.Some of the areas where these technologies may become useful include: sequencing of: gut microbial community, gene metabolic pathways and their expressions, detection of plasmids among others (Harlizius *et al.,* 2004). Whole ecology sequencing can be determined based on metagenomics (Brockhurst *et al*., 2011; Pareek *et al*., 2011; Schadt *et al.,* 2010). At the same time, microbial communities on poultry gut may be studied to reveal changes that affect health, and diseases along with the effectiveness of methodsemployed for their control.

In summary, the aim of the current study was to design a HACCP, determine the nutritional and sensory functionality of IC meat. It looked at nutritional, technological, functional quality of IC from three different ecotypes in Kenya. It further studied characterisation of microbial communities at various nodes of the indigenous chicken value chain in Kenya based on 16s rRNA technology to support efforts aimed at the improvement of ICs' productivity, quality and safety systems.

2.6 The Theoretical and Scientific Gap addressed by the current study

The current study aimed to address the following scientific and theoretical gaps: The first gap was related to HACCP and its applicability to the process and products at the Nakuru Top Market slaughter house. HACCP plans have been in use for many years as a basis of food safety programmes. For a time, implementation and certification schemes have been run by internationally oriented companies especially in developing countries such as Kenya. To this extent, few local suppliers of chicken have operationalised them. Several reasons have been attributed to this vis; perceived and real cost of the initial investment, need for highly competent professionals and attendant cost. In addition,huge technical capacity to operate thesystems among others have also been highlighted (Oloo & Oniang'o, 2018). For IC lack of good will to offer infrastructural services, localised food chains, and lack of market orientation, has been a hindrance to application of HACCP principles. Design of HACCP implementation was therefore customised to the local conditions of the slaughter house in Nakuru. The aim was for thedesigned HACCP plan to address the relevant hazardsin a manner that is scientifically sound and technically applicable. This study designed the HACCP by scrupulously evaluating the state of the current slaughter house then scrutinising the existing HACCP plans and adopting to the Nakuru top market slaughter house (Hofi & Ismail, 2008). It thereforeaddressed the scientific gap about the relevant hazards and their control in IC processing at Nakuru Top Market slaughter house. The Results of metagenomic analysis revealed microbial flux across the IC value chain.The findings input into the decision of relevant hazards and hence design of CCPsacross the IC value chain. This underscores the value of metagenomic studies and their relevance to food safety.

The second scientific and theoretical gap addressed by this study was knowledge functionality and quality of IC chicken in Kenya based on protein characterisation and its implications on IC processability. Utilisation of chicken meat as food depends not just on nutritional quality but also on technological quality. By the time of commissioning the study IC chicken had been clustered into 3 ecotypes based on the microsatellite alleles (Ngeno, 2014). The scientific and theoretical understanding of the relationship between the different ecotypes and their protein functionality was a gap that this study sought to address. Experiments were therefore conducted to determine whether there existeda difference in quality and functionality of the various protein isolates from the different IC ecotypes. The current study has determined and reports the existence of differences in protein quality characteristics and functionality based on the ecotype and body part of chicken.

The third theoretical and scientific gap addressed by the current study was to link the genetics classification of IC into ecotypes to the perceived or experienced nutritional and sensory quality differences among IC as basis of IC improvement programme.From the time of classification of IC into the different ecotypes based on the microsatellite alleles, the relevance to the nutritional and sensory quality attributes remains an area worthy of investigation. To evaluate this relationship an experiment was conductedto determine the nutritional and sensorial quality of IC based on the QDA and JAR scales. The results therefore providedrelationship of nutritional and sensorial quality between the ecotypes, part of chicken, and difference from the broiler which served as the positive control.

The fourth theoretical and scientific gap this study sought to address was in the novel area of potential for use of DNA based techniques in IC microbiome analysis. Until recently, safety of chicken has studied fromthe point of view of pathogen which can be grown by culture techniques (Weinroth *et al*., 2019). Whereas these culture-based techniques of microbial detection have been used extensively in the food industry, its effectiveness is limited to the microbes that can be detected on the different media. DNA based techniques emboldened with HTP sequencing techniques can address this limitation due its robustness in detecting even microbes that are not easy to culture (Weinroth *et al*., 2019). However, the utilisation of the DNA based techniques has been limited to a few areas such as ecology and rarely in food especially to boostthe food safety aspect (Stanley *et al*., 2013). Few of the studies especially on human microbiome projects shed light to the possibility of utilising data from gut microbiome to indicate health status, feed utilisation, and suppression of pathogens. There is potential to use them as tools to address food safety concerns by relating the microbiome flux to unique functions of improved gut health, improved feed utilisation, and suppression of pathogens.The scientific and theoretical gap existed regarding the utilisation of HTP DNA based sequence techniques to study chicken microbiomeand its relationship to food safety interventions. This study has generated important information regarding microbiomeflux across the IC value chain in Kenya.The metagenomic study also reveals the relationships between the microbes and the HACCP control protocols at the different parts of IC value chain. It has also generated information regardingthe potentialapplication of metagenomic studies in enhancing safety and hygienic practice along the IC value chain.

CHAPTER THREE

PREVALENCE OF ENTERIC PATHOGENS AND STAPHYLOCOCCUS AUREUS ON CHICKEN PARTS, SURFACES AND RINSE WATER IN POULTRY SLAUGHTER HOUSE IN NAKURU COUNTY

Abstract

Indigenous chickens in Kenya are reared in free range systems.In some cases, this may expose them to the risks of zoonotic diseases. Slaughter houses especially those located in major towns in Kenya are an important node of the value chain that supplies indigenous chicken to the consumers. The risk of contamination of the poultry carcasses persists into the slaughter houses and can only be prevented through use of strict food safety management systems. Routine surveillance of the most potent pathogenic microorganism in the poultry slaughter houses usually target, *Escherichia coli, Coliforms, Salmonella* and *staphylococcus aureus*. *Escherichia coli and Salmonella* are the two leading organisms responsible for most foodborne illnesses from chicken. A gap audit of the operationalisation of Good manufacturing practices (GMPs) was conducted in one of the largest poultry slaughter houses in Nakuru, County. A total of 33 swab samples from different surfaces (tables, floors, hands, knives, walls, slaughter containers) and process and rinse water were taken from one of the major poultry slaughter houses. The traditional culturing method was used to enumerate for the total viable counts (TVC), and *coli forms* (CC). Biochemical methods were used to identify the specific target microorganisms. There was no significant difference for the TVC and coliforms reported from the different sections. The overall prevalence of *E. coli* was detected at 15.9%, *salmonella* at 24.4 %, and *Staphylococusaureus* at 12.2 %. The de-feathering section had the prevalence of 15.79% of *E.coli, Salmonella* 32%, and *Staphylococcuss aureus* at 10%. The post-mortem area had an *E. coli*, prevalence of 7.69%, *Salmonella* of 23%, and *Staphylococcus aureus* of 15.38%. This study reported values of these pathogenic microorganisms beyond the hygienically acceptable standards. The results provided the basis for the prevalence of these micro-organisms against which the success of the implementation of the HACCP system will be measured. Given that *E. coli, Salmonella, and Stapylococus aureus* are microorganisms of well documented public health concerns, they should be minimised and their prevalence monitored on all surfaces during indigenous chicken processing at the slaughter house.

Key words: *Pathogens, microorganisms, slaughterhouse, prevalence, indigenous chicken.*

3.1 Introduction

According to the Food and Agricultural Organisation of the United Nations (FAO), the pertinent problem facing the world is food insecurity (FAO, 2014). To address this acute food insecurity challenge, the sub-Saharan countries commitment to invest in poultry breeding as an area of focus to address the protein energy malnutrition (FAO, 2014). The trend has hence beenrenewed interest in indigenous chicken (*Gallus domestica*) rearing. Presented evidence confirm that rearing of indigenous chicken (IC) can improve the livelihood of most households in Kenya (Ahlers *et al*., 2009; Kamau *et al*., 2018). Poultry in most rural households in Kenya are managed by women and children leading to availability of income and high protein foods to women and hence, the household members. Poultry products are taunted to assuage the world's increasing demand for animal-based products cheaply, quickly, and safely (FAO, 2009). In Kenya, consumers are expressing an ever increasing demand for IC (Bett *et al*., 2012). However, a steady supply of high-quality IC meat is not yet realised due to lack of a scientifically based processing system that can guarantee the safety and quality of the IC meat products. In major towns in Kenya, consumers rely on already dressed poultry from slaughter houses or from restaurants. The slaughter houses which are strategically situated in these major towns form an important node of the value chain that supplies consumers in the urban centres and its environs.

In Nakuru town, Nakuru Top Market slaughter house supplies indigenous chicken to consumers and retailers.The slaughter house was built in 1961 over an 11 months period commencing from the month of January to the month of November of the same year. The slaughterhouse was built as part of plans to modernise Nakuru by expanding the bus park, creation of a fresh produce open air market. The slaughter house was to supply chicken exclusively to the burgeoning white population around Nakuru includingSubukia and Naivasha. The slaughter house has over the years received bulk of its indigenous chicken supply from, Sondu in HomaBay County, Ahero in Kisumu County, Keroka in KisiiCounty, and Sotik in Kericho County. Currently, the slaughter point sells cockerels, IC, ex-layers and broilers.

The county government of Nakuru has since its inception put emphasis on ensuring continued operationalisation of the premises under hygienic conditions to ensure safety of chicken products from the slaughter house. Toward this end, the county is working in collaboration with the smallholder Indigenous Chicken Improvement Programme (InCIP) programme of Egerton University, to implement a HACCP system. To achieve this, an evaluation of the state of the Good Manufacturing Practices (GMPs) in the premises was

carried out. This was then followed by the evaluation of the prevalence of pathogenic microorganisms from the different surfaces, process and rinse water used at the slaughter house. This chapter therefore presents the report of the finding on the prevalence of *E. coli*, *Coliform* counts, *Salmonella,* and *Staphylococcus aureus* from the swabs taken from surfaces (tables, walls, floors, containers, knives, personnel hands) and from process water and the final chicken rinse water from Nakuru Top market slaughter point. According to the guideline from Centre for Disease Control (CDC , 2010), values for total viable counts (>Log 10>2.41) are interpreted as very contaminated and require immediate corrective action (Table 3.1).

Table 3.1: Guideline for criteria for determination of the hygienic quality of different surfaces for use by inspectors of poultry processing premises

Interpretation	Sponges/Swabs Total cfu	Log conversion	Counts surface based on
Clean	<45	<1.65	<5cfu per cm^2
Contaminated	140 to 260	2.15-2.41	5-10cfu/cm^2
Very contaminated	>260	>2.41	>10 cfu/cm^2

Source: Centre for Disease Control: Food Protection Services; October 5, 2010 Version 3; pg. 6

3.3 Materials and Methods

3.3.1 The Study Area

The study site for HACCP design was in one of the most important slaughter houses within Nakuru County. Nakuru is located in what was formerly the Rift Valley region of Kenya, about 165 km from Nairobi in the North West Direction. The GPS location of the slaughter house lies within the DMS coordinates 0°16'59.99" N 36°04'0.01". Nakuru County covers an area of 7496.5 square kilometres. Nakuru County has had a reliable weathercondition.The county experiencesmoderatetemperatures ranging between 10°C during the cold months (July and August) to 26°C during the hot months (January to March). The county receives between 700mm and 1200mm of rainfall annually, with average annual rainfall being an approximated 800mm. Nakuru County is home to 1, 603, 325 people according to the year 2009, National Census (Kenya Information Guide, 2015).

3.3.2 Justification of the Study Sites

The study was carried out in four counties. Nakuru County was the location of the slaughter house under study (Nakuru Top Market Slaughter house). Nakuru Top Market slaughter house is the oldest indigenous chicken slaughter house in Kenya. It has suffered several embargoes by the Public Health officers due to wanting hygienic conditions. The other Counties (Kakamega, Naivasha, and Taita) were randomly chosen as sampling sites for representative samples of the indigenous chicken ecotypes following previous studies that categorised Kenyan IC into ecotypes based on microsatellite alleles. The ecotype groups were classified as Western, Rift valley and Coastal groups (Ngeno, 2014).All the chicken samples collected were standardised to proximately the same age and farm management practices.

3.3.3 Sampling Procedure and Sample Collection

The sample collection for microbial studies was conducted from different sections. These were the holding (cage), defeathering, slaughtering, and post mortem section by random sampling of sites for swabs. The processing water was collected from the source while, the chicken rinse water was collected from the chicken rinse basin after rinsing of 10-20 chicken. Random sampling of the surfaces was used for collection of swabs from the knives, hands, floors, walls and surfaces within the slaughter house. Observation and administration of questionnaire to the workers and traders based on the gap audit for British Retail Consortium (BRC) good manufacturing practice was used to collect the descriptive data (British Retail Consortium, 2015).

Floors, tables, hands, knives and walls' swabs were collected by selecting an area of approximately 10cm x10cm (100 $cm^{2)}$and standardization by using a reference square. Sterile swabs were soaked into already prepared sterile tubes containing Buffered Peptone water (BPW). Swabbing was done by holding the swab by the end of the stick furthest from the swab to prevent contamination with the fingers. Swabs were then passed backward and forward across the whole selected area and then dipped in to the respective tubes containing the BPW. The tubes were then closed and labelled accordingly. The bottles were shaken several times to mix the content and then placed into a cool box with an internal temperature of 4-6^{o}C (Jørgensen *et al*., 2002). Samples were then transported to the Food microbiology laboratory at Dairy and Food Science and Technology Department of Egerton University for analysis. Serial dilutions were made to power six.

Processing water samples were collected from the tap at point of processing (where the defeathered chicken are first washed and some of the water injected into the veins). The

samples were drawn after first cleaning the mouth of the tap with cotton wool soaked in alcohol and allowing the water to flow out for about 5 minutes. The chicken rinse water was collected by first stirring the water in the chicken wash basins and then collecting into a previously sterilised bottle for transportation to the laboratory for analysis (Lindblad *et al*., 2006).

3.3.4 Microbiological Analysis

For determination of Total Viable Counts (TVC) one millilitre (1mL) of the sample was serially diluted sixfold using 9 mL buffered peptone water. One millilitre (1mL) of homogenate samples was transferred using sterile pipette into sterile labelled petri dishes. Approximately 20 mL of plate count agar (PCA) which had been autoclaved at 121^{o}C for 15minutes, cooled and tempered in a water bath at 45^{o}C, was poured into the petri-dish. The media and samples were mixed gently by swirling in a figure eight manner. The petri dishes were inverted and left to solidify at room temperature and incubated at 37^{o}C, for 24 hours. Plates containing 30-300 colonies were selected and counted. An average count of the duplicate plates was calculated and converted into logarithm for recording and analysis. Discrete colonies grown on plate count agar were selected randomly and purified by repeated plating on the same agar. The colonies were subjected to morphological (cell shape, cell grouping and endospores), biochemical (catalase, Triple sugar iron (TSI)), to identify gram negative organisms (Cunniff, 1996).

The catalase test was conducted as follows:Catalase is an enzyme that breaks down the H_2O_2 and is which is a strong oxidising agent and must therefore be removed from cells by formation of catalase, the test is therefore used as confirmatory for the aerobic microbes. A small amount of growth from the culture was placed on to a clean microscope slide using a toothpick. A few drops of H_2O_2were placed onto the smear and slightly mixed with a toothpick. Rapid evolution of O_2, as evidenced by bubbling was taken as a positive result (Cunniff, 1996).

Further biochemical analysis was conducted using TSI. This is a differential medium that helps to distinguish between gram negative enteric pathogens based on their physiological ability or lack thereof to metabolise lactose, or sucrose, conduct fermentation to produce acid, produce gas during fermentation and generate hydrogen sulphide gas. Reference for identification of the different types of microorganisms was then made to the TSI tables. A loop full of inoculum was taken from the Mackonkey agar and inoculated onto previously prepared and slanted TSI agar slants by stabbing the bottom upwards and finally streaking on the surface. The TSI agar slants were then incubated for 48 hours at 37^{o}C (Cunniff, 1996).

For identification of *E. Coli*, one millilitre (1 mL) of sample was pour plated on to about 15 mL of Violet Red Brilliant Agar (VRBA), and mixed in figure 8 motions. Thereafter, plates

were set at the room temperature on the table then incubated at 37°C. Distinct colonies were picked from the VRBA plates and streaked on Endo Methyl Blue Agar and incubated at 37°C for 24 hours. The plates were then observed for growth of green metallic sheens and reported as positive for *E. coli* (Feng *et al*., 2002).

For identification of *Staphylococcus aureus*, one (1mL) of 10^{-4}samples was aseptically pour plated onto Baird-Parker Agar (BPA) (Oxoid Ltd., Basingstoke, Hampshire, England) plates and incubated aerobically at 37°C for 48 h. Distinct black colonies were counted as representative colonies and were then Gram stained. Those having the characteristic appearance of *staphylococci* colonies were counted and expressed as number of *staphylococci*cfu/mL or cfu/m^2 (Lindblad *et al*., 2006).

Finally, for *Salmonella* spp. identification, one (1 mL) of sample was drawn from the pre-enrichment medium of BPW, and transferred into a selective pre-enrichment medium of Rapparpot-Vassiliadis Soya (RVS) peptone broth and incubated at 42°C for 18 to 24 hours. About 10 µL loop full was transferred from incubated RVS broth (II) and spread onto Xyclose-Lysine Deoxycholate (XLD) and on Brilliant Green Agar (BGA) plates and incubated at 37°C ± 1°C for 18-24 hours. Triple Sugar Iron (TSI) agar slants with a butt of about 1 inch (2.5-3.5 cm) were inoculated by stabbing the butt and carefully streaking of slant using a sterile inoculating needle after slightly touching the centre of a discrete colony on the selective media. The tubes were incubated overnight at 37 °C. An alkaline (red) slant and yellow butt (acid), gas, with or (blackening) were considered positive for *Salmonella enteric* (Feng *et al*., 2002).

3.3.5 Data Analysis

The values of microbial counts were transformed to base-10 logarithm of colony forming units (cfu) per litre of water, per 10m^2 of surfaces or per 100g of weight of carcass then subjected to the different statistical analysis.Analysis of variance (ANOVA) was done using the General Linear Model (GLM) procedure of SAS version 9.1 (SAS Institute, Inc., Cary, NC). Statistical differences were determined after mean separation by DMRT test at $P < 0.05$.

3.4 Results and Discussion

3.4.1 Differences in the Distribution of TVC and Coliforms from the Different Sections of the Slaughter House

The Analysis of variance (ANOVA), statistic of the microbial analysis from the different sections of the slaughter house are shown in Table 3.2 for both Total Viable Counts

(TVC) and total coliform counts (TCC). The values are reported in logs to base 10 (Log_{10}) cfu/mL or cfu/m^2 for process and rinse water and for swabs respectively.

Table 3.2: Concentration of microorganisms from the different sections of the slaughter house

SOURCE	TVC	TCC
Section	1.18^{ns}	4.84^{ns}
Source	0.36^{ns}	13.19^{ns}
Source (section)	0.22^{ns}	2.76^{ns}

The figures followed by the superscript ^{ns} indicate no significant difference for concentration of the microorganisms in the different sections:

The results show that there was no significant difference in the TVC and TCC from swabs taken from the different sections (the cage area, de-feathering and evisceration area, the slaughtering, and the post mortem area). The results also show no significant difference on the TVC and TCC from the different surfaces (the floors, the slaughtering containers, the processing walls, the slaughtering tables, evisceration knives before and after evisceration and finally from the hands of the processors) sampled from the different sections. Total viable counts (TVC), gives an indication of the initial microbial load which is related to initial microbial contamination.

On the other hand, the TCC gives an indication of possible contamination from the contents of gastrointestinal tract. According to CDC (2010), the values of other studies have associated TCC levels > 10^3cfu/cm^2 with poor hygiene practices. All these values indicate a contamination beyond what depicts proper hygienic practices. These values of TVC and TCC clearly demonstrate that the facility requires an implementation of GMPs as well as a safety management system such as HACCP to guarantee consumer safety. The survey conducted through a gap audit of the premise clearly showed that the facility did not operate based on the requirements of GMPs. Contamination of poultry and poultry products should be prevented during handling, slaughter, and processing to protect the public from infections, and diseases. The hygienic quality of poultry processing surfaces, process water, rinse water, as personnel are keys to prevention of cross contamination that may lead to adverse health effect on consumers.

3.4.2 Distribution of Microorganisms from the Different Sections of the Slaughter House

The results indicate no significant difference among the different sections of the slaughter house. In terms of the TVC values, the CAA and the SLA sections had the highest counts while the PMA had the least counts. In a properly run processing facility based on GMPs or HACCP management system, the levels of contamination across the different sections of the poultry process house are supposed to be significantly different. The sections handling raw materials are likely to be heavily contaminated and this is supposed to significantly reduce as production progresses to the finished product section (Sahab *et al.,* 2007). However, these values show that this is not the case (Table 3.3). The lack of significant difference among these values may be attributable to the fact that, the process flow chart of the poultry house showed serious breaches to quality and safety due to the crisscrossing of the raw material as well as finished product.

Table 3.3: Concentration microorganisms isolated from the different sections Concentration in (log to base 10cfu/m^2)

section	TVC	TCC
DES	8.02 ± 0.51^{ns}	5.83 ± 3.24^{ns}
PMA	7.40 ± 1.18^{ns}	5.25 ± 3.24^{ns}
SLA	8.07 ± 0.79^{ns}	6.09 ± 3.15^{ns}
CAA	8.20 ± 0.34^{ns}	5.24 ± 3.80^{ns}

Legend: DES-De-feathering and Evisceration Section; SLA-Slaughter Section; PMA-Post MortemSection; CAA-Cages section. The figures followed by the superscript ^{ns} indicate no significant difference for concentration of the microorganisms in the different sections.

3.4.3 The Distribution of TVC and Coliforms from the Different Surfaces at the Nauru Top Market Slaughter Point

The rinse water, the tables, followed by the floors had the highest concentration of total viable counts of microorganisms at $\log_{10}$ of 8.13, 8.05 and 7.48 cfu/m2 respectively. In all the cases, the levels of TVC were higher than that of CC (Figure 3.1). This is expected as TVC counts brings out all viable cells present irrespective of whether coliforms or just other ubiquitous microorganisms.

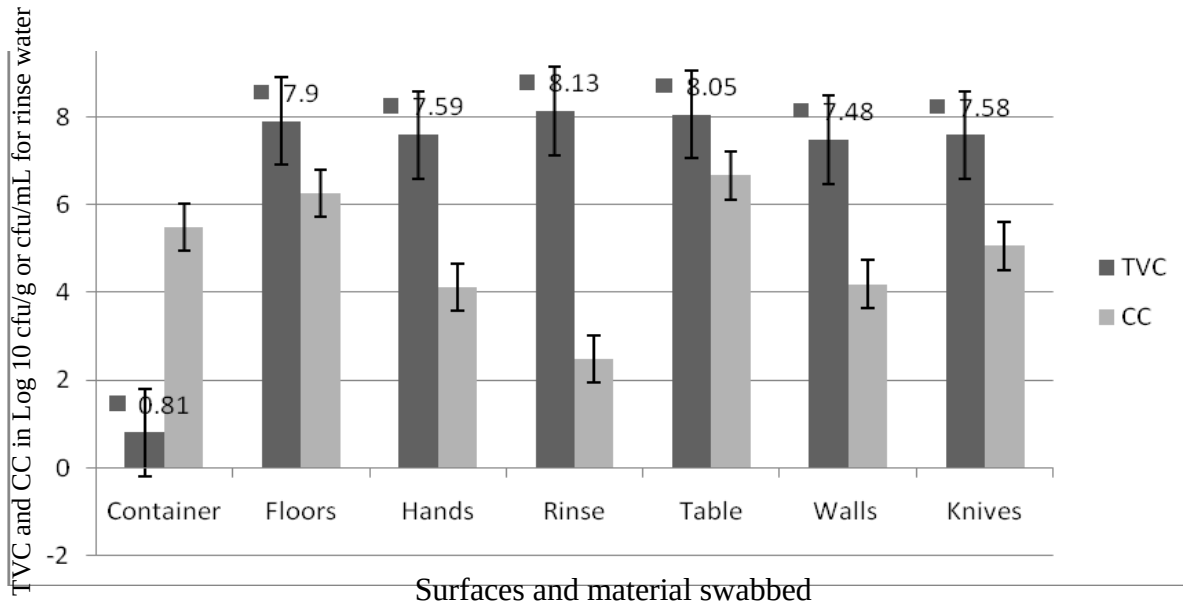


Figure 3.1: Concentration of microorganisms from the different surfaces and rinse water

Concentration is in (Log $_{10}$cfu/m^{2}andcfu/g respectively).

Legend: TVC-Total viable counts; CC-Coli form counts

3.4.4 Prevalence of Enteric Pathogens Isolated from the Different Sections of the Slaughter House

Figures (3.2- 3.4), show the prevalence of the major species of microorganisms isolated from different surfaces of the poultry slaughter house. Figure 3.2 shows the prevalence of the microorganisms isolated from the defeathering and evisceration section of the poultry slaughter house. *Salmonella* remains a global health risk. The vertical transfer of salmonella cells from the brooding hens to the chicks is an important aspect in the epidemiology of salmonella (Bae *et al*., 2013). The two major species of Salmonella are the *S. typhimurium* and the *S. dysenteria*. Both species were recorded at a prevalence rate of 16 % each, totalling to 32%.The infections by these species can lead to significant morbidity and loss of life both in humans and food animalsand result insignificant economic losses when they occur. *Salmonella spp*. are typically transmitted among humans and animals via a faecal-oral route. Often the culpritis the consumption of contaminated food or water (Majowicz *et al*., 2010). Their presence in food is a cause for rejection in many countries. From the studies on the application of GMPs, the slaughter house had a great lapse of hygienic practices. Live birds were brought in to the slaughter house through the same door that was also used for finished product release. Evidently, majority of the food handlers at the slaughter house had no training on hygiene and safety in handling of poultry meat. Neither did any of them have food handler's certificate. These are factors which may have contributed to the contaminations reported in this study. The occurrence of *Salmonella* from hand swabs of the workers is very critical. Currently, there is

no sanitation exercise applied by the workers as they move along the different levels of the chain. Reiter *et al*. (2007) reported higher prevalence of *Salmonella* in the cages, which translated in to higher percentage of *Salmonella* in the scalding water.

The prevalence of up to 16% in the cages and slaughter sections was reflected in the high prevalence of 8% in the post-mortem product areas. This means that the *Salmonella* cells can easily be transferred to the dressed poultry carcasses at the later stages of processing. Salmonella infections result in food poisoning that affects hundreds of people leading not only to hospitalisation but even death. In some countries such as Italy, *Salmonella* food poisoning has been reported to account for as high as 90% of food borne illnesses (Angelillo *et al*., 2001). Due to outbreak of food borne epidemics in the various parts of the country, there is need to institute practices that ensure safety of food products sold particularly for the public. According to the CDC (2010) report on Foodborne Disease Outbreaks, *Salmonella* isolated from chicken was responsible for most illnesses at 700 and 213 hospitalisations. In terms of foods attributed to same ingredients in the cause of disease, chicken was implicated at the third position representing 10% of all reported cases (CDC), 2010). The typhoid fever which is caused by *Salmonella typhi* and *paratyphi*have continued to be great public health concern in Africa (Minton, 2004). In Africa, the highest incidences of *Salmonella* poisoning has been reported in Northern parts (Minton, 2004). Data from Kenya on food borne illnesses resulting from *Salmonella, S. aureus, and E.coli*are scanty. *Staphylococcus aureus* is a gram-positive, catalase-positive, coccoid bacterium and appears in grape-like clusters on stained smears. *Staphylococcus* is part of the normal skin and mucosal flora. Many infections by *Staphylococcus* only occur as a result of wounds or mucosal injuries or both. According to Reiter *et al*. (2007), the prevalence was higher when they used the automated mini-VIDAS system for screening of Salmonella. This may mean that the values reported here could even be much higher if the automated mini-VIDAS system was used instead of the traditional culture method.

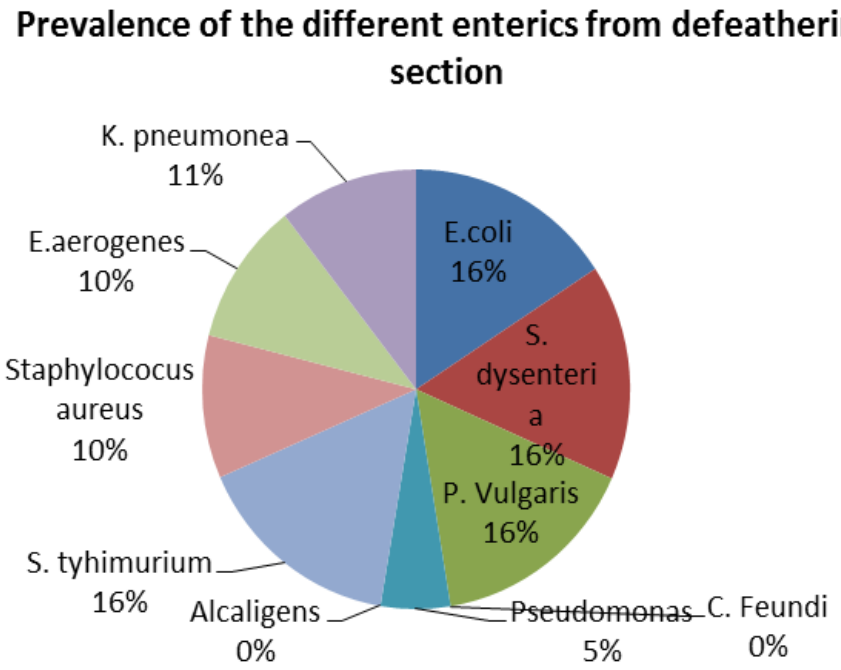


Figure 3.2: Prevalence of the microorganisms Isolated from the defeathering and Evisceration section of the poultry slaughter house

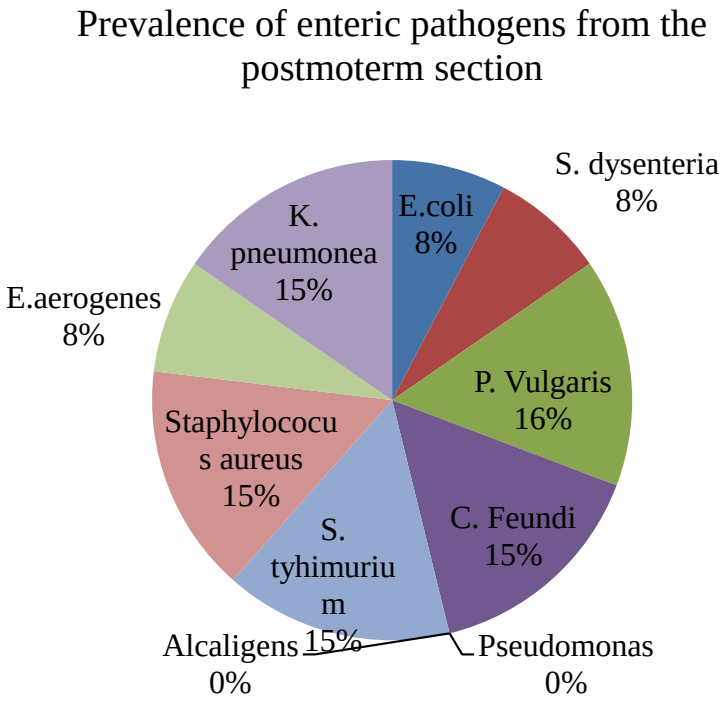


Figure 3.3: Prevalence of the microorganisms Isolated from the Post-mortem section of the poultry slaughter house

Staphylococcus aureus and *Salmonella* are two of the microorganisms most implicated in food borne illnesses worldwide (Aydin *et al*., 2011; Costa *et al*., 2012). One hundred 100% of the samples showed TVC of over Log $_{10}$ of cfu/m^2 or cfu/mL. Baseline surveys conducted in Sweden and Canada show a totally different picture. Based on the results figure 3.2. *E.coli*

are in the order as to cause serious health risks to the consumers of poultry products from the slaughter house. The overall prevalence of *E.coli* from the samples was at 12.2% while at the de-feathering and Evisceration and the post-mortem area was at 15.79 and 7.69 %, respectively Figures 3.2. *E.coli* belongs to the large family of *Enterobacteriaceae* which are hygienically sensitive group of microorganisms. These microorganisms reside in the intestinal tract and therefore found in large quantities in faeces. Their presence, demonstrate possible contamination with dirt or faecal material and they must be controlled due to their possibility of food poisoning. According to a report by Odwar *et al*. (2014a), a majority of poultry products sold in Nairobi had infections from pathogenic microorganisms *E.coli*, and *Salmonella*. The present study suggests high contamination by the same in the slaughter house but not necessarily on the rest of the poultry meat sold at different selling points within Nakuru.

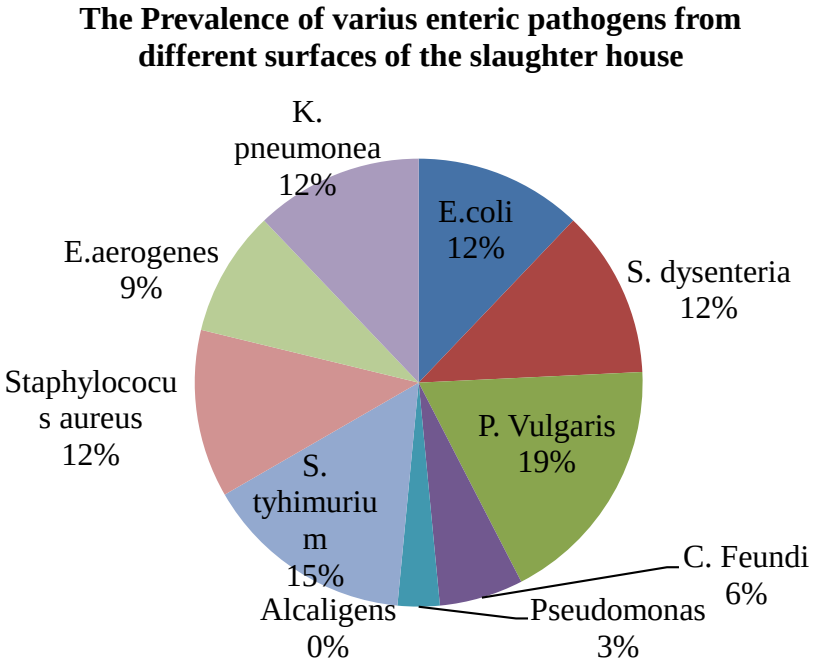


Figure 3.4: Prevalence of the major microorganisms Isolated from all the sections of the poultry slaughter house

The overall prevalence of *Staphylococcus aureous* of 12.12 % recorded in (Figures 3.2-3.4). The post-mortem and evisceration sections of the slaughter house had a prevalence of *Staphylococcus aureous* at 10% and at post-mortem section at 15%. The levels of Staphylococcus reported in this study reveal numbers that could easily lead to infections. These results support the findings from the survey of GMP that demonstrated great gaps in compliance to GMP procedures. The results of this study showed a prevalence rate of about 15% from the different surfaces and rinse water. The Staphylococcus poisoning is a leading

cause of illnesses worldwide and affects thousands of people each year (Hazariwala *et al*., 2002). It is commonly found in the skins of birds, mammals and formites. Humans are a major source of *S. aureus* poisoning. It is often found in nasal passages, throat, and skin of carcasses. In year 2000 in the USA *Staphylococcus* poisoning was estimated to be the second leading cause of food borne illness in the USA. Due to its ubiquitous nature, the staphylococcal poisoning can only be kept at bay through maintenance of clean sanitised environment (CDC), 2010). The second measure to deal with *Staphylococcus* infections will ensure hygienic handling of food at every stage of production to consumption.

3.4.5 Prevalence and Microbial Presence on the Different Parts of Chicken

The microbial load from different body parts of chicken expressed in (log_{10}cfu/g) per gram is presented in Table 3.4. There was no sisnificant difference in the two parameters TVC and TCC as presented in Table 3.4, for the different body parts. This was true for broiler as well as for the IC.

Table 3.4: Microbial load of different parts of indigenous chicken and broilers

		Microbial load (log_{10}cfu/g)	
Chicken	**Part**	TVC	TCC
Broiler	Head	7.81±0.40[a]	7.99±0.23[a]
	Breast	8.08±0.14[a]	8.17±0.05[a]
	Skin	7.38±0.72[a]	7.64±0.47[a]
	Thigh	7.54±0.68[a]	7.41±0.67[a]
	Leg	7.13±0.01[a]	7.30±0.28[a]
Indegenous	Head	7.24±0.24[a]	7.68±0.24[a]
	Breast	7.82±0.22[a]	7.63±0.25[a]
	Skin	7.25±0.29[a]	7.23±0.23[a]
	Thigh	6.87±0.21[a]	7.08±0.15[a]
	Leg	7.42±0.20[a]	7.68±0.21[a]

Means with the same letter along the column are not significanty different at P<0.05, TVC: Total Viable Count, TCC: Total Coliform Count, cfu/g: colony forming unit per gram, SE: Standard Error of the mean

Prevalence of E. *Coli* in different parts of the indigenous and broiler chicken are presented in Figure 3.5. Except for the skin, the rest of the parts had significantly different E.Coli numbers.

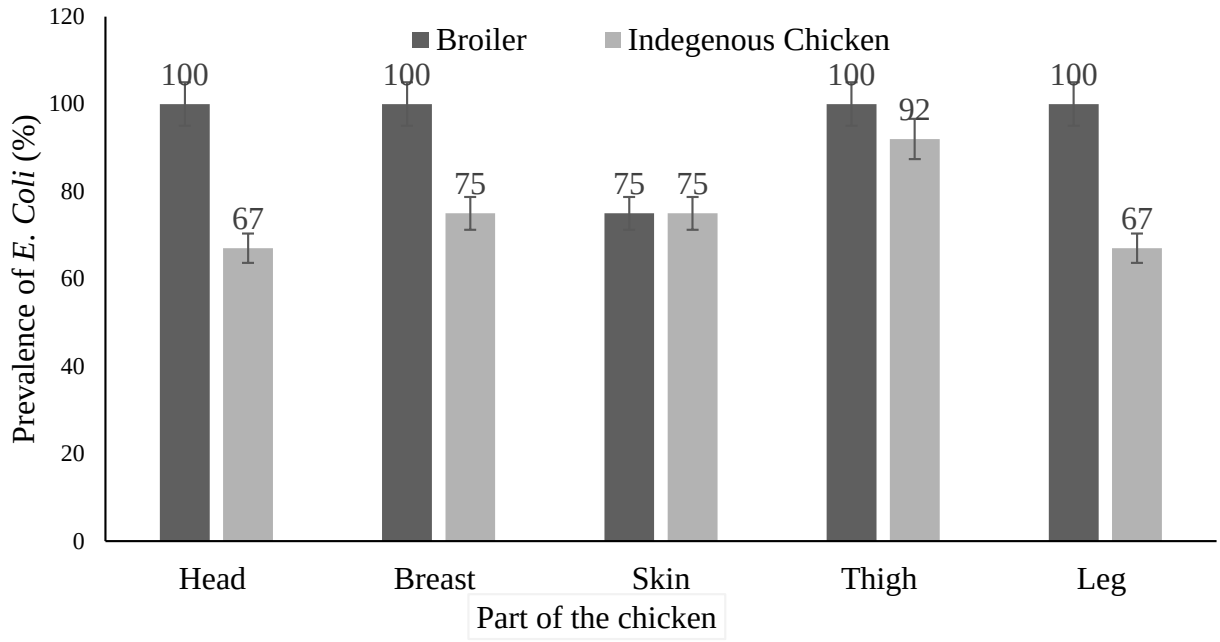


Figure 3.5: Prevalence of E. *Coli* in different parts of the indigenous and broiler chicken

Prevalence of *Staphylococcus aureus* in different parts of indigenous chicken and broilers is depicted in Figure 3.6. In all samples the levels of *Staphylococcus aureus* in broiler was higher than that of IC.

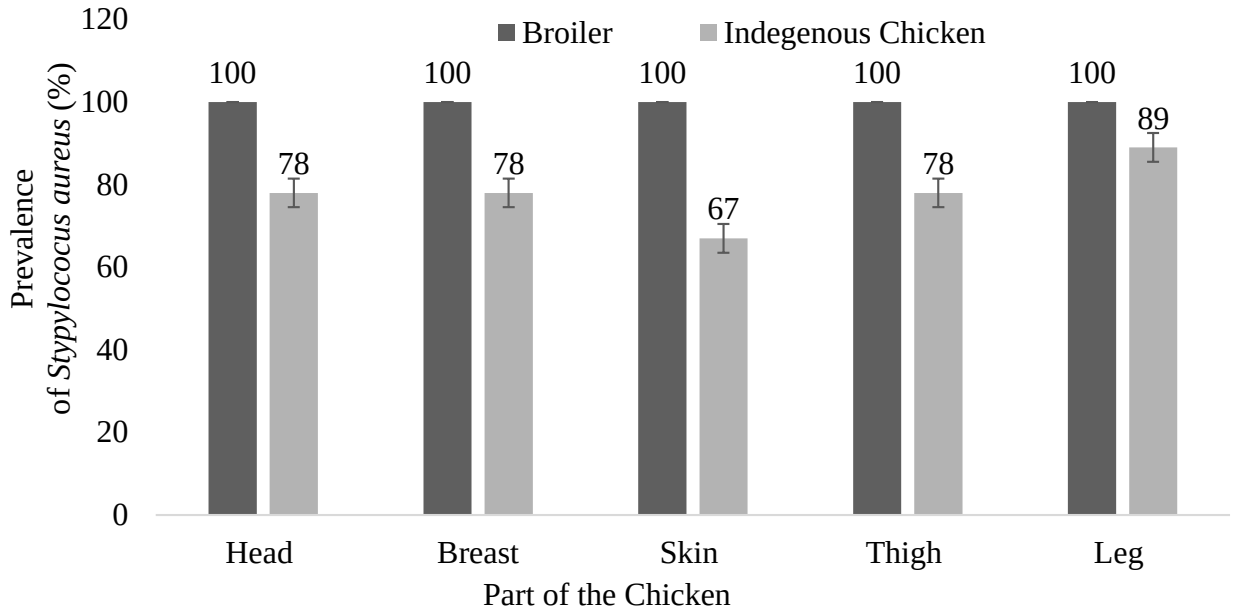


Figure 3.6: Prevalence of *Staphylococcus aureus* in different parts of indigenous chicken and broilers

The mean concentration of *Staphylococcus aureus* in different parts of indigenous chicken and broilers was presented in Table 3.5. There was no significant difference among the body parts.

Table 3.5: Staphylococcus aureus in different parts of indigenous chicken and broilers.

		***Staphylococcus aureus* load (log_{10}cfu/g)**			
Chicken	**Part**	Median	Minimum	Maxmum	Mean±SE
Broiler	Head	7.48	6.08	7.48	7.01±0.47[a]
	Breast	7.48	7.48	7.48	7.48±0.00[a]
	Skin	7.48	6.30	7.48	7.09±0.39[a]
	Thigh	7.48	7.48	7.48	7.48±0.00[a]
	Leg	7.48	7.46	7.48	7.47±0.01[a]
Indegenous	Head	7.41	5.78	7.48	7.07±0.17[a]
	Breast	7.14	5.60	7.48	6.94±0.18[a]
	Skin	7.29	6.36	7.48	7.11±0.12[a]
	Thigh	7.08	5.78	7.48	6.91±0.19[a]
	Leg	7.48	6.90	7.48	7.38±0.06[a]

Means with the same letter along the column are not significanty different at P<0.05, cfu/g: colony forming unit per gram, SE: Standard Error of the mean.

3.5 Conclusion

Indigenous chickensin Kenya are reared in free range systems. Slaughter houses especially those located in major towns in Kenya are an important node of the value chain that supplies indigenous chicken to the consumers. The Nakuru Municipal slaughter house supply important dressed indigenous chicken meat to the burgeoning urban population in and around Nakuru. The risk of contamination of the poultry carcasses persists into the slaughter houses. This therefore can only be prevented through use of strict food safety management systems. The prevalence rates realised from this study are alarming (67-89%).This high prevalence was recorded for the pathogenic microorganisms (*Salmonella*, *E.coli*, and *Staphylococcus aureus- and the other enterics*). There was clearly high prevalence of pathogenic microorganisms at the Nakuru top market slaughter house to warrantee an intervention. A quick intervention involving all stakeholders is necessary to address the food safety challenges posed by the high prevalence of the evaluated pathogenic microorganisms at the Nakuru top market slaughter house.

CHAPTER FOUR

DESIGN OF A HACCP PLAN FOR INDIGENOUS CHICKEN SLAUGHTER HOUSE IN KENYA

Abstract

Indigenous chicken rearing is seen as a poverty alleviation and food security strategy especially in rural households in Africa. Chicken meat is a delicacy in almost every household in Kenya. It is a common food in restaurants and hotels that serve fast foods in urban areas. Demand for and consumption of indigenous chicken meat in Kenya has been on the rise. Many slaughterhouses have been set up in strategic locations close to towns or in towns to allow for quick supply of the dressed chicken carcass to consumers. Poultry meat is a low acid food and has been associated with the presence of foodborne pathogens such as *Campylobacter*, *Escherichia coli*, *Salmonella enteritidis*,and *Staphylococcus aureus*, especiallywhen processing conditions are not hygienic. Hazard Analysis and Critical Control Point (HACCP) is based on a scientific verifiable process to identify, control, reduce or eliminate any potential hazards to guarantee food safety. The current study was conducted based on the actual production conditions of the slaughter house. It was initiated through a survey that looked in to the operations of the slaughter house on the basis of good manufacturing practices, as well as standard operation and sanitation procedures. From the results of the study and the gap audit analysis based on a checklist, the HACCP study was commissioned. The study aimed at developing a HACCP system; based on the seven HACCP principles and a critical scrutiny of several existing models. Four Critical Control Points (CCPs) were identified and a HACCP plan, complete with perquisite programs was presented to deal with the identified hazards and therefore present the consumers with high quality and safe products. Design of a model for the application and operationalisation of HACCP system was undertaken as an important step in ensuring consumers enjoy safe products from the indigenous chicken meat prepared from the slaughter house.

Key words: *Indigenous chicken, slaughter house, HACCP, Critical control points (CCPs)*

4.1 Introduction

There is currently a high demand nutritious and safe food products (Hofi,& Ismail, 2008). The need for provision of high quality and safe indigenous chicken (IC) products is therefore no exception. Many systems have been put in place by manufacturers to achieve this aim. Hazard Analysis and Critical Control Point (HACCP) is a scientific system which was developed to assure pathogen free foods (The National Advisory Committee on Microbiological Criteria for Foods, 1992). Ropkins and Beck indicated that HACCP has been widely recognised as a rational and effective means of achieving safe food products through its application of 'from the farm to the fork' approach. It is aimed at providing a step by step control of pathogens throughout processing (Ropkins & Beck, 2000). It forms the basic preventive measure for the control of pathogens in foods (Jackson *et al*., 1996). Its success lies in its reliance on preventive approaches in dealing with potential pathogens in foods (Ropkins & Beck, 2000). The HACCP concept was first developed in the 1950s, through a collaboration of the Pillsbury Company, the US Army's Natick laboratory, the US National Aeronautics, and Space Administration (NASA), and the US Army's Airforce Space Laboratory (Hessing *et al*., 2015). It was developed in response to the failure of end-use product testing to assure food safety and decrease final product wastage (Bardic, 2001; Bennet& Steed, 1999; Mortimore, 2001).

The HACCP was first presented to the public as a food safety system in 1985 following a paper report by the National Academy of Sciences. From then on, the concept gained worldwide recognition as an integral food safety assurance practice (Mortimore, 2001). The first widely recognised international definition of HACCP was given in 1993' when codex Alimentarius Commission presented its HACCP standard. By the year 2000, many factories and companies had developed a number of safety standards which led to problems in implementation of third-party audits and certification. This led to the development of the ISO standards, especially ISO 22,000 which became known as a food safety management system (Codex Alimentarius Commission, 2003). The core of ISO 22,000 standard is the development and operationalisation of a HACCP system. The HACCP has become globally recognised as a food safety system based on its preventive approach to eliminate potential chemical, biological, and physical, hazards. Unlike end-use product testing, HACCP leads to reduction in the occurrence of foodborne illnesses. The HACCP is used to control occurrence of food-borne diseases by applying control systems at points within the production chain where food safety hazards could be controlled, eliminated, or reduced to within acceptable levels. It is a system of evaluation and control over the whole process to guarantee safe foods to consumers (Jackson

et al., 1996). The HACCP maintains the wholesomeness and safety of meat and poultry products because all potential hazards are anticipated, identified, characterised, eliminated or reduced to an acceptable level at each stage of the process. The HACCP is a product and facility specific system (Hofi & Ismail, 2008). Each facility is required to institute a HACCP system for each of the products or product lines it produces. Poultry meat processing industry in Kenya is only slightly developed and is dominated by the Kenchic industries which mostly process the exotic broiler. Processing of IC in Kenya is at its infancy and none of the slaughter houses dealing with the IC are currently HACCP certified for the production of high-quality meat products to the consumers.

Poultry meat is a low acid food and has been associated with the presence of foodborne pathogens such as *campylobacter*, *Escherichia coli, Salmonella enteritidis*, and *Staphylococcus aureus* (Meng & Doyle, 2002; Rabsch *et al*., 2001). The design of a model for the application and operationalisation of HACCP system was viewed as an important step in ensuring that safe chicken meat products prepared in these slaughter house reach the consumers. The National Advisory Committee on Microbiological Criteria for Foods outlined five (5) preliminary steps and seven (7) principles in the development of a HACCP plan (The National Advisory Committee on Microbiological Criteria for Foods, 1997).

This study was carried out to develop a HACCP model based on the actual processing conditions of the slaughterhouse. HACCP system was developed based on seven HACCP principles for operation in a poultry slaughter house in Nakuru County, Kenya. The successful implementation of the HACCP plan aimed to lower the risk of food safety hazards in the final IC product.

The slaughterhouse's quality objective was to supply its customers with safe IC products of highest quality by implementing the HACCP system that ensures a safe processing environment free from all important potential contaminants. To achieve this, it will create and maintain strong relations with farmers, employees, the county administration, and other business partners while operating within the national and international regulations relevant to the business.

4.2 Materials and Methods

This study was conducted in a poultry slaughter house which processes indigenous chicken, located in Nakuru County in Kenya. The slaughter house is jointly owned by the municipal council and over thirty traders who supply it with chicken for processing (dressing). It has employed a dozen to half a dozen casual labourers to slaughter and dress the chicken. The slaughter house is a small-scale processing unit with a capacity to process about one thousand chickens per day.The National Advisory Committee on Microbiological Criteria for Foods outlined five preliminary steps and seven principles in the development of a HACCP plan. These steps were the basis of the HACCP development plan.

4.2.1 Preliminary Steps

The support of the District veterinary officer, the senior management of the facility, and the county director of veterinary services was sought and obtained in writing.

A team was formed which included: production in charge, representatives of the traders, resident veterinarian, consultant of food hygiene and sanitation, consultant of food microbiology and a technician from Egerton University laboratory. The team engaged in the product description in terms of raw material (live birds), ingredients, processing, packaging, storage, and distribution.

4.2.2 Product Description Records Table

The product was described as: dressed frozen or chilled indigenous chicken '*kuku kienyeji'* prepared based on HACCP principles for cooking before consumption. The detailed summary of the product description is presented in Table 4.1.

4.2.3 The Development of the Flow Diagram

Each step in the process was outlined in sequence in the flow diagram (Figure 4.1) from raw materials through processing, packaging, and storage. The hazards were identified via observations of operations followed by measuring the operations' parameters.

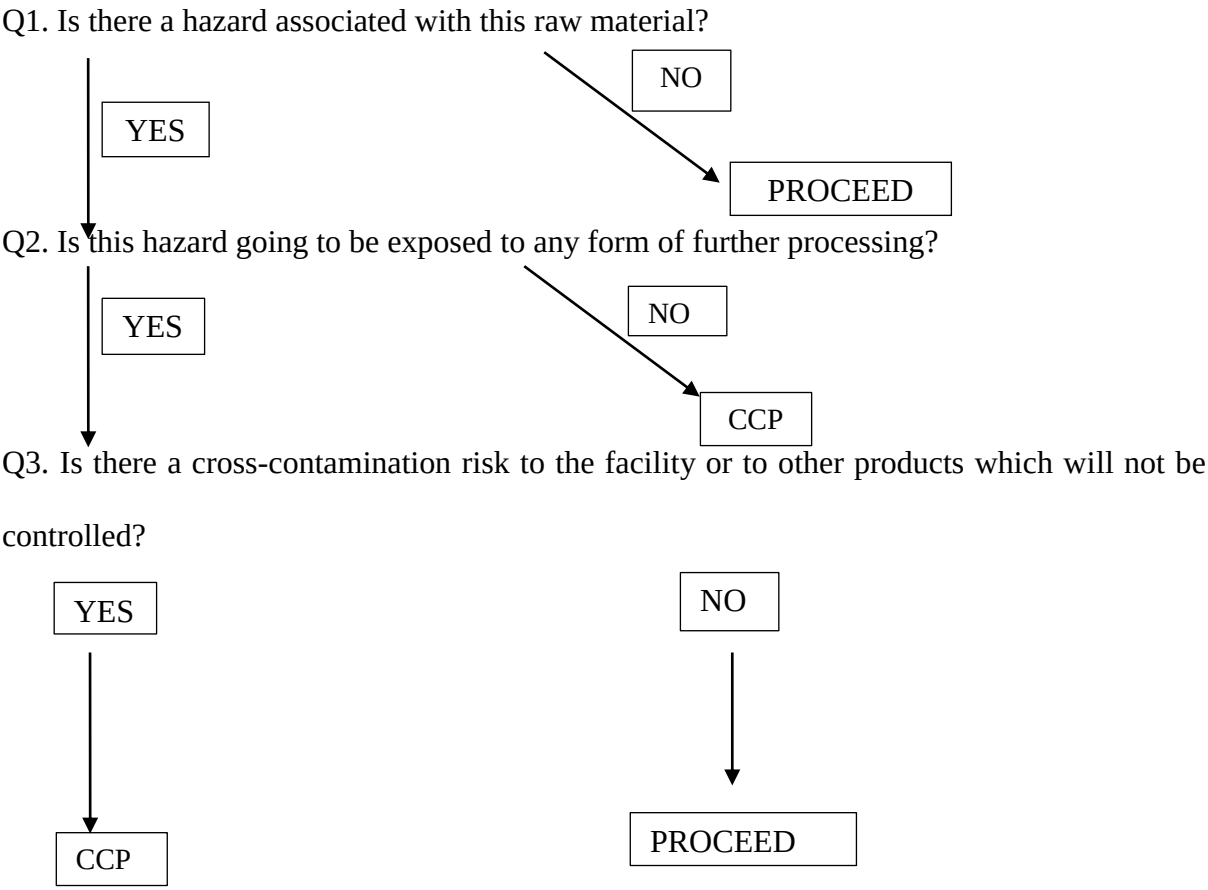


Figure 4.1: The decision tree for identification of CCPs for the Raw materials and ingredients

Source: (Khaliduzzaman, 2017) with modification

4.2.4 Observation of Operations

Each product preparation process was observed: receipt of raw materials, storage, heat treatment, cooling, and packaging. Furthermore, the use of any additives, temperature, and packaging and storage conditions was thoroughly scrutinised. A structured questionnaire was developed and administered to the traders and suppliers. This was used to interrogate the history and nature of the raw material (live birds) received. The personnel parameters (hygiene, education, health, cleanliness, habits), premises parameters, (equipment, floors, walls, and ventilation) and working conditions, were scrutinised and recorded.

4.2.5 Measuring Operations Parameters

The measured parameters included adequate time and temperature to be applied. This was recommended and recorded during the production storage, display, and distribution of the poultry carcass on the flow diagram. The other measurements included the evaluation of the physical, chemical, and biological hazard and were conducted based on the HACCP guidelines.

4.2.6 Critical Control Point (CCP) Determination

A HACCP plan requires the identification of a CCP which is defined as a step in the process at which a control measure must be applied to prevent occurrence of, eliminate, or reduce the risk of occurrence of a hazard to an acceptable level (Dutch National Board of Experts HACCP, 2012). In the identification of CCPs, experts recommend the use of a given set of questions to help in the decision-making process (Codex Alimentarius Commission, 1993; Riswadkar, 2000). These questions constitute the decision tree. Figure 4.2 presents the decision tree for identification of CCPs for the raw materials and ingredients.

The decision tree Figure 4.3 was used to identify the CCPs for the raw materials listed in Appendix C, while the decision tree in Figure 4.4 was used to identify CCPs for the process steps in appendix D. Decision Matrix questions for raw materials and ingredients as well as the decision matrix for the process steps are also present in Appendices C and D respectively.

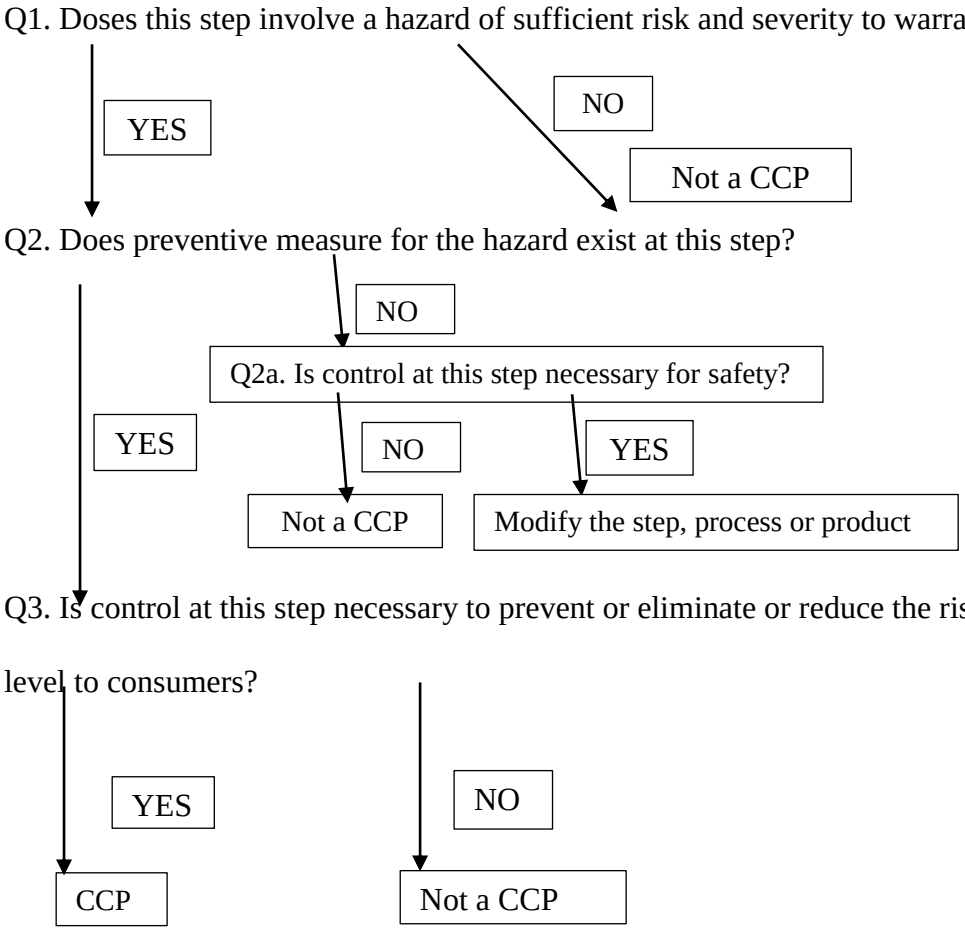


Figure 4.2: The decision tree for identification of CCPs for the process steps-: (Khaliduzzaman, 2017)

The raw materials and ingredients decision matrix is presented in Appendix C while decision matrix for process steps, is Appendix D

4.2.7 Research Method

This study was a follow up to quantitative research that was used to survey and enumerate the occurrence and prevalence of foodborne pathogens by swabs from the different surfaces of the slaughter house (Chapter 3). This acted as the baseline survey that helped to set the target for the developed HACCP (Mortimore, 2001). The current study did not use quantitative research, but qualitative techniques. The aim of this work was to design a HACCP model for implementation in an actual food operation situation. According Patton (2003), qualitative approaches are preferred where it reveals complex details of phenomena which are not easy to compile and convey quantitatively. A HACCP plan (Table 4.2), based on the actual

conditions of the premise was developed with the aim to improve the chicken quality and safety using the HACCP principles. Comparison was made with various generic models (Codex Alimentarius Commission, 2005).

4.3 Results

4.3.1 *Prerequisite Programmes (PRPs)*

An assessment of the PRPs was conducted according to *Codex Alimentarius*general principles of food hygiene and GMPs (Codex Alimentarius Commission, 2005). The study revealed the basic conditions of the premise for suitability of producing and handling safe chicken at every stage of the product. The major areas for consideration were as follows.

Location of the Slaughterhouse. The location of the poultry slaughter house presents serious threats to food safety given the bustling of activities surrounding the slaughter house. However, additional security by cages and a fence, and its positioning at the corner of the market enhances its security as every unauthorised person to the premise can easily be monitored and stopped. Pest control operations for the premise benefit from the municipal council's regular pest control services. However, the premise can benefit more from a contracted focused pest control system.

Physical Condition of the Premise and Room. The premise was originally designed to permit good food hygiene practices and protection from cross contamination. The walls are water, insect and rodent proof; however, deterioration of the premise and ageing of the facility presents a critical challenge to food safety. Renovations were suggested in all the major areas to create a slope of 0.1m per 6m on the floor and installation of a self-closing door. In addition, further improvements to replace all the surfaces coming into contact with food with stainless steel equipment were agreed upon. A suitable sanitation procedure for personal hygiene and cleaning was also generated in agreement with the management.

State of Equipment. Table surfaces were made of cement, a mixture of plastic, and some painted surfaces. It was recommended all food contact surfaces be made of stainless steel to provide for ease of cleaning, adequate sanitation, and disinfection. All cracks and dents were recommended to be totally covered.

Water Supply. The slaughter house is fed with water from the municipal council water treatment plant and has make-shift storage tanks for water. The capacity of the storage tank

(about 450 litres)was inadequateshould the main supply system experience any challenges or shortages. A 10,000-litre capacity tank, should be installed to provide for adequate water reserve. However, due to its location in Nakuru's Central Business District (CBD), the premise has never experienced any loss of water supply. The water quality was portable and conformed to Kenya Bureau of Standards for drinking water.

Maintenance and Cleaning. The establishment and equipment were cleaned occasionally. Repairs had not been undertaken for a long time and the facility was run down in several areas. The maintenance, sanitation, and cleaning procedures were drawn and put in place for implementation by the management.

Pest Control. The slaughter house has not specifically engaged an independent pest control contractor nor does it practice its own eradication. However, it is served with Nakuru municipal council pest control programme for the whole municipal council market. A pest control schedule/procedure and an alternative of outsourcing of this process was suggested and documented.

Waste Management. A daily waste disposal system is run through the county garbage collection scheme. Liquid waste is connected directly to the county council sewerage treatment unit. This calls for a mechanism for prevention of any accidental contamination from reverse flow of waste. Regular waste (feathers and other process refuse) collection, covering/separation, and classification of waste were advised. The quality control checks on national standards for releasing of waste and discharge of waste water should also be done and standards adhered to.

Sanitisation System. There is a sanitation (toilet) facility accessible to the public as well; hence it is difficult to control personnel use of the facility. Remodelling of the premise to include tight sanitation and cleaning of hands and feet, and to allow proper handling of Proper Protective Equipment (PPE) was recommended.

Personal Hygiene. Implementation of hygienic practices for: personnel handling, production, packaging, storage, sale of chicken and products occurred. The code to use aprons, head covers and foot wears were in place. Sanitisers for hands and foot baths were recommended. Medical check-up and issuance of a food handler's certificate for all workers was proposed and its adherence monitored. The personnel cleanliness and hygiene monitoring

wereobserved and recorded. The same was proposed for visitors and any other contractors who may come into contact with the facility.

Storage and Transportation. The storage facility lacked temperature and humidity-controlled conditions that could possibly expose chicken to fast deterioration and microbial proliferation. Most of the products were sold within the day, but the food safety concern remained real. A cold store display unit, complete with a temperature monitoring gauge and temperature/humidity log was proposed. General cleanliness and separation of food items from the slaughtered chicken unit was also implemented.

Traceability. Currently, the only traceability in place is in terms of business owner but not individual chicken product, which leaves a gap in implementation of any recall procedures in case of a food-borne illness outbreak. The First in First Out (FIFO) approach to traceability was incorporated with a traceability index including batch number, incoming date, production date, and live and dressed weight was recommended. A formal procedure for handling of customer complaint was also drawn.

Training. A training schedule was drawn for employees.The training included training for new comers, re-training for those re-joining the premise after some time, and mandatory periodic training after every 3 months. The training content will include on personnel hygiene, Occupational Health and Safety (OHS), production procedures, food safety, cleaning and sanitation, waste management, and environmental health management.

Production Process. The raw chickens are received at the reception bay from traders who transport them by bicycles, cars, or by hand. The chickens are first inspected by the traders, followed by the veterinary officer for general health and any manifestations of pests. Once passed, the chickens are held in cages of a capacity of about 10 birds each, even though these cages are often over crowded due to their limited capacity. The chickens remain in the cages until the time for slaughter and dressing according to demand. Processing occurs according to the flow diagram given in Figure 4.3.

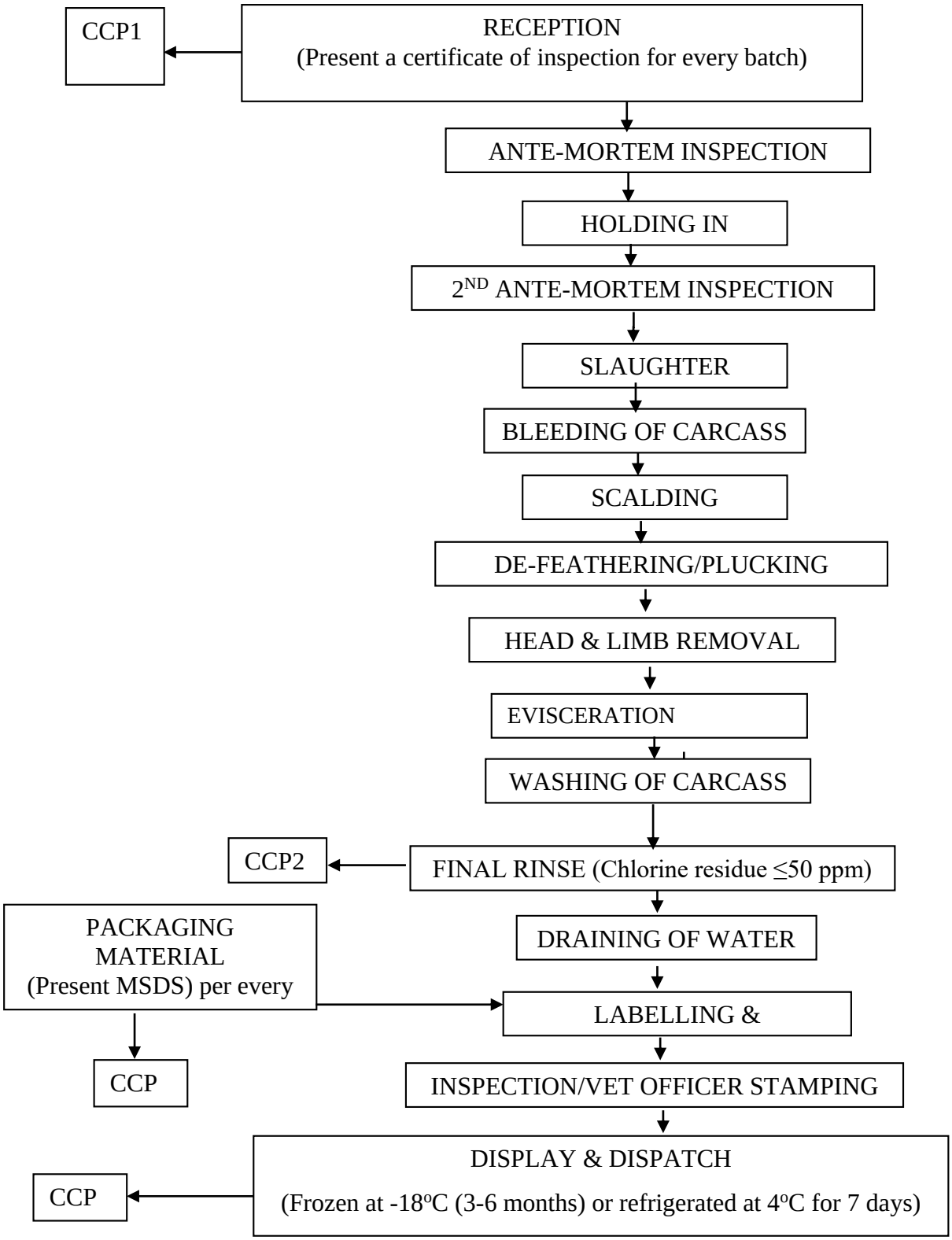


Figure 4.3:Flow diagram for the processing of indigenous chicken at the Nakuru top market slaughter point.

4.3.2 Detailed Product Description

Table 4.1 shows detailed summary of the product description. It encompasses the common name, packaging type, and intended use. It also shows labelling instructions and offers shelf life, information on the special distribution controls needed to ensure product safety.

Table 4. 1: Detailed summary of the product description records

#	Item	*Description*
1	Common name	*Dressed indigenous chicken (Kuku kienyeji)*
2	How is it to be used	Ready to cook carcass and parts
3	Type of packaging	Carcass packed individually or in parts in polythene bags or boxed in bulk.
4	Shelf-life	3-6 months at -18 ^{o}C or 4^{o}C for 7days.
5	Where will it be sold? Consumers or intended use	Hotels, restaurants, Wholesale to distributors, retailers and to individual consumers.
6	Labelling instruction	Safe food handling labels (keep refrigerated or frozen; cook thoroughly before eating).
7	Is special distribution control needed	Keep refrigerated or frozen.

The product was aptly described as indicated in the Table 4.1. The description encompasses how to handle and use product by customers. It also entails details of proper product labelling. The product common name was given as dressed indigenous chicken (*kuku kienyeji*) Swahili word for IC.

4.3.3 The HACCP Plan

Table 4.2a: HACCP Plan for Indigenous Chicken Processed at a Slaughterhouse in Nakuru

CCP Nos	Process Step;	Hazard	Critical Limits	Monitoring Procedure	Frequency	Preventive measure	Corrective action	Record	Responsible person	Verification Procedures
CCP No 1	Reception	Antibiotic residues	No chicken delivered within recommended days of withdrawal	Inspection of certificate of compliance for every batch signed by an authorised veterinary officer	Every delivery	Withholding the flock awaiting approval of an authorised veterinary officer	Reject the batch if the certificate of compliance is not produced	Certificate of compliance Records	QA officer in charge	Check the certificate of compliance records/get quarterly MRLs reports from accredited laboratories.
CCP 2	Final Rinse	Pathogens (*E.coli*, *Salmonella*, *Campylobacter*)	Residual chlorine of 50ppm	pH and Chlorine residue measurements using the strips.	After every batch/lot of chicken.	Withhold the carcass,	Re-adjust chlorinator and take samples to confirm full operationalisation.	Final rinse/carcass quality records.	QA/C officer in charge	Verify final rinse water quality records/daily residual chlorine checks/titration and Obtain quarterly chlorine analysis reports.

Table 4. 2b: HACCP Plan for Indigenous Chicken Processed at a Slaughterhouse in Nakuru

CCP Nos	Process Step;	Hazard	Critical Limits	Monitoring Procedure	Frequency	Preventive measure	Corrective action	Record	Responsible person	Verification Procedures
CCP 3	Packaging material	Toxic materials	No unqualified product used	Supplier audits and quality assurance	Each supply	Qualified packaging material supplied and proof of check of MSDS; approved supplier list and specifications agreed.	Change supplier or brand of non-conforming material.	Raw material reception records	QA/C officer in charge	Verify list of qualified suppliers, conduct regular supplier audits, and check MSDS for every material.
CCP 4	Display/Dispatch	Pathogens (*E.coli, Salmonella, Campylobacter*)	≤4ºC for 7 days	A calibrated thermometry and a temperature coding log	Internal temperature to reach 4ºC in 4hrs after processing	Withhold product, and adjust the temperature to the correct reading for the adequate amount of time.	Freeze product and observe as an internal temperature of 4ºC is arrived at.	Dispatch records/Final product temperature logs and records	QA/C officer in charge	Review the temperature logs daily/before dispatch. Calibrate thermometer daily. Check and record temp once per batch.

4.4 Discussion

4.4.1 HACCP Plan

The HACCP control chart (Table 4.2a and b) gives an organised list of the hazards and documentation of all the CCPs, which is the most important document of the HACCP plan. By enumerating any step as a CCP it follows that the process will be emphasised and scrutinised during production (Hofi & Ismail, 2008). Tables in Appendices C and D show all the potential hazards and classification, in terms of chemical (C), physical (P) or biological (B), at the process steps at which they could occur, and the number of CCP so that it is specific and documented. The HACCP control chart, further gives the critical limits, monitoring procedures, frequencies of monitoring, preventive measures, and corrective action for all the hazards listed. Finally, it presents the documented records, the persons responsible and the verification procedures. The CCPs were identified in the raw material especially on reception of chicken with possible contamination of antibiotic and pesticide residues as well as on the process steps. Another CCP was identified as occurrence of toxic chemicals in packaging material. On the process steps, the reception, final rinsing and display/dispatch process steps were noted as CCPs. Earlier (Burson, 2015)reported the process step records and procedures for verification in their developed control chart for meat products (Burson, 2015). Zhao reported the process steps in the HACCP plan that is similar to the findings in this study (Zhao, 2003). Codex advises on the importance of monitoring and documentation procedures in an HACCP plan for meat and meat products (FAO, 2013).

From this study, the various monitoring procedures for the different hazards at CCPs are presented in the HACCP plan. The importance of development of monitoring procedures was advocated by Northcutt and Russell (2010). In considering monitoring procedures, emphasis was put on those methods that are implementable and appropriate for online use (Seuli *et al*., 2015). All the four CCPs identified in this study had monitoring procedures.

4.4.2 The CCP 1-Reception

The CCP1 was identified at reception and the target hazard as residues of antibiotics. This was based on experiences where the veterinary officers and traders have reported incidences where ignorant or malicious farmers have presented for slaughter chicken treated and before the withdrawal period of the drugs lapsed. In many studies, the MRLs are cited by many companies as the critical limit. However, under the circumstances, regular analysis of MRLs would not suffice due to costs involved. Instead, a due diligence form followed by a

certification from the veterinary officer in charge was adopted. The critical limit was zero acceptance of any lot of chicken without a certificate. Monitoring procedures were outlined as inspection of the certificate of compliance. Verifications of the effectiveness of this CCP1, was designed to involve quarterly laboratory checks on MRLs, and the regular check on the records of certificate of compliance right before dispatch. The responsibility for supply of high quality and safe product must begin from the farm. This includes rearing and particularly with regard to chicken feed and water. The HACCP system designed here is to work in an environment of control and formal registration. It may not be applicable to farmers who only grow for own consumption or those that sell to their immediateneighbours and local shops. Even within the European Union, such farmers and local suppliers are excluded from the compliance to the legal food laws (Food Safety Authority of Ireland, 2011). The main reason for this is that legal requirements dictate that a facility has a traceability plan and this is not often applicable in a small, disenfranchised system which is what local neighbour to neighbour slaughter houses do.

Operationalisation of CCP1. Rationale why it was found to be a CCP:Studies by Kenya Medical Research Institute (KEMRI), have indicated that most of the antibiotics used in Kenya are not capable of eliminating altogether the *E.coli* and the Salmonella spp. which have been found to be resistant (Odwar *et al.,* 2014b). As a result, the antibiotic residues if present in the final chicken will lead to development of resistance to drugs and easily leave patients vulnerable from attack by bacteria and typhoid. Farmers are a critical link to this CCP (Odwar *et al.*, 2014b). Theyregularly practice adding antibiotics into commercial feeds or drinking water for birds hence leading to exposure of birds to human drugs. The recording system must be up to date and farmers assisted to fill them judiciously. Records regarding every handling, treatment, management types of diseases and kind of drugs used must be kept. The site visits of veterinary officers and recommended suppliers of the drugs to justify the efficacy must be brought to bear. A correct historical record of the farm animals mostly as a batch must be put in place. Then orders for supply of indigenous chicken must then only be given to the contracted farmers who must have signed a binding agreement to operate according to the regulations. The first line of defence is to ensure that Poultry showing clinical signs of disease or known to be contaminated are not slaughtered for human consumption (Food safety Authority of Ireland, 2011). This is a novel addition to HACCP implementation and practice. It lays a burden on the shoulders of farmers too and hence make them collaborators in the implementation of safety of their IC. Since their contribution is directly related to safety of IC

and relates to their profitability, there is a higher likelihood of adherence to this code of conduct.

Challenges to its Implementation and Suggestions to Overcome them. The effectiveness of this CCP like all other quality management systems depends on integrity and practice of due diligence on the side of all actors in the value chain. A formal legal registration and operation at the farm level and adequate record keeping by farmers is a necessity. The veterinary personnel are key to the success of effective control of this CCP. Any breach to ethical code of conduct and acting in due diligence will render this CCP difficult to manage.Currently, the use of sensitivity tablets for bioassays will allow for enforcement of this CCP at the reception.When affected this can lead to rejection of affected batches at reception even though by due diligence such a loss would have been prevented.

4.4.3 The CCP 2-Final Rinse Water

The second Critical Control Point (CCP2) was identified at the final rinse step. The responsibility for ensuring safety of a product lies with the manufacturer rather than the consumer of the said product. Due to the fact, there is no heat treatment on chicken meat carried out at the slaughter house, the final rinse process provides the only opportunity for reducing or eliminating any pathogens that may be present on the surfaces as well as on the product. The critical limit was set as nil occurrence of pathogenic microorganisms, especially (*E.coli),* in any lot of chicken. Observing the strength of the residual chlorine in the final rinse water provided a system of monitoring. Verification of the effectiveness of this process is based on the inspection of the final rinse water records, and the routine use of chlorine test strips (Najjar & Meng, 2009). The concentration of Chlorine that ensures safety of poultry has been a subject of a wide range of studies (Najjar & Meng, 2009). Effective control against important pathogenic microorganisms is achieved by chilling of carcasses in chlorinated water.

Rationale.

The responsibility for product safety lies with the manufacturer not the consumer. Even though the consumer will cook the food, the handling of product in a manner that doesnot pass any contaminating microorganisms to other foods at the consumers' end cannot be guaranteed. All that can be guaranteed by the manufacturer is a safe food devoid of pathogenic microorganisms even when the consumers mishandle the food. Chlorine residue is known to destroy the cell wall of the pathogenic microorganisms killing them in the process. Its

effectiveness is dependent on concentration of the acidic pHranging 2.3-2.9 (Najjar & Meng, 2009). But to be effective the dosage and contact time must be observed. Studies have indicated that Chlorine at 50ppm concentration is effective,capable of reducing pathogenic microorganisms. This concentration of chlorinewill control cross contamination, contribute to safety of chicken within a contact period of 15 minutes, and where residual Chlorine is kept at 5ppm (Najjar & Meng, 2009). Higher concentrations of chlorine can discolour the meat rendering it whitish, while lower will not be effective. The minimum residue for chlorine is also put at 5ppm so that it does not become an environmental hazard. Chlorine at these levels will also be broken down by heat to its components and gaseous forms will be liberated. Hence, risks due to exposure to chlorine is estimated to be 0.3-1%. This therefore has no danger whatsoever to human health, whereas potential exposure from chlorinated drinking water is 99% (Najjar & Meng, 2009).

Challenges to its Implementation and How to Overcome them. For effective implementation an automatic chlorinator that is calibrated regularly should be in place. The correct contact time and concentration must be maintained each time and for every batch. The parameters must be adhered to because too much concentration of the Chlorine will lead to discoloration while too little will not be effective at killing target pathogenic micro-organisms.

4.4.4 The Third CCP-3

The third Critical Control Point (CCP3) was identified as toxic chemicals in the packaging material. This is a critical step because any such toxicity will probably end up in the plate of consumers. To ensure that all the packaging material used gives no chance for contamination, it is best to pre-qualify the suppliers of the packaging material. This way, only those who can produce material safety data sheets (MSDS) that are acceptable should be given the responsibility to supply. By monitoring every supply to ensure only pre-qualified supplies effectiveness of this will be verified through the quarterly supplier audits and review of all relevant records.

Rationale why it was Identified as CCP. Packaging material gets into contact with the food. After packaging a pre-dispatch inspection is conducted. This will only take care of the physical hazard but not any embedded chemical and microbiological hazards. The packaging material used is normally polythene bags. They come in different thickness and gauges. It should be established that the product has not been treated by any illegal substance that has not been specified in legal standards according to Codex Alimentarius Commission.

Control of packaging material that comes into contact with the given food product is one step to ensure the food is safe for consumption. Safety of packaging material is the prerogative of the producers. For this control to work effectively the packaging manufacturer bears the greatest responsibility and it is the work of the poultry slaughter house to verify that the product they have acquired is not hazardous to their product.The poultry slaughterhouse must ensure that the choice of the manufacturer is one that can deliver safe packaging material. The manufacturer must be able to produce evidence that they are operating in a manner such as to guarantee they produce safe materials. This can be verifiedthrough the company producing third party evidence (audit status) that they operate in strict adherence to legally acceptable practice. Thiscan be validated through a regular supplier or third-party audits by the poultry slaughter house. The slaughterhouse must be able to have at least a list of three such approved pre-verified suppliers for continued operations should any of the suppliers run out of stock or face serious challenges. In addition, every batch of packaging material supplied must be accompanied by a material safety data sheet. This safety data sheet must comply with legal requirements.

4.4.5 Fourth Critical Control Point (CCP-4)

The CCP4 was identified at the display and dispatch of dressed carcass. Literature and experience indicates that there is a possibility of cross contamination with pathogens when temperatures rise beyond 4ºC for chicken products (Tompkin, 1994). To monitor the temperature, a temperature monitoring device is installed. The effectiveness of this CCP is to be verified based on the daily calibration of the thermometer, the review of all temperature logs before dispatch, and reading of the product temperature once per lot/batch. Verification of the implementation of the HACCP programme is critical to the success of the HACCP. Often this is a role played by the regulatory agencies (The National Advisory Committee on Microbiological Criteria for Foods, 2006).

Rationale why it was Identified to be a Critical Control Point (CCP). Chicken meat is rich in nutrients. As a result, microbial growth and proliferation would occur in chicken meat after slaughter and dressing. Immediate action should be taken to bring the product under control. This is the reason why processing and handling should be done under chilled water or on ice flakes. Even if the previous processes had removed or reduced to acceptable levels all the pathogenic micro-organisms leaving the chicken meat under room conditions would lead to spoilage within only a few hours. The spoilage would be caused by growth of spoilage micro-

organisms as well as the throughoxidative changes to fats in meat leading to rancidity and putrefaction. The later will be due to deterioration of fat and protein resulting in rancid and putrefied smell respectively. These actions can be prevented or slowed down significantly through refrigeration or freezing. Studies have demonstrated that to keep chicken meat longer (3 months and above) the freezing conditions must be at or below -18^{o}C. At these temperatures all three major changes that may lead to deterioration in product quality will be slowed down. This means there will be no significant quality drop within this keeping period.

The Innovation in this HACCP System. In literature there is no documented HACCP plan in existence for the processing and dressing of indigenous chicken in Kenya. A majority of HACP plans available have been implemented for exotic chicken. Even so, a majority give at least 6 CCPs. This may be good as it offers more possibilities for control. But this becomes tedious and difficult to control from a SMEs like the Nakuru Top Market Slaughterhouse. This work presents only 4 CCPs and it puts emphasis in area that are able to assure safety of indigenous chicken just the same way that the HACCP plans with 6, 7 or 8 CCPs could be able to do. Furthermore, based on results of metagenomic analysis of IC across the value chain, pit makes a case for review of CCP at the farm. It proposes that the target hazard should be chemical antibiotic residues rather than microbial.

4.5 Conclusion

The design and implementation of a HACCP system, still presents the best way for assurance of safe meat and poultry products. In this study, the crucial need for existence and operationalisation of GMPs, and Standard Sanitation Operation Procedures (SSOP) was revealed and hence the suggestions for improvement of premises to support these basic tenets of a HACCP plan. The product description was done to alert consumers of the nature of products and best handling practices. It also revealed the potential hazards in the final product, and how to handle the chicken products in a manner to prevent the occurrence of the relevant hazards. This is a critical food safety safeguard. Itis an important segment of any food safety assurance practice. Potential hazards were recorded both in the raw materials as well as in the process steps and relevant control measures presented. The decision tree was then used to identify the CCPs. A HACCP control chart was finally drawn for the processing of indigenous chicken at the poultry slaughterhouse based on all the principles of HACCP. The designed HACCP is novel and appropriate and can be most effective way of handling the safety of IC

from the slaughterhouse. The Four CCPs were identified: supply and reception of raw material, supply of packaging material, final rinsing of carcass, and low temperature storage of carcass during display and dispatch. These show a massive reduction in number of CCPs compared to most available HACCP plans for chicken worldwide. The innovation in the developed HACCP plan does not just guarantee safety of IC but also does so in a manner that cuts costs and is easy, and effective for the target farming communities to implement.County governments that desire to invest in the processing of indigenous chicken in Kenya should be ready and willing to invest in the design of and facilitate the implementation of HACCP system for the slaughter houses they set up. This will evidently improve safety of the indigenous chicken meat and offer a competitive advantage through marketing to high value markets and branding of IC.

CHAPTER FIVE

QUANTITY AND FUNCTIONALITY OF PROTEIN FRACTIONS ISOLATED FROM 3 ECOTYPES OF INDIGENOUS CHICKEN IN KENYA

Abstract

The aim of this study was to evaluate the effect of the cluster ecotype and the part of chicken on nutritional composition, and functionality of sarcoplasmic and myofibrillar proteins that are most relevant to the technological features of chicken meat. Over 50 chickens from each ecotype cluster purchased, slaughtered and the meat stored under refrigeration at -20 ^{o} C and later on transferred in cooler box on ice and flown to Durban University of Technology, South Africa. Protein fractions were extracted with a cocktail of Sodium Chloride buffer (50mM NaCl, 50mM Tris HCl; 75mM DTT and 1mM EDTA at pH 7) and quantified by Bradford method. One dimensional Sodium Dodecyl Polyacrylamide Gel Electrophoresis (SDS PAGE) was applied to separate protein fractions. Emulsifying capacity, emulsifying stability, solubility, and in vitro digestibility were determined on the total protein isolates. Significant differences in band expressions were recorded for the myofibrillar and the sarcoplasmic proteins. The three ecotypes had high quality proteins with all the limiting and essential amino acids at concentrations higher than FAO/WHO recommended daily allowance for adults and children. Distinct protein bands at larger molecular weight proteins >100 kDa, corresponding to Myosin Heavy Chain, medium fractions 75 kDa and 45 kDa and even lower molecular weight fraction <25 kDa were present in the chicken breast and the thighs. It concludes that Indigenous chicken protein isolates' nutritional and functional properties are affected by part of chicken and ecotype clusters.

Keywords*:indigenous chicken, protein isolate, functionality, sarcoplasmic, myofibrillar*

5.1 Introduction

Indigenous chickens (IC) are preferred to the broiler in many parts of the world and particularly in Kenya due to a range of reasons. In terms of investments, they require less feed, are forage scavengers, they are resistant to pests/diseases and serve as a source of income for many households especially in rural areas. Consumers on the other hand, prefer IC because the IC are *tastier*, juicier, and have more intense chicken flavour. Kenya has a great pool of genetically diverse IC. Kenyan IC has been classified genetically into 3 ecotype clusters (Ngeno *et al*., 2015). Many studies have recently used the microsatellite markers for the genetic evaluation of chicken (Qu *et al*., 2006). Limited work if any has been done to evaluate any differences in meat quality based on this classification (Ngeno *et al*., 2015). Poultry meat is made up of water, proteins, fat, minerals, and carbohydrates (Bender, 1992; Omana *et al*., 2010). These components determine the functional and sensory quality of the meat. Chicken is a source of high-quality protein. Chicken meats contain all the essential amino acids needed by human body. Scientific consensus is that the protein composition/functionality affects overall meat characteristic of appearance, texture, and mouth feel (Mudalal *et al*., 2014). Two major types of chicken muscle proteins (myofibrillar and actin are responsible for muscle texture and water holding capacity (WHC) (Ooizumi & Xiong, 2004). The sarcoplasmic proteins on the other hand play only a minor role (Petracci *et al*., 2013). The solubility of sarcoplasmic and myofibrillar proteins on the other hand are reported to be correlated to water retention capacity of chicken meat resulting in low cooking losses (Li- Chan *et al.,* 1987; Warner *et al*., 1997). Lower protein solubility has an influence on the functionality/defects of chicken proteins such as the pale soft exudative (PSE) condition (Mudalal *et al*., 2014).

Meat proteins are generally categorised into three in terms of their solubility in salt, water, or alcohol. Chicken protein composition especially of breast have a crucial impact in processability of meat, its nutritional, and sensory quality (Mudalal *et al*., 2014).The knowledge of protein composition and structure is important to explaining functionality and applications. Protein composition and structure may vary across the different ecotypes or genotypes and this is likely to affect their functionality (Arise *et al*., 2017). The structure and functionality relationships of proteins are dependent on pH, ionic strength, and temperature. The process of protein fractionation, takes advantage of the fact that various protein components have various solubility in salt withdiffering isoelectric points or pH ranges. The gel chromatography method applied in protein isolation and fractionation is based on size exclusion principles and separates the protein fractions based on the differences in their relative

molecular weights (Mudalal *et al*., 2014). Studies have demonstrated the fact that the different sizes of proteins contribute distinctly to their functionality. To achieve this separation, native protein must be denatured and their chargesneutralised (Warner *et al*., 1997).

5.2 Materials and Methods

5.2.1 Sample Collection

At least five chicken from each of the three IC ecotype was collected from Taita, Naivasha and Kakamega. Sample size for this aspect of the study was limited by the cost of amino acid profiles. The analysis was carried out at Durban University of Technology in South Africa. Samples were collected and flown to South Africa in ice within 24hours. They were then immediately transferred to a blast freezer at -80◦C at the Food Science Laboratory in South Africa awaiting further analysis.

5.2.2 Moisture Content Determination (AOAC Method 960.46)

Ten (10) grams of chicken meat was weighed into a crucible and dried in an oven set at 100-102m^{o}C to a constant weight (16 hours). The crucibles were then cooled in a desiccator and moisture content of the samples determined by difference. The analysis was performed in triplicate. The formula was as as shownbelow:

% Moisture content=[Weight of wet meat samples-Weight of dried samples]/Weight of wet samplesX100

5.2.3 Ash Determination (Method 13.002, AOAC, 1984)

Chicken samples (10 g) were weighed and dried for 16 hours at 105^{o}C. The samples were then ashed in a muffle furnace (Gallenkamp size 2) at 550^{o}C for 3 hours. The ash was cooled in a desiccator to room temperature and weighed. Ash content was then calculated as a percentage of the dry sample. That is;

$$\% \, Ash \; = \frac{Mass \, of \, Ash}{Mass \, of \, Sample} \; x \; 100.$$

This was done in triplicates.

5.2.4 Determination of Total Lipids (AOAC Method Number; 960.39)

The total lipidswere extracted by gravimetric measurement based on Official Methods of Analysis (2000) (AOAC; method 960.39). Slight Modification where extraction was started in mortar and pestle and finished off in the Soxhlet extractor was employed. About five gram

of chicken sample was ground in a motor and pestle and placed into the thimble to which 1-1.5 gram of sand had been added.The sand and the chicken samples were mixed with a glass rod. The glass rod was wiped by a piece of cotton wool in the top of the thimble.The pieces of cotton wool were retained. The samples were dried in an oven at 102ºC for 5 hours. The samples were then allowed to cool in a desiccator. The piece of cotton wool was removed from the bottom of the beaker and placed on the top of the thimble. The thimble was then inserted in a Soxhlet liquid/solid extractor. A clean, dry 150 mL round bottom flask was weighed and filled with about 90 mL of petroleum spirit into. The extraction unit was assembled and heated over an electric heating mantle. The heating was applied to the solvent and the content brought to boil. The heat source was be adjusted so that solvent dripped from the condenser into the sample chamber at the rate of about 6 drops per second.

The extraction was continued for 6 hours, then the extraction unit was removed from the heat source. The extractor and condenser were detached from each other. The flask was then be removed from the heat source and the remaining solvent evaporated off. The flask was then placed in an oven at 102ºC and its contents dried until a constant weight is reached (1-2 hours).

The flask wascooled in a desiccator and weighed together with its contents.

Weight of empty flask (g) = W1

Weight of flask and extracted lipids (g) = W2

Weight of sample = S

% Crude fat = (W2 – W1) x 100 S

The percentage crude fat was calculated according to the formula below.

$$\%\ Crude\ fat = \{\frac{W2 - W1}{S}\} x\ 100$$

5.2.5 Crude Protein Analysis

This was undertaken using micro-kjeldhal (Bionic Scientific Technology, India) Official Methods of Analysis (2000) (AOAC, micro-kjeldal method: 960.52). Chicken samples, 1 g was weighed into a *digestion flask* and then digested by heating it in the presence of conc. Sulphuricacid (an oxidising agent which digests the non-protein matter), anhydrous sodium sulphate (to speed up the reaction by raising the boiling point) and selenium as a catalyst (protein extraction catalyst). After the digestion, the flask was connected to a receiving flaskby a tube. The solution in the digestion flask was neutralised by adding Sodium hydroxide. The Ammonia gas formed was distilled using steam and collected in a flask containing excess

Boric acid. The nitrogen content was then estimated by titration of the ammonium borate formed with standard hydrochloric acid, using a phenolphthalein indicator to determine the end-point of the titration. The amount of nitrogen was calculated by the amount of acid added that changed the colour of the distilled sample solution.

% Crude protein = (blank titre) x 1.401 x 6.25 x 20 x Normality of NaOH/Sample Weight

Where the 6.25 is the Nitrogen-conversion factor.

5.2.6 Shear Force Determination

Breast fillets were cooked on racks in aluminium lined, covered pans in a preheated convection oven to an internal temperature of 76 °C for 10 min. The fillets were cooled to room temperature, individually wrapped in aluminium foil and stored overnight at 4°C. A Meullenet-Owens razor shear (MORS) method was then used to evaluate the shear force of the cooked breast and thigh fillets (Guan *et al*., 2013; Meullenet *et al*., 2004). Shear force was determined on intact fillets using a texture analyser (TA-XT2i; Scarsdale, NY, USA) fitted with a razor blade (24 mm in height and 8.9 mm in width) with a 100-g load cell set to 20-mm penetration depth. The Crosshead speed was triggered by a 10-g contact force with 10/5 mm/s. Breasts and thigh muscles were punctured across muscle fibres (Fanatico *et al*., 2007a).

5.2.7 Determination of Cooking Loss

Ten (10) fillets with the average fillet weight 118 g) were removed from the packaging bags and placed in 2 glass 23 × 33 × 5 cm Pyrex baking dishes (5 fillets/dish) covered with aluminium foil. The samples were cooked in the preheated Vulcan electric (commercial) oven Vulcan model E60F, Vulcan-Hart, Louisville, Ky., U.S.A., with internal dimension 56 × 66 × 34 cm) set at 163°C. The core temperature (endpoint internal cooking temperature) was set to reach 80 ◦C. The fillets were removed and allowed to rest at room temperature. After cooked samples cooled to 76°C, they were weighed for determining cooking loss.Slices were then removed for the WB shear force measurements (Fanatico *et al*., 2007a).

Cooking loss was calculated by dividing the difference between raw fillet weight and cooked fillet weight by raw weight and multiplying by 100:

Cooking Loss = {(Raw Weight−Cooked Weight)/Raw Weight}x100

5.2.8 Colour Determination

Colour measurement of the freeze-dried portions of IC was carried out using colour flex (A60-1014-593; Hunter Associates Laboratory, Reston, VA, USA). The colour values were on the basis of lightness (L*), red-green (a*) and yellow-blue (b*) values. The instrument was calibrated against white and black colour tiles before colour measurement (Arise *et al*., 2017). Total colour difference (DE) was calculated as shown below:

$$\Delta E = [\ (\Delta L^*)2 + (\Delta a^*) + (\Delta b^*)2]^{1/2}\ .$$

While Hue angle = $\tan^{-} 1^{\ b/\ a}$

where: ΔE was change in colour difference, ΔL*= change in Lightness, Δa*=change in red-green and Δb*=change in yellow-blue.

5.2.9 Myofibrillar Protein Preparation

Myofibrillar protein fractions were extracted by the procedure of Mudalal *et al*. (2014), with some modifications (pH and DDT concentration). The minced meat samples were extracted in a buffer containing (50 mM $NaCl_2$, 50 mM Tris-HCL (pH=7.0); DTT (75mM DTT) and 1mM EDTA (pH=7). The extraction was conducted by homogenising samples through a desk top Homogenizer at 8,000 rpm, then extracted for 30 minutes. The extract was then centrifuged at 10 rpm for 10 minutes. The residue was dissolved in 10 mls of fresh extraction buffer and the process repeated to obtain the 2 resultant fractions. The Myofibrillar and sarcoplasmic protein extracts were stored at -80 °C in rigor buffer (75 mM KCl, 10 mM KH_2PO_4, 2mM $MgCl_2$, and 2 mM EGTA pH-7.0) containing 50% (v/v) glycerol until ready for protein quantification. The total content of myofibrillar and sarcoplasmic proteins was measured by Bradford assay according to (Bradford, 1976), that involves binding the proteins to Coomassie brilliant blue G-250 dye. The absorbance of the blue protein dye complex was detected at 595 nm using UV Spectrophotometer installed with Cary win software.

5.2.10 Amino Acid Composition Determination

The amino acid contents of the breast and thigh of IC ecotypes were determined using Pico-Tag method (Bidlingmeyer *et al*., 1984). This method is based on the principle of reverse phase chromatography with pre-column derivatization following acid digestion. Protein samples were hydrolysed with 6 M HCl at 116^{o}C for 24 h prior to chromatographic analysis.

5.2.11 SDS PAGE -Analysis

Frozen chicken breast and thigh muscles were selected for the extraction and separation of proteins using SDS-PAGE analysis according to the procedure by Mudalal *et al*. (2014). Samples were mixed at a ratio of 1:1 with standard sample buffer that contained 8 M urea, 2 M thiourea, 3% (wt/vol) SDS, 75 mM dl-dithiothreitol, and 25 mM TrisH-Cl at pH 6.8 (Fritz *et al*., 1989), and heated at 100°C for 5 min in a water bath, cooled, and applied to the gel. Fifteen microliters (15 µL) of myofibrillar protein extract were loaded on 12 % Mini-Protean TGX Stain-Free Gel (Bio-Rad). A similar amount of sarcoplasmic ex-tract was loaded onto a 12% Mini-Protean TGX Stain-Free (Bio-Rad). The separated protein bands were identified by comparing their mobilities against those of a molecular standard weight marker. The reservoir buffer used in the Mini-protean II cell small electrophoresis unit (Bio-Rad) was made of 50 mM Tris, 0.384 M glycine, and 0.1% (wt/vol) SDS. Small gels were set to run at a constant voltage of 180 V for stacking and running gel, respectively. Sarcoplasmic and myofibrillar protein gel images were captured by a ChemiDoc MP tabletop scanner with Image Lab Rev 4.0 software (Bio-Rad) and based on UV Illumination. The exposure time was less than 1 minute. One dimensional SDS-PAGE analysis was used to evaluate the molecular weight profile of sarcoplasmic and myofibrillar proteins.The concentration of each band was expressed in 2 ways as absolute (mg/g of meat) and as relative abundance (%). The latter was calculated based on the sum of protein concentration in all bands within the same lane to avoid the small differences due to protein loading among lanes. In each band, the dominant protein was determined based on molecular weight and relative abundance. Electrophoretic protein bands were assigned by comparison with data reported in literature using mass spectrometry.

5.2.12 Foam Capacity

Foam capacity and foam stability was determined according to the procedure of Chan Omana and Betti (2011). Known volume of proteins at different concentrations were whipped in a vortex mixer (Fisher Scientific, ON Canada) at a speed of 10 g for 1 minute. Foamability or foam expansion was expressed as percentage volume increase after mixing using the equation below:

$$\%\ Foam\ expansion\ (FE)\ = \frac{\text{Foam volume}}{\text{Initial liquid volume}}\ x\ 100$$

Volumes of the suspension were recorded before and after homogenisation. Foaming capacity was determined as follows using the mean of three measurements.

$$Foam\ Capacity\ (FC) = \frac{Volume\ of\ Foam\ after\ homogenization - Volume\ before\ Homogenization}{Volume\ before\ homogenization}\ x\ 100.$$

Foam stability was determined as the volume of foam that remained after 1hr at room temperature expressed as percentage of the initial volume. The following equation was used.

% Foam stability={Volume of foam after 1 hour-volume of foam before homogenisation}/volume before homogenisationx100

5.2.13 Emulsifying Activity and Stability

Emulsifying activity and stability were determined using the method described by Lawal *et al*. (2007). Five millilitre portions of known protein solutions were homogenised with five mL of sunflower oil. The emulsions were centrifuged at 1100 g for five minutes. The height of the emulsified layer and that of the total contents in the tube was determined. The emulsifying activity (EA) was calculated using the expression below.

EA (%) =[Heigh tofemulsifiedlayerinth etube- Height of original mixture]/ height of the original mixture ×100.

Emulsion stability was determined by heating the emulsion at 80 °C for 30 minutes after which it was centrifuged at 1100 g for 5 minutes.

$$Emulsion\ Stability\ \ (ES) = \frac{Height\ of\ Emulsified\ Layer\ after\ Heating - Height\ of\ emulsified\ layer\ before\ Heating}{Height\ of\ original\ mixture}\ x\ 100.$$

5.2.14 Protein Solubility

Protein solubility was estimated according to differences in extractability of proteins in different ionic strength solutions Warner *et al*. (1997) and modified by Mudalal *et al*. (2014). Sacroplasmic protein solubility was measured in three replications by weighing 1g of chicken meat sample. Ten millilitres of ice cold 25 mM potassium phosphate buffer (pH 7.2) were added to the samples and homogenised witha high-speed blender on 11,000 rpm/min. The homogenised samples were kept under refrigeration conditions of 4^{o}C for 20 h and then centrifuged at 2600xg (RCF = 1.12 x Radius x (rpm/1000)^{2}) for 30 minutes at 4^{o}C. The supernatant was decanted and protein concentration measured using the Bradford assay (Bradford, 1976) with bovine serum albumin as a standard. Total protein solubility was

determined in' a 1.1 M KI/0.1M potassium phosphate (pH 7.2) buffer. Myofibrillar protein solubility was calculated by the difference in solubility of total and sarcoplasmic proteins.

5.2.15 In vitro GI Digestion

In vitro digestion of boiled chicken meat was performed according to Garrett *et al.* (1999) and modified by Sangsawad *et al*. (2017) with slight modifications. The boiling of chicken meat was done with six times volume of hot water in a temperature-controlled media MK2102 electromagnetic furnace (Media Group, China) at temperature of 95±3°C for 15 minutes (equals to well done). Cooked samples (2 g dry solid) were homogenised (IKA Works Asia, Bhd, Malaysia) in 100 mL of deionised water (DI) for 1 min. The pH was adjusted to 2.0 ± 0.02 with 1M HCl and pepsin (2.86% of substrate, dry basis) was added. Digestion was performed at 37 ^{o}C for 1 h in a shaker at 95 rpm speed. Subsequently, the pH was increased to 7.5 ± 0.02 by adding 5 M NaOH. Pancreatin (4.00 % of substrate, dry basis) was added and the reaction was carried out at 37 ^{o}C for 2 h in a shaker at 95rpms. The enzymatic digestion was terminated by submerging the sample in a 95 ^{o}C water bath for 10 min and cooling on ice to room temperature (Sangsawad *et al.*, 2017). The digested mixtures were then centrifuged at 10,000 g for 10 min. The peptide content of the supernatant was determined using Bradford assay, with Bovine Serum Albumin (BSA) as the standard.

5.2.16 Data Analysis

Numerical Data was analysed using SAS version 9/1. ANOVA was used to infer differences in data while means were separated using the DMRT at a $p \leq 0.05$. SDS page results were visualised using Image Lab Rev 4.0 software (Bio-Rad) and based on UV Illumination and band positions observed based on molecular weight.Quantities and band positions were compared to standard protein marker (10-190 kda).

5.3 Results and Discussion

5.3.1 Chemical Composition of Indigenous Chicken Ecotypes in Kenya

No significant difference was reported in moisture content between the thighs and the breasts (71.37±1.73 and 71.62±0.57) among the ecotypes respectively. In all the ecotypes the thighs recorded higher moisture content than breasts. The mean moisture content reported for the IC ecotypes ranged from 69-72 % (Table 5.1 a) .

Table 5.1a: Proximate composition of breast and thigh portions

Chicken Part	**Moisture Content**	**Fat Content**	**Protein Content**	**Ash Content**
Breast	71.62±0.57[a]	2.12±0.50[a]	23.21±0.74[a]	4.73±0.39[a]
Thigh	71.37±1.73[a]	3.12±0.49[a]	21.46±1.05[a]	3.66±0.34[a]
Kakamega	72.98±0.82[a]	2.75±0.68[a]	20.11±1.25[a]	4.15±0.64[a]
Naivasha	70.97±0.92[a]	2.51±0.62[a]	23.42±0.90[a]	4.43±0.30[a]
Taita	70.78±2.41[a]	2.78±0.61[a]	23.14±1.10[a]	4.07±0.57[a]

The mean values recorded (breast 71.6 % and thigh 71.3%) were lower than those reported by Chumngoen and Tan (2015a) who got an average moisture of 74.73% among the Taiwan native chicken. Moisture content is critical to yield and also determines functionality. In the current study this was similar among all the ecotypes. Protein content was the main focus of this study. Though, no significant differences were noted for crude proteins among the different parts of the ecotypes, yet the breasts recorded higher values for all the ecotypes than the thigh muscles. These results are supported by the study conducted by Sirri *et al*. (2010) who noted that the breast chicken muscle had higher protein content. The breast and thigh muscle composition has been found to differ in some studies but with regard to proximate analysis, these differences are not significant. This is however in contrast to Guan *et al*. (2013) who reported that the genotypes of birds influenced their chemical composition.

The chemical composition of different chicken ecotypes are presented in Table 5.1b.

Table 5.1b: Proximate composition of IC ecotypes

Ecotype	**Chicken Part**	**Moisture Content**	**Fat Content**	**Protein Content**	**Ash Content**
Kakamega	Breast	72.53±1.00[a]	1.75±0.86[a]	21.21±1.50[a]	4.43±1.03[a]
	Thigh	73.32±1.32[a]	3.59±0.95[a]	19.02±2.04[a]	3.88±0.96[a]
Naivasha	Breast	70.40±0.91[a]	1.64±0.31[a]	24.08±1.11[a]	4.61±0.38[a]
	Thigh	71.69±1.83[a]	2.95±0.89[a]	22.99±1.35[a]	4.23±0.49[a]
Taita	Breast	72.46±0.79[a]	2.78±1.00[a]	24.31±0.91[a]	5.00±0.83[a]
	Thigh	69.09±4.97[a]	2.77±0.80[a]	21.97±1.99[a]	2.96±0.40[a]

Values given as mean ± standard deviation (n=) Values are expressed in (g/100g) of sample.

[a]-Means and standard deviations within the same column with no common superscript are significantly different at ($P<0.05$)

The rate of growth of chicken also affects their protein content (Fanatico *et al*., 2007a; Guan *et al*., 2013). Indigenous chicken are all slow growing and hence record high quality protein content ranging from 19.0±2.04to 24.3±0.91%. (Khiari *et al*., 2014), reported protein content of 19.7% among the commercial Turkey purchased from the market. Protein content determines nutritional quality of chicken meat. Similar crude protein content of native chicken meat were reported by Guan *et al*. (2013). Fanatico *et al*. (2007b) reported slightly higher values from Slow- and Fast-Growing Chicken Genotypes raised indoors or with outdoor access in the United States. Chumngoenand Tan (2015a) recorded higher protein values both for the broiler and Taiwan native chicken. With regard to fat content, the same trend was observed as higher values of fat content was recorded for the thighs (3.12 %) than for the breasts (2.12 %). This was across all the ecotypes. These values were however higher than those reported by Chumngoen and Tan (2015a). Fat content determines functionality, sensory quality and nutrition of poultry meat (Aronal *et al*., 2012a). The difference in fat content is normally dependent on the genotype, age, production system,and feed (Guan *et al*., 2013). The results of fat content from this current study agree with other researchers with regard to average fat content for chicken (Fanatico *et al*., 2007; Sirri *et al*., 2010). With regard to the Ash content, the same trend was observed. Higher values of Ash content were reported on the breasts than on the thigh muscles for all the ecotypes. Ash content shows mineral content but chicken meat is not critically targeted toward this (Sirri *et al*., 2010). These values were however higher than values reported by Khiari *et al*. (2014).

5.3.2 The SDS-PAGE Results

The SDS PAGE for Kakamega ecotype reveals distinct bands between the myofibrillar and sarcoplasmic proteins. Up to twelve (12) bands were noticeable on the sarcoplasmic proteins and a maximum of 10 were reported on the myofibrillar protein for the breast muscle (Figure 5.1). This confirms the report by Mudalal *et al*. (2014) that SDS-PAGE analysis for meat proteins from normal and white striated fillets showed different patterns for sarcoplasmic and myofibrillar proteins. The myosin heavy chain (MHC) proteins are dominant and prevalent on the myofibrillar proteins on the thighs as well as on the breast muscles. These proteins are shown to be smeared at the wells evidence of their inability to disintegrate into lower molecular weight proteins and thus their movement down the gel is hindered. The Actin (42-45 kDa) molecular weight proteins is a distinct poultry muscle protein as is visibly recorded both on the breast and thigh muscles of the Kakamega ecotype (Figure 5.1). Sarcoplasmic protein fraction

separated into at least 12 distinct bands. This was similar for protein fractions either from the breast or thigh. This was also reported by Mudalal *et al*. (2014) though they stated that only 11 of these bands having molecular weight of between 25 and 90 kDa were quantified. A band is observed at the 75 kDa mark. This band however,was not as prominent and distinct as the Actin band observed at 42-45 kDa.

Myofibrillar proteins on the other hand had prominent HMC at the top and some of these were too large as to enter the gel wells for better separation. This may be as a result of the difficulty in reduction in disulphide bonds which results in higher molecular weight species that are transferred out of the gels with greater difficulty than those of lower molecular weight (Fritz *et al*., 1989). There is a prominent band at 135 kDa which separates into two with one band spotted at the 100 kDa mark, representing (C-protein). This separation must have been aided by the application of the DTT and then denaturation of the muscles by boiling for 5 minutes at 95ºC (Mudalal *et al*., 2014). The IC protein isolates from the current study also express very prominent band at the 45 kDa (Actin). The sarcoplasmic protein from breast muscle had seven fully expressed protein bands (lane 2) compared to only 5 for thigh muscle (lane 4) for Kakamega ecotype. The concentration of these specific bands is indicative of the nature of the poultry muscle from those affected by white stripping (Mudalal *et al*., 2014).

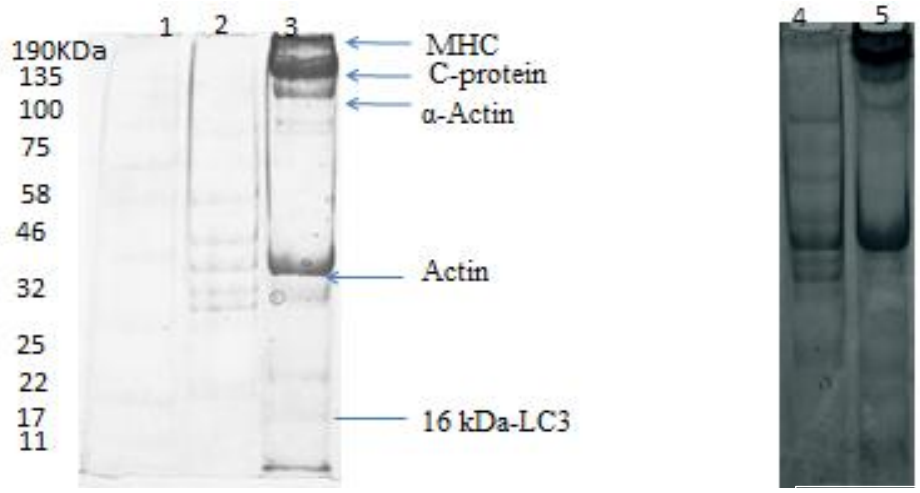
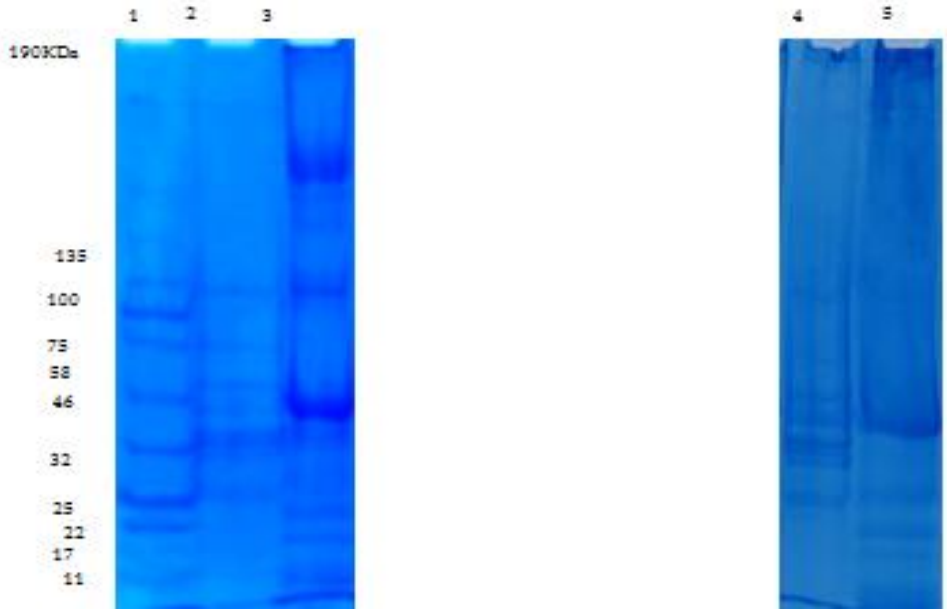


Figure 5.1: SDSPAGE profile of Sarcoplasmic and myofibrillar proteins for Kakamega IC

Legend: Lane 1: marker; Lane 2: Sarcoplasmic; Lane 3: Myofibrillar; Lane 4: Sarcoplasmic; Lane 5: Myofibrillar

The SDS PAGE of the Taita ecotype reveals that myofibrillar proteins of both the breast and thighs have distinct and similar band profiles or characteristics (Figure 5.2). There was less smearing at the top of the gel and this may have been as a result of the denaturation for 5 minutes at 95 ^{o}C (Fritz *et al*., 1989). The dominant bands are still the MHC (molecular weight above 135 kDa) and actin of 42-45 kDa molecular weights. The observation agrees with the report of Omana *et al*. (2010) about the location of this abundant actin band. The myofibrillar proteins isolated from the thighs however show least separation of the Heavy Myosin Chain proteins which indicates a difficulty in their separation or reduction to lower molecular weight proteins. More bands were expressed in sarcoplasmic proteins than were expressed in the myofibrillar proteins for both the thigh and breast muscle.



Breast Thigh

Figure 5.2: SDS-PAGE profile of Taita ecotype of Kenyan IC

Legend: Lane 1: Marker; Lane 2: Taita Breast (sarcoplasmic); Lane 3: Taita breast (myofibrillar); Lane 4: Taita thigh (sarcoplasmic); Lane 5: Taita thigh myofibrillar

With regard to the Naivasha ecotype, the same trend is observed. More bands totalling 12 are visible for the sarcoplasmic proteins on both the breast and thigh muscles. The myofibrillar proteins expression for breast and thighs are very similar (Figure 5.3). The breast's myofibrillar protein separates better in to 4 distinct bands whereas the thigh myofibrillar proteins have mainly the bands corresponding to MHC and the C-proteins and alpha-actin. This is supported by the work of Omana *et al*. (2010). At the same time, it is evident based on the sizes of the bands that hydrolysis of proteins in this process was greatly reduced. This would

suggest intact action of the protein functionality particularly with regard to gel-forming capacity (Kristinsson, 2001). The poor separation or mobility observed on the myofibrillar proteins from the thigh may suggest the occurrence of disulphide bonds just before sample applications particularly in the presence of DTT (Fritz *et al*., 1989). Though they reported that this problem could be eliminated by heating or denaturation and use of 2-mercaptoetanol for longer than 4 minutes. In the current work denaturation was done but use of 2-mecarptoethanol was omitted for this study.

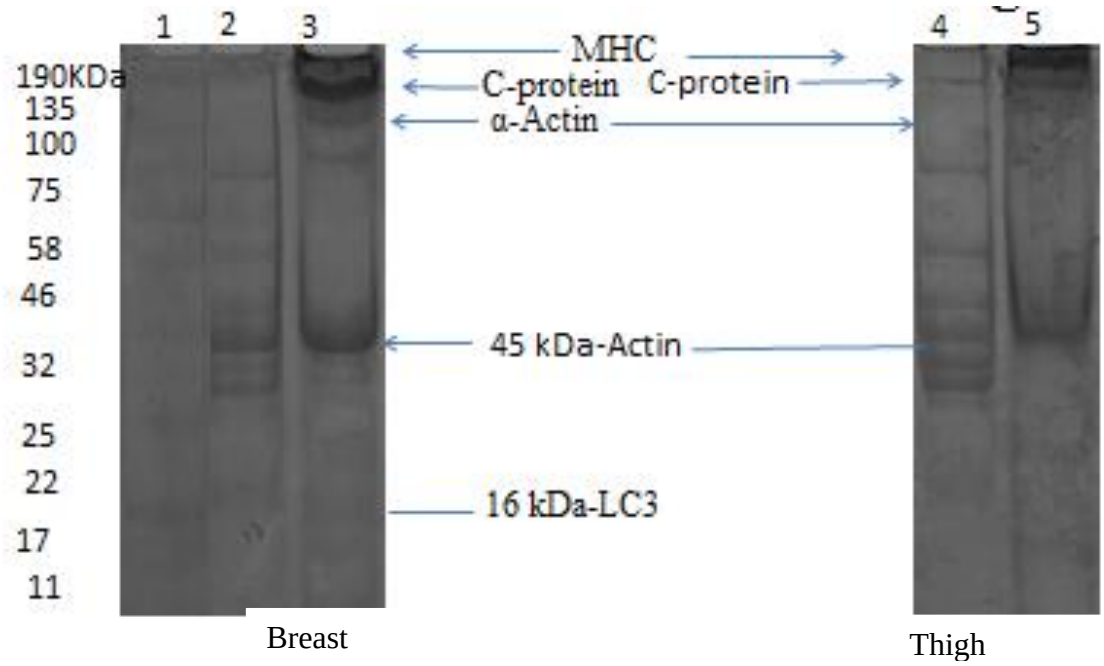


Figure 5.3: SDS-PAGE profile of Naivasha ecotype of Kenyan indigenous chicken (a) Breast (b) Thigh

Legend: Lane 1: Marker: Lane 2: Naivasha breast (sarcoplasmic); Lane 3: Naivasha breast (myofibrillar); (b) Lane 4: Naivasha thigh (Sarcoplasmic); Lane 5: Naivasha thigh (myofibrillar).

5.3.3 The Characteristic Bands of Myofibrillar and Sarcoplasmic Muscles

The SDS PAGE pattern for all the ecotypes and the parts of chicken demonstrate the presence of sarcoplasmic and myofibrillar proteins. This is similarly reported by several studies (Fritz *et al*., 1989; Zanetti *et al*., 2013). The SDS-PAGE for the myofibrillar proteins isolated from the three ecotypes show three distinct bands at a molecular weight above the 135 kDa as (MHC, C-proteins and the alpha-Actin). Protein fractions are determined based on their solubility on a chosen ionic strength of buffer in this case 50mM NaCl was used. The SDS-PAGE patterns revealed the characteristic soluble proteins mostly enzymes and regulatory

proteins from the structural muscle of the contractile nature particularly the abundant actin and myosin. This is in line with the reports by Zanetti *et al*. (2013). From these bands, the next prominent band is Actin which is very indicative of poultry proteins at molecular weight of 42-45 kDa. The slow twitch myosin chains LC1 and LC2 and the fast twitch light chain myosin precede these at molecular weights ranging from 27-16 kDa (Figure 5.4). The muscles are part of contractile fibre (Mudalal *et al*., 2014). Zanetti *et al*. (2013) reported that the fast twitch fibres are major components of breast muscles. This study confirms this finding based on the distinct bands of LC1 and LC2.

The SDS-PAGE of the sarcoplasmic proteins shows the MHC, C-proteins and the Alpha-Actin as very distinct bands and which are well separated. The presence of alpha actin is a clear indication of the ability of this protein to be extracted by the NaCl. This is supported by the report by Xiong *et al*. (2000). The next prominent bands visible on the electrophoretograms are located at the 90kDafor glucose pyruvate and pyruvate kinase at 68kDa. The lowest molecular weight protein fraction is the phosphoglycerate mutase at 25 kDa. In total at least 12 distinct bands are observed for sarcoplasmic protein as opposed to 8-10 (maximum) bands observed for the myofibrillar protein fraction for all the ecotypes. This was irrespective of whether the SDS-PAGE was conducted on breast or thigh muscle.

The two protein fractions (sarcoplasmic and myofibrillar) have the highest relevance to nutritional quality and processability of chicken meat (Xiong *et al*., 2000). There is a clear demonstration in profile of protein sub fractions between the myofibrillar and sarcoplasmic proteins. For the myofibrillar proteins, the actin and alpha actin seemed to be the most extractable proteins (Xiong *et al*., 2000). The results of this work also support the finding of Zhang *et al*. (2015) who reported the most intense myofibrillar protein bands as containing the MHC at 200 kDa, actin at 43kDa and tropomysin subunit at 35kDa.

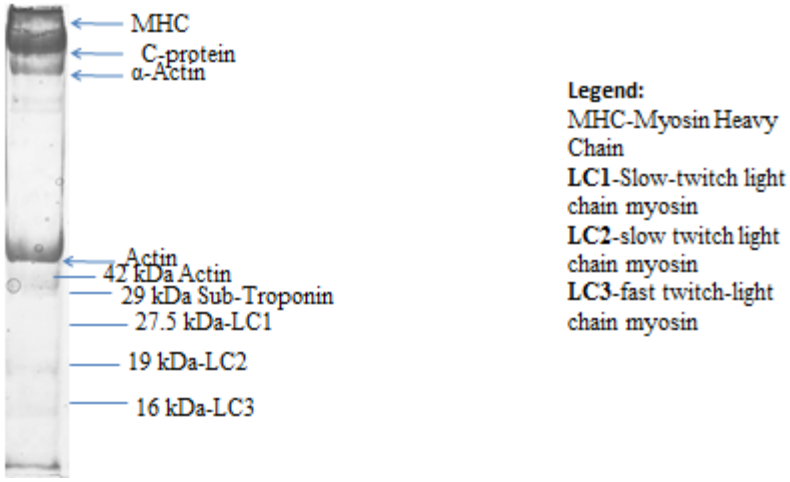


Figure 5.4:SDS-PAGE profile of myofibrillar protein from Kenyan indigenous chicken showing prominent bands identified.

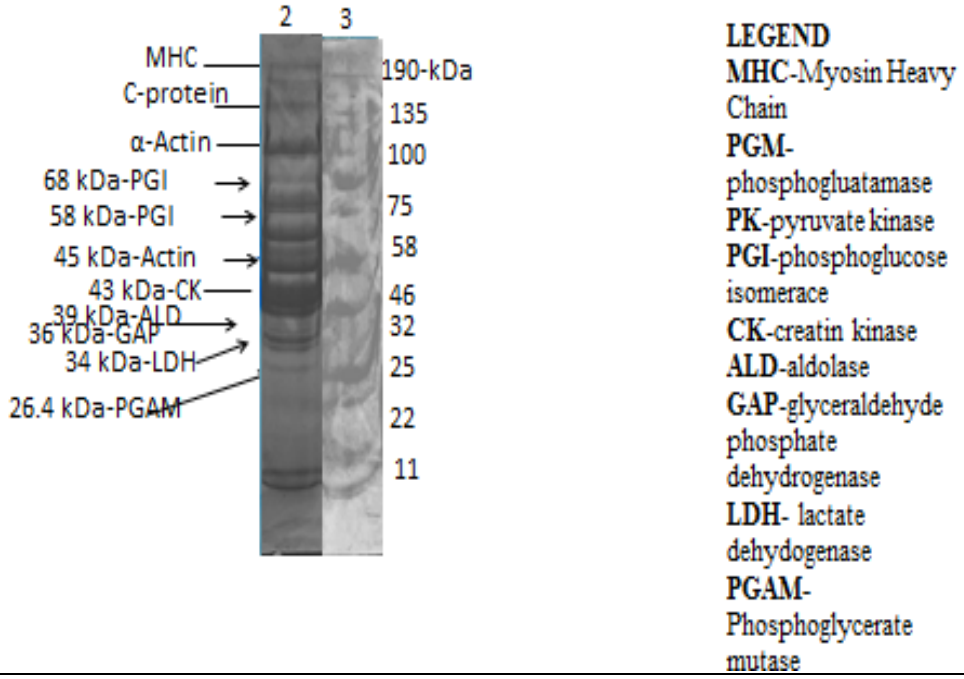


Figure 5.5: SDS-PAGE profile of sarcoplasmic proteins from Kenyan indigenous chicken displaying some of the most prominent bands identified

Legend: Lane 2: marker; Lane 3: Sarcoplasmic protein fraction

Proteins involved in energy metabolism were identified in the sarcoplasmic proteins (Figure 5.5). Notably these were expressed by bands corresponding to the Enolase, pyruvate kinase (PK), and the glyceride phosphate dehydrogenase (GAP). This confirms the studies of

Zanetti *et al*. (2013) who reported an increased expression of five proteins involved in the energy metabolism among these were phosphoglycerate kinase and beta-enolase.

5.3.4 Amino Acids Composition of the Kenyan Indigenous Chicken Ecotypes

Histidine and Lysine amino acids were higher in the breasts than in the thigh ($P<0.05$) muscles for all the ecotypes.There were no significant differences reported on the other amino acid profile of the breasts and thighs. Among the three ecotypes, the essential and non-essential amino acids were not significantly different (Table 5.2a and 5.3b).

Table 5.2a: Amino acid profiles of the breast and thigh portions (g/100 g protein)

Amino acid	Chicken parts		Ecotype			FAO/WHO2007 recommendations for adults	FAO/WHO2007 recommendations for children
	Breast	Thigh	Kakamega	Naivasha	Taita		
His	2.96±0.03[a]	2.15±0.13[b]	2.51±0.40	2.46±0.52	2.69±0.30	1.6	1.9
Lys	9.02±0.26[a]	7.76±0.24[b]	7.95±0.56	8.43±0.75	8.79±0.58	1.6	5.8
Met	7.96±0.19	6.77±0.89	6.79±0.84	6.90±1.09	8.42±0.13	1.7	2.7
Val	4.33±0.02	3.91±0.22	4.04±0.33	3.99±0.31	4.34±0.01	1.5	3.5
Ile	3.91±0.04	3.62±0.17	3.67±0.29	3.69±0.16	3.94±0.02	1.3	2.8
Leu	7.25±0.10	6.70±0.41	6.81±0.60	6.73±0.35	7.41±0.11	1.9	6.6
Phe	3.42±0.26	3.50±0.27	3.50±0.42	3.25±0.17	3.63±0.38	1.6	6.3
Thr	4.20±0.09	4.04±0.23	4.03±0.35	4.01±0.05	4.33±0.16	0.9	3.4
Total	43.05±	38.45	39.3	39.46	43.55		

[ab]-Means and standard deviations within the same column with no common superscript are significantly different at ($P<0.05$

The results of amino acid profile of breat and thigh portions (g/100 gprotein) is shown in Table 5.2b. Taita breast had highest values of Glutamic acid (14.89%) while Naivasha ecotype had the lowest (13.96%).

Table 5.2b: Amino acid profile of breast and thigh portions (g/100 gprotein)

Amino Acid	Breast	Thigh	Kakamega	Naivasha	Taita
Ser	3.42±0.1	3.46±0.19	3.32±0.12	3.39±0.05	3.62±0.22
Arg	5.73±0.06	5.69±0.34	5.64±0.19	5.51±0.23	5.99±0.37
Gly	3.89±0.17	4.55±0.37	4.60±0.70	4.20±0.02	3.87±0.27
Asp	9.09±0.20	8.62±0.39	8.76±0.72	8.68±0.23	9.13±0.24
Glu	14.23±0.27	14.36±0.71	14.05±0.72	13.96±0.08	14.89±0.83
Tyr	2.82±0.07	2.79±0.24	2.74±0.22	2.67±0.08	3.02±0.26
Als	5.19±0.05	4.93±0.09	5.04±0.15	5.04±0.25	5.11±0.83
Pro	2.98±0.07	3.44±0.18	3.42±0.36	3.10±0.06	3.12±0.26
Total	47.35	47.74	47.57	46.55	33.86

[ab]-Means and standard deviations within the same column with no common superscript are significantly different at ($P<0.05$

Remarkably, all the amino acids tested were higher than those recommended by WHO/FAO for adults as well as for children(FAO/WHO/UNU expert consultation (WHO Technical Report), 2007). The values of Tryptophan and Cysteine were not quantified in this work. The values reported for Methionine (at least 6.77 g/100g), and Lysine (at least 7.76 g/100g) which are limiting amino acids in cereals and legumes were very high in chicken. This supports the assertion that chickens are a good source of high-quality protein. It alsoconfirms that Kenyan indigenous chickensreared under free range are equally a good source of the same. High amounts of Lysine, Aspartic acid and Glutamic acid were found in all the three ecotypes. The same trend was observed for the breasts and thighs. This result is in line with other findings of Wattanachant *et al*. (2004) and Aronal *et al*. (2012b). These researchers reported high values for these three amino acids. They also reported very high values of leucine in the breast and thighs of chicken, a result which is supported by our findings. Protein quality is closely related to the efficiency of the protein in human digestive system. The protein digestion efficiency further plays an important role in determining the level of protein required for consumption or type of protein source required to satisfy the nutritional requirements of an individual (Aronal *et al*., 2012b). This information is vital for the choice of the quantity of a given food or food source that is required for digestion. Clearly, IC meat does not only supply adequate protein but also high-quality protein that supplies in adequate amounts all the essentials amino acids.

5.3.5 Emulsion and Foaming Capacity and Stability

Emulsion capacity and emulsion stability of protein isolates from the breasts and thighs of the Kenyan IC ecotypes are presented in Table (5.3). Only the Kakamega ecotype had a significantly different ($P<0.05$) emulsion capacity. This difference is also expressed in the stability of the emulsion. The Naivasha ecotypes' emulsion capacity is not significantly different between the breast and the thigh protein isolates. Though there is a significant difference in the stability of the emulsions for the Naivasha ecotype, there were no significant differences on the emulsion capacity and emulsion stability of protein isolates for the Taita ecotype. The stability of the emulsions is dependent on the ease of solubility of proteins and this is a function of the protein hydrophobicity.

Table 5.3: Emulsion, Foaming, capacity and stability of Kenyan Indigenous Chicken ecotypes

Ecotype	Chicken Part	Emulsion Capacity (ml oil/g protein)	Emulsion Stability (ml oil/g protein)
Kakamega	Breast	22.43±1.38^{b}	41.13±8.87^{a}
	Thigh	30.16±0.61^{a}	41.65±3.81^{b}
Naivasha	Breast	31.07±0.51^{a}	40.23±4.93^{b}
	Thigh	28.75±0.98^{a}	44.31±9.02^{a}
Taita	Breast	28.04±0.54^{a}	28.13±3.13^{c}
	Thigh	32.11±2.11^{a}	25.54±3.04^{c}

5.3.6 Foaming Capacity and Stability

Foaming capacity was significantly different (higher in thigh muscle) for protein isolates from the Kakamega ecotype (P<0.05). The foaming stability followed this trend as well (Table 5.4). With regard to Naivasha ecotype, the breast recorded higher value of foaming capacity than that of the thigh. However, the stability of the foam was not different. Taita ecotype on the other hand, recorded higher foaming capacity on the thigh than on the breast and the stability of the foam followed the same trend.

Table 5.4: Foaming Capacity and Stability of Kenyan Indigenous Chicken ecotypes

Ecotype	Part of chicken	Foaming capacity (volume of air: volume of protein isolates)	Foaming stability (volume of air to volume of protein isolates)
Kakamega	Breast	82.51±0.00^{bc}	59.70±7.60^{c}
	Thigh	86.31±11.41^{b}	78.71±11.41^{a}
Naivasha	Breast	93.92±3.80^{a}	45.25±8.37^{d}
	Thigh	67.30±0.00^{c}	44.49±7.60^{d}
Taita	Breast	74.90±0.00^{c}	67.30±0.00^{b}
	Thigh	86.31±3.80^{b}	48.29±3.80^{d}

^{ab}-Means and standard deviations within thesame column with no common superscript are significantly different at (P<0.05)

Foam is flexible, cohesive film that entraps air bubbles. Compared to foams from milk and eggs, meat based foam products exist in different parts of the world (Omana *et al*., 2010). The current study suggests the difference in foaming capacity among the ecotypes and the type of muscle to depend on both factors. The results show a positive correlation between the protein solubility and the foam capacity as was the case of Kakamega ecotype (Table 5.4). The foaming stability however is not found to correlate with increasing hydrophobicity which is pH dependent. The reason for this was suggested as the improper balance of flexibility and higher rigidity of proteins at the air-water bubble interface (Kinsella, 1979).

5.3.7 Protein Digestibility and Solubility

The digestibility of the protein isolates shows a significant difference ($P<0.05$) between the thigh and the breast muscles for Taita and Naivasha ecotypes (higher digestibility of thigh protein isolates). For Kakamega ecotype, the breasts had a higher digestibility. With regard to solubility, only the Kakamega ecotype showed a difference between the breast and the thigh. The solubility of breast protein isolates was 60.9 % compared to 43.8 % for the thigh protein isolates (Table 5.5). Naivasha and Taita ecotypes had no significant difference with regard to protein isolates' solubility for the breast and the thigh. The protein solubility has been shown to correlate with process properties such as WHC, represented by cooking loss, drip loss or moisture uptake (Warner *et al*., 1997). In the current studies cooking loss was determined and results presented in Table 5.5.

Table 5.5: Protein Digestibility and Solubility

Ecotype	Chicken Part	Protein digestibility (%)	Solubility (%)
Kakamega	Breast	63.50 ± 4.50^{b}	60.90 ± 2.08^{b}
	Thigh	60.00 ± 6.10^{b}	43.84 ± 6.89^{c}
Naivasha	Breast	66.00 ± 6.10^{b}	65.88 ± 0.63^{a}
	Thigh	75.50 ± 2.50^{a}	66.94 ± 0.06^{a}
Taita	Breast	63.90 ± 3.50^{b}	67.32 ± 0.02^{a}
	Thigh	75.50 ± 0.50^{a}	67.22 ± 0.06^{a}

[ab]-Means and standard deviations within the same column with no common superscript are significantly different at ($P<0.05$)

Sun and Holley (2010), demonstrated that protein solubility also relates to the texture. In another related study, Omana and others demonstrated the foam capacity of proteins have an optimal pH of about 11.5 from whence an increase in pH results in a reduced foam expansion (Omana *et al*., 2010). All the extraction of current isolates were done at a pH around neutral where protein solubility is optimal and hence the differences observed in foaming capacity can be ascribed to differences in part of chicken muscle as well as differences in ecotypes.

Protein solubility is also a factor that determines the quality of meat and is in particular an indicator of different meat defects such as PSE, and Dark, firm and dry (Warner *et al*., 1997). Protein solubility is a key index of functionality as a direct demonstration of the denaturation and aggregation for proteins. Whereas Tang *et al*. (2006) suggested that changes in conformation of proteins could be due to formation of soluble protein aggregates (Xiong *et al*., 2000),on the other hand, suggested that the increasing exposure of internal hydrophilic sites could be responsible. The higher solubility observed on the breasts for the Kakamega and Taita ecotypes could precisely be due to this very reason. As supported by digestibility and texture results, the breasts muscles were better broken down and therefore isolates exposed more hydrophilic sites responsible for the increased solubility. Rasale and Das (2015) suggested that cavitation including shear and turbulence induced when they used pulsed ultra sound could disrupt the hydrophobic interactions hence molecular association of protein aggregates thus improving solubility. In our case, the disruptive force was provided by the homogeniser blender and the disruption must have been greatest in breast muscle than on the thigh muscle.

5.3.8 Colour of Ecotypes and Part of Indigenous Chicken

Significant differences were observed on all the ecotypes with regard to lightness (L*), redness-greenness (a*), and yellowness-blueness (b*) values as well as the chrome and the hue values ($P<0.05$) (Table, 5.6). Higher values of the L* were reported in all the ecotypes for the breasts. The reverse was true with regard to the a* values which were lower in the breasts than in the thighs ($P<0.05$). The values of b* were higher on the thighs than on the breasts for all the ecotypes (Table, 5.6).

Table 5.6: Colour of the breast and thigh portions

Ecotype	Chicken Part	L*	a*	b*	Chrome	Hue
Kakamega	Breast	82.74±0.50[a]	2.33±0.05[b]	17.31±0.08[b]	40.27±0.97[c]	0.46±0.18[a]
	Thigh	70.60±0.52[b]	5.19±0.17[a]	21.07±0.36[a]	109.52±5.31[a]	0.77±0.10[a]
Naivasha	Breast	80.50±0.63[a]	2.57±0.07[b]	15.21±0.19[c]	39.06±1.57[c]	-3.31±1.09[a]
	Thigh	65.89±0.51[c]	5.81±0.04[a]	18.13±1.66[b]	105.49±10.28[a]	2.17±1.89[a]
Taita	Breast	82.48±0.15[a]	1.87±0.07[c]	13.66±0.20[c]	25.61±1.33[d]	0.68±0.27[a]
	Thigh	74.35±0.47[b]	3.16±0.17[b]	17.14±0.33[b]	54.23±3.43[b]	-1.23±0.67[a]

Data expressed as mean ±SD n=18

[ab]-Means within the same column with no common superscript are significantly different at ($P<0.05$)

To this extent the results demonstrate that colour is a differential of the thighs and breast muscles of chicken meat. This supports the finding reported that colour provides one of the most important factors considered by consumers in making choices of meat for consumption. This trend is similar to the results obtained by Chumngoen and Tan (2015a). Among the ecotypes, the Kakamega recorded highest value for L* while Naivasha showed the lowest value of L* and hence highest Hue. Meat colour has been determined to depend on the species, diet, age, type of muscle, and exercise the animal is exposed to. In some cases, handling pre and post slaughter also affects the colour. The distinction observed in colour difference in this study between muscles of breast and thigh of the same chicken confirms this assertion. At the same time the ecotype differences has also determines the colour difference between chicken even of the same muscle. Chumngoen and Tan (2015b) also observed a difference in the colour of the native Taiwan chicken and those of commercial broiler, even under the same feeding regime. The correlation of these results (objective/instrumental) to those of subjective analysis supports this assertion as has been previously supported by Sow and Grongnet (2010). The meat and poultry industry and even research fraternity relies on the a*/b* to establish quality of chicken. Colour affects acceptance and the purchasing decisions of consumers.

5.4.9 Cooking Loss and Texture

Highest cooking losses were observed among the thigh muscles for all the ecotypes 29.61 % (Table 5.7) at ($P<0.05$). Breast had the least cooking loss for all the ecotypes at 20.43 %. The cooking losses recorded were also significantly different with Taita, Naivasha, and Kakamega (35.32%, 30.64% and 22.86%) having the highest cooking loss respectively. The lowest cooking losses were recorded for Naivasha breast at 15.47 %. These findings show that the breasts have lowest cooking loss and hence better water holding capacity. Cooking results in denaturation of meat proteins evidenced in shrinkage in dimension and weight loss due to expression of water and melting of fat (Ikhlas *et al*., 2011; Khiari *et al*., 2014). This is one of the major sources of consumer dissatisfactions with meat products. The determination of cooking loss among these ecotypes is very crucial to the poultry industry in Kenya and its consumers. For processors, evaluation of cooking loss is a quick way to establish economic value of their goods especially where the sale is on weight basis. The ability of meat products to retain moisture is dependent on the gel network microstructure (Trout, 1988). This they do by regulating the capillary suction force thus the water holding capacity. The improvement of water holding capacity of meat products was the subject of the research by Khiari *et al*. (2014). These researchers successfully used protein isolates to achieve improved water retention in chicken patties. The presence of water is associated with less resistance. The Kakamega ecotype which showed least resistance was also observed to have relatively higher moisture content. This observation is supported by the work of Youssef and Barbut (2011). As a result, the protein isolates from Kakamega ecotype show promise for use in enhancing water retention in processed meat products such as patties.

With regard to texture, the thigh muscles recorded significantly higher values than the breasts (Tables 5.7). Among the ecotypes, Naivasha had the highest value of hardness followed by Taita and Kakamega ecotype. A positive correlation was observed between the cooking loss and the hardness of the chicken parts and ecotypes, (Tables5.7, 5.8 and 5.9). This finding confirms the results of Khiari *et al*. (2014). Compared to the protein content of these meats, the breasts registered higher protein content than the thighs. According to Youssef and Barbut (2011), this would suggest that the breasts ought to give higher values for texture; this was however not validated in our study, even though such a correlation was derived for minced meats (Omana *et al*., 2010).

Table 5.7: Cooking loss and hardness of breast and thigh portions

Chicken Part	Cooking loss (%)	Hardiness- force
Breast	20.43±2.06[b]	50.48±7.33[b]
Thigh	29.61±2.37[a]	75.27±7.26[a]

Data expressed as mean ±SD n=36

[ab]-Means within the same column with no common superscript are significantly different at (P<0.05).

The relationship between cooking loss and texture of the different ecotype clusters of IC are reported in Table 5.8. Taita had highest cooking loss (30.77%) while Naivasha reported the highest hardness (75.56%) with regard to texture parameter.

Table 5.8: Cooking loss and hardness/texture of the Kenyan indigenous chicken ecotypes

Ecotype	Cooking loss (%)	Hardness- force-newton
Kakamega	21.24±1.11[b]	48.67±8.32[a]
Naivasha	23.06±4.48[b]	75.56±10.22[a]
Taita	30.77±2.72[a]	64.51±9.03[a]

Data expressed as mean ±SD n=36

[ab]-Means within the same column with no common superscript are significantly different at (P<0.05).

The relationship between cooking loss and texture of the different body parts of ecotype clusters of IC are reported in Table 5.9. Taita breast had highest cooking loss (35.32%) while Taita thigh reported the highest hardness with regard to texture parameter (85.26%).

Table 5.9: Relationship of cooking loss and texture (hardness) for IC ecotype

Ecotype	Chicken Part	Cooking loss (%)	Hardness-(force-Newton)
Kakamega	Thigh	19.60±1.12[e]	62.37±13.46[a]
	Breast	22.87±0.91[d]	34.96±6.94[a]
Naivasha	Thigh	15.48±1.04[e]	78.19±13.09[a]
	Breast	30.65±2.06[b]	72.92±16.24[a]
Taita	Thigh	26.22±1.65[c]	85.26±10.39[a]
	Breast	35.32±0.32[a]	43.56±8.78[a]

Data expressed as mean ±SD n=36

[ab]-Means within the same column with no common superscript are significantly different at ($P<0.05$).

5.5 Conclusion

The expression of proteins from the three Kenyan IC ecotypes showed distinct differences between myofibrillar and sarcoplasmic proteins based on SDS PAGE but very small differences among the ecotypes and their body parts. With regard to functionality, there are differences in emulsification, digestibility, and foaming capacity among the three ecotypes and also on the different parts. Texture of the chicken meat from the ecotypes was found to be inversely related to the cooking losses. This study concludes that the functionality of protein isolates from indigenous chicken in Kenya depends on several factors including the part of the body, the ecotype cluster and quantity of the total protein content.Further investigation on the functionality of the studied protein isolates for indigenous chicken reared under intensive system of farming for complete comparison with the IC reared under free range is recommended.

CHAPTER SIX
DESCRIPTIVE SENSORY QUALITY OF KENYA'S INDIGENOUS CHICKEN MEAT FROM DIFFERENT ECOTYPE- CLUSTERS REARED UNDER AN INTENSIVE SYSTEM

Abstract

Indigenous chicken (IC) in Kenya performs a major food security and socio-economic function for most households, especially of the rural poor. The trend has been to move from rearing IC on free-range systems, to more intensive and semi-intensive systems. This study was conducted by use of Quantitative Descriptive Analysis (QDA) and the Just About Right (JAR) scale scores to quantify the appeal of the IC meat reared under intensive systems. The IC used in the study had been obtained from Taita, Kakamega and Narok ecotype clusters kept under the intensive system at Indigenous Chicken Improvement Programme (InCIP) unit at Egerton University. During the intensive rearing, the chickens were given the same treatment in terms of feed, disease control at all the stages and water was given *ad-libitum*. The chickens were slaughtered at the same age and only cocks were used to control for sensorial differences accruing due to sex. Five cocks from each ecotype cluster were slaughtered after a feed withdrawal period of 8-10 hours and their meat prepared by boiling for sensory evaluation after ageing on ice for 3-6 hours. A trained panel of tasters 13 was used to evaluate the descriptive and JAR sensorial quality of indigenous chickens' meat from the breast and thighs. One commercial broiler (Kenbro) was used as a control. Results showed that there was significant effect at ($P<0.05$) of the ecotype of the IC on meat aroma, flavour and brown colour intensity. The JAR scale showed that the consumers' scores for the colour, flavour, juiciness, tenderness of indigenous chicken was 'just about right' compared to broiler which was described by colour as too light, flavour as too strong, too juicy in terms of expression of juiciness. With regard to texture, broiler meat was describes asbeing rather too tender. The Principal Component Analysis results showed that there were two principal components (colour and texture) that accounted for 55.4 % and 11.6% and 53.9 and 19% for both descriptive scores and JAR scores for IC meat respectively. This study indicates sensorial differences among the Kenyan indigenous chicken ecotypes (of different genetic characteristics) under intensive systems and demonstrates significant difference among various attributes from the commercial broiler.

Key words: *Descriptive, sensory evaluation, Kenyan Indigenous chicken, ecotype cluster, intensive system*

6.1 Introduction

In Kenya, the first most comprehensive genetic evaluation of genetic material of indigenous chicken was conducted through the InCIP programme of Egerton University. It used microsatellite markers to analyse genetic variability among the Kenyan indigenous chicken from all over the country. The result was that the Kenyan indigenous chicken could be classified into Coastal group represented by Taita, Western Kenya Group represented by Kakamega and Rift valley group represented by Narok, Naivasha ecotype clusters (Ngeno, 2014). Livestock producers are in a consensus that to be able to improve on productivity and quantity of indigenous chicken, it will be paramount to introduce them to a mix of intensive and semi-intensive systems (Chaiban *et al*., 2020). Descriptive sensory evaluations are vital in sensorial studies as they often bring almost quantitative results comparable to objective methods. This is because QDA makes use of well-trained panellists The Just about right (JAR) are useful in predicting and explaining consumer acceptance.The results provide useful information in supplementing for product optimisation (Jahnke, 2005). Using both in a sensory study usually gives a more complete assessment and better explanation to the responses obtained from the panellists (Jahnke, 2005).

In spite of these marked advantages of IC in Kenya, there is not yet a documented systematic study that has attempted to validate this perception and enumerate reasons responsible for the desirable qualities of indigenous chicken over the exotic broilers. Though there is a great genetic variation among indigenous chicken in Kenya, limited studies have been done to determine whether this genetic variation affects the sensory quality of indigenous chicken particularly those growing under controlled environment (intensive systems). The increasing consumer demands and increasing intensification of the indigenous chicken rearing, calls for a sensory evaluation for consumer acceptability on sensorial quality that consumers feel are most important. Therefore, the aim of this study was to determine the sensory appeal of the IC from different cluster ecotypes in Kenya reared under the intensive system. The results inform on the effect of genetic variability brought about by cluster ecotypes on the sensory appeal of the IC meat to the consumers.

6.2 Materials and Methods

6.2.1 Sample Preparation and Determination of Sensory Quality of IC Meat

Indigenous chicken from the 3 cluster ecotypes (Kakamega, Taita and Naivasha) were reared under intensive units at InCIP Egerton University, Kenya. Five healthy cocks from each ecotype cluster were selected for conducting of sensory evaluation. The birds were slaughtered after observing a feed withdrawal period of 8-10 hours, before slaughter. After slaughter the carcass was thoroughly bled; then followed by scalding at 60 ^{o}C for two minutes. The carcasses were then chilled at 12 ^{o}C for 30 minutes and aged on ice for 1>2.5 hours before deboning. The cooked samples of the left drum sticks from the indigenous and exotic chicken were presented to screened and trained panellists for descriptive sensory evaluation. The descriptive sensory analysis was conducted on the breast and thigh meats at the sensory evaluation room (Dairy and Food Science Department, Egerton University). The boiled meat was prepared by first deboning the meat and cutting into small pieces approximately of 2 x 2 cm. Meat from each carcass was cooked individually. The IC meat pieces were put into the cooking pot and water added to cover the meat. Cooking was done for 45 min-60 minutes. A sample of broiler meat prepared the same way was presented as a control. Salt was then added to taste. After tempering at room temperature for about 10 minutes, samples were presented for descriptive sensory analysis. Samples were randomised by product type (ecotype cluster) and then by meat type (breast meat or thigh). Each panellist was presented with 6 pieces on white sensory evaluation plates labelled with 3-digit blinding codes.

6.2.2 Screening of Panellists and Training

A trained meat descriptive panel of (13) members as recommended by sensory spectrum Inc., Chatham, NJ conducted quantitative descriptive analysis (DQA). Written and in a few cases, verbal informed consent was sought before admitting the panellists. The QDA method as described by Stone *et al*. (2004), was used followed for the descriptive test. The panel was trained according to the ISO procedures (ISO, 2012). In the pre-screening testing, the assessors were trained in developing sensory descriptors and the definition of the sensory attributes. They developed and agreed on vocabulary, words, and intensities. The panel was screened through affective tests and acuity test on relevant sensory attributes. An orientation was done to familiarise the tasters with the colour, flavour, and texture definitions of the IC meat sample. The panel reduced from a list of about 20 attributes, into 9 on a hedonic scale as shown in Table 6.1.

Table 6.1: Descriptive of the 9-point hedonic scale used for quantitative descriptive sensory evaluation

	Attribute	Subjective Ranking
1	Aroma intensity	(1 =Extremely bland to 8 = Extremely intense)
2	Initial impression of juiciness (moisture release)	(1 = Extremely dry to 8 = Extremely juicy)
3	First bite (initial hardness)	(1 = Extremely tough to 8 = Extremely tender)
4	Cohesiveness of mass	(1=Extremely loose to 8=Extremely compact)
5	Sustained impression of juiciness	(1 = Extremely dry to 8 = Extremely juicy)
6	Muscle fibre and overall tenderness (chewiness)	(1 = Extremely tough, to 8 = Extremely tender
7	Amount of connective tissue (fibrousness)	(1= Extremely abundant to 8 = none
8	Overall chicken flavour intensity	(1= Extremely bland to 8 = extremely intense)
9	Brown colour intensity	(1= None to 8 = Extremely intense)

The trained panel used descriptive textural attributes to evaluate tenderness characteristics of breast and thigh meat. Initial hardness, cohesiveness, and moisture release was evaluated in the first bite stage, whereas hardness of mass, cohesiveness of mass, fibrousness, and number of chews to swallow were evaluated in the chew down stage. Just-About-Right (JAR) scales were used to assess the appropriateness of colour (1 = much too light, 2 = too light, 3 = just about right, 4 = too dark, 5 = much too dark), the appropriateness of tenderness (1 = much too tough, 5 = much too tender), the appropriateness of juiciness (1 = much too dry, 5 = much too juicy), and the appropriateness of flavour (1 = much too weak, 5 = much too strong). The JAR scales were used because they were useful for diagnostics, while hedonic scales do not allow determination of the appropriateness of intensity of the attribute (SAS, 2012).

6.2.3 Statistical Analysis

Data from the hedonic scales and JAR scales was analysed using SAS version 9.1.For the descriptive statistics, Analysis of Variance, correlation, and principal component analysis (PCA) were conducted. Means were separated using Duncan's Multiple Range Test (DMRT) in a nested experimental design procedure at P<0.05. The PCA data was analysed using the PRINCOMP procedures of the SAS standardised data to zero and unit variance (Allen *et al*., 1998).

6.3 Results and Discussion

6.3.1 Descriptive Quality Analysis

The chickens' breasts scored higher in aroma, toughness at first bite, and overall tenderness as shown in Figure 6.1. On the other hand, thighs scored highest in initial impression of juiciness, cohesiveness of mass, sustained expression of juiciness, tissue fibrousness, flavour intensity and brown colour intensity. However, these differences were not significant at P≤0.05.

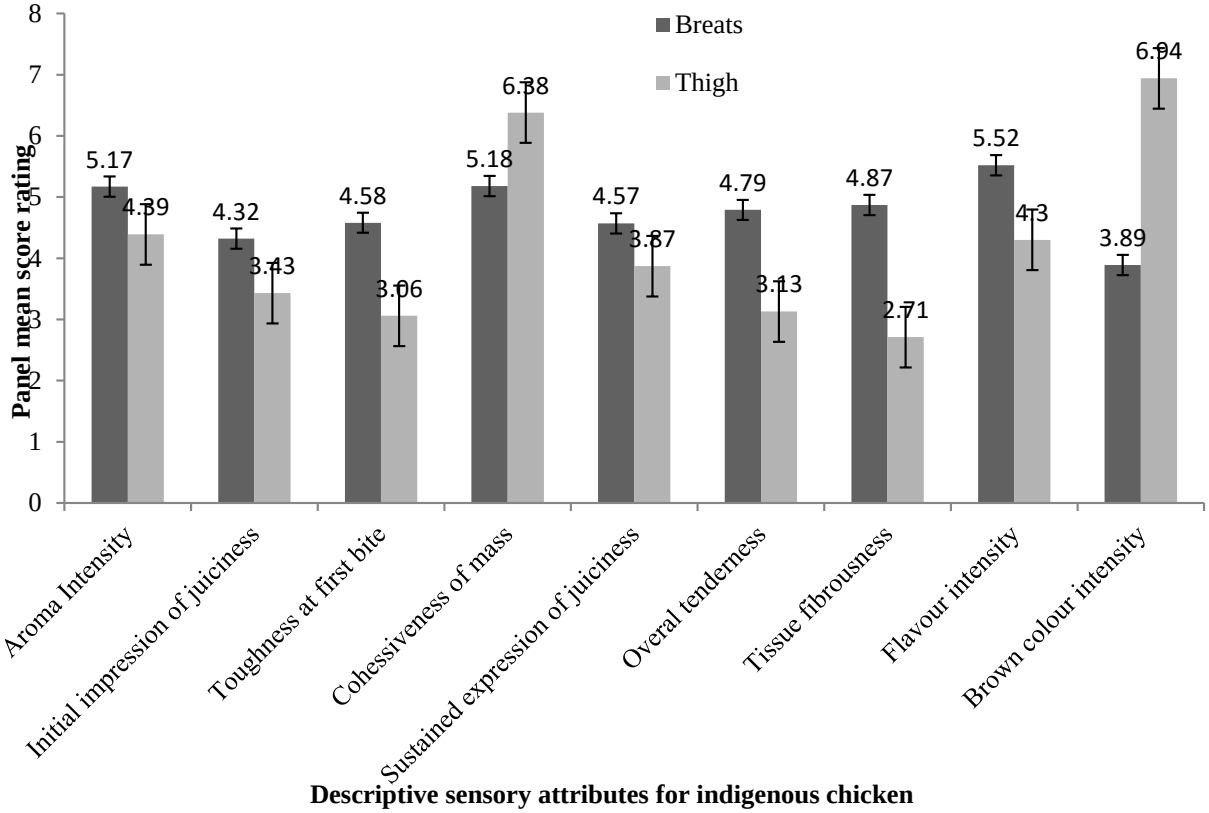


Figure 6.1: The mean descriptive analysis results from breast and thigh of broiler.

With regard to indigenous chicken; very significant differences were noted between the sensory attributes from the thighs and the breast meats. Figure 6.2 presents overall descriptive sensory scores for all the three different types of IC ecotypes. The breasts scored higher for aroma, initial impression of juiciness, toughness at first bite, sustained expression of juiciness, overall tenderness, tissue fibrousness, and flavour intensity (Figure 6.2). The thigh meat scored highest for cohesiveness of mass, and the brown colour intensity.

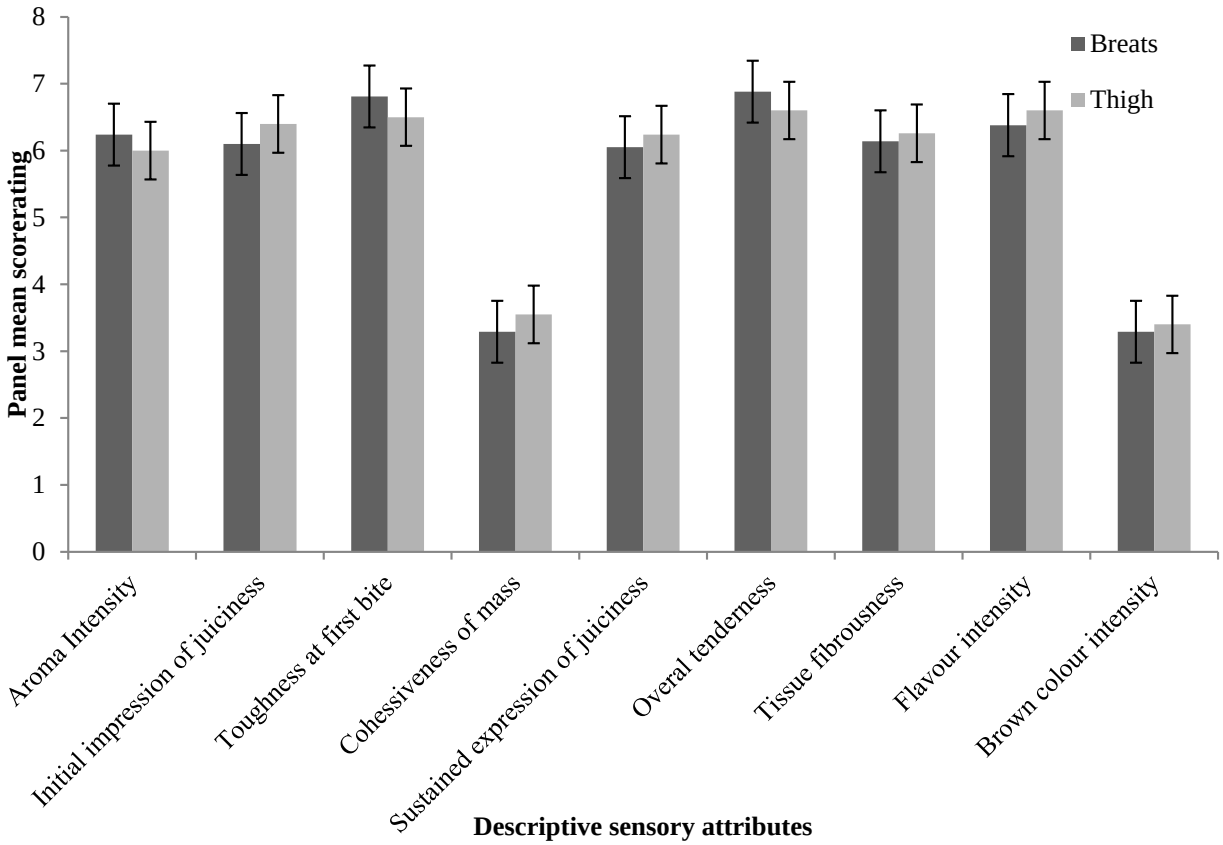


Figure 6.2:The mean descriptive analysis results of the different attributes from breast and drum sticks (thigh) of IC meat

The JAR attributes for broiler chicken breasts and thighs are given in Figure 6.3. The values for Just About Right (JAR) scores for these varied significantly from the expected figure of 3.0. Colour attribute scored the weakest at 2.12 and 2.44 for breasts and thighs respectively. The rest of the attributes; tenderness (3.85, 3.8), juiciness (3.46, 3.49) and flavour (3.49 and 3.29) for breast and thighs respectively scored significantly above the JAR scale of 3.0, shown in Figure 6.3.

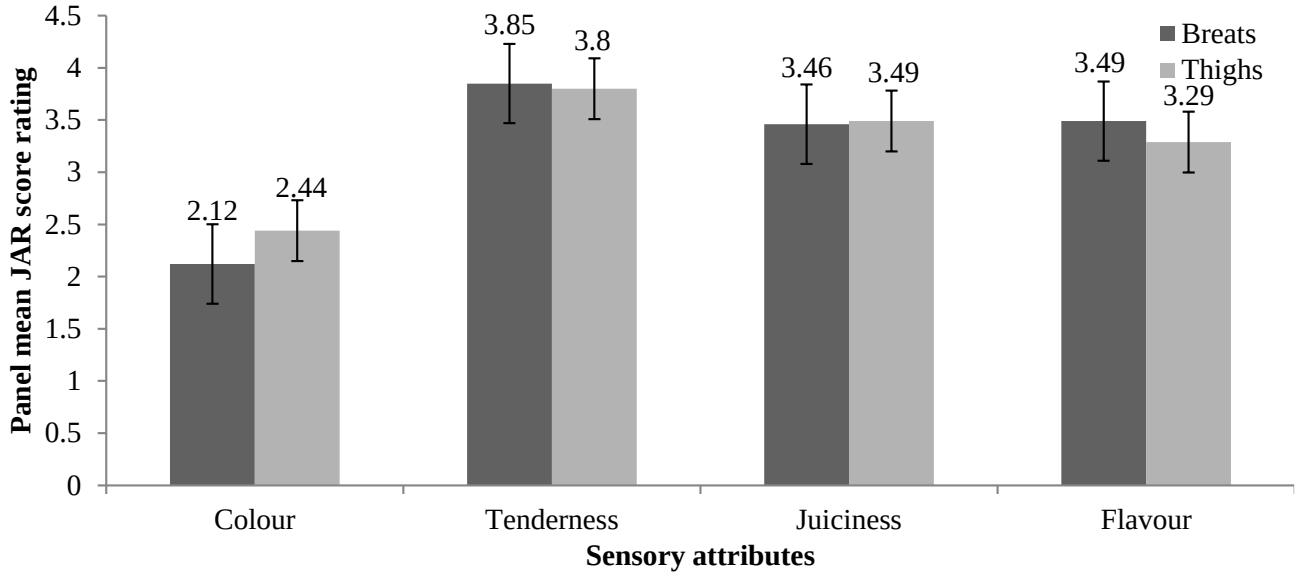


Figure 6.3:The JAR scores for colour, tenderness, juiciness, and flavour attributes for broiler chicken breasts and thighs

Legend

Appropriateness of colour (1 = much too light, 2 = too light, 3 = just about right, 4 = too dark, 5 = much too dark), Appropriateness of tenderness (1 = much too tough, 2=too tough, 3 = just about right, 4=too tough, 5 = much too tender), Appropriateness of juiciness (1 = much too dry, 2=too dry, 3 = just about right, 4=too juicy, 5 = much too juicy)Appropriateness of flavour (1 = much too weak, 2=too weak, 3 = just about right, 4=too strong, 5 = much too strong).

The JAR scores for the IC chicken breast and thighs are given in Figure 6.4; the breast colour (2.59) was lower significantly compared to JAR of 3.00 while the thigh colour (4.11) was significantly more intense than the JAR score of 3.00. With regard to tenderness, both breast and thigh scores of 2.73 and 2.05 respectively, were significantly lower than the just about right score of 3.00. This indicates that the scores tended toward too tough. The juiciness score for both breast and thigh was scored at 2.72 and 2.29 respectively, these were significantly lower than the JAR score of 3.00 and indicating that the meat was tending toward too dry. Finally, the flavour score for IC chicken breast was at the JAR position. This underscores a very fundamental issue why most consumers prefer indigenous chicken. The thighs flavour was at 2.45 only slightly below the JAR scale. This means the flavour was recorded as weak.

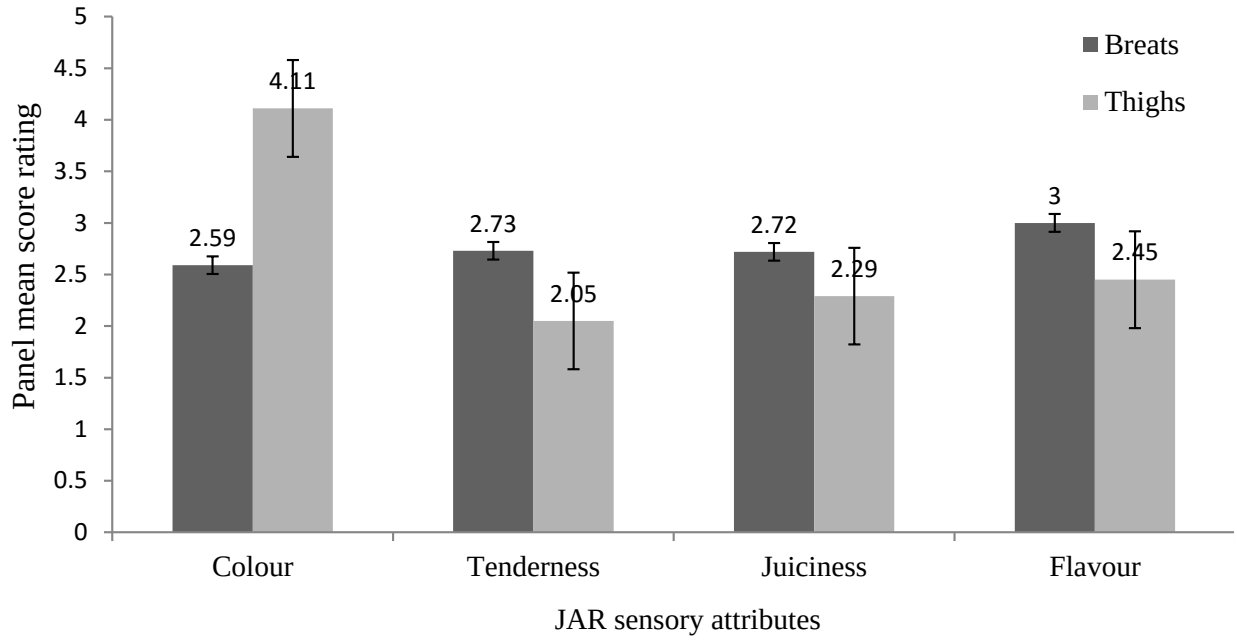


Figure 6.4: The JAR scores for colour, tenderness, juiciness and flavour attributes for IC chicken breasts and thighs

Appropriateness of tenderness (1 = much too tough, 2 = too tough, 3 = just about right, 4=too tough, 5 = much too tender), Appropriateness of juiciness (1 = much too dry, 2=too dry, 3 = just about right, 4 = too juicy, 5 = much too juicy) Appropriateness of flavour (1 = much too weak, 2 = too weak, 3 = just about right, 4 = too strong, 5 = much too strong).

ANOVA of mean scores for the sensory attributes on a 9-point hedonic scale for the broiler and the 3 IC ecotypes are reported in Table 6.2.

Table 6.2: Comparison of the Sensory attributes of thighs and breasts of the broiler and IC ecotypes

Part	Variable	Ecotype			
		Broiler (n=42)	Naivasha (n=30)	Taita (n=28)	Kakamega (n=28)
Breast (part 1)	Aroma	6.24±1.48^{a}	4.70±1.78^{b}	5.42±1.58^{b}	5.43±1.62^{b}
	Juiciness	6.10±1.51^{a}	4.30±1.18^{b}	4.19±1.41^{b}	4.46±1.79^{b}
	First bite	6.81±1.23^{a}	4.23±1.61^{b}	4.81±1.30^{b}	4.75±1.82^{b}
	Cohesiveness	3.29±1.83^{b}	5.23±1.55^{a}	5.04±1.43^{a}	5.25±1.35^{a}
	Impression	6.05±1.45^{a}	4.50±1.50^{b}	4.62±1.60^{b}	4.61±1.31^{b}
	Tenderness	6.88±1.17^{a}	4.83±1.53^{b}	4.65±1.35^{b}	4.86±1.78^{b}
	Fibrousness	6.14±2.03^{a}	4.93±2.18^{b}	5.27±1.82^{b}	4.43±2.03^{b}
	Flavour	6.38±1.48^{a}	5.00±1.64^{b}	5.77±1.37^{ab}	5.86±1.53^{ab}
	Colour	3.29±2.10^{a}	3.43±1.45^{a}	4.12±1.24^{a}	4.18±1.66^{a}
Thigh (part 2)	Aroma	6.00±1.67^{a}	3.43±1.74^{c}	5.00±1.62^{b}	4.86±1.74^{b}
	Juiciness	6.40±1.42^{a}	2.93±1.48^{c}	4.19±1.74^{b}	3.25±1.73^{b}
	First bite	6.50±1.57^{a}	2.53±1.78^{b}	3.35±1.65^{b}	3.36±2.04^{b}
	Cohesiveness	3.55±1.73^{b}	6.77±1.25^{a}	6.12±1.31^{a}	6.21±1.23^{a}
	Impression	6.24±1.54^{a}	2.93±1.68^{c}	4.85±1.74^{b}	3.96±1.53^{b}
	Tenderness	6.60±1.19^{a}	2.90±1.92^{b}	3.42±1.55^{b}	3.11±1.62^{b}
	Fibrousness	6.26±1.55^{a}	2.37±1.79^{b}	2.85±1.59^{b}	2.96±1.86^{b}
	Flavour	6.60±1.06^{a}	3.50±1.80^{b}	4.85±1.67^{b}	4.64±1.70^{b}
	Colour	3.40±1.81^{b}	7.03±0.72^{a}	7.04±1.18^{a}	6.75±1.38^{a}

Means with same letter are not significantly different at P<0.05. Means separated by Duncans' Multiple Range test (DMRT).

The comparison between the sensory attribute (aroma) on the breasts show a significant difference from the broiler and the 3 different ecotype clusters (Naivasha, Taita and Kakamega). The same case is noted for juiciness, first impression of hardness, tenderness, and fibrousness with regard to the breasts.The broiler had the highest score from the 3 IC ecotypes. On the cohesiveness of mass, the broiler breast scored lowest significantly than for all the 3 IC ecotypes. The value for cohesiveness of mass among the 3 IC ecotypes was however not different significantly at p>0.05. The scores for breast colour between the broiler and the 3 IC ecotypes was significantly different. The comparison of the same attributes from the thighs of

the broiler and the 3 IC ecotypes are also presented in Table 6.2. The broiler thighs scored for aroma was significantly higher than for that of the 3 IC ecotypes. Among the IC ecotypes, the Taita and Kakamega had highest scores for aroma. The same trend was observed on the attribute of sensory juiciness. However, for the attribute on first bite, the Naivasha ecotype had lowest score than the other two ecotypes and the broiler. Only the score on broiler was significantly different from Naivasha ecotype for this attribute (first bite).

The broiler thigh had the lowest score for the attribute; cohesiveness of mass compared to the 3 IC ecotypes. Among the IC ecotypes, the Naivasha variety had the highest score for cohesiveness of mass but this was not significant from the scores of other IC chicken. With regard to tenderness, fibrousness, and flavour attributes the broiler had a significantly higher score than the values recorded for the 3 IC ecotypes. Finally, on the colour attributes, the broiler thigh had the lowest score than the scores recorded for colour from the thighs of all the IC ecotypes in the study.

6.3.2 JAR Rating

The Just About Right (JAR) scale scores for the breasts and thighs with regard to attributes (colour, tenderness, juiciness and flavour) for the broiler and the 3 IC ecotypes are given in Table 6.3; part one and two respectively. The broiler breast had the lowest JAR score on colour of 2.12 (too light) while Taita ecotype had the highest JAR score of 2.88 closest to the reference JAR score of 3.00, on the attribute of tenderness, the broiler had the highest score at 3.85 toward too tender, while the Narok ecotype had the highest score for the same attribute among the 3 IC ecotypes at JAR score of 2.85. This was closest to JAR reference of 3.00. The broiler JAR score for juiciness attribute was highest overall at 3.46 tending toward too juicy, while the highest score for juiciness among the IC was for Kakamega at the 2.93 which was closest to the JAR reference of 3.00. The Narok ecotype JAR score for flavour was lowest overall at 2.46 tending toward too weak. This was significantly different from the JAR score for broiler which was highest at 3.49 ($P<0.05$). The Taita and Kakamega ecotypes had JAR scores of 3.15 and 3.36 for the same attribute. These two ecotypes had the closest score to the JAR reference score of 3.00 for the flavour attribute.

Part two of the same (Table 6.2) presents the JAR scores for the thighs of the chicken samples with regard to attributes (colour, tenderness, juiciness, and flavour). The JAR score for broiler thigh on colour attribute was the lowest at 2.44 (almost too light), this was significantly different from the scores from the 3 IC ecotypes. The Naivasha ecotype had the highest score among the IC ecotypes with a JAR score of 3.96 (tending towards too dark). The JAR crores on tenderness were significantly different between the broiler at 3.80 (almost too

tender) and the 3 IC ecotypes. Taita ecotype had lowest JAR score of 1.96 (almost too tough). With regard to juiciness, there was a significant difference between the JAR for the broiler at 3.49 (toward too juicy), and the 3 IC ecotypes. The Narok ecotype had the lowest score on juiciness among the three IC ecotypes at 2.04 which was too dry. However, among the 3 IC ecotypes, the JAR scores for this attribute for the thigh meat was not significant. The JAR scores for flavour, for the broiler, Taita and Kakamega ecotypes were not significantly different. Narok ecotype had the lowest JAR score on flavour attribute at 1.96 which was toward too weak.

Table 6.3: Sensory attributes rated using Just-About-Right (JAR) scale for chickens from different ecotypes

Part	Variable	Ecotype			
		Broiler (n=42)	Narok (n=28)	Taita (n=28)	Kakamega (n=28)
Breast	Colour	2.12±0.87^{a}	2.19±0.75^{a}	2.88±0.43^{a}	2.68±0.61^{a}
	Tenderness	3.85±1.04^{a}	2.85±0.54^{b}	2.77±0.71^{b}	2.57±1.23^{b}
	Juiciness	3.46±1.00^{a}	2.81±0.49^{b}	2.38±0.57^{b}	2.93±1.25^{b}
	Flavour	3.49±0.95^{a}	2.46±0.58^{b}	3.15±0.61^{ab}	3.36±1.03^{ab}
Thigh	Colour	2.44±0.74^{b}	3.96±0.88^{a}	4.35±0.49^{a}	4.04±0.74^{a}
	Tenderness	3.80±1.05^{a}	2.11±1.10^{b}	1.96±0.66^{b}	2.07±1.05^{b}
	Juiciness	3.49±0.98^{a}	2.04±0.96^{b}	2.50±0.51^{b}	2.36±1.09^{b}
	Flavour	3.29±1.03^{a}	1.96±0.92^{b}	2.65±0.63^{ab}	2.75±1.35^{ab}

Means with same letter are not significantly different at $P<0.05$. Means separated by Duncans' Multiple Range Test (DMRT).

6.3.3 Principal Component Analysis (PCA)

Principal Components Analysis (PCA) results of the sensory attributes of the chicken meat showed that Factor I and Factor II accounted for 55.4% and 11.6% of the variation in the hedonic scale rating respectively as shown in Figure 6.5. Factor I had positive loadings for meat fibrousness, meat tenderness, initial juiciness on first bite, meat flavour, meat sustained impression of juiciness and meat aroma.

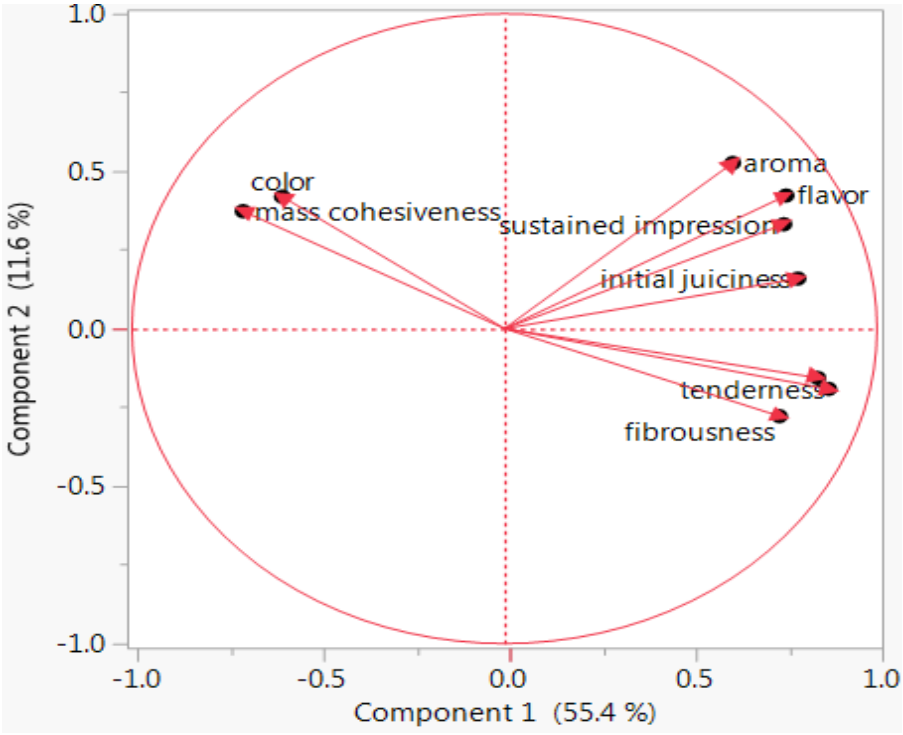


Figure 6.5: Plot of the principal component analysis showing the relationship among sensory attributes of chicken meat on hedonic rating

Factor I had negative loadings for meat colour and meat cohesiveness of mass. Factor II had positive loadings for meat aroma, meat colour, and negative loadings for meat tenderness and meat fibrousness as shown in Table 6.4.

Table 6.4: Loading matrix of the sensory analysis of the chicken meat on hedonic scale rating

	Prin1	Prin2	Prin3	Prin4	Prin5	Prin6	Prin7	Prin8	Prin9
Aroma	0.612	0.526	-0.471	0.182	-0.066	-0.165	0.186	-0.149	0.062
Initial juiciness	0.787	0.158	0.335	-0.163	-0.155	-0.309	0.136	0.269	0.065
First bite	0.840	-0.156	0.135	0.098	-0.026	0.371	0.187	-0.014	0.256
Mass cohesiveness	-0.701	0.372	0.082	-0.330	0.440	0.056	0.230	0.001	0.042
Sustained impression	0.749	0.331	0.359	-0.230	-0.041	.0130	-0.209	-0.314	-0.018
Tenderness	0.870	-0.191	0.071	0.036	0.084	0.086	0.271	-0.043	-0.332
Fibrousness	0.737	-0.277	0.037	0.245	0.489	-0.228	-0.129	-0.025	0.081
Flavour	0.755	0.422	-0.194	-0.018	0.127	0.245	-0.243	0.264	-0.080
Colour	-0.596	0.419	0.409	0.543	0.007	0.040	0.027	0.024	-0.046

Key: Prin=Principal component.

The correlation coefficients between the sensory attributes in chicken meat are presented in Table 6.5. The most important correlations were found between meat cohesiveness of mass and meat impression of juiciness at first bite (–0.6064), meat cohesiveness of mass and meat tenderness (–0.5986), meat tenderness and meat impression of juiciness at first bite (0.7690), meat tenderness and meat fibrousness (0.6686). The coefficients indicate that a high indirect dependence exists between these variables.

The PCA for sensory attributes based on the JAR score are presented in Figure 6.6. It shows that Factor I and II accounted for 53.9 % and 19 % respectively of all the variation in the JAR scores. Factor I had positive loading for tenderness, juiciness, and flavour. It had a negative loading for colour. Factor 2 had positive loading for flavour, juiciness, colour, and negative loading for tenderness.

The correlation coefficients between the sensory attributes in the JAR scale in chicken meat are presented in Table 6.6. The most important correlations were noted between colour and meat tenderness (-0.4741), tenderness and juiciness (0.5345) and flavour and juiciness (0.3795). The PCA scores of chicken meat on JAR rating is as shown in 6.6.

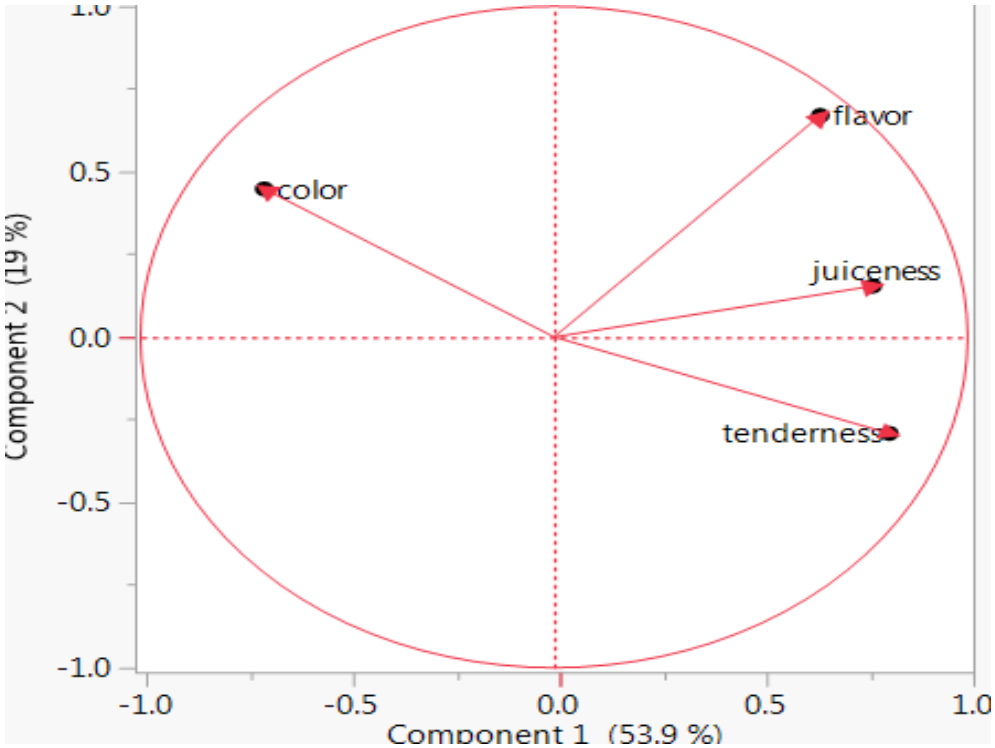


Figure 6.6: Plot of the principal component analysis showing the relationship among sensory attributes of chicken meat on JAR rating

Table 6.5 presents the correlation coefficients of the sensory attributes on the hedonic scale rating. It shows notable negative correlations between cohesiveness of mass.

Table 6.5: The correlation coefficients of the sensory attributes on the hedonic scale rating

	Aroma	Initial juiciness	First bite	Mass cohesiveness	Sustained impression	Tenderness	Fibrousness	Flavour	Colour
Aroma	1.00	0.42	0.38	-0.32	0.42	0.42	0.32	0.63	-0.24
Initial juiciness		1.00	0.59	-0.46	0.68	0.63	0.48	0.53	-0.36
First bite			1.00	-0.60	0.57	0.76	0.59	0.55	-0.45
Mass cohesiveness				1.00	-0.36	-0.59	-0.52	-0.37	0.43
Sustained impression					1.00	0.56	0.42	0.60	-0.29
Tenderness						1.00	0.66	0.54	-0.52
Fibrousness							1.00	0.45	-0.42
Flavour								1.00	-0.34
Colour									1.00

Table 6.6 shows the correlation coefficients of the sensory attributes on the JAR scale. Juiciness and tenderness had the highest positive correlation while the colour and tenderness had highest negative relationship.

Table 6.6: The correlation coefficients of the sensory attributes on the JAR scale

	Colour	Tenderness	Juiciness	Flavour
Colour	1.00	-0.47	-0.30	-0.29
Tenderness		1.00	0.53	0.29
Juiciness			1.00	0.37
Flavour				1.00

Table 6.7 shows the Loading matrix of the parameters of the JAR scale. The principal components I and 2 accounted for over 60% for tenderness, juiciness and falvour.

Table 6.7: Loading matrix of the parameters of the JAR scale

	Prin1	Prin2	Prin3	Prin4
Colour	-0.700	0.447	0.503	0.235
Tenderness	0.810	-0.291	0.235	0.450
Juiciness	0.770	0.154	0.501	-0.361
Flavour	0.643	0.669	-0.349	0.122

Sensory quality is very vital to the realisation of a consumer's food preference. It consists of qualitative, quantitative, or hedonic quality measurement. Ideal poultry meat is considered to have high nutritive value and great functional roles such as flavour, tenderness, juiciness of the cooked product among others (Santé *et al*., 2001). For chicken, the main sensory features are: colour, tenderness, juiciness and flavour (White *et al*., 1964).The current study gave descriptive hedonic quality measurements as well as JAR and PCA for the main attribute of Kenyan IC meat as described by a trained panel. The descriptive test is often very reliable and correlated to instrumental analysis (Krapoth, 1987; Lyon & Lyon, 2000; Palmer *et al*., 1965). However, the method is extensive and time consuming, and hence more time was taken by the panellists in this study to agree on the descriptive terms.

6.3.4 Flavour/Aroma

Both flavour and aroma are complex attributes of meat and are affected by; species, age, fatness, type of tissue, locality, gender, diet and method of cooking (Muchenje *et al*., 2010). Many studies have demonstrated the close relationship between the flavour preference of chicken and overall acceptance (Chepkemoi *et al*., 2017). Tables 6.2 shows significant difference in flavour and aroma attributes not just between broiler and IC chicken but also among the different IC ecotypes. This study confirms the finding of Baracho *et al*. (2006) and Shen *et al*. (2005)that flavour of chicken depends on breed, and cut of meat. Even though in their study (Guèye & Dieng, 1997), found no significant difference between broiler and IC at $p>0.05$, for the specific sensory attributes of interest , it must be borne in mind that their study was based on same age for both broiler and Ethiopian IC. They also did not find any significant in flavour among the consumers from the different parts of chicken. With increasing age however, significant differences were found between broiler and IC at $p<0.05$ as well as for breast or for thigh meats of the broiler and IC.

In the current study, the ages of the IC and the broilers were different. This may also explain the significant difference reported between the IC and broiler meats as well as from breast and thighs from the IC meat and broiler. The panellists also noted clearly that the part of the meat whether thigh or breast, has a distinct influence on flavour and aroma (Table 6.2 and 6.3). Flavour is one of the primary attributes of consumer choice for chicken; Flavour attributes are often expressed as flavour and overall flavour intensity (Moreng,& Avens, 1985). The panellists recorded higher flavour intensity for the broiler compared to the IC chicken. However, JAR responses indicated that the IC was most acceptable compared to broiler flavour which tended toward the too strong mark. Moreover, the panellists recorded a pattern of higher flavour intensity from the breast of both IC and broilers than from the thighs. This could be explained by the fact that, the thigh often performs most of chicken movement functions.The thighstherefore possess leaner meat (less fat tissue deposits) compared to the breasts. Thighs muscles are involved in more exercises (walking, running, fight) hence have deep colour, more capillaries, blood, pigment and fat (though they could be leaner) (Deatherage, 1963). Among the different ecotype clusters of indigenous chicken, the significant differences especially between Taita and the other two (Kakamega and Naivasha) ecotypes can be traced to the fact that, the Taita ecotype happens to be generally a smaller and learner IC based on carcass and dressed weights compared to the two other ecotypes. This study therefore posits that the Taita ecotype may be targeted to the consumers that prefer leaner chicken meat even though that may mean compromising on the overall chicken flavour intensity. On the other hand, the Kakamega ecotype should be a delicacy for consumers who prefer a much juicier and high flavour intensity IC meat. The majority of IC chickens are enjoyed by cooking, boiling and later frying and addition of a preferred set of spices. The current study prepared chicken by boiling and adding same amount of salt just to taste. This cancelled any flavour differences that may accrue from different methods of preparation and by use of different spices.

6.3.5 Texture Attributes

Texture is also one of the most important determinants of consumer preference for poultry meat. Many terms are used by quantitative descriptive sensory panellists to describe textural attributes of poultry meat. These terms could include; fibrousness, first bite hardness, cohesiveness of mass, and overall tenderness. Descriptive tests are commonly used in assessing attributes related to texture. Texture is described in different terms in most studies based on the consumers and their knowledge of the product. In the current study, overall tenderness, toughness of meat at first bite, tissue fibrousness, and cohesiveness of mass were terms used to

describe texture attributes of the chicken meat samples. Of these terms, tenderness is most important sensory characteristic of meat and has drawn lots of interest from researchers (Takahashi, 1996). The tenderness of meat is the sum total of the mechanical strength of skeletal muscle tissue and it's weakening during the post-mortem aging of meat. The former depends on species, breed, age, sex and individual skeletal muscle tissue of animals and fowls (Fletcher, 2002).

Meat tenderness originates in structural and biochemical properties of skeletal muscle fibres, especially myofibrils and intermediate filaments, and of the intramuscular connective tissue, (the endomysium and perimysium), which are composed of collagen fibrils and fibres. There was a significant correlation (though negative) between the cohesiveness of mass and the impression of first bite for all the samples of chicken and parts used. Many factors account for tenderness or toughness of meat. The presence of connective tissues, the nature and size of muscle fibre, method of preparation and ante-mortem and post-mortem ageing conditions all contribute. Muchenje *et al.* (2008) observed that collagen cross linking increased with age of chicken and was generally associated with toughness. The presence of abundant collagen tissue means that the initial hardness (first bite) will be significantly higher. On the other hand, due to the presence of abundant collagen material, the meat sample will tend to be more cohesive through the chewing process hence the negative correlation noted between these two variables (Table 6.5).

At the same time, the cohesiveness of mass is also negatively correlated to the tenderness. Meat tenderness has been described as a function of age, breed, and feeding regime of poultry. In the present study, all IC ecotypes were kept under the same feeding regime and were approximately same age. The difference in texture reported here may therefore be the result of the difference in their genetic makeup. The time of ageing from animal slaughter and meat consumption and other components also determine meat tenderness (Xazela *et al.*, 2011). In the present study, all the chicken meat, were treated and aged the same. Studies show that muscle size increases with the biological age and poultry meat may be tougher with age. Other factors that may affect tenderness include: fat content, muscle fibre, composition, electrical stimulation, ageing regime, cooking (Muchenje *et al.*, 2008). According to Muchenje *et al.* (2008), tender poultry meat rapidly releases juices and fewer residues remains in the mouth after chewing. This is clearly supported from the results of this study in which the tenderness is negatively correlated to cohesiveness of mass (Table 6.5).

6.3.6 Juiciness

Juiciness is another important factor for determining consumers' preferences for indigenous chicken (Latter-Dubois, 2000). Juiciness may be determined by tasting panels and described either as initial impression of juiciness, or sustained expression of juiciness. The panellists reported very significant differences in the juiciness of the different types of chicken meat tasted. There was a pattern between the initial and sustained expression of juiciness. The samples that had higher initial expression of juiciness got to retain it upon the subsequent chews to the chew down stage. An interesting observation is that the broiler thigh was found to be more juicer compared to the breasts. This was the reverse for all the indigenous chicken; in which panellists observed the breasts to be juicer. This was the case both for breasts as well as the thighs of the indigenous chicken meat tasted.

Juiciness is directly related to the intra-muscular lipids and moisture content of the meat.Broiler meat both from the thigh and the breasts were significantly juicer than meat from the IC. This confirms reports by Fanatico *et al*. (2005), who found that breast meat from slow growing chicken, were considered too dry compared to fast growing ones. Two factors may be responsible for this. The first is the higher drip losses which may be due to the large surface area of breast meat compared to the muscle size. The other factor could be the lower content of intra-muscular fat due to the tendency of the lower growing IC chicken to have leaner meat. Although Chartrin *et al*. (2006) found out that higher fat content was related tenderness; he however did not find a correlation of the tenderness to juiciness. In the present study, there is a very clear positive correlation between tenderness and juiciness. All samples that scored highest in terms of tenderness also recorded higher scores for juiciness. Fanatico *et al*. (2007a) recommended an evaluation of both initial and sustained impression of juiciness and the current study makes this investigation. The panellists' results showed a positive correlation between the initial and sustained impression of juiciness. However, with regard to JAR scale, the broiler meats were found to tend toward;too juicy. The Kakamega ecotype IC was closest to JAR score on juiciness. The Narok ecotype was found to be too dry. The JAR in this case helps put the descriptive scores into perspective by bringing out exactly how the panellists and consumers would respond to the given product.

6.3.7 Colour

Colour is one of the most used consumer attributes in making choice of purchase of poultry products. Trained panellists describe colour of chicken meat using many terms that range from cream-white to deep dark brown in descriptive analysis. In the current study the

panellists described colour in terms of brown colour intensity. Colour of poultry meat may be affected by age, sex, strain, diet, intra-muscular fat, moisture content, and pre-slaughter handling (Marcoulides & Hershberger, 2014). The colour is a function of the muscle pigments myoglobin and Haemoglobin (Hb). Colour of poultry meat also varies steadily from slaughter through the different ageing processes as a result of the changes in the chemical state of these pigments. Overall, the breast muscle discolour more than thigh meat because it not only contribute to the largest percentage of live weight, its light colour render any slight discolourations more noticeable(Northcutt, 2000). The panellists reported a pattern in colour difference between the breast meat and the thigh meat for both broiler and the IC. The breast had more intense brown colour for IC though this was not significantly different from the colour of IC breasts. But on the thigh scores, the colours of IC meat were significantly darker than the broiler. This may explain the reason for the lower score recorded for thigh meats for both the IC and the broiler compared to breasts.

The JAR scores reported colour for broiler breast meat as too light compared to the IC meat samples which were closest the JAR scale. The multivariate PCA was applied to the descriptive sensory attributes. The PCA indicated that colour has had a negative loading for the first principal component and a positive one for the second principal component. This study showed that presence of an intense brown colour is almost in all cases related to lower degree of meat tenderness, lower flavour intensity, and less juiciness. Since meat colour is an easier attribute to assess, this attribute may be very helpful to manufactures and consumers in making decisions about the suitability of a given poultry product for a given process or its acceptability. Principal component analysis is an effective approach for determining the most important sensory factors hence helps producers in product development. This study shows that colour was an important attribute in distinguishing among the samples. In this respect, it reinforces the more subjective JAR scale measures (Heycox, 2007; Yu *et al*., 1998). The PCA provides an objective way of aggregating indicators so that the variation in data can be accounted for as concisely as possible. The PCA ignores any attributes that did not distinguish significantly among the samples.

6.4 Conclusion

The descriptive analysis results indicated significant difference in scores between breasts and thigh. These differences were registered as: highest score in aroma, initial impression of juiciness, toughness at first bite, sustained expression of juiciness, overall tenderness and flavour for breasts. The thighs registered: highest scores on cohesiveness of

mass, and brown colour intensity. There were also significant differences among the different ecotypes with regard to aroma, flavour, and brown colour intensity. The JAR scale showed the consumers' scores for the colour, flavour, juiciness, tenderness of indigenous chicken as 'just about right' compared to broiler which was described as too light, flavour too strong, too juicy in terms of expression of juiciness and too tender with regard to texture. The PCA results showed that two Principal Components were responsible for 55.4 % and 11.6% and 53.9 and 19% for both descriptive and JAR scores for IC meat respectively for all the ecotypes. It shows colour as the most important attribute for distinguishing among the different samples of Kenyan IC. This study shows a very interesting relationship between the JAR scores and the descriptive quantitative tests, with JAR scores giving best indication in developing a product that would be most liked by the consumers. This study presents pioneeringresults of sensorial differences among the Kenyan indigenous chicken ecotypes (of different genetic characteristics) grown under intensive systems. It has also brought out significant difference among various attributes (particularly colour, tenderness, and flavour) between IC and the broiler. We recommend further studies to be conducted to evaluate the differences in sensory quality amongthe three ecotype clusters reared by free range from those reared under intensive systems.

CHAPTER SEVEN
FATTY ACID AND AMINO ACID PROFILES OF INDIGENOUS CHICKEN IN KENYA

Abstract

An experiment was conducted to determine the nutritional quality (amino acid and fatty acid profile) of Indigenous chicken (IC) in Kenya reared under free range system. Five cocks of each (ecotype) were purchased, slaughtered and transported to Jomo Kenyatta University of Agriculture and Technology (JKUAT)and Durban University of Technology, South Africa at the shortest time in ice cooler box. Amino acid profile was conducted using Pico Tag where samples were hydrolysed with acid for 24 hours, then derivatized, and analysed by reverse phase HPLC. Fatty Acid Methyl Esters (FAME) were synthesised by a direct or one-step extraction transesterification method. Fatty Acid Methyl Esters (FAME) were then separated and quantified using a Gas Chromatography (GC) system. The amino acid and fatty acid profile of IC in Kenya was affected by the ecotype cluster as well as the part of chicken (breast or thigh). The amino acid profile of experimented chicken revealed values higher than Recommended Daily Allowance (RDA) by FAO/WHO. The ratio of omega -6: omega -3 for the IC were higher than 4:1. The values reported here were still very favourable (9:1) compared to other meat sources and demonstrates that the consumption of IC meat can therefore reduce the risk of cardiovascular diseases.

Key words: *Fatty Acid Profile, Amino Acid Profile, Indigenous Chicken, Ecotypes*

7.1 Introduction

Poultry in Kenya play an important socio-economic, food and nutrition security roles in most households and especially in rural areas. Kenyan poultry population was estimated at 42.4 million birds (FAO, 2014). This figure currently stands at about 46 million birds according to report presented by ILRI 2019 out of which 75% are local indigenous chicken. The remaining 22 % are broilers while the rest are breeding stock and spent layers. Meat quality and palatability is affected by several factors. Most important of these is the fatty acid composition in the muscles and adipose tissues (Hoffman *et al.,* 2005). The amino acid profile is also responsible for nutritional and functional quality of chicken meat. The most common rearing method for indigenous chicken is through the free-range backyard system. According to Pavlovski *et al*. (2013) chicken meat from intensive broiler production differs in quality from native breeds and those in a free-range system. Intensification of the native/ indigenous chicken

has been on the increase for the past 20 years. Rearing native chicken under intensive in Serbia has been studied by Bogosavljevic-Boskovic *et al*. (2010), Milošević *et al*. (2003), Blagojević *et al*. (2009) and Pavlovski *et al*. (2013). In Europe and the USA, there are broilers kept under free range production system since they are considered to have a more intense flavour. This concept is also supported by the strong animal welfare group (Dawkins *et al.*, 2015). The demand for poultry meat has been estimated to triple in Africa a trend likely to be replicated in other parts of the world by the year 2030.

Food nutrition data is important to international organisations, private individual, food aid programmes, and epidemiologist. These groups often relate patters of disease with the dietary components and nutritional assessment of individual intake and diet counselling (Almeida *et al*., 2006; Rand *et al*., 1991). The USDA and FAO have published papers with nutrition tables of chicken. However, these are from the data collected from the chicken reared in the USA and Europe. According to the American Diabetes Association (2005) it is advisable to limit total daily energy intake from fat to <30%. They also recommend that the SFA and Trans-fatty acids should contribute no more than 10%. It has already been demonstrated that replacing red meat with chicken may result in significant decrease in apolipoprotein B and total cholesterol levels in type 2 diabetic patients (Gross *et al*., 2002). This effect is probably an attribute of the higher PUFA content of chicken meat as compared to beef. At the same time, the beneficial attributes of PUFA are dependent on the ration of omega 6 fatty acids (n-6) to that of omega 3 (n-3). An ideal ratio is often agreed as 4:1. This ratio is often favourable in poultry meat depending on diet (Alagawany *et al*., 2019).

With regard to quality of protein food sources, the value of the amino acids measured in terms of the amino acid scores, essential amino acid index, and the protein digestibility corrected amino acid scores is more important to the understanding of the nutritive value of a given protein in the diet (FAO/WHO, 1985). At the same time, the low levels of collagen in poultry meat is a good indicator of its digestibility and hence biological value (Marangoni *et al*., 2015). In spite of the apparent benefits of poultry meat to nutrition and especially with regard to provision of essential fatty acids and amino acids, the nutritive composition of these factors in indigenous chicken in Kenya is yet to be reported and documented. This study therefore evaluated the nutritive value of indigenous chicken meat in Kenya by determining the fatty acid and amino acid profiles of the three ecotypes.

7.2 Materials and Methods

7.2.1 Fat Content Determination

The crude fat content was determined by Soxhlet extraction, using the standard method of the Association of Official Analytical Chemists (AOAC, 2006).

7.2.2 Fatty Acid Analysis

Fatty profile acid analyses was carried out according to (AOAC, 2006): method 996.06 and modified by (Indarti, 2015). Fatty Acid Methyl Esters (FAME) were synthesised by a direct or one-step extraction transesterification method. Briefly, 0.1-g of sample was mixed with 2 ml of a mixture of methanol and sulphuric acid (85:15, v/v) and 2 ml of chloroform. Samples were heated to 100°C for 30 min and cooled to room temperature in a desiccator. Then, 1 ml of distilled water was added to the mixture, followed by vortexing for 1 min. The mixture was allowed to separate and the organic phase containing FAME was then transferred and dried with anhydrous Na_2SO4. Samples were stored in a freezer (-20°C) while awaiting Gas Chromatography (GC) analysis.

Fatty Acid Methyl Esters (FAMEs) were separated and quantified using a Gas Chromatography system (Automatic System XL, Perkin Elmer, Norwalk, Connecticut, USA) equipped with a flame ionisation detector and a 30-m x 0.25-mm fused silica capillary column (Omegawax 250, Supelco, Bellefont, USA). Helium was used as the carrier gas while hydrogen and compressed air was used for Flame Ionisation Detection (FID). The oven temperature was programmed to rise from 50-220°C at a rate of 4°C min^{-1} and then held at 220°C for 35 min. The injector and detector temperatures were set to 250°C and 260°C, respectively. Individual fatty acids were identified by comparison to known standards (Supelco 37 Component FAME Mix; Supleco) and the areas beneath the identified chromatographic peaks calculated by integration. Saturated Fatty Acids (FSA), Mono-Unsaturated Fatty acids (MUFA), and Poly-Unsaturated Fatty acids (PUFA) and their ratios were be calculated from the overall fatty acid composition data.

7.2.3 Determination of Amino Acid Profile

The total amino acid content of the 17 different amino acids in the sample were evaluated in duplicate and reported as g/100g sample. Total amino acid profile (excluding Tryptophan, Cysteine, and Methionine) were determined on lyophilised, ground and homogenous samples by Central Analytical Facilities at Stellenbosch University and based onSouth Africa Grain Laboratories (SAGL), in-house method 009: as adopted from Pico-Tag

method as described by Bidlingmeyer *et al*. (1984). Samples were hydrolysed with HCl acid for 24 hours, then derivatized and analysed by Reverse phase High Performance Liquid Chromatography (HPLC) with RF2000 Fluorescence detector and a Nova-Pak C18 4 μm, 3.9 × 150 mm column using Chromeleon 6.80 software. The samples were first freeze dried before shipping to Central Analytical Facilities at Stellenbosch University, South Africa for the analysis.

7.3 Results and Discussions

Table 7.1 shows the amino acid profiles of the indigenous chicken ecotypes, from different body parts, and the total essential and non-essential amino acids. Except for the amino acids Histidine and Lysine which were higher in the breasts than in the thigh muscles for all the ecotypes, there were no significant differences reported on the amino acid profile of the breasts and thighs ($P<0.05$). The values reported for Methionine (at least 6.77 g/100g), and Lysine (at least 7.76 g/100g) were reported in this study. These two are limiting amino acids in cereals and legumes but were very high in the chickens. This reinforces the assertion that chickens are a good source of high-quality protein. It also confirms that Kenyan indigenous chickensgrown under free range are a good source of the high-quality proteins. High amounts of Lysine, Aspartic acid, and Glutamic acid were found in all the three ecotypes. The same trend was observed as well for the breasts and thighs.

Table 7.1:Amino acid profile for the different ecotypes

Total amino ac**id content within:**		**Concentration (%)**
Ecotype (A)	Kakamega	5.43 ± 0.52^{b}
	Naivasha	5.37 ± 0.53^{b}
	Taita	5.77 ± 0.57^{a}
Chicken part (B)	Breast	5.65 ± 0.44^{a}
	Thigh	5.39 ± 0.43^{b}
Type (C)	Essential	4.84 ± 0.27^{b}
	Non-essential	6.67 ± 0.65^{a}

The study reported high levels of Glutamic acid (Figure 7.1). The glutamic acid has been demonstrated to correspond to the delicious chicken taste and is theorised to be a

contributor to the exclaimed better taste in indigenous chicken. This result is in line with the findings of Wattanachant *et al*. (2004) and Aronal *et al*. (2012a) who reported high values for these three amino acids. They also reported very high values of leucine in the breast and thighs of chicken, a result which is supported by our findings. Protein quality is closely related to the efficiency of the protein utilisation in human digestive system. This is usually a factor of protein digestibility and quantity and quality of the amino acid (either essential or none essential). The IC reported high quality proteins and with their recorded delightful taste, they will continue to contribute to the protein diets of many consumers.

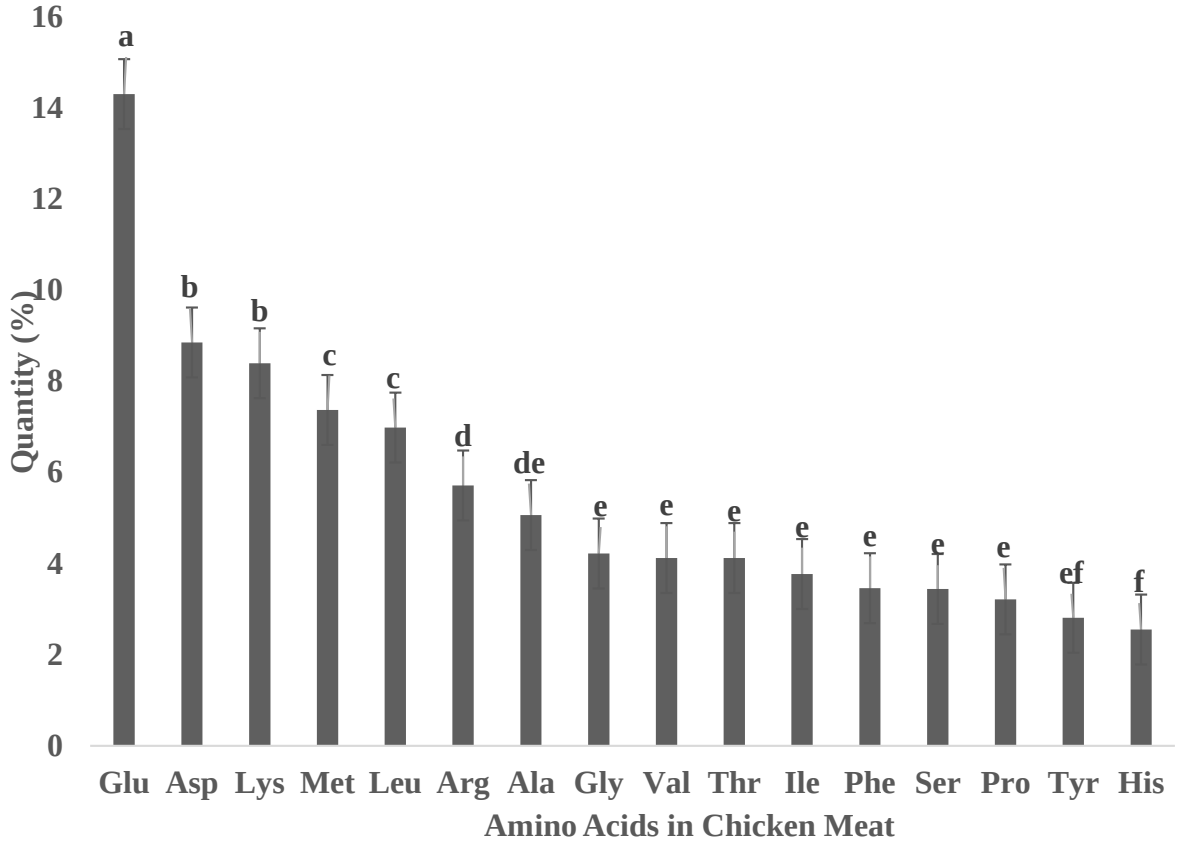


Figure 7.1: Total composition of the amino acids in chicken meat

Glutamic acid has been reported to have very significant effects on the taste of meat. The higher levels reported in this study is supportedby reports from others studies. It may be one of the factors that are responsible for the better taste already reported in many parts of the world when comparing the indigenous chicken and the broiler (Farmer, 1999). Histidine value even though being the lowest was still reported at a value that is more than the recommended value by the WHO/FAO, 2007 for both adults and children.

7.3.1 Amino Acid-Chemical Scores

The amino acid -chemical scores score of a food product reflects its amino acids (AA) content in comparison with the ideal protein (Aronal *et al*., 2012b). Normally, egg white, and milk proteins are considered the ideal proteins due to their high digestibility. The rest of the amino acids are compared against these two protein sources (FAO/WHO, 1985). When there is a need to know the utilisation of AA by the organism it is necessary to do a correction of the score value by protein digestibility. This is what is often referred to as the Protein Digestibility Corrected Amino Acid Sore (PDCAAS) (Marangoni *et al*., 2015). Poultry meat like that of milk and egg are referred to as the high-quality proteins. They both have a PDCAAS of 1 or very close to one. This is unlike that of legumes and vegetable sources which have a low PDCAAS value of up to 0.5 for wheat and for beans at 0.75 (Marangoni *et al*., 2015). In the current study the values of the PDCAAS for all the amino acids from the IC chicken had a value greater than unity for both the breasts and the thighs. This is testament to their versatility as a source of excellent protein quality.

7.3.2 Fatty Acid Profile of Indigenous Chicken in Kenya

The fatty acid profile of the breast meat of the three ecotypes are presented in Table 7.2. Poultry meat has a major component of TAGs which are PUFAs, MUFAs, SFAs.These are vital nutritional components. Within the Kakamega ecotype, the palmitic acid composition was highest followed by the oleic and linoleic fatty acid compositions, respectively. The value of Caprylic acid at 0.04 % was the lowest. For Naivasha ecotype, the oleic acid content was highest followed by palmitic and then linoleic acid. The lowest was the composition of Arachidonic fatty acid. For Taita ecotype, oleic acid, acid composition was highest followed by palmitic and then linoleic acid composition. Among the three ecotypes, the composition of the palmitic acid was significantly different in Naivasha than in Kakamega and Taita ecotypes. Palmitic acid is a saturated fatty acid and one of the most abundant in nature. The composition of stearic acid was equally significantly higher in Naivasha than in Kakamega but not in Taita ecotypes. Factors affecting fatty acid content of poultry: animal breed, external and internal fat levels, climate, breed, and rearing methods employed (Bragagnolo & Rodriguez-Amaya, 2001). These factors are more often than not most determined by the regions and resultant cultural, management and feeding practices of the people.

Table 7.2: Fatty acid composition ofthe breasts

	Ecotype		
Fatty acid	Kakamega	Naivasha	Taita Taveta
Caprylic	0.04±0.02[a]	0.88±0.51[a]	0.14±0.07[a]
Capric	0.57±0.28[a]	0.42±0.11[a]	0.02±0.01[a]
Lauric	0.42±0.17[a]	0.26±0.03[a]	0.18±0.08[a]
Myristic	0.76±0.19[a]	0.76±0.04[a]	0.99±0.14[a]
Palmitic	20.20±0.71[a]	17.88±0.59[b]	20.02±0.64[a]
Palmitoleic	1.73±0.14[a]	1.51±0.15[a]	1.74±0.16[a]
Stearic	9.81±0.16[b]	11.65±0.78[a]	10.76±0.37[ab]
Oleic	19.55±0.60[a]	20.59±0.67[a]	20.33±0.59[a]
Linoleic	15.06±0.95[a]	13.12±0.59[a]	15.75±0.75[a]
Linolenic	0.89±0.19[a]	0.84±0.05[a]	0.58±0.08[a]
Arachidic	0.32±0.21[a]	0.19±0.09[a]	0.13±0.04[a]
Arachidonic	10.22±0.73[b]	13.11±0.29[a]	10.13±0.29[b]
EPA	0.76±0.14[a]	0.80±0.10[a]	1.10±0.05[a]
DHA	2.31±0.36[a]	1.40±0.10[b]	1.66±0.10[b]

Fat content and fatty acid profiles of Triacylglycerols (TAGs) in muscles strongly correlate to meat quality especially tenderness, juiciness, and flavour. Though Wattanachant *et al*. (2004) reported higher content of saturated fatty acids lower PUFAs, in indigenous chicken muscle than in broiler. Jaturasitha *et al.* (2008b) found different results. In the current study, the fatty acid profile was found to differ per ecotype ($p \leq 0.05$) possibly due to the different feeds fed to the different genotypes based on the unique conditions of the farmers. This suggestion was also made by Cherian *et al*. (2002). In their study, they set to determine muscle fatty acid composition and thiobarbituric acid-reactive substances in broilers fed on different cultivars of sorghum. They noticed significant difference in these values for chicken fed on different cultivars. The fatty acid profile of the thigh meat among the three ecotypes is stated in Table 7.3.

For the thigh meat, oleic acid was highest ($p \leq 0.05$) in the Kakamega ecotype followed by palmitic acid and then linolenic acid. The lowest was capric acid at 0.18±0.05 %. With regard to Naivasha ecotype, oleic acid was highest followed by palmitic and then linoleic acid. The lowest fatty acid composition was for capric acid at 0.06± 0.03 %. Lastly, for the Taita

ecotype, oleic acid was the highest followed by linoleic and then palmitic acid and the lowest was capric acid at 0.1±0.05 %. Compared across the ecotypes, significant differences ($p \leq 0.05$) were noted on Caprylic acid between Naivasha-Taita and Kakamega ecotype. Lauric acid composition was also significantly higher in Kakamega than in Naivasha and Taita ecotypes. Myristic fatty acid composition was significantly different for Naivasha and Kakamega ecotypes from Taita ecotype. Finally, the acid docosahexaenoic acid (DHA) composition was significantly higher in Kakamega than in Naivasha and Taita. The DHA fatty acid is part of the long chain omega 3 fatty acids that also includes Eicosapentaenoic (EPA), Docosapentaenoic (DPA) (Haug *et al.,* 2011).

Table 7.3: Fatty acid composition of thigh meat

Fatty acid	**Ecotype**		
	Kakamega	Naivasha	Taita Taveta
Caprylic	0.27±0.07^{b}	0.81±0.19^{a}	0.44±0.16^{ab}
Capric	0.18±0.05^{a}	0.06±0.03^{a}	0.10±0.05^{a}
Lauric	0.23±0.03^{a}	0.10±0.02^{b}	0.13±0.04^{ab}
Myristic	0.63±0.10^{ab}	0.84±0.06^{a}	1.12±0.14^{b}
Palmitic	18.34±0.61^{a}	16.58±0.49^{a}	18.47±0.70^{a}
Palmitoleic	2.00±0.15^{a}	2.20±0.18^{a}	2.17±0.25^{a}
Stearic	12.97±0.33^{a}	13.72±0.53^{a}	12.23±0.48^{a}
Oleic	19.85±0.63^{a}	23.53±1.31^{a}	22.44±1.35^{a}
Linoleic	15.73±0.30^{a}	15.98±0.97^{a}	19.18±2.06^{a}
Linolenic	0.59±0.04^{a}	1.51±0.51^{a}	0.78±0.11^{a}
Arachidic	0.15±0.03^{a}	0.18±0.03^{a}	0.22±0.10^{a}
Arachidonic	10.64±0.40^{ab}	12.19±0.67^{a}	9.29±0.88^{b}
EPA	1.18±0.24^{a}	0.60±0.05^{a}	0.91±0.18^{a}
DHA	2.45±0.21^{a}	0.90±0.10^{b}	1.55±0.24^{b}

Among the three ecotyes the values of SFA, MUFA, and PUFA were not significantly different ($p \geq 0.05$). However, the value of omega 3 and Omega 6 fatty acids recorded a significant difference at ($p \leq 0.05$). Kakamega ecotype recorded the highest value of omega 3 as well as omega 6 composition. This was followed by Taita and finally, the Naivasha ecotype. Though many factors may have contributed to this including ecotype (Pavlovski *et al*., 2013),

the other likely one is that the feeding regime for chicken in Kakamega consists chiefly of free range in which they chicken scavenge on grasses, leaves, worms and so forth. It is been reported that the ratio of omega 3 to omega 6 fatty acids is higher in the feeds scavenged by chicken from natural diets than in what they are fed in cereals and legumes (Haug *et al*., 2011). In the other regions particularly Naivasha, the town influence resulted in farmers relying heavily on chicken waste from the hotels in town and some confessed to the use of waste from the dump site.

The SFA content of chicken meat was contributed to majorly by palmitic, myristic, and stearic fatty acids both in breast and thigh (Table 7.2 and 7.3). Meat in general is associated with the supply of fatty acids especially the SFA which have been associated with the risk of cardiovascular diseases especially in the phase of the modern life styles especially in the developed countries (Pavlovski *et al*., 2013). The SFA has been reported to be significantly different between breeds for example naked neck and the broiler (hybro G+ and Cobb 308) (Pavlovski *et al*., 2013). Fatty acid profiles were also reported to differ significantly ($p \leq 0.05$) based on the part of the body by other authors (Adulyatham *et al*., 2006; Pavlovski *et al*., 2013). Poultry meat is considered healthier because of a considerable low-fat content compared to other meats. Lipids of animal origin typically contain triglycerides (glycerol), cholesterol, and phospholipids (Abdulla *et al*., 2015). A similar data expressing the values of these fatty acids on the breast muscles of the three ecotypes is presented in Figure 7.2. There was a significant difference ($p \leq 0.05$) in the MUFA content of the thighs of the ecotypes (Naivasha and Taita) had higher values than that of Kakamega. Which had a lower concentration.

As for the composition of *omega* 6 fatty acids, Kakamega ecotype had significantly higher value than that of (Naivasha and Taita) ecotypes. Wheareas Naivasha ecotype had higher omega 6 to omega 3 ratio than Taita ecotype (Figure 7.4).

Table 7.4: Ratio of omega -6 to omega-3 fatty acids

Ecotype	**Part of chicken**	**Omega 6**	**Omega 3**	**Ratio**	**Recommended**
Kakamega	Breast	12.64±0.81	1.32±0.19	9.5:1	4:1
	Thigh	13.18±0.63	1.41±0.18	9.0:1	
	Overall	12.91±0.51	1.36±0.13	9.5:1	
Naivasha	Breast	13.12±0.32	1.01±0.07	13.0:1	4:1
	Thigh	14.09±0.72	1.00±0.18	14.0:1	
	Overall	13.60±0.40	1.01±0.10	13.5:1	
Taita	Breast	12.94±0.76	1.11±0.09	11.7:1	4:1
	Thigh	14.24±1.57	1.08±0.12	13.2:1	
	Overall	13.59±0.87	1.10±0.08	12.4:1	

The three categories are saturated fatty acids (SFA), Mono unsaturated fatty acids (MUFA), Poly unsaturated fatty acids (PUFA) and omega 3 and Omega 6 fatty acids. These are represented in Figure 7.2.

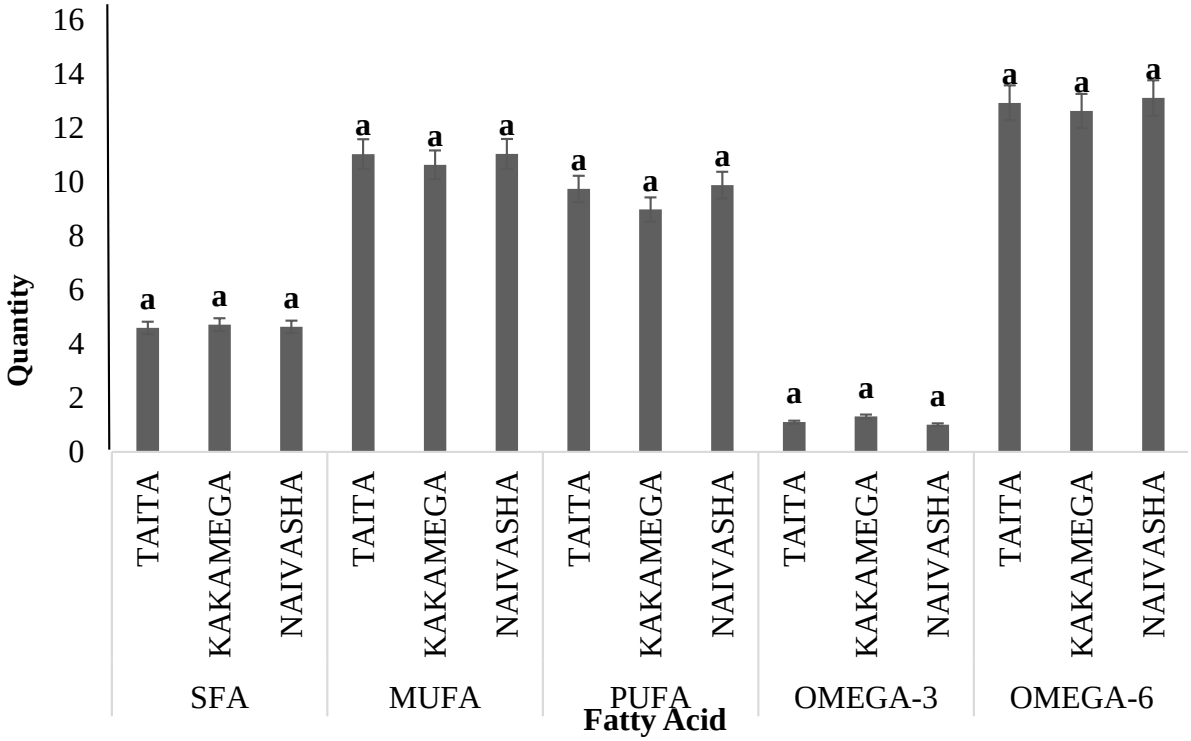


Figure 7.2: Fatty acid composition of the breast meat grouped in terms of their levels of saturation

Figure 7.3 reprsents the differences in (SFA, MUFA, PUFA; omega-3, omega-6) composition in Thigh (T) Breast (B) portions for the three different ecotypes. Significant differences were recorded in MUFA for Taita and Naivasha Ecotypes.

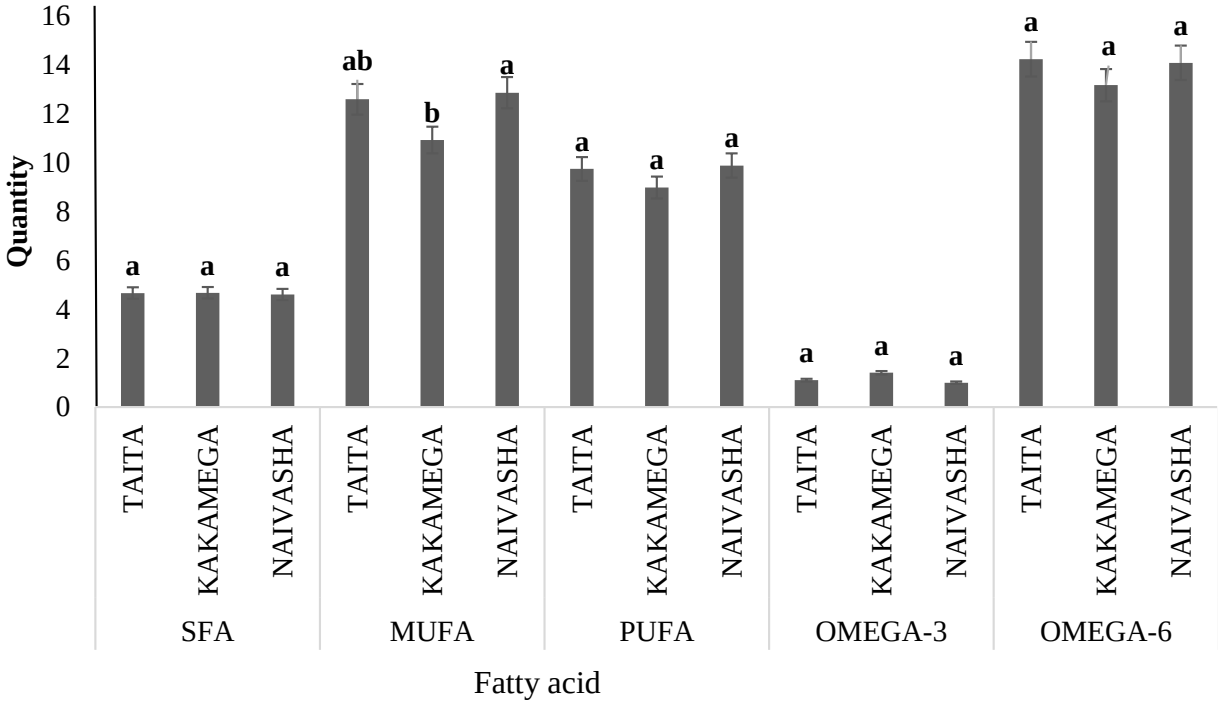


Figure 7.3: Differences (SFA, MUFA, PUFA; omega-3, omega-6) composition in Thigh (T) Breast (B) portions

7.4 Conclusion

This study revealed significantly higher amino acid and fatty acid profiles for different ecotypes of indigenous chicken in Kenya. The Glutamic acid was the highest amino acid while the lowest value recorded was for Histidine, however all the reported values exceeded the recommended intakes by WHO/FAO for adults and for children. There was no significant difference among the values of PUFA, MUFA, and SFA among between the breast and thigh. The value of Glutamic amino acid was highest and this could be correlated to the good taste proclaimed by consumers of indigenous chicken. Ratios of omega -6: omega -3 are above the ratio of 4:1 and Kakamega had the lowest ratio among the 3 ecotypes. Free range rearing applied to indigenous chicken results in lower omega -6 :omega3.Where possible free-range rearing should be encouraged so that the poultry meat may in addition to conferring nutritive value, contribute to the lowering of risk of communicable diseases.

CHAPTER EIGHT

MOLECULAR CHARACTERISATION OF MICROBIAL COMMUNITIES ISOLATED AT DIFFERENT NODES OF INDIGENOUS CHICKEN VALUE CHAIN IN KENYA

Abstract

Poultry represents 30% of Kenya's Agricultural GDP which accounts for about 25% of overall GDP. Kenyan poultry population is estimated at about 42.4 million birds according to (FAO, 2014). This has now been raised to 46 million birds by the year 2019 as has been reported by ILRI.Indigenous chicken (IC) accounts for 75% of this overall poultry population whereas broiler contributes 22%. Though only scanty reports exist in Kenya about the specific causes of food borne illnesses, the available data already points to a serious food borne disease burden due to the contamination of foods by a number of pathogens. Limited work has been done to establish microbial quality based on DNA sequencing of these micro-organisms at different nodes of the value chain and from the different ecotype clusters of indigenous chicken in Kenya. This study was conducted to evaluate microbial ecology of three different ecotypes of indigenous chicken from three different body parts (breast, thigh and caecum) and compared to that of the broiler. It has also revealed the different taxonomic classifications of microbial communities at different nodes of IC value chain.Diversity metric measurements revealed clusters within samples and among different samples. Indigenous chicken microbiome consists of novel bacteria that can be associated with improved health, better performance, antagonism to pathogenic microbes, and improved food intake. The node of value chain, IC ecotype, and part of chicken does not significantly determine microbiome abundance. However, there is a marked difference in microbial abundance, and diversity between the broiler, and indigenous chicken. Microbiome of IC (Naivasha and Kakamega, are clustered together) while that from Taita clusters closer to the broiler microbiome. No strains of pathogenic bacteria were identified in the samples.This may suggest that CCP at farm should potentially be set to address other hazards other than microbial. Indigenous chickens'gut are rich in microbial communities and most of them are beneficial. Through HT-NGS technologies some bacteria that have never been cultured were identified including; TM7 and *Prevotella.* The huge abundance of *Prevotella* especially in Kakamega ecotype indicates the chickens'guts' adaptation to utilising plant-based material. It was observed that such greens were a regular part of these chickens' diet under free range system and few mixed systems.

Key words: *Indigenous chicken, 16S rRNA, Ecotypes, microbiome, microbial communities, value chains.*

8.1 Introduction

Over the past few years, food safety risks have become magnified due to the impact of globalisation, and urbanisation. Food may be produced in one part of the world and be delivered in a record time, to the other part of the world.Such food may carry with it any would be contaminants from a part of the world to the next. The given level of infrastructuraldevelopment can easily allow products to be rapidly distributed thus enhancing the impact of this kind of foodborne illness should it arise. The most common pathogenic microorganisms associated with chicken meat are *E.coli, Salmonella spp*., *Staphylococcus Spp*. and *Campylobacter spp*. (Duggett, 2015). Though only scanty reports exist in Kenya about the specific causes of food borne illnesses, the available data already points to a serious food borne disease burden due to the contamination of foods by these pathogens (Hoffmann, & Baral, 2019). It also suggests that different nodes of the indigenous chicken value chain present different conditions for the thriving of these micro-organisms. Limited work has been done to establish microbial quality based on DNA sequencing of these micro-organisms at different nodes of the value chain and from the different ecotype clusters of indigenous chicken in Kenya. Indigenous chicken (IC) (*Gallus domestica*) meat products are generally considered to be of high quality by consumers. This has resulted in increased demand with consumers showing a willingness to pay premium prices for IC meat products. To improve the safety of the IC meat products, the meat must be healthy, and free from disease causing pathogens that are a risk to human health. The Illumina sequencing platform is the most current addition to the HT-NGS technologies. It results in longer and deeper reads at less cost estimated at about 0.5 USD per Mega base (Mb) against the USD 31 per Mb which is the cost of the 454 GS Junior installation (Kozich *et al*., 2013). The current study was conducted to evaluate microbial ecology of three different ecotypes of indigenous chicken from three different body parts (breast, thigh and caecum) and compared that to the broiler. It has also revealed the different taxonomic classifications of microbial communities at different nodes of IC value chain and the diversity metric measurements within samples, and between different samples. The study presents the results of High through Put Next Generation Sequencing Technology to provide scientific data for action of the public health departments in curbing foodborne illnesses.

8.2 Materials and Methods

8.2.1 Sampling Plan

Chicken samples were purchased from random households from the different regions of ecotypes based on the research of Okeno *et al.* (2013). Structured questionnaires were ministered to collect information on management practice relevant to microbial control and other relevant information. A total of 90 chicken from the three 3-Clusters/ecotypes of indigenous chicken from (Taita, Kakamega and Naivasha) and broiler. From each ecotype, at least 8 chicken (male and female were collected) to represent each of the 3 population structures (Mwacharo *et al*., 2013) each of the two nodes of the value chain (farm and market) (Mathuva, 2005) and transported to laboratory for slaughter and analysis.

8.2.2 Indigenous Chicken sample handling

Farmer's traders and end market operators or processors were identified through District Livestock officers within the areas of interest (regions-corresponding to target ecotypes). Relevant questionnaire on handling and management practices of the chicken were then administered by researcher. Both verbal and written consent was sought from the respondents before administration of the questionnaires and before sample collection. The IC chicken, same age, and sex were randomly purchased from operators identified by snowballing. The chickens were slaughtered by precision incision of jugularvein with a sharp knife at one strike/on a single stroke to dislocate the head.After that, chicken were held upside down to thoroughly drain off blood. Care was taken to prevent any cross contamination between the parts of chicken. Hands, knifes, and equipment were constantly sterilised with 70% ethanol, a longitudinal incision was made with a sterile sharp knife from breast and thigh and the edges pulled back to drive about 1-2 g of sample per chicken. Care was also taken to collect part of the skin. Samples were carefully inverted in to a sterile container of BPS under aseptic conditions and tightly closed. The carcasses were then open after removal of feathers by sterile hands around the gut region of the chicken. This was followed by a careful incision to reveal the intestinal components then the caecum was carefully pulled out, its contents carefully pulled back and then cut out using the sterile knife. The caecum portion of the intestines was targeted and about 1-2 g of its contents inverted aseptically into the previously sterile sampling tubes containing BPS. Samples were immediately cooled in ice and carried in a cooler box at less than 4 ^{o}C straight to BeCA-Hub laboratory. Once in the laboratory, the samples were transferred to a blast freezer (-80^{o}C) awaiting DNA extraction.

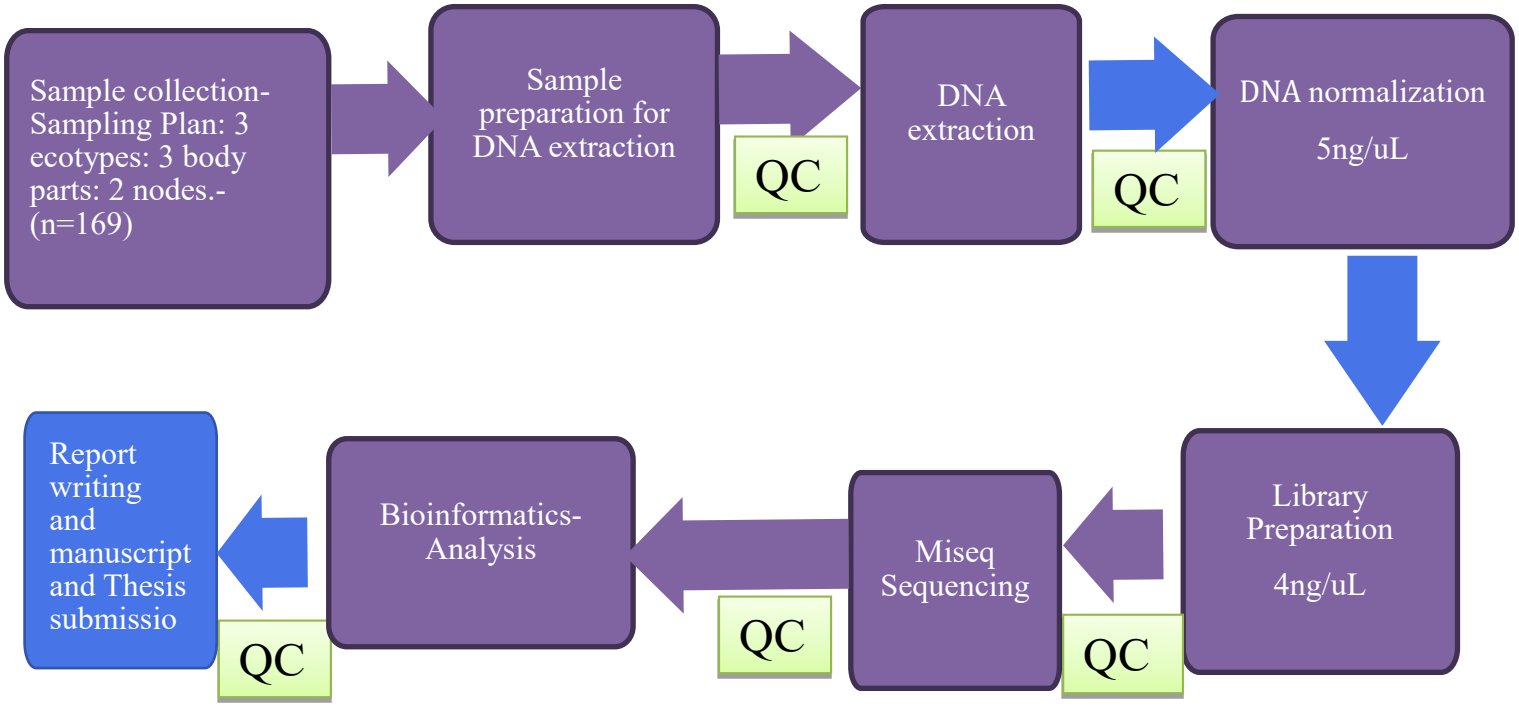


Figure 8.1: Flow diagram of the major steps in the methodology

8.2.3 DNA Extraction, PCR Amplificationof 16S rRNA Amplicon Sequence

Samples were freeze dried and then crushed individually in fume hood-(bio-cabinet) and 25 mg of the homogenised samples was used for DNA extraction. DNA Extraction was conducted by the procedures of PureLink™ Genomic DNA Kits for purification of genomic DNA Catalog nos. K1820-01, K1820-02, K1821-04 (Invitrogen, 2007) with slight optimisation. Quality control of genomic DNA was conducted on Nanodrop and by gel electrophoresis on 0.8% E-Gel® agarose gel running at 100 volts for 45 minutes. The 1kb Plus Lambda DNA was loaded with samples as marker.

8.2.4 First and Second PCR Amplification

First step of PCR was used to amplify template out of a DNA sample using region of interest- specific primers with overhang adapters attached. The 16SPCR1_Fwd and 16SPCR_Rev primer were obtained from Bioneer Inc. Oakland USA (order No 10017918) and the primer sequences as:

TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGNWGCAG (Forward) and

GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATACC (Reverse).

The Primary PCR reaction conditions remained the same thus: Two and a half (2.5 ul) of the total DNA was used for the first PCR reaction in a final volume of 25 ul using the 2xKAPA HiFi Hot start Ready mix. The thermocycler condition for first PCR was as follows:

Thermocycler conditions

Step1- 95^{o}C/3 min

Step2- 95^{o}C/30 sec

Step3- 63^{o}C/30 sec 35X

Step4- 72^{o}C/30 sec

Step5- 72^{o}C/5 min

Step6- 20^{o}C/10 min

From the results of the PCR optimisation (Gradient PCR), the temperature of 61^{o}C was chosen for scaling up the process of 16s DNA Library preparations. The annealing temperature was 63 ^{o}C/30 secs and the number of PCR cycles was 35X. 3 µl of the PCR product was electrophoresed on a 1.5% agarose gel.

Step 1: 95 ^{o}C for 3 mins

Step 2: 95 ºC for 30 secs
Step 3: 61 ºC for 30 secs } X 9 Cycles
Step 4: 72 ºC for 30 secs

Step 5: 72 ºC for 5 Mins

Step 6: 20 ºC final holding temperature

DNA library preparation was based on the procedure of Illumina16S Metagenomic Sequencing Library Preparation '*Preparing 16S Ribosomal RNA Gene Amplicons for theIllumina MiSeq System'* (Illumina Inc. USA). Slight modification especially by increasing incubation time from 2-5 minutes during the clean-up steps was adopted. The Amplicon library was normalised according to the same protocol and loaded to Miseq for analysis.

8.2.5 The Final Library Quality Analysis

The tape station Agilent 2200 was used for additional quality control (quantification and Library peak sizing) using High Sensitivity (HS) DNA assays kit. Two (2 µl) of a library DNA amplicons was mixed with equal volume of HS Buffer (D 1000). Two (2 µl) of DNA ladder was run along the samples to act as an internal standard for sizing and quantification of the library amplicons.This is because high quality DNA yield and integrity was necessary to produce a reliable and reproducible assay result.The results confirmed the suitability of the genomic DNA results for downstream applications.

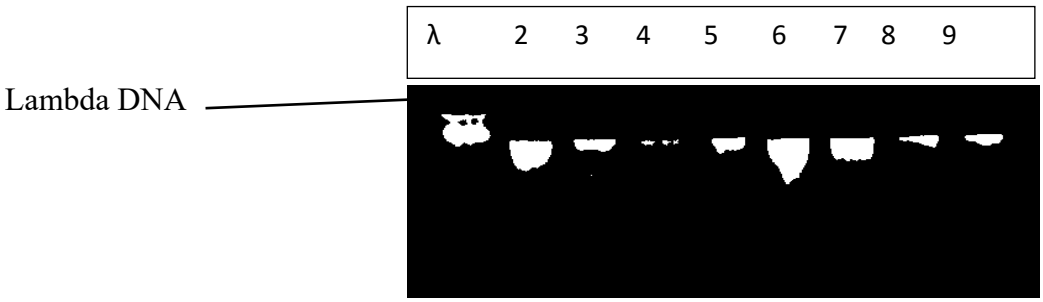
8.2.6 Illumina Miseq Sequencing

MiSeq library preparation and 271 × 271 paired-end sequencing (Illumina, San Diego, CA, USA) were performed by BeCAILRI hub next Generation Sequencing platform using a protocol and primers (obtained from Bioneeer targeting the v3 and v4 regions). These primers were initially recommended and previously described by the Earth Microbiome project (Gilbert *et al.,* 2014). The Miseq Platform generated, demultiplexed FASTQ files were converted to QIIME 2 Artifact for downstream analysis. The final QIIME2 batch script has been attached an appendix and will also be uploaded to GIT hub for sharing with the wider bioinformatics community. Figure 8.2 shows the flow diagram of the major steps in the methodology.

8.3 Results and Discussion

8.3.1 Effect of Ecotype, Node of Value Chain and Part of Chicken on DNA Extraction and Quality

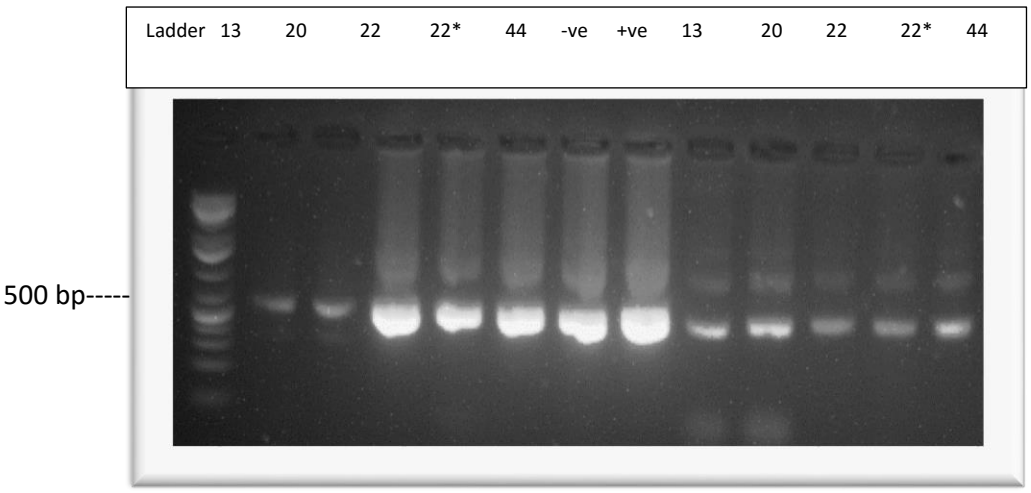
The results of the quality of genomic DNA after extraction and running on 0.8% agarose gel at 100v for 45 minutes is as depicted in figure 8.3. The quality of DNA extracted from the gel showed the extraction kit and conditions were able to avail DNA of good integrity as the Lambda DNA. Figure 8.4 shows the DNA quality of amplicons after 1st and second PCR amplifications on 1.5% Agar rose gel and run at 199 minutes at 70 volts. The library quality as indicated on the gel were reported and approximated at 500 base pairs and this was well indicated by the 1kb ladder used. Quality of DNA is often a function of concentration and purity or integrity. DNA quantitated by use of Nano drop and Qubit reagents. While the Nano drop measurements gave quality of total DNA (double stranded and single stranded) the qubit reading was more useful as it gave only the value of the double stranded DNA in nanograms per µl. The DNA quality was not affected by two factors under study (ecotype, node of value chain) however the part of chicken had an effect on DNA yield and quality. The caecum samples had the most concentrated DNA and had more smears hence their DNA extraction required use of RNAse to remove any smears and enhance the quality.



Legend: Lanes 2-9 select genomic DNA samples

Figure 8.2: Gel image quality of Genomic DNA of few selected samples from chicken

Legend: on 8% agar rose gel.



Legend: Lane 13-44-Amplicon library samples

Figure 8.3: Gel image quality of DNA Amplicon library of few selected samples

Legend: on 1.5 % agar rose gel for 90 minutes at 75 volts.

Figure 8.4 shows the results of library quality as read from the Agilent tape station on High Sensitivity (HS) buffer and Ladder. Most of the Agilnet DNA quality after adjusting by the ladder ranged from 425-600 base pairs that covers the expected region of the DNA library fragments. Both the results of the Agilent trace and accompanying Electrophoregram of the selected samples showed appreciable correlation. The quality of DNA library is determined by the size of the peak and the Qubit concentration. Both factors are crucial to the determination of the concentration of the library and the final concentration of the combined library before loading into Miseq Sequencer for generation of FASTQ files.

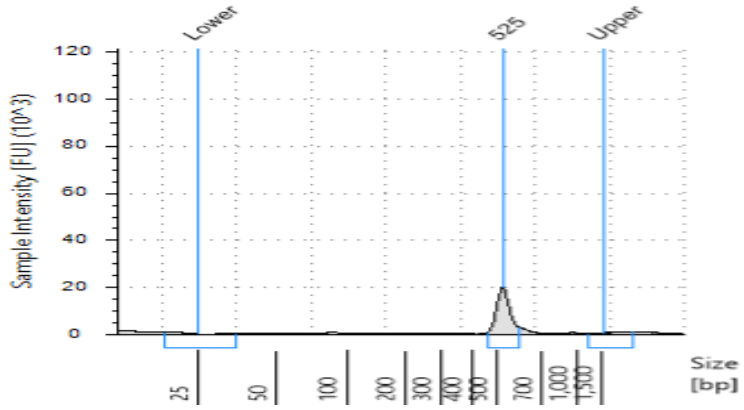


Figure 8.4: Sample library quality from the tape station Agilent 2200-HS Buffer (D 1000)

8.3.2 Effect of Ecotype, Node of Value Chain and Part of Chicken on the Quality of Illumina Miseq Reads

The quality of Illumina reading of multiplexed samples DNA samples were not affected by any of the factors under study. The quality and number of reads generated from Illumina Miseq was however affected by the sequencing depth and the choice of read lengths. For uniformity, the results presented here were obtained from de-multiplexed sequences of DNA libraries at 270 base pairs of paired end reads. The statistical analyses of the resulting sequences were conducted by Quantitative Insights into Microbial Ecology (QIIME2) (version 2018/6).

The QIIME2 DADA2 denoising statistics of the Illumina generated demultiplexed sequences is presented in Table 8.2. The quality of the forward and reverse reads was not significantly different up to the 200 base pair points. However, from here the quality drop was higher for the reverse reads than for the forward reads. QIIME2 uses DADA2 or the DEBLUR tools to generate an interactive quality of sequenced bases. The resulting figure for trimming the sequences at 220 was as below to ensure only high-quality reads were used for further downstream analysis.

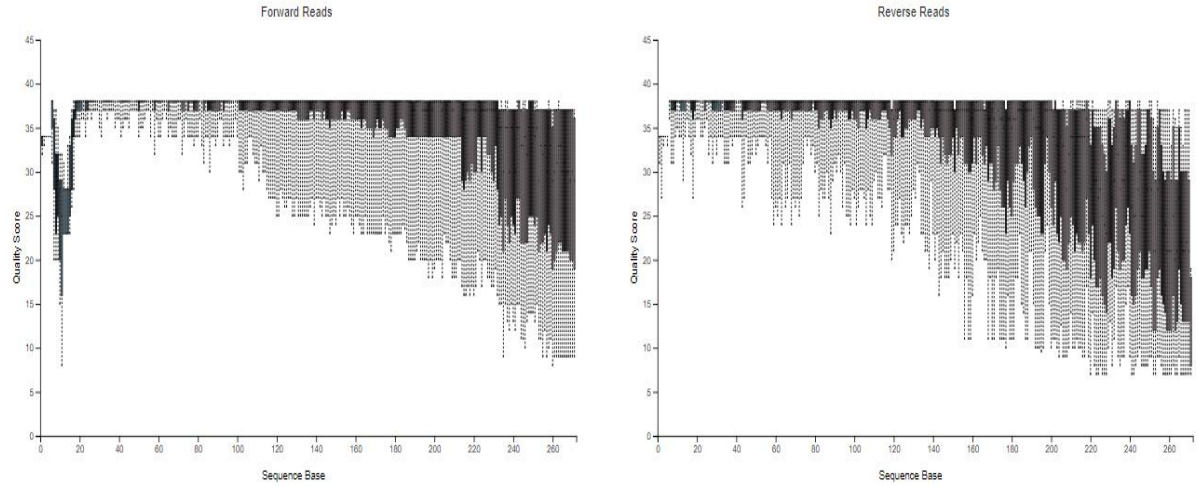


Figure 8.5: QIIME2 generated interactive quality control of reads

8.3.3 Quality of Filtering and Denoising Based on QIIME2 Tools

DADA2 tool in QIIME2 was used for further quality filtering of all the reads by conducting a filter at a specified base pair as shown in Table 8.1 and the script in the appendix. Based on the interactive curves in Figure 8.5, the left trimming parameter was set at 0 while that of the right was set at 200 base pairs to take care of the bigger quality drop in the reverse reads so as to have equal quality of reverse and forward reads for the downstream analysis.

Average number of reads was the total number of reads for the 1st and second runs which were 14,901,364 and 6,990,486 respectively.

Table 8.1: Showing Selected Read Quality Statistic for different samples

Sample Id	Input reads	Filtered	Denoised	% Filtered
13	146820	126886	126886	86.42%
14	191431	166869	166869	87.16%
15	143171	124406	124406	86.89%
16	136242	119378	119378	87.62%
Total	14,901,364	14,900,246		99.9%

8.3.4 Effect of Ecotype on Diversity Distance Matrices of Microbes Isolated from IC and Broiler

Figure 8.6 represents the beta diversity which is used to compare multiple communities to determine the number of OTUs or taxa shared among them. It determines functional dissimilarities between multiple populations. It is calculated as Similarity Index: (Jaccard Bray-Curtis)-and it shows shared populations between microbial profiles of multiple communities. Axis 1 explains 19.99% of the variation as Taita and broiler contain microbiota most distinct from the Kakamega and the Naivasha ecotypes. Two reasons can be advanced for this important observation. First, that the Taita Ecotype and broiler have same feeding regime based on supplementation of the cereal based diet. Second, Taita County is generally a dry area receiving limited rainfall (average 650 mm annually). This means that even when chickens are allowed to free range, their food sources are limited to few greens and lots of dry matter. Axis 2 and three explain 5.71 and 5.22% of the variation and the Kakamega and Naivasha ecotypes are captured in these two axes. The species evenness, and diversity are important indicators of microbial analysis. The two indices are a pointerto health of livestock (gut microbiome). Diversity of gut microbiome decreases with antibiotic use and nutrients imbalance (Lozupone *et al*., 2012). The less diversity expressed in broilers can be an indication of the reduction caused by use of antibiotics over time.

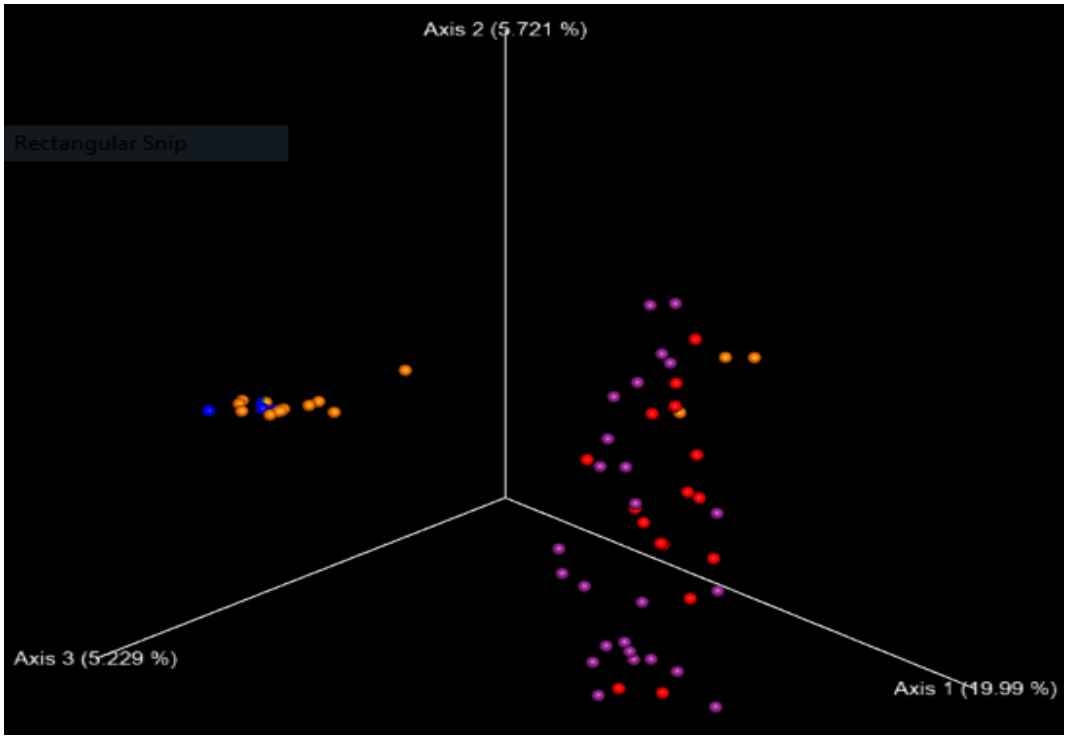


Figure 8.6: Jaccard emperor-plots-measure of diversity distance among

There are different techniques that ecologists use to describe diversity observed in different communities.These techniques have been successfully adapted by food microbiologists as well as geneticists interested in better understanding of microbial communities and their involvement in none-specific resistance (Duggett, 2015). Either the diversity is measured within a community (Alpha diversity) or between a collections of samples (Beta diversity) (Kuczynski *et al.*, 2012). For gut microbiome the diversity within the community is associated with enhanced functionality, feed intake, diseases and pathogenic resistance among others. The beta Diversity represents the explicit comparison of microbial (or other) communities based on their composition. Some of the Alpha diversity estimates tested by QIIME2 include Chao1 and ACE for richness, Pilou's, Shannon Wiener's and Simpson's evenness estimates. Beta diversity estimates are based on weighted UniFrac distances and Bray-Curtis dissimilarity indices(Sinclair *et al.*, 2015). The diversity noted among different

ecotypes in this work is similar to the results obtained by Stanley *et al*. (2013). Though in their case the flocks were reared under similar conditions. The fact that there are no unique microbiota colonising the gut of chicken is an indication that the inherent gut microbiome fails to colonise the gut.Instead, other microbial communities picked along the value chain are responsible for this observation.

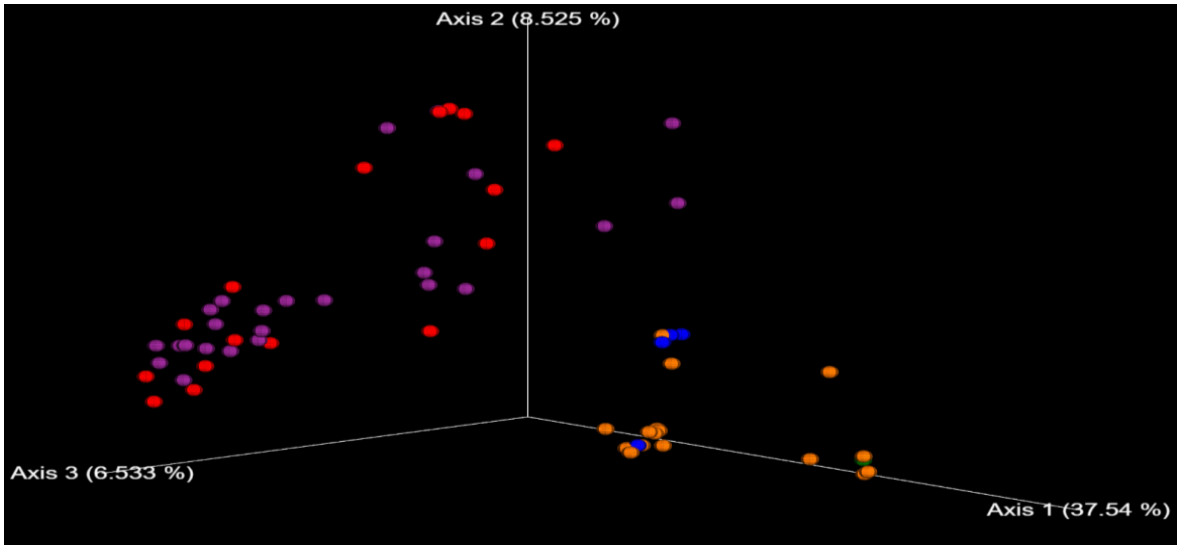


Figure 8.7: Weighted unifrac distance among the different IC ecotypes

8.3.5 Core Diversity Metrics Measurements for Microbial Communities Isolated from IC and Broiler

Weighted unifrac distance- (quantitative measure of community dissimilarity that incorporates phylogenetic relationships between the features) is presented in (Figure 8.7). Beta diversity can be measured by Unweighted UniFrac (qualitative) and weighted UniFrac (quantitative) distances (Lozupone *et al*., 2012). UniFrac calculates distance in genetic diversity based on phylogenetic diversity (tree). Studies using UniFrac shows that Beta diversity is influenced by environmental factors including: temperature, pH and substrate. In our case, Figure 8.8 shows higher diversity in Kakamega followed by Naivasha. The lowest diversity was recorded in the broiler microbes followed by Taita ecotype.

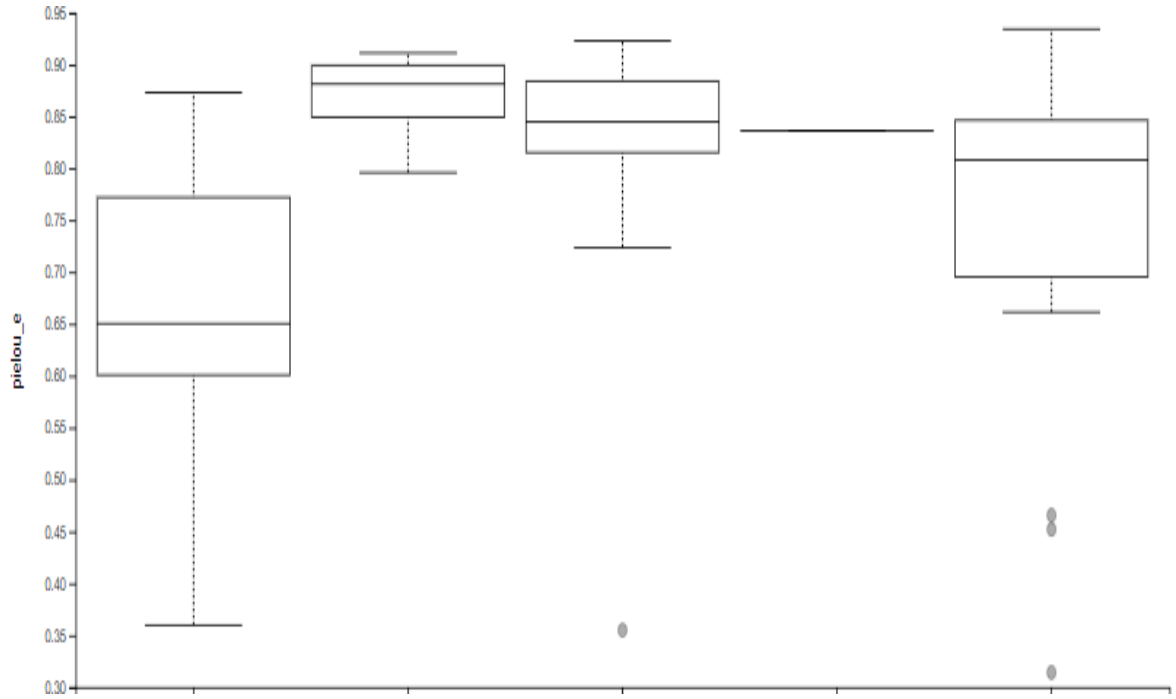


Figure 8.8 rsity] di of chicken

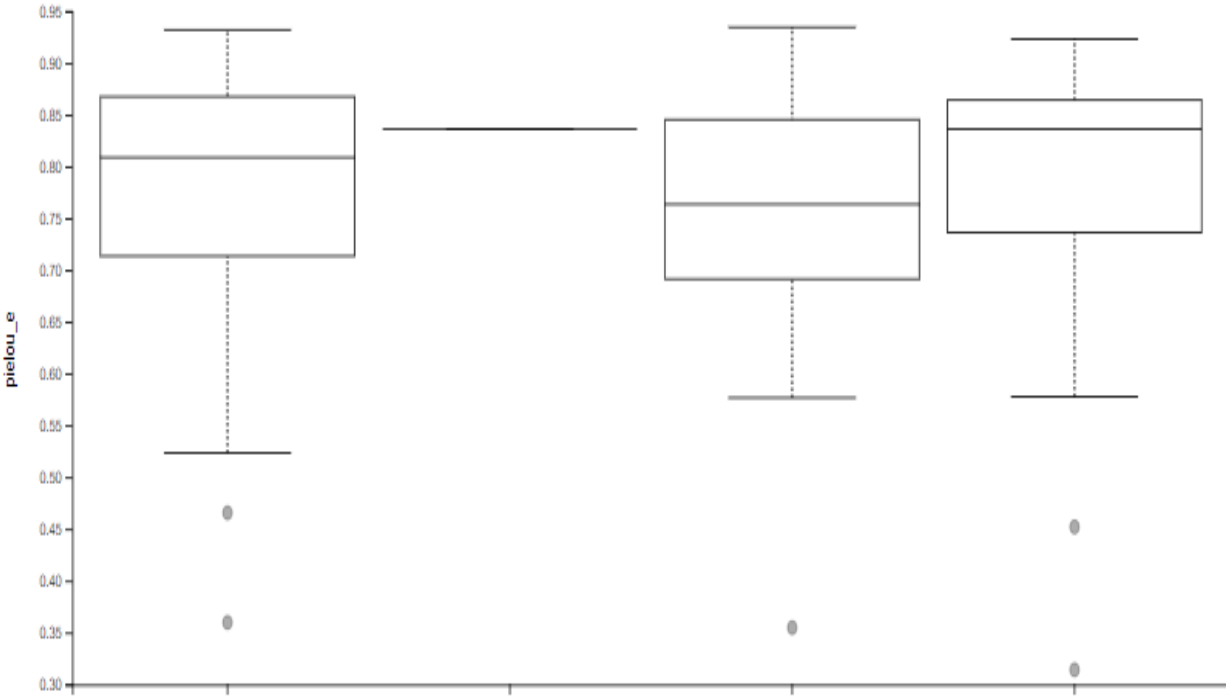


Figure 8.9: Alpha Diversity Box Plot for the different parts of chicken

Alpha diversity box plots show statistical summary of diversity in a single population. Microbial alpha diversity with a box plot exhibiting the community diversity (The Shannon

estimator) is presented in Figure 8.9. The boxes have lines at the lower quartile, median, and upper quartile values (Figure 8.9).

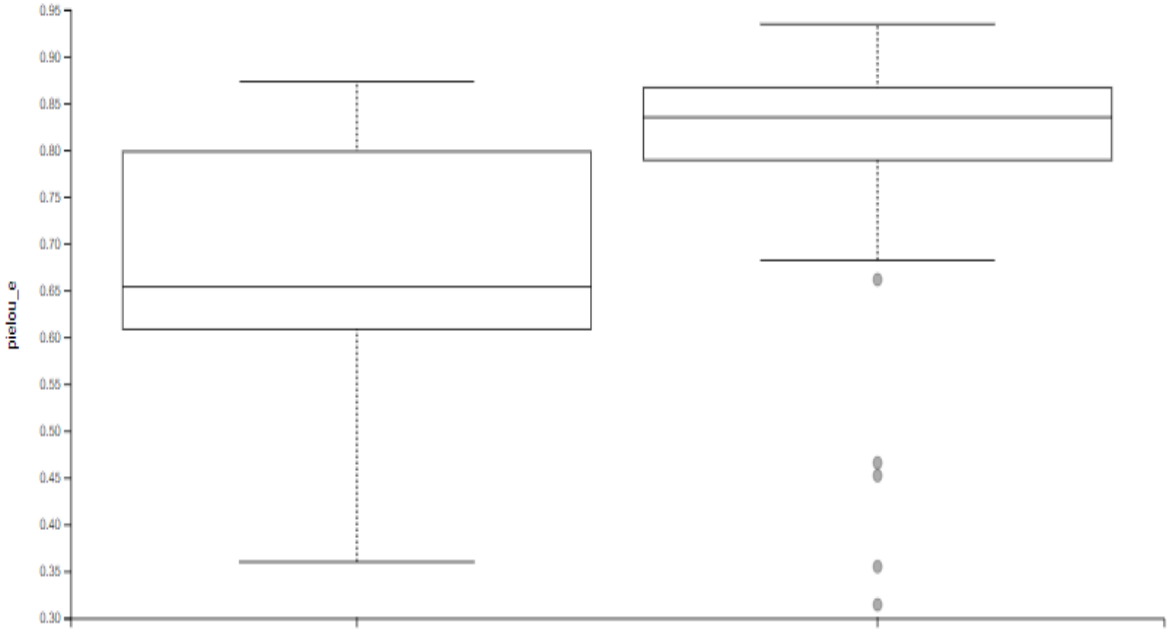


Figure 8.10: Alpha Diversity Box Plot for the different breeds of chicken

Figure 8.10 shows the alpha diversity box plot for the different breeds of chicken while Figure 8.11 shows the Jaccard Emperor plot for the different breeds (indigenous ecotypes and the broiler). There is greater variability in the microbial community in the indigenous chicken than in the broiler. At the same time, it shows that there are more microbial communities that are shared among the indigenous chicken than are within the broiler populations. Most of the OTUs isolated from the broiler are clustered closer together than those from IC which have a wider divergence. Over 80% of bacteria isolated from the broilers belonged to the domain of *k_Bacteria* and this was followed by *Coriobacteriaceae* at 16.5 %. The phyla *Bacteroidetes* was reported at about 5.6 % in some of the samples. The family of *Ruminococcaceae* was also reported at 6.14% and*Provetella* at 1.21%. The order *Streptophyta*and *Alopobium* genus were two OTUs identified at a relative abundance of 1.22 and 0.8%. The *Streptophyta* genus *streptococcus* is known to be capable of causing disease in chicken (Messier *et al*., 1993). Indigenous chicken however had the most dominant bacterial OTU as *Bifidobacterium* at over 80% in some samples. *Bifidobacterium* is always associated with better gut health and antagonism to *Campylobacter jejuni*, an important pathogen associated with chicken(Mookiah

et al., 2014). K_Bacteria domain was still most dominant at about 53% in some samples. The *Coriobacteriaceae* family had a 20.5% andbacteroides at 17% in some of the samples. Some of the OTUs identified belonged to the unassigned group which are either totally novel bacteria or simply a different organisms and possibly viruses. The *Veillenellaceae*family was observed at 2.6 in the samples where their presence was most noticed. In addition there were the *Megasphaera*and *Bacillus* species. The *Bacillusfamily;*particularly the genus *Lactobacillii* have been found to confer antagonistic effect on *Campylobacter jejuni* and *E. Coli* (Duggett, 2015). These gut microbiota act by forming a protective barrier against pathogen bacteria; preventing them from attaching to host cells through competitive exclusion. Some novel isolated OTUs included the *Cyanobacteraeceae* (class YS2) and 0.97% of *Sphaerochaetaceae*and *Mycoplana* at 0.68%, *Staphylococus* family and vadinCA 11, Atopobium at 0.19% and *Mogibacteriaceae*. *Staphylococus* genus is a part of common enteric pathogen with most common potent species being aureus. These enterics can cause loses, reduce chicken welfare, lead to increased mortality, and increased risk of contamination that can lead foodborne illnesses in humans.

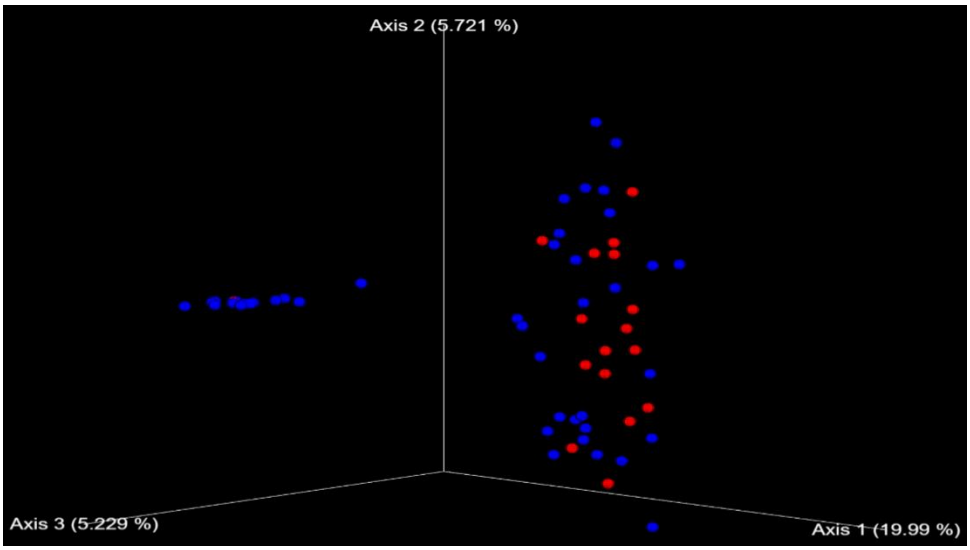


Figure 8.11: Jaccard Emperor plot for the different breeds of chicken

Legend

Broilers

Indigenous chicken

8.3.6 Effect of Sex of IC Chicken on Microbial Community Abundance Compared to Broilers

Appendix F shows the kingdom taxa of all the ecotypes. Only broilers and Taita ecotypes had archaea. This indicates that the feed given to these chickens require them develop their gut microbiome and to accumulate archaea. The archaea genera despite being closely associated with bacteria have unique gene pathways that are closely related to eukaryotes. Uniquely, they possess enzymes useful in translation and transcription. They have been observed to have more energy sources than eukaryotes. For example, they can rely on either lipids on their cell membranes or even on archaeols (Stoeckenius, 1981).

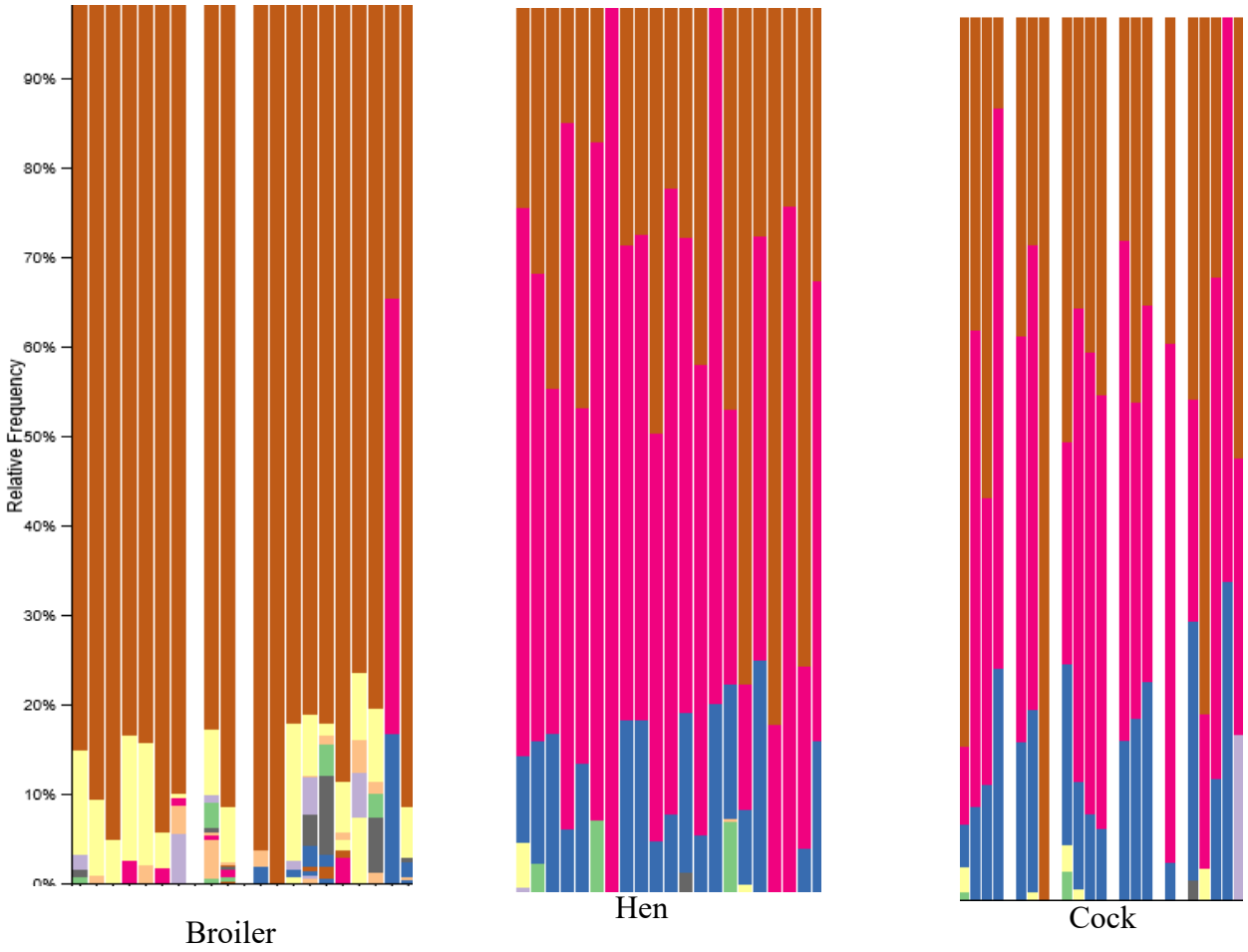


Figure 8.12: Phyla abundance based on sex of the chicken

The archaea utilise and drive energy from a variety of unique sources including organic compounds such as sugars, ammonia, or even metal ions. Their salt tolerant variants utilise sunlight while some use carbon. Their presence in broilers and Taita ecotypes, can indicate a feeding regime that shows adaptation to utilise energy from different sources such as salty chicken matter, or lipid containing foods. Archaea are also methanogens that can produce methane and have been exploited in biogas production (Bang & Schmitz, 2015). Appendix G gives the taxa at level 2 for all the ecotypes (phyla). The major phyla under bacteria were Actinobacteria, Bacteroidetes, Cyanobacteria, *Firmicutes, Proteobacteria, Synergistetes, TM7, Deferribacteres, Spirochaetas*and *Lentisphaerae* and the unclassified. The phyla of Archaea arerepresented by the *Euryarchaeota family*.

The unassigned OUT represented the rest of the microbiota isolated from all the chicken samples. Siegerstetter *et al*. (2017) observed the phyla *Cyanobacteria, Bacterioidetes, Actinobacteria, Tenericutes, Proteobacteria* and *Firmicutes.* Ding *et al*. (2017) on the other hand found the most abundant phyla in all stages of gut inheritance and establishment were *Proteobacteria, Firmicutes,* and *Bacteroidetes,* followed by *Actinobacteria,Cyanobacteria,* and *Synergistetes.*Our results affirm that these are some of the most abundant microbial species found in gut chicken. The Kenyan indigenous chicken however, had additional unique microbial phyla including the *Deferibacteres, Spirochaetas* and *Lentisphaerae.* An important genus of *Deferibacteres*is the *Mucisplirillum* which has previously been associated with gut of rodents. Its presence in the chicken samples affirms the observation in some of the sample collection households where the chicken were kept in close proximity to domestic pets.

Figure 8.12 shows the taxonomic abundance of IC based on sex and compared to broiler as a control. Cocks are predominantly inhabited by *Bifidobacterium* genus with some samples recording as much as over 52% relative abundance of this genus. The second most abundant group is the k_Bacteria with some samples recording up to 32% of the kingdom of bacteria whose other taxonomic units are not identified based on the Green Genes classifier data base. The *Coribacteriaceae* family followed with about 23% of the relative abundance recorded in some samples. *Provetella* family was also recorded in some samples and going up to 25% in a single sample. *Bacterioides, Bacteroidales* were almost equally abundant at about 11-15 % depending on individual samples. There were some novel groups of microbial OTUs like *Ruminococcaceae, Barnesiellaceae* and the *Methanomas siliicocaceae* (vadinCA11 genus).

Hens also had predominantly abundant OTUs as the *Bifidobacterium* genus with samples having up to 60% relative abundance. This is also followed in abundance by the unidentified bacterial community k_Bacteria. The family of *Coriobacteriaceae* follows closely

with samples recording a relative abundance of 19.67%. The *Bacteroidales* phyla and the unassigned microbial OTUs were next most abundant. The novel *Veillonallaceae* was reported in lower relative abundance with the highest being in a sample at 2.64%. Under this family the genus *Megasphaera* was most abundant at 1.6%. *Provotella* genus and *Porphyromonadaceae* were also recorded. On the other hand, the broiler had the most abundant OTU as the k_Bacteria and *Bacteroidales* with some samples having up to 80%. The next most abundant OTUs was *Bacteroideles* at 15.4 % and next abundant as *Ruminococcaceae* with a sample recording up to 8.9 % of this family. The nature of microbial composition between hens and cocks based on the most abundant OTUs was not different. The *Bifidobacterium* is among few microorganisms that have been used successfully as probiotics in broilers to enhance performance. Other genera that have been used include*; Lactobacillus, streptococcus, Bacillus, Enterococcus, Aspergillus,* and *Saccharomyces* (Bhogoju *et al*., 2018).

The broiler has very low diversity in the top 5 most abundant organisms compared to the indigenous chicken (Figure 8.12). *Pophyromonadacea*_species in this family are commonly associated with a variety of human and animal infections.Such infections include; glucose level regulators and HIV related inflammations. The candidate div-TM7-has never before been isolated relation to human microbiome yet has known-genome size of 705base pairs. The *Privotella*-genus has been associated with plant rich diets. This genus was most abundant in Kakamega ecotype. This indicates the adaptation of the ecotype to utilising plant based diets. The *Asteroplasma* genus which was never annotated yet and neither featured in any community according to encyclopaediaof Life, was reported in a few of the samples. The K-bacteria-representing the unknown and unassigned group of bacteria present an opportunity for continued exploitation of bacteria species that may be useful in the long run. The *Bifidobacterium* family was present in abundance in the IC microbiome. The *Bifidobacterium* has been exploited asprobiotics. They have been used after antibiotic for treat of bowel syndrome. The other novel family reported was that of *Cytophagacea.* Thisfamily candigest indigestible cellulose by use of glycil hydrolases. This ability becomes vital especially for chicken that are reared on free range and on limited green matter such as the Taita Ecotype.

8.4.7 Effect of Node of Value Chain on Microbial Communities Isolated from IC and Broilers

Figure 8.13 shows relative taxa abundance based on the node of the value chain (market or farm). The Bifidobacterium was most abundant OUT isolated from the farm while the market is dominated by k_Bacteria-. A novel and rare OTU (*Asteroleplasma*) was also isolated

at the farm with a sample reportingit at a frequency of 8.57%. In the marketing centres, *Rikenellaceae* at 0.67 frequency and the genus *Coprobacillus, Barnesiellaceae and Desulfovibrio* were some of the novel bacterial OTUs isolated. Assembling of reads accruing from 16S rRNA studies require complete or near complete recovery. This is usually because unassembled reads in metagenomic data usually lack usable phylogenetic signal (Yuan *et al*., 2015). The large frequency of the unrecognised set of microbial communities registered in all the samples may be a result of this phenomena. But it is also possible that these present unknown microbes that should be further explored.

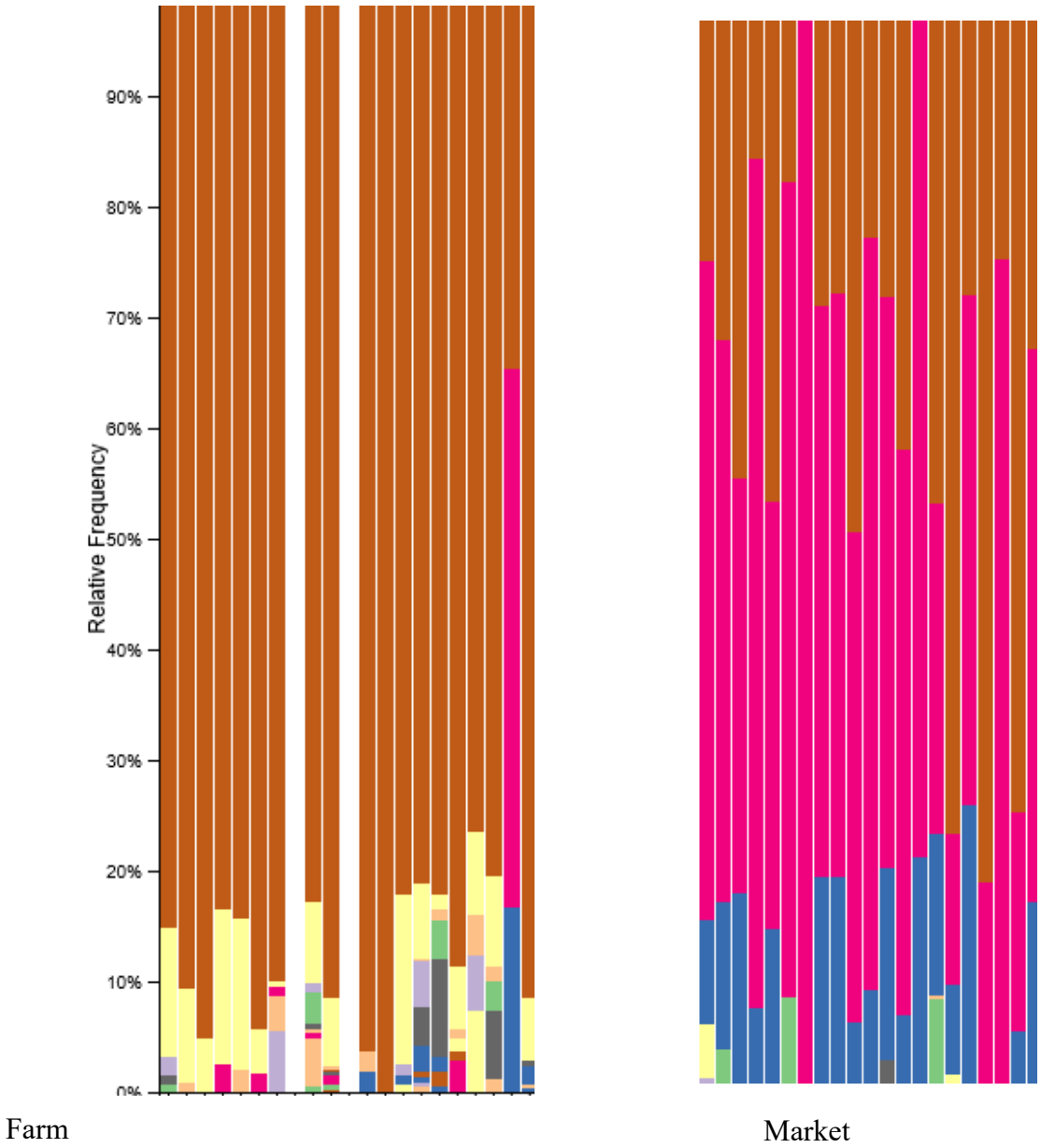


Figure 8.13: Phyla abundance based on node of value chain

8.3.8 Effect of Ecotype on Microbial Community Abundance

Figure 8.14 shows the microbial community abundance for every ecotype of indigenous chicken. The unknown bacterial domain dominated the type of microbial community in broilers with samples showing up to 80% of these bacterial OTUs. This was followed in abundance at a distance by the *Bacteriodes* which was recorded at about 11% and *Cyanobacterium* at 5.4 %. *Streptophyta,* and *Ruminococcacea* were reported at 8.9% in just one of the samples. As for the Kakamega ecotype, the *Bifidobacterium* was the most abundant genus at 52 % in most of the samples.

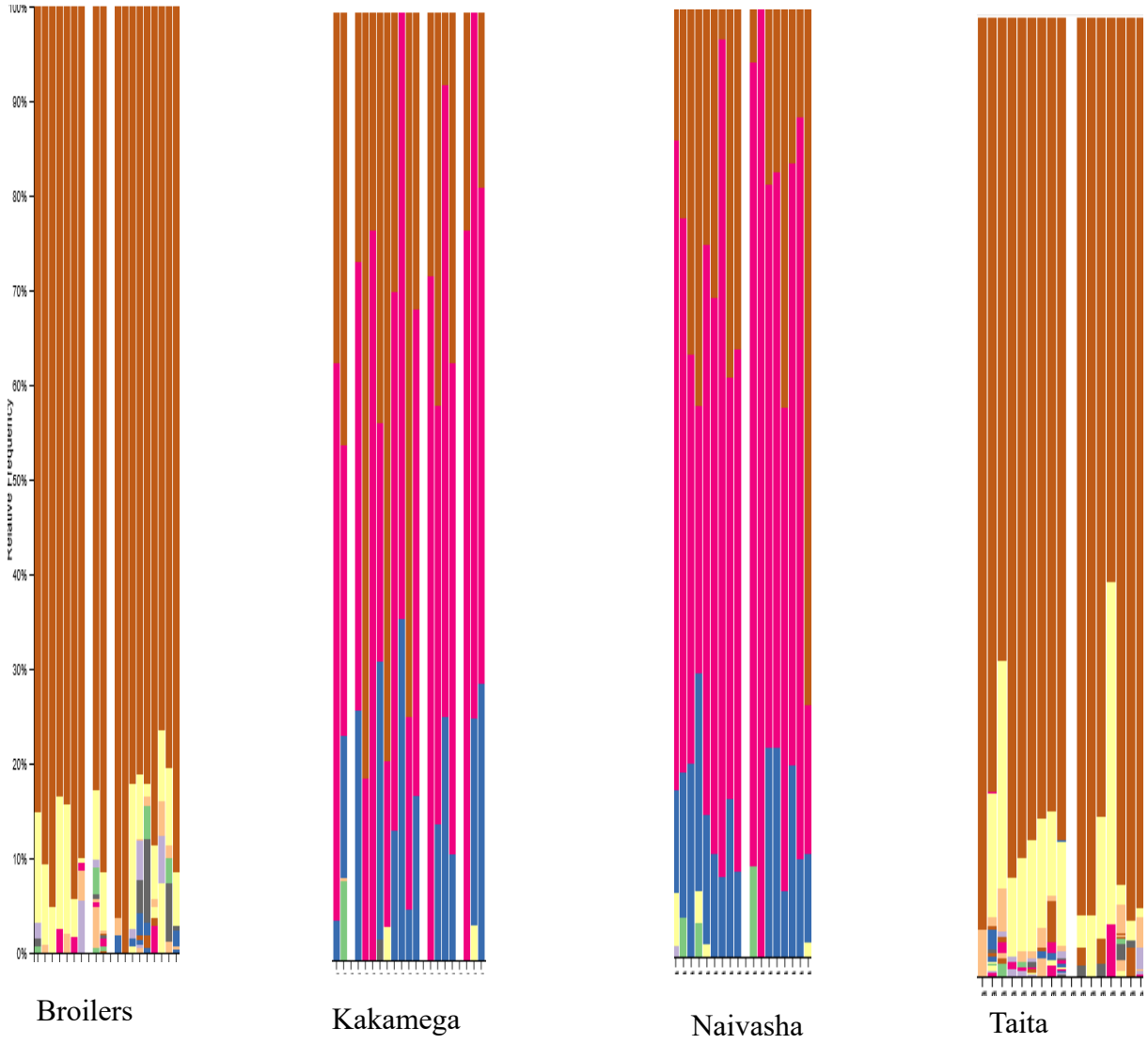


Figure 8.14: Phyla abundance based on Ecotype of chicken

The unknown (unclassified) bacteria domain (k_Bacteria) was second most abundant at 32 % and *Coriobactericeae* was third at relative abundance about 20.5%. There was also an unassigned OTUs with a relative abundance of about 8% in some of the samples of this ecotype. The *Prevotella*genus of bacteria was also present in some of samples of Kakamega ecotype, reporting up to 2.7%. Naivasha ecotype samples were also dominated by *Bifidobacterium e*ven though at a lower percentage than that reported for the Kakamega ecotype. The k_Bactereria was reported at about 29.68% and *Coriobacteriaceae* followed with relative abundance of 17.9%. The unassigned OTU was recorded at 8.53% of relative abundance. *Bacteriodale* was observed OTU at relative abundance of about 2.8%. A novel OTU *Parabacteroides* was also isolated in some of the samples at only 1.92 % relative abundance. With regard to Taita ecotype, the *k_Bacteria* was recorded at 60% while that of *Bacteroides* was reported at 7.8%. The *Bacteroidales* was reported at 3.83% while *Porphyromonadaceae* was reported at 4.87% in some few samples. Taita ecotype recorded the *Methanomassiliicoccaceae-vandin CA11 genus.*Studies have affirmed that the caecum had the most diverse set of microbial communities (Duggett, 2015). They also found out that *the Ruminococcaceae, Lachnospiraceae,* and *Alistipes* were isolated from the gut microbiome. The gut of poultry is resident to a wide range of microbial communities with differing functions and contribution to poultry health and physiology (Fuller & Perdigon, 2003; Parker *et al*., 2007). A majority of species which have never been previously isolated by culturing technologies are uncovered using HT-NGS technologies only. Bjerrum *et al*. (2006) also observed that 85% of 557 cloned were not closely related to those previously identified by culturing methods.


Legend
k__Bacteria;__;__;__;__;__;__
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Bifidobacteriales;f__Bifidobacteriaceae;g__Bifidobac
k__Bacteria;p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacteriaceae;__
k__Bacteria;p__Bacteroidetes;__;__;__;__
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;__;__
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides
Unassigned;__;__;__;__;__
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__;g__
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Porphyromonadaceae;__
k__Archaea;p__Euryarchaeota;c__Thermoplasmata;o__E2;f__[Methanomassiliicoccaceae];g__vadinCA1
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Legen
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k__Bacteria;p__Cyanobacteria;__;__;__;__
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;__;__
k__Archaea;p__Euryarchaeota;c__Methanomicrobia;o__Methanomicrobiales;f__Methanocorpusculaceae;g__Methanocorpusculum

8.4.9 Selected Novel and Significant Microbial Communities Isolated from Kenyan IC and Broilers

Table 8.2 shows a select number of some unique microbial communities recovered from the IC and broiler samples. It also describes the significance of these bacteria in the human and or poultry gut ecosystem. A bacteria family associated with abdominal sepsis; *Parabacteroides* was isolated from the chicken at the farm. There are also those bacteria whose presence suggest adaptation to plant-based feeds such as greens. This may suggest that the environment and hereditary adaptation of chicken play a key role in the microbial communities that reside in the gut (Thibodeau *et al*., 2015).

Table 8.2: Summary of select novel/pathogenic microbes from Kenyan IC Ecotypes

Identity	Ecotype	Node of value chain	Part of chicken	Significance to human and chicken health
Bifidobacterium	Both Kakamega and Naivasha	Most abundant in the farm	Caecum	Prevent erosion of 'good' bacteria due to antibiotics Probiotics. Control of diarrhoea and bowel syndrome
Parabacteroides	--	Farm	--	Abdominal sepsis clinical specimens
Prevotellacea	Kakamega	Bulking point	Caecum followed by breast	Rich in plant diets: beneficial-tolerance to glucose fluctuations-but inflammation associated in HIV
Candidate div TM7-candidatus saccaribacteria	Kakamega	Only in the farm	Thigh and breast mostly	New phyla-never grown in the lab-Full genome-705 (parasitic). Relation to human health yet fully established
Dyadobacter		Both farm and bulking points	--	Remediating soil contaminated with crude oil,
**K-Bacterium*	Both Kakamega and Naivasha	Relatively highest at farm	Highest in breast	Potential gold mine for microbes of novel importance or pathogens?

8.5 Conclusion

Indigenous chicken microbiome consists of novel bacteria that can be associated with improved health, better performance, antagonism to pathogenic microbes, and improved food intake. The node of value chain, IC ecotype and part of chicken does not significantly determine microbiome abundance. However, there is a marked difference in microbial abundance and diversity between the broiler and indigenous chicken. Microbiome of IC (Naivasha and Kakamega, are clustered together) while that from Taita clusters closer to the broiler microbiome. Up to the data already analysed, no strains of pathogenic bacteria were identified in the samples and this may suggest that CCP at farm should potentially be set to address other hazards other than microbial. Indigenous chickens are rich in microbial communities most of which are beneficial. Through HT-NGS technologies some bacteria that have never been cultured were identified including: TM7 and *Prevotella.* The huge abundance of *Prevotella* especially in Kakamega ecotype indicates these chickens' gut adaptation to utilising plant-based material. It was observed that plant-based materials are a regular part of these chickens' diet as they are majorly reared under free range system and few mixed systems. Further work to reveal the connection between some of the novel bacteria particularly pathogenic and their association with public health data will be a good step in helping to tackle any future foodborne outbreaks that may be caused by these microorganisms.

CHAPTER NINE

GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

9.1 General Discussion

9.1.1 Introduction

Poultry farming in Kenya is dominated by indigenous chicken (IC) which accounts for 76 % of the total poultry population (NAFIS-National Farmers Information Services, 2018). Studies show that many consumers prefer the IC to broiler for their perceived superior taste flavour and texture (Chepkemoi *et al.*, 2017). Commercial processing of poultry in Kenya is mostly dominated by broiler processors. This means that in spite of the high demands for IC, access to processed indigenous chicken remain a challenge to consumers especially in urban centres. To address this matter, slaughter houses are currently being constructed in several counties to avail dressed indigenous chicken to urban dwellers. To fully benefit from this initiative, the safety and quality of chicken dressed in these slaughterhouses must be addressed. This requires an understanding of prevalence of pathogenic microorganisms in slaughter houses. These enteric pathogens should be targeted eliminated or reduced to ensure safety of indigenous chicken produced from the slaughter house.

The International Commission on the Microbial Safety of Foods (ICMSF) developed and promoted HACCP system to address this situation (InternationalCommission on Microbiological Specifications for Food, 2006). HACCP system lays great emphasis on prevention of hazards.It combines this with detection of hazards at the steps in the food chain where the food safety problem is most likely to occur. The advantage of running a HACCP plan is the fact that, even in the event control is lost at a CCP, it will still be detected in time and appropriate corrective action instituted to prevent unsafe food from reaching the consumer (InternationalCommission on Microbiological Specifications for Food, 2006).

A wide spread adoption of the HACCP processing system will enhance the consumer confidence in the IC products and reduce barriers to international trade. HACCP has been described as the most cost-effective management tool for producing the safest food possible with the existing technology. A complete HACCP plan minimises quality problems hence maintaining consumer confidence while protecting the business. The establishment of a HACCP plan is also very educative since the personnel must participate in drawing out the plan. They have an in depth understanding of the limitations of the facility, equipment, people, and other relevant factors. An effective HACCP plan must begin from the farm because some

safety concerns may not be adequately dealt with at or beyond the slaughtering stage. All this focus aims in ensuring quality of IC for consumer preferences (Riswadkar, 2000).

In describing poultry meat quality; four factors are of great importance viz: technological quality (presented in Chapter 5), sensorial quality (presented in Chapter 6), nutritional quality (presented in chapter 7) and safety-hygienic quality (presented in Chapters 4 and 8). Safety is most affected by of handling and hygienic practices along the value chain of the chicken products (Lindblad *et al*., 2006). The technological/functional quality, nutritional and sensorial qualities depend heavily on the molecular structures and composition of the chicken muscle. This was discussed in Chapter 5. Chicken muscles consist of water, lipids, proteins, minerals, connective tissues to a large extent. Water/moisture content determine mostly functional/technological or processability quality of properties of meat as well as sensorial quality. The lipids are very crucial in conferring processability safety and shelf-life functions to chicken meat. In combination with protein, they determine the nutritional properties of chicken meat. The most important constituent of poultry meat with regard to nutrition is the protein particularly their digestibility, and their amino acid profiles as discussed in Chapter 5 and Chapter 7.

9.1.2 Determination of IC Quality in Relation to Ecotype Clusters

The overall aim of this study was to contribute to food safety and quality of IC meat processing for improved productivity, profitability, and better livelihoods to ensure reliable supply of safe IC meat that satisfies a wider market. To do this, the study aimed at conducting an overall survey of the quality and safety of the processing facility in the Nakuru municipality to evaluate prevalence and occurrence of pathogenic organisms. The microbial quality and load of IC from different body parts was equally determined and reported. At the same time, it instituted a HACCP design to realise the critical control points and the critical limits that ought to be observed to ensure safety of IC processed at the slaughterhouse. The aim was to identify CCP along the IC processing line that would be easy, to manage, and monitor and easy to implement based on the knowledge and experience of the stakeholders and backed with training.

With regard to quality, the study looked at the sensory, microbial, nutritional and technological quality aspects of indigenous chicken and compared that to the quality of the broiler meat. To avoid the incessant fluctuations, based on the different raring methods, the sensory quality was determined on IC reared under intensive system and at approximately same

age and feeding regimen. With regard to technological quality, the physical parameters including proximate analysis were determined on the major parts of significance to the consumers and that is the thigh and the breast of IC. Various parameters including texture, colour, and protein distribution among others were determined and results presented.Since nutritional quality is very vital especially for IC which act as a source of amino acids, these were determined. The amino acid profile and fatty acid was done on breast and thigh for all the 3 different ecotypes under study. To explore the microbiome of IC and their abundance as well as to identify novel microorganism that inhabit the gut of the IC, metagenomics based on 16s rRNA was conducted forIC and compared to that of broiler. The caecum part of GIT was targeted as studies have shown this to have the highest concentration and diversity of the microbial in the gut system (Mookiah *et al*., 2014).

9.1.3 Designing HACCP Systems for IC Processing in Kenya

There is urgency to reverse the decline witnessed in quality of processed IC slaughter houses by instituting HACCP plans that act to safeguard the IC meat from would be contaminants and guarantee safety-and quality of IC in Kenya. To increase value for IC farmers and open IC markets wider than is currently observed, functional HACCP plans is an important necessity. The results of the survey of the Nakuru slaughter house premise revealed a process that allows for high prevalence of microbial communities especially *Salmonella, Staphylococcus aureus* and even *E.coli.* Though this prevalence may not directly contribute to unsafe food because of subsequent steps of cooking, salting, marinating or even spicing, the prevalence warranted an intervention. With regard to HACCP development, typical HACCP plans for chicken usually involve more than 6-8 at times 10 CCPs. Such a large number of CCPs though vital to ensuring food safety, may prove burdensome. This is especially so in the scenario under study where majority of the players had no formal training in food safety. Alsothe service providers such as transporters among others were not necessarily regulated with regard to food safety parameters.

The HACCP study resulted in creation of 4 CCPs only and how to monitor them to ensure that the chicken produced under this system were of safety standards meeting the national and international regulations. One of the major limitations of this study was the inability to implement this HACCP plan in the premise due to lack of buy in and resources allocation from the county. However, based on the researchers experience and the design of HACCP system, there is a very high likelihood of this designed HACCP contributing to

safeguard the safety and quality of IC and remove or reduce to manageable levels all the hazards identified during the study. A concerted effort among all stakeholders to ensure that all the people involved are enlightened and brought on board is necessary. An initial investment especially in availing the basic pre-requisite requirements such as water, hygienic premises, working surfaces and other infrastructures should be done either by County/National government cooperation or by public private sector partnerships and inclusive of international institutions such as United Nations Industrial Development Organisation (UNIDO). This is a chance that remains open for implementation of this HACCP plan and its adoption or a similar approach to similar slaughter houses across the country and in the region.

9.1.4 Making HACCP System Work in Kenya

A key challenge to the implementation of HACCP systems in developing countries is quantifying value of HACCP systems in monetary terms. This is mainly because quality management related costs are often indirectly relatable to the increase in quantity of material but rather in the increase in quality. Furthermore, in some cases, whereas the quality production costs more to the producers, the consumers who do not necessarily appreciate the extra effort leaves processors discouraged to continue to invest in producing quality (Oloo & Oniang'o, 2018). Until recently, there were limited tools to monitor progress and even the parameters to monitor as key performance indicators with regard to quality improvements. This has changed and a number studies now demonstrate monetary gains resulting from implementation of HACCP. The gains more often than not come from increased efficiency that comes with the system, repeat customers who enjoy quality, participation in high niche markets among other indirect benefits (Romano *et al*., 2005). Apart from the direct financial gains that come from use of HACCP systems, studies now demonstrate high magnitudes of financial losses and consequences that may result from outbreak of foodborne diseases and illnesses that result from neglect or lax in the implementation and adherence to food safety management systems (Hoffmann & Baral, 2019).

The study by WHO in which they estimated the global burden of disease is the first one of its kind. It did not just estimate the cost of foodborne disease burden as expressed in medical bills for hospitals but more so in the context of the Disability-adjusted life years (DALYS), which is a measure of overall disease burden, expressed as the number of years lost due to ill-health, disability or early death. The report by the World Bank group, 2019 affirmed this and expounded on the estimated average DALYS particularly in the developing countries (middle-

and low-income economies). In their publication of the *Safe food imperative'* the group stated that 'Unsafe food costs low- and middle-income economies US$ 110 billion in lost productivity and medical expenses each year (Jaffee *et al*., 2019).

The recently estimated cost of Listeriosis outbreak in South Africa, from 2017-2018 is a clear relatable case (Hoshide, 2019). Making HACCP systems work in the Kenyan context is to have an approach that does not just appeal to the safety aspects but also financial. The approach must include a demonstration of measurable promise of implementation of HACCP system especially to the financially inclined or Agri-business entrepreneurs working in this space. The regulatory top-down approach that has been implemented in several countries has not worked well. In fact, based on my experience with this study, it is the one reason why quality and safety systems fail (Oloo & Oniang'o, 2018). This because businesses aim to do things right only in the eyes of the regulator. What they do beyond the scenes some that boarder on food fraud that could even lead to food intoxication and infection are usually only known to them. The second hurdle to making HACCP systems work is inadequate infrastructure that is pre-requisite to implementation of HACCP system. From the results in Chapter 4, the slaughter house was reported to have inadequate water storage facility, dilapidated processing plant, and other items that denote infrastructure. Without the provision of the basic infrastructural platform, the process of implementing HACCP systems become too burdensome on the Small and medium Enterprises (SMEs) who have to invest in infrastructuresfirst that support HACCP implementation. There should be government effort to provide the needed infrastructure and where this is not sufficient, engagement of Public private partnerships (PPPs) should be invoked to provide necessary infrastructure. This should not be a difficult job if only both parties begin by appreciating the massive benefits that assuring food safety brings to the development of the nation (Romano *et al*., 2005).

Participatory innovations to the HACCP plan are the third aspect which is necessary to make HACCP work in our context. Whereas in almost all poultry HACCP plans indicate the farm as a CCP and where the target hazard is often microbial, this CCP seems unnecessary for the IC. The assumption is usually that because of the way that IC feed through scavenging that this exposes them to pathogenic microorganisms that are potential hazards. Whereas this assumption may be true, the results presented in Chapter 8 of this work casts doubts on this long-held belief. The results of the chicken microbiome from the gut simply demonstrates, a composition of GIT microbiome that is self-regulating. In essence healthy IC gut exhibits novel bacterial communities that are involved in many functions such as better feed utilisation,

improved gut health through production of bacteriocins, among others. Based on these findings, whereas there could be a hazard and a CCP control at the farm, was most likely to be chemical rather than microbiological. Even then, only limited studies have been done in Kenya to either refute or substantiate this claim. This why the only way to make HACCP work in the Kenyan IC context is to innovate based on sound science.This exercise should involve coming up with manageable HACCP plans that are unique and customised to a specific business.

9. 1.5 Linking the IC sensory quality to ecotype characteristics and its applications

In Chapter 6, the quantitative descriptive Analysis (QDA) and the Just About Right (JAR) technique was used to evaluate the sensory parameters of IC. Sensory analysis based on QDA methodology gives results comparable to the machine parameters for sensorial studies. The study revealed that colour and texture were the two most important quality that distinguished between the broiler and the IC and also among the IC themselves. Colour, texture and flavour are the most important features that consumers use for deciding or marking purchasing decisions (Chepkemoi *et al*., 2017). This study reveals for the first time that the ecotypes of IC, portray different measures of these and could be used as a tool for market differentiation and price determination. These parameters were also significantly different from the broiler. The JAR scores were proven to be very critical for determining preference of IC compared to the broilers. The results revealed that the trained panel, expressed the values of IC sensory attributes as just about right as opposed to the broilers which were to either extremes based on the parameter. Value proposition is a critical factor in spurring agribusiness development of IC in Kenya. This information regarding correlation of ecotypes and the sensory quality should be exploited as a value proposition that can allow farmers fetch premium prices and open up wider markets inclusive of export. However, to do this, there must be an integrated system with HACCP as developed that assures consumers of the safety of the sold chicken.

9.1.6 Nutritional Quality and Metagenomics Frontiers in IC Meat Production

Chicken meat has been hailed as being nutritionally superior to other sources of animal and plant proteins. In recent past accompanying lifestyle diseases associated with saturated fatty acid consumption from chicken especially the first growing broilers have been reported. This can be a counterproductive phenomenon in proven for IC. This necessitates that an analysis of fatty acid profiles among IC ecotypes be established to relay more information to consumers and processors on the quality of IC in this regard or to improve IC meat.Fatty acid

and amino acid profiles were least varied among the IC. However, there was high quantity of Glutamic acid an amino acid that is associated with the flavour of IC and this result suggested the reason for better JAR scores as opposed to the broilers. The values of the omega 6 and omega 3 ratio was higher (9:1) than the recommended 4:1. This indicated the perceived better nutritional quality of IC compared to that of the broiler. Whereas this realisation is key to advancing healthy eating of IC meat, it also goes to send a warning to farmers and feed producers in ensuring that they provide feed that results in healthier IC meat. This may mean assuring diets rich in MUFA and PUFA that are healthier to the IC and can be translated to consumers as well. The association of free-range rearing and IC from Kakamega ecotype with best ratio of omega 6 and omega 3 is good news that the stakeholders should work together to ensure no erosion happens. Combined with the observed benefits of highest sensory evaluation scores and most diverse OTUs, these results present evidence that should be exploited by the stakeholders to ensure enhanced productivity, enhanced higher value and better livelihoodsfor Western region IC farming community.

The microbiome studies of IC microbiota revealed a very diverse association of microbial communities. Some novel microorganisms that have never been isolated and hence reported through culture techniques were also observed. There was evidence based on the microbiome to suggest that there are many beneficial microorganisms in the gut of IC that aid their adaptability to feeding on grass or other green matter. It was also observed that there are abundant microbial communities that aid in maintaining IC's healthy gut,enhance suppression of pathogens while increasing their protection from diseases. All these major findings are to the best of knowledge not reported before for Kenyan IC. There is an opportunity to invest in a manner that exploits the necessary microbiome and produced in large scale as pro-biotics to be used to improve the adaptation and health of IC and even broilers.

9.1.7 Linking Metagenomics to Food Safety and Quality of IC based on HACCP Principles

The 16s rRNA metagenomics utilises specific primers that target the 9 hypervariable regions for bacteria. The resulting reads are then amplified through PCR and blasted in data base (green genes) for OUT identification and characterisation at 97% similarity index project (Gilbert *et al*., 2014). As opposed to short gun metagenomics which quantifies all DNA from sample including the host; the 16s rRNA reduces possibilities of the dangers of masking of microbiome DNA with the large amounts of host DNA. The results of the metagenomic study

identified novel bacteria especially at the farm node of the IC value chain. Some of the microorganisms such as: *Bifidobacterium, Provetella*, are highly beneficial to healthy gut of chicken. The microorganisms have been known to suppress the pathogenic microorganisms such as *Campylobacter jejuni and caecal E.coli* (Mookiah *et al*., 2014).

Feeding regimes that increases colonisation of these microorganisms and reduction of antibiotic use can be a novel way that does not just suppress pathogens but allow for better utilisation of feed, healthier and gut. Evidently, where the chicken has a healthy set of microbiomes as was the case of IC under study, the microbial hazards at the farm level is greatlyreduced. For this reason, this study identified the hazard as chemical rather than microbial. That is why the CCP at the farm was designed for control of chemical hazards. The knowledge of microbiome flux can therefore be utilised in ensuring that feeding of chicken exploits prebiotics and probiotics. This will lead to reductionof diseases, and improve IC health for better safety.

9.1.8 Take-home Message to Farmers, Policy Makers, Food Processors, and Consumers

This section forms the basis of a policy brief that has been drafted for dissemination of finding to most relevant stakeholders. To assure safety of IC from 'Farm to Fork' the role farmers cannot be overemphasised. Farmers ought to observe best practice on feeding and comply with treatment regimens and withdrawal period of antibiotics used for IC. In liaison with extension officers, the knowledge of microbiome of IC gut and how providing a healthy chicken gut supports resulting product safety should be emphasised. This inclination will prove more affordable, result in healthier poultry heard, and IC farming sustainability.

The policy recommendations from this study is that provision of safety and high-quality IC is attached to the hygienic conditions and handling during and after processing. Infrastructural investments to ensure that slaughter houses operate under GMPs is the first step toward full exploitation of the benefits of HACCP programs. The county Governments, Public, Private Partnerships (PPPs) should engage to shoulder this burden. The benefit will be that farmer communitieswill reduce costs of assuring safety. This will translate into farmers and traders optimising their profits.

For food processors, they have a vital role ensuring hygienic quality of meat that can be achieved through successful HACCP implementation. This is because the most important microbial contamination in poultry occurs at this node. In addition, the important relationship between the technological quality (protein functionality) and sensorial quality provides insights

into capacity to develop new products or conduct product branding and differentiation. For example, the protein digestibility, omega 6 to omega 3 ratios are linked to the superior quality of IC from Kakamega ecotype. This knowledge can be used by stakeholders to market the products to high value markets thus fetching better prices for improved livelihoods. This study showed that presence of an intense brown colour is almost in all cases related to lower degree of meat tenderness, lower flavour intensity, and less juiciness. Since meat colour is an easier attribute to assess, this attribute may be very helpful to manufactures and consumers in making decisions about the suitability of a given poultry product for a given process or its acceptability. Principal Component Analysis offers an effective approach for determining the most important sensory factors and can help producers in product development. Furthermore, our study shows that Taita ecotype can be targeted by consumers who prefer leaner meat even though they may have less flavour intensity. On the other hand, Kakamega ecotype presents a juicier and more flavour intense IC for those consumers who prefer these.Finally, consumers should continue to put a demand on high quality and safe IC. They should therefore seek to get their produce from slaughter housespracticing GMP and where possible HACCP certified. Consumers must also adhere to the handling and cooking instructions offered by manufacturers to avoid any microbial contamination from IC products.

9.2 General Conclusions

i. Prevalence of IC in Kenyan slaughter house as presented in the current study was falling into the unhygienic conditions.The application of HACCP is an appropriate intervention to address the food safety especially where the food is taken far beyond the immediate utilisation of the consumers. Where the slaughter house supplies individuals as was the current case, proper labelling ‘keep under refrigeration or cook thoroughly’ is a good intervention when additionally printed on the labels.

ii. The Nakuru Top market slaughter house would benefit greatly from implementation of designed system which takes into account appropriate GMPs based on the premise and competence of the personnel at the slaughter house. Whereas the hygienic conditions were deteriorated, the most critical factor was lack of sufficient infrastructural support for operationalisation of the GMPs. When bolstered with the additional implementation of the HACCP system, the CCCPs identified in this study should be sufficient to address the safety issues reported. Its effectiveness is based on ability of the team and personnel

to sufficiently implement it. The other factor is that it has few CCPs and practicable monitoring and corrective action procedures for the IC processors.

iii. With increased demand for healthier meat sources byconsumers, more innovative products aredemanded as is the case in many parts of the world already. Product development is best based on our understanding of the technological quality of IC especially the functionality of protein isolates. The protein quality characteristics of myofibrillar and sarcoplasmic proteins based on SDS PAGE provides this information. The functionality of the protein fractions depends on ecotype and part of chicken and determines some important consumer demanded qualities such as texture and cooking loss. The IC have distinct protein band patterns for the sarcoplasmic and myofibrillar proteins for both the breast and the thighs.

iv. Indigenous chicken is a delicacy in most parts of the world and especially in Kenya. Consumer acceptability indices are dependent on several factors. Among the 3 ecotypes; the JAR and QDA results indicated that the IC are preferred for their scores on aroma flavour and texture which were superior to that of broilers. The attribute of colour was shown to be most distinguishing attribute among different chicken ecotypes and body parts. It is also correlated with texture and the two can be used for modelling of attributes that correlate to consumer acceptability and preference for indigenouschicken meat.

v. In addition to having a great taste IC are seen as being good sources of high-quality nutritious food especially protein. The results show IC have higher values of essential amino acids than the FAO/WHO recommended daily intake. In addition, they provide healthy sources of protein based on their fatty acid profile. Their specific ratio of omega 6 to 3 supportsthat they are a source of leaner and healthier meat.

vi. Metagenomic analysis is important for best understanding of microbial flux at different nodes of IC value chains,different ecotypes, and different parts of chicken. It can also aid pathogens diagnosis. Though the part of chicken and IC ecotype does not determine the microbial abundance, there is a significant difference between diversity and abundance of broiler and IC microbiome. In addition, distinct abundance and clustering patterns of IC microbiome depends on ecotype and relates to the feeding regimen. IC had novel gut microbiomeassociated with disease control, gut health, improved feed utilisation, decreased the caecal Escherichia coli among other benefits.The knowledge of microbial fluxfeeds back in to HACCP based systems. It supported the fact that

microbiome composition of IC gut can be a toolfor pathogenic microbial control at different IC processing steps.

9.3 General Summary and Recommendations for Further Research

We recommend further studies to understand and identify the role and functions of gut microbiome in suppression of pathogens and for use as probiotics and the success of their application. Though this work did not explore the genetic expression of the desirable sensory and quality characteristics, the results can be used in creating markers for enhancing indigenous chicken's genetic production and improvement. Further studies are therefore recommended to explore this possibility.

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APPENDICES

Appendix A: Detailed summary of the product description record

#	Item	*Description*
1	Common name	*Dressed indigenous chicken (Kuku kienyeji)*
2	How is it to be used	Ready to cook carcass and parts
3	Type of packaging	Carcass packed individually or in parts in polythene bags or boxed in bulk.
4	Shelf-life	3-6 months at 0^{o}C or below 4^{o}C for 7days
5	Where will it be sold? Consumers or intended use	Hotels, restaurants, Wholesale to distributors, retailers and to individual consumers.
6	Labelling instruction	Safe food handling labels (keep refrigerated or frozen; cook thoroughly before eating).
7	Is special distribution control needed	Keep refrigerated or frozen.

Appendix B: Raw materials and ingredients Decision Matrix

Materials and Ingredients	Hazard	Class	Q1	Q2	Q3	CCP	Remarks
Raw material (Indigenous chicken)	Pathogen	B	Y	Y	N	Not CCP	Scalding temperatures would kill external pathogens/Chlorinated portable rinse water will keep off pathogens.
	Antibiotics & pesticide residues	C	Y	N		CCP1	Certificate of inspection of chicken by approved/registered government officer.
	Foreign matter	P	Y	Y	N	Not CCP	De-feathering/plucking and physical inspection and personnel hygiene.
Process Water	Chlorine residues	C	N			Not CCP	Chlorine residue test reports.
	Pathogen	B	N			Not CCP	Water testing sample reports and records of water treatment by the county government.
Packaging material	Toxic Ingredients	C	Y	N		CCP 2	Supply of quality packaging material is critical (approved suppliers).
	Foreign matter	P	Y	Y		Not CCP	Physical examination/inspection and personnel hygiene.

Legend: B –Biological; C-Chemical; P-Physical: Y-Yes; N-No; CCP-Critical Control Point

Appendix C: Decision matrix for Process Steps

Process step	Hazard	Class	Q1	Q2	Q2a	Q3	CCP	Remarks
Reception	Pesticide /antibiotics residues	C	Y	Y		Y	CCP 1	Inspection of individual chicken by qualified government veterinary officer followed by issuance of certificate of clearance.
	Pests and rodents	B	Y	Y		N	Modify step	
	Pathogens (E.coli)	B	Y	N	N		Not a CCP	
First Inspection	None							
Holding in cages	*Salmonella/S. aureus*	B	Y	N	Y		Modify step	Only healthy birds are collected and caged. Cleaning and disinfection of cages at least twice a day and records kept
	Dust, soil,	P	Y	Y		N	Modify steps	Cleaning and disinfection of cages at least twice a day and records kept and personal hygiene
	Droppings	P	Y	Y		N	Not CCP	Cleaning and disinfection of cages at least twice a day and records kept
	Foreign matter	P	N					Cleaning and disinfection of cages at least twice a day and records kept

Second Inspection	None							
Slaughter	*Salmonella* and *E.coli*	B	Y	N		N	Not CCP	All workers must take medical examinations from Government health facilities and produce valid *food handlers'* certificate before being allowed to step into the slaughter house.
	Detergent residues	C	Y	N	Y		Modify step	Thoroughly clean rinse and sanitize all knives ready for use in the premise. Keep record of all the knives and the cleaning and rinsing done.
	Blood	P	N				Not a CCP	Observe proper stunning and slaughtering procedure including production of evidence of training of personnel involved.
Bleeding of Carcass	Blood clots in veins hence (pathogenic microorganisms' growth).	B	Y	N	Y		Modify Step	Training of personnel and adherence to proper bleeding procedures and inspected by QAM/QC/veterinarian and records kept.
Scalding	Smoke	P	No				Modify step	Modify step to use electricity and other non-smoke producing sources of energy.
	Peeled skin	P	No					Proper timing and temperature of the scalding water and a monitoring device and records kept.

	Dirt	P	Y	N	Y		Modify Step	Regular change of scalding water and records kept.
De-feathering	*E.coli, Salmonella and Campylobacter*	B	Y	Y		N	Not a CCP	Proper timing and temperature of scalding water (records). Correct procedure and pressure for de-feathering and inspected by the Qc in charge and records kept. Personnel hygiene??
	Damaged skin	B	N				Not a CCP	Correct procedure and pressure for d-feathering and inspected by the Qc in charge and records kept.
Removal of head/limbs	Metal chippings	P	Y	Y	N		Not CCP	Use of stainless-steel knives and inspection of the knives at least twice a day and records kept.
	Detergent residues		Y	Y	N		Not CCP	Training of personnel on proper cleaning & rinsing procedures rinsing records kept.
Evisceration	Pathogens (faecal and ingest contamination from gut breakage). *Intestinal contamination (*E.coli,*	B	Y	N	Y		Not CCP modify the steps	Proper setting of evisceration equipment. Training presentation on evisceration procedure and training records kept. Visual examination of carcass for faecal contamination. Maintenance of correct strength of chlorination for the rinse water.

	Salmonella, Campylobacter)							
Washing	Detergent residues	C	Y	Y	N		Not CCP	Final rinse water strength is crucial.
	Chorine residues	C	Y	Y		N	Not CCP	Determination of chlorine residue on a quarterly basis and records kept.
	Foreign matter (feathers, skin, dirt)	P	N				Not CCP	Visual examination of carcass for any foreign matter and inspection rerecords kept.
Final rinse	Foreign matter	P	N				Not a CCP	Visual exam will ensure no foreign matter goes beyond this process.
	Chlorine residue		Y	N	Y		Modify the process step	
Draining of water	Residual rinse water in veins of chicken	P	No					Training presentation on correct drainage of carcass and inspection of carcass for proper drainage before rereleased.
Labelling		None						

Inspection/vet officer stamping	Ink	P	No				
Packaging material	Toxic material	C	Y	N		CCP3	Possibility of toxic material persists and must be control through HACCP plan.
Display, dispatch	Biological (*E. coli, Salmonella and S. aureus*)		Y	Y	Y	CCP4	Proper chilling of product to prevent proliferation of pathogenic bacteria. Installation of a chilling monitoring equipment (log)

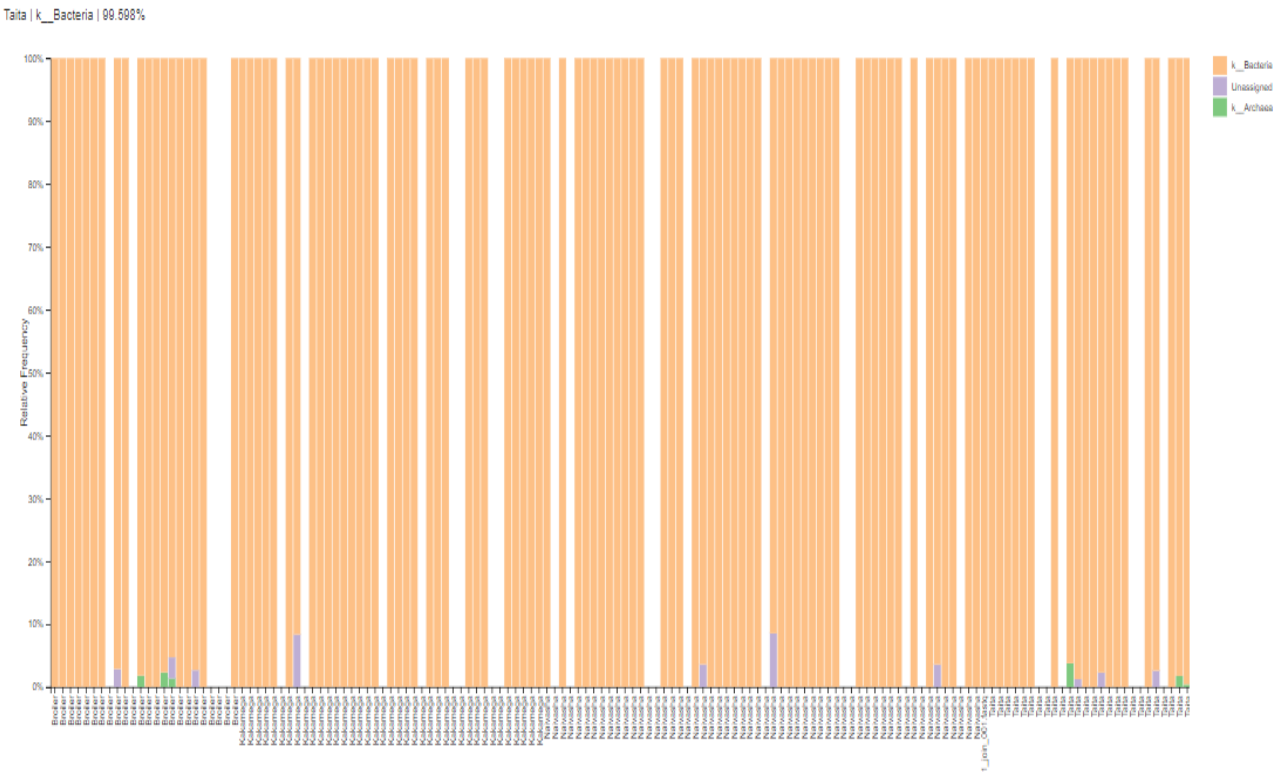
Legend: B –Biological; C-Chemical; P-Physical: Y-Yes; N-No; CCP-Critical Control Point

Appendix D: HACCP Plan for Indigenous Chicken Processed at a Slaughterhouse in Nakuru

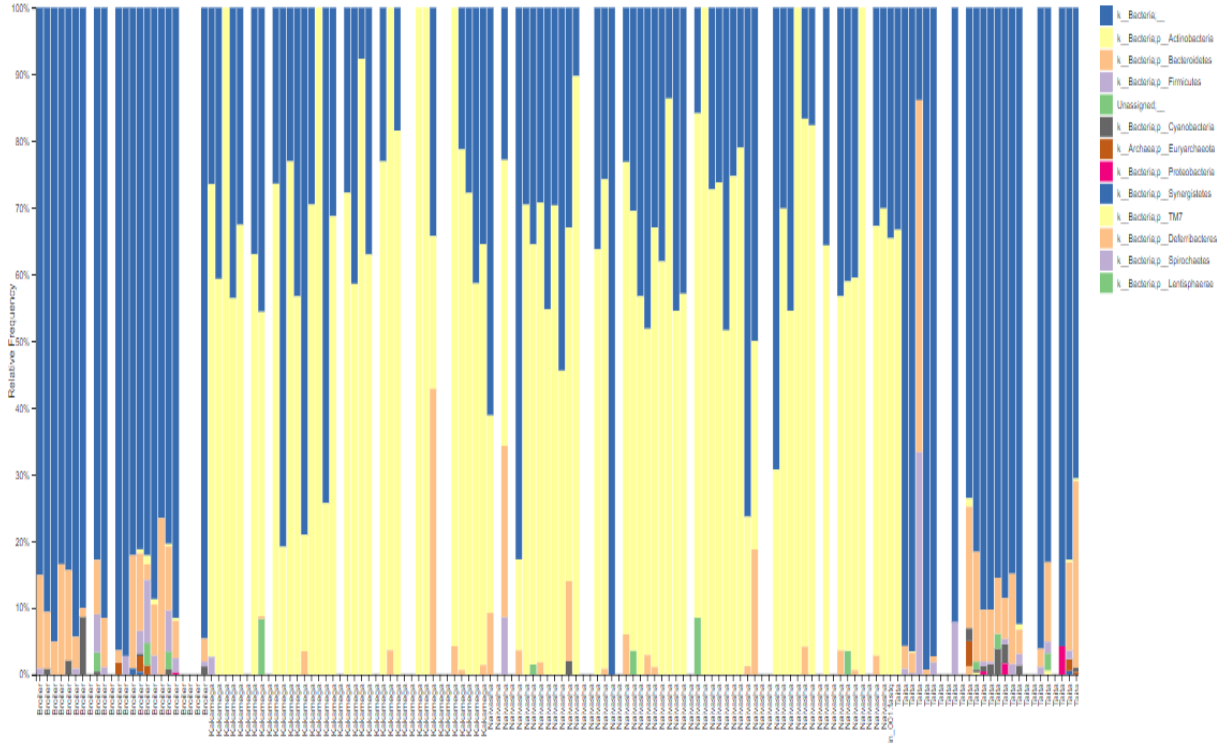
CCP Nos	Process Step;	Hazard	Critical Limits	Monitoring Procedure	Frequency	Preventive measure	Corrective action	Record	Responsible person	Verification Procedures
CCP No 1	Reception	Antibiotic residues	No chicken delivered within recommended days of withdrawal	Inspection of certificate of compliance for every batch signed by an authorized veterinary officer	Every delivery	Withholding the flock awaiting approval of an authorized veterinary officer	Reject the batch if the certificate of compliance is not produced	Certificate of compliance Records	QA officer in charge	Check the certificate of compliance records/get quarterly MRLs reports from accredited laboratories.
CCP2	Final Rinse	Pathogens (*E.coli, Salmonella, Campylobacter*)	Residual chlorine of 50ppm	pH and Chlorine residue measurements using the strips.	After every batch/lot of chicken.	Withhold the carcass,	Re-adjust chlorinator and take samples to confirm full operationalization.	Final rinse/ carcass quality records.	QA/C officer in charge	Verify final rinse water quality records/daily residual chlorine checks/titration and Obtain quarterly chlorine analysis reports.

CCP3	Packaging material	Toxic materials	No unqualified product used	Supplier audits and quality assurance	Each supply	Qualified packaging material supplied and proof of check of MSDS; approved supplier list and specifications agreed.	Change supplier or brand of non-conforming material.	Raw material reception records	QA/C officer in charge	Verify list of qualified suppliers, conduct regular supplier audits, and check MSDS for every material.
CCP4	Display/ Dispatch	Pathogens (*E.coli, Salmonella, Campylobacter*)	≤4^{o}C for 7 days	A calibrated thermometry and a temperature coding log	Internal temperature to reach 4^{o}C in 4hrs after processing	Withhold product, and adjust the temperature to the correct reading for the adequate amount of time.	Freeze product and observe as an internal temperature of 4^{o}C is arrived at	Dispatch records/Final product temperature logs and records	QA/C officer in charge	Review the temperature logs daily/before dispatch. Calibrate thermometer daily. Check and record temp once per batch.

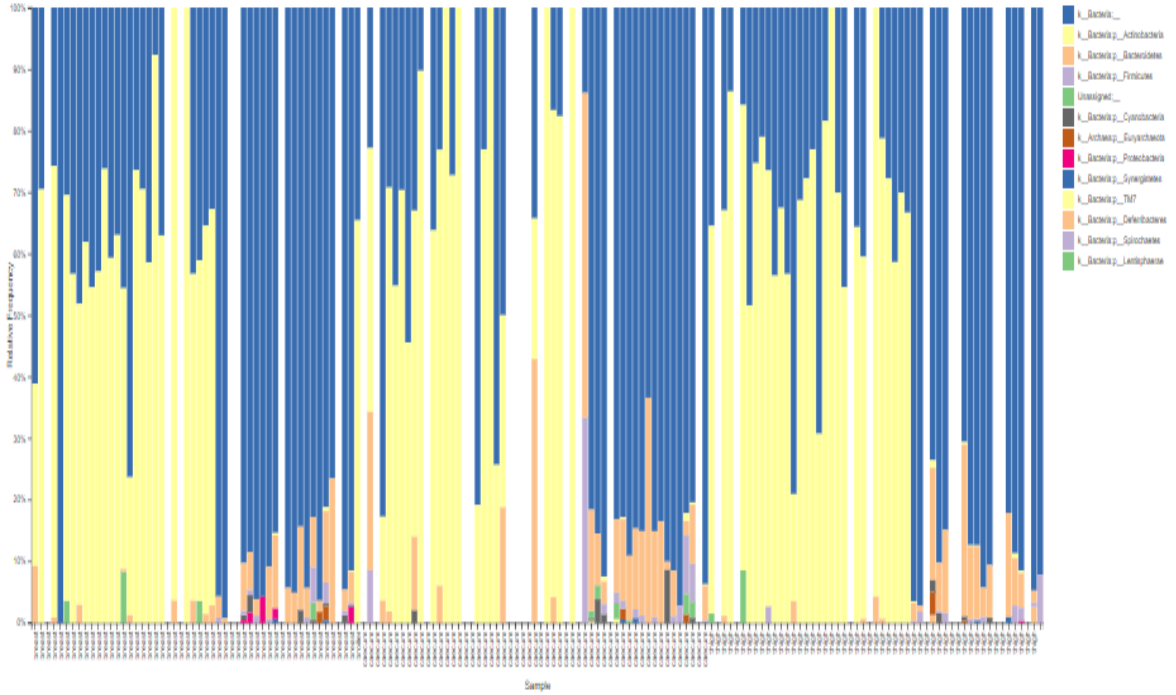
Appendix E: Level1 Kingdom OUT abundance of ecotypes



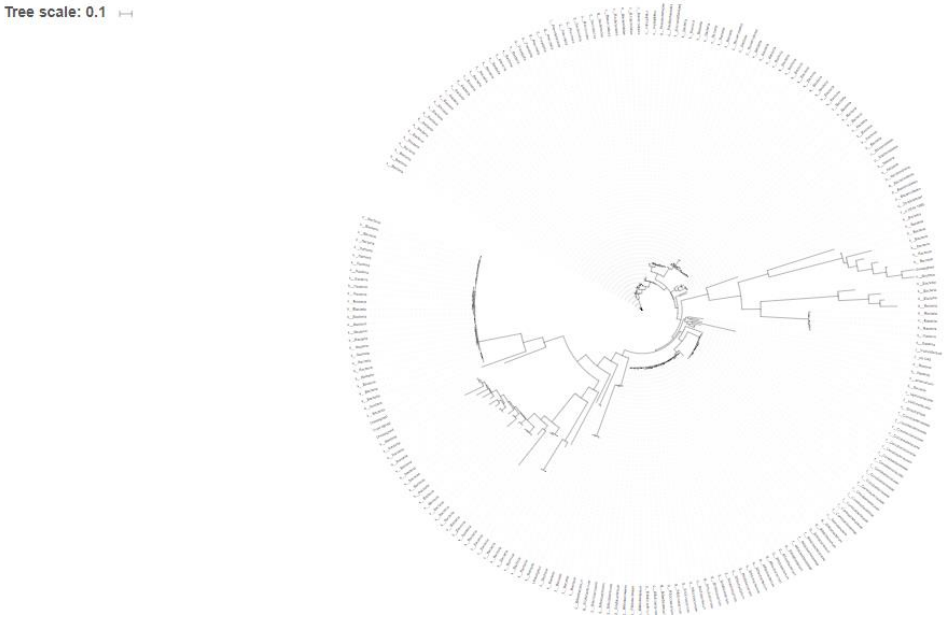
Appendix F: Level 2 Phyla OUT abundance of ecotypes.



Appendix G: Phyla abundance based on different parts of chicken



Appendix H: Tree diagram of OTUs isolated from IC and broiler



Appendix I: Sample data analysis outputs

Hedonic rating

Ecosystem	chicken	part	N Obs	Variable	Mean	Std dev
Narok	IC	Breast	30	Aroma	4.70	1.78
				Juiciness	4.30	1.18
				First bite	4.23	1.61
				Cohesiveness	5.23	1.55
				Impression	4.50	1.50
				Tenderness	4.83	1.53
				Fibrousness	4.93	2.18
				Flavour	5.00	1.64
				Colour	3.43	1.45
		Thigh	30	Aroma	3.43	1.74
				Juiciness	2.93	1.48
				First bite	2.53	1.78
				Cohesiveness	6.77	1.25
				Impression	2.93	1.68
				Tenderness	2.90	1.92
				Fibrousness	2.37	1.79
				Flavour	3.50	1.80
				Colour	7.03	0.72
Taita	IC	Breast	26	Aroma	5.42	1.58
				Juiciness	4.19	1.41
				First bite	4.81	1.30
				Cohesiveness	5.04	1.43
				Impression	4.62	1.60
				Tenderness	4.65	1.35
				Fibrousness	5.27	1.82
				Flavour	5.77	1.37
				Colour	4.12	1.24
		Thigh	26	Aroma	5.00	1.62
				Juiciness	4.19	1.74
				First bite	3.35	1.65
				Cohesiveness	6.12	1.31
				Impression	4.85	1.74
				Tenderness	3.42	1.55
				Fibrousness	2.85	1.59
				Flavour	4.85	1.67
				Colour	7.04	1.18
Broiler	br	Breast	42	Aroma	6.24	1.48

				Juiciness	6.10	1.51
				First bite	6.81	1.23
				Cohesiveness	3.29	1.83
				Impression	6.05	1.45
				Tenderness	6.88	1.17
Fibrousness	6.14	2.03				
Flavour	6.38	1.48				
Colour	3.29	2.10				
		Thigh	42	Aroma	6.00	1.67
				Juiciness	6.40	1.42
				First bite	6.50	1.57
				Cohesiveness	3.55	1.73
				Impression	6.24	1.54
				Tenderness	6.60	1.19
Fibrousness	6.26	1.55				
Flavour	6.60	1.06				
Colour	3.40	1.81				
Kakamega	IC	Breast	28	Aroma	5.43	1.62
				Juiciness	4.46	1.79
				First bite	4.75	1.82
				Cohesiveness	5.25	1.35
				Impression	4.61	1.31
				Tenderness	4.86	1.78
Fibrousness	4.43	2.03				
Flavour	5.86	1.53				
Colour	4.18	1.66				
		Thigh	28	Aroma	4.86	1.74
				Juiciness	3.25	1.73
				First bite	3.36	2.04
				Cohesiveness	6.21	1.23
				Impression	3.96	1.53
				Tenderness	3.11	1.62
Fibrousness	2.96	1.86				
Flavour	4.64	1.70				
Colour	6.75	1.38				

JAR RATING

Ecosystem	chicken	N part	Obs	Variable	Mean	Std Dev
Narok	ic	breast	26	Colour	2.1923077	0.7493587
				Tender	2.8461538	0.5434930
				Juice	2.8076923	0.4914656
Flavour	2.4615385	0.5817745				
		Thigh	28	Colour	3.9642857	0.8811669
				Tender	2.1071429	1.1001443

				Juice	2.0357143	0.9615629	
Flavour	1.9642857	0.9222413					
Taita	ic	breast	26	Colour	2.8846154	0.4314555	
				Tender	2.7692308	0.7103629	
				Juice	2.3846154	0.5710988	
Flavour	3.1538462	0.6126864					
		Thigh	26	Colour	4.3461538	0.4851645	
				Tender	1.9615385	0.6621643	
				Juice	2.5000000	0.5099020	
Flavour	2.6538462	0.6287962					
Broiler	br	breast	41	Colour	2.1219512	0.8716399	
				Tender	3.8536585	1.0382913	
				Juice	3.4634146	1.0024361	
Flavour	3.4878049	0.9518916					
		thigh	41	Colour	2.4390244	0.7432624	
				Tender	3.8048780	1.0540283	
				Juice	3.4878049	0.9778024	
Flavour	3.2926829	1.0306285					
ic	breast	2	Colour	2.5000000	0.7071068		
				Tender	3.0000000	1.4142136	
				Juice	3.0000000	0	
Flavour	3.0000000	0					
Kakamegaic	breast	28	Colour	2.6785714	0.6118322		
				Tender	2.5714286	1.2301331	
				Juice	2.9285714	1.2450962	
Flavour	3.3571429	1.0261141					
		Thigh	28	Colour	4.0357143	0.7444681	
				Tender	2.0714286	1.0515798	
				Juice	2.3571429	1.0959280	
Flavour	2.7500000	1.3505829					

---

1. **AMINO ACIDS**

data AMINOACID;

input sample $ ecotype $ Part $ His Ser Arg Gly Asp Glu Thr Ala Pro Lys Tyr Met Val Ile Leu Phe;

cards;

KAT	KKMG	thigh	2.11	3.20	5.44	5.30	8.04	13.33	3.68	4.89	3.77	7.39	2.52	5.95	3.71	3.38	6.21	3.08
NAT	NAIV	thigh	1.94	3.34	5.28	4.22	8.45	14.04	3.96	4.79	3.16	7.68	2.58	5.81	3.68	3.53	6.38	3.42

```
TAT  TAIT thigh 2.39  3.83  6.36  4.14  9.37  15.71  4.48  5.11  3.38
     8.21  3.28  8.55  4.35  3.96  7.52  4.00
KAB  KKMG breast 2.91  3.43  5.83  3.90  9.48  14.77  4.38  5.18  3.06
     8.51  2.96  7.62  4.37  3.96  7.40  3.92
NAB  NAIV breast 2.98  3.44  5.73  4.18  8.90  13.87  4.06  5.28  3.04
     9.17  2.75  7.98  4.30  3.84  7.07  3.08
TAB  TAIT breast 2.99  3.40  5.62  3.60  8.88  14.06  4.17  5.10  2.85
     9.37  2.76  8.29  4.33  3.92  7.29  3.25
;
procglm;
class ecotype part;
model Lys = ecotype part/ss4;
means ecotype part/tukey;
run;
```

2. **FAT**

```
DATA FAT;
INPUT SAMPLE $ ECOTYPE $ PART $    FAT;
CARDS;
TAB  TAITABREAST      1.731
TAB  TAITABREAST      6.167
TAT  TAITATHIGH       2.652
TAT  TAITATHIGH       2.842
TDB  TAITABREAST      0.833
TDT  TAITATHIGH       2.0201
TCB  TAITABREAST      3.944
TCT  TAITATHIGH       5.631
TBT  TAITATHIGH       0.726
TBB  TAITABREAST      1.217
NAB  NAIVAS    BREAST     2.248
NAT  NAIVAS    THIGH      0.283
NBB  NAIVAS    BREAST     1.377
NBT  NAIVAS    THIGH      4.135
NCT  NAIVAS    THIGH      4.099
NAB  NAIVAS    BREAST     1.281
NAT  NAIVAS    THIGH      5.988
NET  NAIVAS    THIGH      2.338
NDT  NAIVAS    THIGH      0.828
KAB  KKMG      BREAST     4.436
KAB  KKMG      BREAST     3.085
KAT  KKMG      THIGH      7.27
KAT  KKMG      THIGH      3.23
KDT  KKMG      THIGH      0.206
KDB  KKMG      BREAST     0.111
KEB  KKMG      BREAST     0.358
KEB  KKMG      BREAST     0.738
KET  KKMG      THIGH      4.139
KET  KKMG      THIGH      4.139
KET  KKMG      THIGH      2.544
;
```

PROCGLM;
CLASS ECOTYPE PART;
MODEL FAT=ECOTYPE PART PART(ECOTYPE)/SS4;
MEANS ECOTYPE PART PART(ECOTYPE)/TUKEY;
RUN;
procmeans mean stderr maxdec=**2**;
class ecotype part;
var PROTEIN;
run;

3. **ASH**

DATA ASH;
INPUT SAMPLE $ ECOTYPE $ PART $ ASH;
CARDS;
TAB TAITABREAST 3.018518519
TAT TAITATHIGH 3.239316239
TDB TAITABREAST 3.675324675
TDT TAITATHIGH 2.209150327
TEB TAITABREAST 4.08
TET TAITATHIGH 2.135
NAB NAIVAS BREAST 4.17037037
NAT NAIVAS THIGH 2.555555556
NBB NAIVAS BREAST 3.694300518
NBT NAIVAS THIGH 4.705521472
NCB NAIVAS BREAST 3.689873418
NCT NAIVAS THIGH 4.111764706
KAB KKMG BREAST 2.373134328
KAT KKMG THIGH 1.964285714
KBB KKMG BREAST 5.521008403
KBT KKMG THIGH 4.675
KCB KKMG BREAST 5.382293763
KCT KKMG THIGH 4.990566038
TAB TAITABREAST 5.12345679
TDB TAITABREAST 5.373831776
TEB TAITABREAST 8.745454545
TET TAITATHIGH 2.904761905
NAB NAIVAS BREAST 4.762195122
NAT NAIVAS THIGH 4.206896552
NBB NAIVAS BREAST 6.052173913
NBT NAIVAS THIGH 5.557692308
NCB NAIVAS BREAST 5.26446281
TDT TAITATHIGH 4.308641975
;
PROCPRINT;
PROCGLM;
CLASS ECOTYPE PART;
MODEL ASH=ECOTYPE PART PART(ECOTYPE)/SS4;
MEANS ECOTYPE PART PART(ECOTYPE)/TUKEY;
RUN;
PROCMEANS MEAN STD STDERR CV MAXDEC=**2**;
CLASS ECOTYPE;

```
VAR ASH;
RUN;
PROCMEANS MEAN STD STDERR CV MAXDEC=2;
CLASS PART;
VAR ASH;
RUN;
PROCMEANS MEAN STD STDERR CV MAXDEC=2;
CLASS ECOTYPE PART;
VAR ASH;
RUN;
```

4. EMULSIONS

```
DATA EMULSIONS;
INPUT Sample $ ecotype $ part $ Protein FCFE      FS;
CARDS;
KCT   KKMG        THIGH       369.45 97.72  90.11
KCT   KKMG        BREAST      507.30 74.90  67.30
KCB   KKMG        BREAST      588.25 82.51  52.09
KCB   KKMG        BREAST      629.85 82.51  67.30
NEB   NAIVAS      BREAST      652.59 90.11  36.88
NEB   NAIVAS      BREAST      665.10 97.72  53.61
NET   NAIVAS      THIGH       668.82 67.30  36.88
NET   NAIVAS      THIGH       670.00 67.30  52.09
TBT   TAITATHIGH        671.63 82.51  44.49
TBT   TAITATHIGH        672.78 90.11  52.09
TBB   TAITABREAST       673.05 74.90  67.30
TBB   TAITABREAST       673.37 74.90  67.30
;
PROCPRINT;
RUN;
PROCGLM;
CLASS ECOTYPE PART;
MODEL Protein FCFE        FS=ECOTYPE PART PART(ECOTYPE)/SS4;
MEANS ECOTYPE PART PART(ECOTYPE)/TUKEY;
RUN;
PROCMEANS MEAN STD STDERR CV MAXDEC=2;
CLASS PART;
VAR Protein FCFE    FS;
RUN;
PROCMEANS MEAN STD STDERR CV MAXDEC=2;
CLASS ECOTYPE;
VAR Protein FCFE    FS;
RUN;
PROCMEANS MEAN STD STDERR CV MAXDEC=2;
CLASS ECOTYPE PART;
VAR Protein FCFE FS;
RUN;
PROCCORR;
VAR Protein FCFE FS;
RUN;
```

5. COLOUR

```
data color;
input sample $ ECOTYPE $ PART $L        a        B C hue;
cards;
TAT   TAITATHIGH        73.43   2.84    16.79   47.68   -2.57
TAT   TAITATHIGH        74.63   3.4     17.43   59.26   -0.44
TAT   TAITATHIGH        75      3.24    17.21   55.76   -0.68
TAB   TAITABREAST       82.29   1.92    13.9    26.69   0.71
TAB   TAITABREAST       82.78   1.97    13.8    27.19   1.14
TAB   TAITABREAST       82.38   1.73    13.27   22.96   0.19
KAT KKMG  THIGH         69.61   4.89    20.51   100.29  0.57
KAT KKMG  THIGH         70.81   5.23    20.95   109.57  0.85
KAT KKMG  THIGH         71.39   5.46    21.74   118.70  0.90
KAB   KKMG        BREAST      83.37   2.4     17.26       41.42   0.78
KAB   KKMG        BREAST      83.1    2.35    17.47   41.05   0.45
KAB   KKMG        BREAST      81.76   2.23    17.19   38.33   0.15
NAT   NAIVAS      THIGH       64.86   5.73    14.82   84.92   -1.61
NAT   NAIVAS      THIGH       66.4    5.85    19.79   115.77  4.06
NAT   NAIVAS      THIGH       66.4    5.85    19.79   115.77  4.06
NAB   NAIVAS      BREAST      81.13   2.59    15.42   39.94   -2.92
NAB   NAIVAS      BREAST      81.13   2.68    15.38   41.22   -1.65
NAB   NAIVAS      BREAST      79.24   2.43    14.82   36.01   -5.36
;
procprint;
procglm;
class ECOTYPE PART;
model L         a b C hue=  ECOTYPE PART(ECOTYPE);
means ECOTYPE PART(ECOTYPE)/tukey;
run;
procmeans mean std stderr cv maxdec=2;
class ecotype;
var L a b C hue;
run;
procmeans mean std stderr cv maxdec=2;
class part;
var L a b C hue;
run;
procmeans mean std stderr cv maxdec=2;
class ecotype part;
var L a b C hue;
run;
proccorr;
var L a b C hue;
run;
procreg;
model C hue=a b;
run;
```

6. EMULSION STABILITY

```
data emustable;
input Sample $ ecotype $ part $ ESSlant  ESH;
cards;
KCT   KKMG        thigh   24.48979592   37.83783784
KCT   KKMG        thigh   10.20408163   45.45454545
KCB   KKMG        breast  25.53191489   250.
KCB   KKMG        breast  43.01075269   32.25806452
NEB   NAIV  breast  28.57142857   35.29411765
NEB   NAIV  breast  24.10714286   45.16129032
NET   NAIV  thigh   22.60869565   53.33333333
NET   NAIV  thigh   25.0       35.29411765
TBT   TAIT  thigh   15.68627451   22.5
TBT   TAIT  thigh   20.83333333   28.57142857
TBB   TAIT  breast  12.0     25.0
TBB   TAIT  breast  26.41509434   31.25
;
procmeans mean stderr maxdec=2;
class part;
var ESSlant  ESH;
run;
procmeans mean stderr maxdec=2;
class ecotype;
var ESSlant  ESH;
run;
procmeans mean stderr maxdec=2;
class ecotype part;
var ESSlant  ESH;
run;
```

7. EMULSION CAPACITY

```
data capacity;
input Sample $ ecotype $  part $ ECslants     ECHoriz;
cards;
KCT   KKMG        thigh   25.0     29.54545455
KCT   KKMG        thigh   26.78571429   30.76923077
KCB   KKMG        breast  25.45454545   23.80952381
KCB   KKMG        breast  10.0       21.05263158
NEB   NAIV  breast  23.68421053   31.57894737
NEB   NAIV  breast  24.07407407   30.55555556
NET   NAIV  thigh   26.41509434   27.77777778
NET   NAIV  thigh   25.45454545   29.72972973
TBT   TAIT  thigh   22.64150943   34.21052632
TBT   TAIT  thigh   19.23076923   30
TBB   TAIT  breast  19.60784314   28.57142857
TBB   TAIT  breast  22.68041237   27.5
;
procmeans mean stderr maxdec=2;
class part;
var ECslantsECHoriz;
```

run;
procmeans mean stderr maxdec=**2**;
class ecotype;
var ECslantsECHoriz;
run;
procmeans mean stderr maxdec=**2**;
class ecotype part;
var ECslantsECHoriz;
run;

8. PROTEINS

data proteins;
input sample $ part $ ecotype $ rep protein;
cards;

TET	THIGH	TAITA1	28.908	
TET	THIGH	TAITA2	13.77	
TDT	THIGH	TAITA1	23.294	
TDT	THIGH	TAITA2	22.396	
TAT	THIGH	TAITA1	20.925	
TAT	THIGH	TAITA2	22.508	
TAB	BREAST	TAITA1	26.92	
TAB	BREAST	TAITA2	27.207	
TDB	BREAST	TAITA1	22.992	
TDB	BREAST	TAITA2	23.427	
TEB	BREAST	TAITA1	23.526	
TEB	BREAST	TAITA2	21.766	
NAT	THIGH	NAIVAS	1	27.474
NAT	THIGH	NAIVAS	2	26.565
NBT	THIGH	NAIVAS	1	22.327
NCT	THIGH	NAIVAS	1	21.971
NCT	THIGH	NAIVAS	2	19.673
NCT	THIGH	NAIVAS	3	19.927
NAB	BREAST	NAIVAS	1	21.143
NBB	BREAST	NAIVAS	1	24.187
NCB	BREAST	NAIVAS	1	26.503
NCB	BREAST	NAIVAS	2	24.471
KAT	THIGH	KKMG	1	19.656
KBT	THIGH	KKMG	1	21.299
KBT	THIGH	KKMG	2	23.451
KCT	THIGH	KKMG	1	19.256
KCT	THIGH	KKMG	2	11.414
KAB	BREAST	KKMG	1	23.266
KBB	BREAST	KKMG	1	15.829
KBB	BREAST	KKMG	2	22.768
KCB	BREAST	KKMG	1	24.068
KCB	BREAST	KKMG	2	20.12

;

```
procprint;
run;
procglm;
class part ecotype rep;
model protein= part ecotype part(ecotype) rep/ss4;
means part ecotype part(ecotype)/tukey;
run;
procmeans mean stderr std cv maxdec=2;
class part;
var protein;
run;
procmeans mean stderr std cv maxdec=2;
class ecotype;
var protein;
run;
procmeans mean stderr std cv maxdec=2;
class ecotype part;
var protein;
run;
```

9. PROTEIN DIGESTIBILITY

```
dataprodiges;
input Sample $ PART $ ecotype $ Digestib;
cards;
KCT   thigh KKMG   50.19623659
KCT   thigh KKMG   62.69827972
KCB   breast KKMG 72.94435841
KCB   breast KKMG 78.00481704
NEB   breast NAIVS 76.25281378
NEB   breast NAIVS 75.2956079
;
procglm;
class PART ecotype;
model Digestib= PART ecotype/ss4;
means PART ecotype PART(ecotype)/tukey;
run;
```

10. MOISTURE

```
DATA MOISTURE;
INPUT SAMPLE $ ECOTYPE $ PART $    MC;
CARDS;
TAB   TAITABREAST       71.50537634
TAT   TAITATHIGH        74.67532468
TDB   TAITABREAST       72.79151943
TDT   TAITATHIGH        72.08029197
TEB   TAITABREAST       74.55470738
TET   TAITATHIGH        54.33789954
NAB   NAIVAS       BREAST       70.26431718
```

```
NAT   NAIVAS      THIGH     73.29376855
NBB   NAIVAS      BREAST    68.04635762
NBT   NAIVAS      THIGH     74.00318979
NCB   NAIVAS      BREAST    69.01960784
NCT   NAIVAS      THIGH     73.22834646
NAB   NAIVAS      BREAST    73.25349301
KAT   KKMG        THIGH     73.38403042
NCB   NAIVAS      BREAST    71.42857143
NCT   NAIVAS      THIGH     66.22222222
KAT   KKMG        THIGH     70.61403509
KAB   KKMG        BREAST    72.10300429
KBT   KKMG        THIGH     72.4137931
KBB   KKMG        BREAST    74.43946188
KCT   KKMG        THIGH     76.88679245
KCB   KKMG        BREAST    71.04072398
TCB   TAITABREAST     71.00760456
TCT   TAITATHIGH      75.27975585
;
PROCPRINT;
PROCGLM;
CLASS ECOTYPE PART;
MODEL MC=ECOTYPE PART PART(ECOTYPE)/SS4;
MEANS ECOTYPE PART PART(ECOTYPE)/TUKEY;
RUN;
PROCMEANS MEAN STD STDERR CV MAXDEC=2;
CLASS ECOTYPE;
VAR MC;
RUN;
PROCMEANS MEAN STD STDERR CV MAXDEC=2;
CLASS PART;
VAR MC;
RUN;
PROCMEANS MEAN STD STDERR CV MAXDEC=2;
CLASS ECOTYPE PART;
VAR MC;
RUN;
```

11. FOAMING CAPACITY AND STABILITY

```
DATA FOAMING;
INPUT ECOTYPE $ PART $ Sample $ Protein FC_FE FS;
CARDS;
KKMG THIGH KCT 369.452      97.71863118   90.11406844
KKMG THIGH KCT 507.301      74.90494297   67.30038023
KKMG BREAST KCB      588.247      82.5095057    52.09125475
KKMG BREAST KCB      629.847      82.5095057    67.30038023
NAIVA BREAST NEB     652.591      90.11406844   36.88212928
NAIVA BREAST NEB     665.104      97.71863118   53.6121673
NAIVA THIGH NET 668.823      67.30038023   36.88212928
NAIVA THIGH NET 669.998      67.30038023   52.09125475
```

```
TAIT THIGH TBT     671.632        82.5095057    44.48669202
TAIT THIGH TBT     672.777        90.11406844   52.09125475
TAIT BREAST TBB    673.052        74.90494297   67.30038023
TAIT  BREAST TBB   673.366        74.90494297   67.30038023
;
PROCGLM;
CLASS ECOTYPE PART;
MODEL Protein FC_FE FS= ECOTYPE PART  PART(ECOTYPE)/SS4;
MEANS ECOTYPE PART  PART(ECOTYPE)/TUKEY;
RUN;
PROCMEANS MEAN STDERR MAXDEC=2;
CLASS PART;
VAR Protein FC_FE FS;
RUN;
PROCMEANS MEAN STDERR MAXDEC=2;
CLASS ECOTYPE;
VAR Protein FC_FE FS;
RUN;
PROCMEANS MEAN STDERR MAXDEC=2;
CLASS ECOTYPE PART;
VAR Protein FC_FE FS;
RUN;
```

OUTPUT OF FOAMING CAPACITY AND STABILITY

Dependent Variable: Protein

Source	DF	Sum of Squares	Mean Square	F Value	Pr> F
Model	5	85227.79478	17045.55896	9.79	0.0075
Error	6	10446.13611	1741.02268		
Corrected Total	11	95673.93089			

R-Square	Coeff Var	Root MSE	Protein Mean
0.890815	6.727949	41.72556	620.1825

Source	DF	Type IV SS	Mean Square	F Value	Pr> F
ECOTYPE	2	55986.78922	27993.39461	16.08	0.0039
PART	1	8652.35885	8652.35885	4.97	0.0673
PART(ECOTYPE)	2	20588.64671	10294.32336	5.91	0.0381

Dependent Variable: FC_FE

Source	DF	Sum of Squares	Mean Square	F Value	Pr> F
Model	5	891.536189	178.307238	3.36	0.0859
Error	6	318.061559	53.010260		
Corrected Total	11	1209.597748			

R-Square	Coeff Var	Root MSE	FC_FE Mean
0.737052	8.892512	7.280815	81.87579

Source	DF	Type IV SS	Mean Square	F Value	Pr> F
ECOTYPE	2	38.5529163	19.2764581	0.36	0.7095
PART	1	43.3720308	43.3720308	0.82	0.4006
PART(ECOTYPE)	2	809.6112418	404.8056209	7.64	0.0224

Dependent Variable: FS

Source	DF	Sum of Squares	Mean Square	F Value	Pr> F
Model	5	1909.333179	381.866636	3.47	0.0809
Error	6	660.411456	110.068576		
Corrected Total	11	2569.744635			

R-Square	Coeff Var	Root MSE	FS Mean
0.743005	18.31345	10.49136	57.28771

Source	DF	Type IV SS	Mean Square	F Value	Pr> F
ECOTYPE	2	1185.887705	592.943852	5.39	0.0458
PART	1	0.192765	0.192765	0.00	0.9680
PART(ECOTYPE)	2	723.252710	361.626355	3.29	0.1087

The SAS System 16:33 Saturday, July 22,

Tukey's Studentized Range (HSD) Test for Protein

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	6
Error Mean Square	1741.023
Critical Value of Studentized Range	4.33902
Minimum Significant Difference	90.524

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	ECOTYPE
A	672.71	4	TAIT
A			
A	664.13	4	NAIVA
B	523.71	4	KKMG

Tukey's Studentized Range (HSD) Test for FC_FE

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	6
Error Mean Square	53.01026
Critical Value of Studentized Range	4.33902
Minimum Significant Difference	15.796

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	ECOTYPE
A	84.411	4	KKMG
A	80.608	4	NAIVA
A	80.608	4	TAIT

Tukey's Studentized Range (HSD) Test for FS

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	6
Error Mean Square	110.0686
Critical Value of Studentized Range	4.33902
Minimum Significant Difference	22.761

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	ECOTYPE
A	69.202	4	KKMG
B A	57.795	4	TAIT
B	44.867	4	NAIVA

Tukey's Studentized Range (HSD) Test for Protein

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	6
Error Mean Square	1741.023
Critical Value of Studentized Range	3.46046
Minimum Significant Difference	58.947

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	PART
A	647.03	6	BREAST
A			
A	593.33	6	THIGH

The GLM Procedure

Tukey's Studentized Range (HSD) Test for FC_FE

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	6
Error Mean Square	53.01026
Critical Value of Studentized Range	3.46046
Minimum Significant Difference	10.286

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	PART
A	83.777	6	BREAST
	A		
	A	79.975	6 THIGH

The GLM Procedure

Tukey's Studentized Range (HSD) Test for FS

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	6
Error Mean Square	110.0686
Critical Value of Studentized Range	3.46046
Minimum Significant Difference	14.821

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	PART
A	57.414	6	BREAST
A			
A	57.161	6	THIGH

Level of PART	Level of ECOTYPE	N	Protein Mean	Protein Std Dev	FC_FE Mean	FC_FE Std Dev	FS Mean	FS Std Dev
BREAST	KKMG	2	609.047000	29.4156421	82.5095057	0.0000000	59.6958175	10.7544758
THIGH	KKMG	2	438.376500	97.4739627	86.3117871	16.1317136	78.7072243	16.1317136
BREAST	NAIVA	2	658.847500	8.8480272	93.9163498	5.3772379	45.2471483	11.8299233
THIGH	NAIVA	2	669.410500	0.8308505	67.3003802	0.0000000	44.4866920	10.7544758
BREAST	TAIT	2	673.209000	0.2220315	74.9049430	0.0000000	67.3003802	0.0000000
THIGH	TAIT	2	672.204500	0.8096373	86.3117871	5.3772379	48.2889734	5.3772379

N

PART	Obs	Variable	Mean	Std Error
BREAST	6	Protein	647.03	13.51
		FC_FE	83.78	3.63
		FS	57.41	5.03
THIGH	6	Protein	593.33	52.14
		FC_FE	79.97	5.07
		FS	57.16	7.77

ECOTYPE	Obs	Variable	Mean	Std Error	N
KKMG	4	Protein	523.71	57.37	
		FC_FE	84.41	4.78	
		FS	69.20	7.84	
NAIVA	4	Protein	664.13	3.98	
		FC_FE	80.61	7.84	
		FS	44.87	4.62	
TAIT	4	Protein	672.71	0.38	
		FC_FE	80.61	3.64	
		FS	57.79	5.70	

The MEANS Procedure

ECOTYPE	PART	Obs	Variable	Mean	Std Error	N
KKMG	BREAST	2	Protein	609.05	20.80	
			FC_FE	82.51	0.00	
			FS	59.70	7.60	
	THIGH	2	Protein	438.38	68.92	
			FC_FE	86.31	11.41	
			FS	78.71	11.41	
NAIVA	BREAST	2	Protein	658.85	6.26	
			FC_FE	93.92	3.80	
			FS	45.25	8.37	
	THIGH	2	Protein	669.41	0.59	
			FC_FE	67.30	0.00	
			FS	44.49	7.60	
TAIT	BREAST	2	Protein	673.21	0.16	
			FC_FE	74.90	0.00	
			FS	67.30	0.00	
	THIGH	2	Protein	672.20	0.57	
			FC_FE	86.31	3.80	
			FS	48.29	3.80	

Appendix J: Sample QIIME analysis batch Script

Ben_Qiime2_commnad_BenforJB - Notepad

File Edit Format View Help

```
#Commands we have executed so far with qiime2:
#!/usr/bin/env bash
#SBATCH -p batch
#SBATCH -w taurus
#SBATCH -J qiime2_grouped
#SBATCH -n 4
#In case I want to bring or copy my raw multiplexed reads from Illumina machine-
#>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
# under /var/scratch/jb/ben_long_reads on taurus:
WKDIR=/home/boloo/Q
cd $WKDIR
#mkdir original_dat
#first create a local directory with only the R1 and R2 files:
#cp /export/data/ilri/miseq/users/BenOloo/pool2_repeat/*R[12]*.fastq.gz
#original_dat/
# load the qiime2 module
module load qiime2/2018.4
# first step: import data from .fastq.gz files into a Qiime2 .qza file:
# under /var/scratch/jb/ben_long_reads on taurus:
mkdir original_dat

#first create a local directory with only the R1 and R2 files:
cp /export/data/ilri/miseq/users/BenOloo/pool2_repeat/*R[12]*.fastq.gz
original_dat/

module load qiime2/2018.4

# first step: import data from .fastq.gz files into a Qiime2 .qza file:
qiime tools import --type 'SampleData[PairedEndSequencesWithQuality]'
--input-path original_dat/ --source-format
CasavaOneEightSingleLanePerSampleDirFmt --output-path
longreads-demux-paired-end.qza

# second step: quality filtering of the reads
qiime quality-filter q-score --i-demux longreads-demux-paired-end.qza
--o-filtered-sequences longreads-demux-filtered.qza --o-filter-stats
demux-filter-stats.qza


# DO NOT PERFORM this second step: quality filtering of the reads
#qiime quality-filter q-score --i-demux longreads-demux-paired-end.qza --o-filtered-sequences longreads-demux-filtered.qza --o-filter-stats demux-filter-stats.qza
#qiime quality-filter q-score --i-demux longreads-demux-paired-end.qza --o-filtered-sequences  longreadsrun2-demux-paired-end.qza --o-filter-stats demuxrun2-filter-stats.qza
```

Appendix K : **Published** Papers

ajfand SCHOLARLY, PEER-REVIEWED AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT
Volume 18 No. 1 April 2018
PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST
ISSN 1684 5374

Afr. J. Food Agric. Nutr. Dev. 2018; 18(1): 13202-13225 DOI: 10.18697/ajfand.81.16900

DESCRIPTIVE SENSORY QUALITY OF KENYA'S INDIGENOUS CHICKEN MEAT FROM DIFFERENT ECOTYPE- CLUSTERS REARED UNDER AN INTENSIVE SYSTEM

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Oloo Benard

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ajfand SCHOLARLY, PEER REVIEWED
AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT
Volume 17 No. 1 March 2017
PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST
ISSN 1684 5374

Afr. J. Food Agric. Nutr. Dev. 2017; **17(1):** 11616-11638 **DOI: 10.18697/ajfand.77.16765**

DESIGN OF A HACCP PLAN FOR INDIGENOUS CHICKEN SLAUGHTER HOUSE IN KENYA

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Benard Oloo

*Corresponding author e-mail: olooo.odhiambo@gmail.com

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[2]Department of Animal Science, Egerton University, P.O Box 536, Egerton, Kenya

Journal of Food and Nutritional Sciences Research 2019, 1(1), 1-11.

Fatty Acid and Amino Acid Profile of Indigenous Cluster Ecotypes of Kenyan Free-ranging Chicken

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https://doi.org/10.37512/100

ABSTRACT

An experiment was conducted to determine amino acid and fatty acid profile of indigenous chicken reared in Kenya under a free range feeding system. Five cocks of each ecotype were purchased, slaughtered and transported to JKUAT and Durban University of Technology, within 12 and 24 hours respectively in an insulated box containing ice. Amino acid profile was estimated using Pico Tag by hydrolysis with acid for 24 hours, derivatized and analyzed by reverse phase HPLC. Fatty Acid Methyl Esters (FAME) were synthesized by a direct/one-step extraction transesterification method. FAME were then separated and quantified using gas chromatography. The amino acid and fatty acid profile of indigenous chicken in Kenya was affected by the ecotype as well as the part of the chicken (breast or thigh). The amino acid profile of experimental chicken revealed values higher than the RDA. The ratio of □-6:□-3 though higher than 4:1, demonstrated that the consumption of indigenous free ranging chicken meat is likely to give favourable health outcomes.

KEY WORDS: Fatty acid, amino acid, indigenous chicken, Kenya

INTRODUCTION

Poultry in Kenya play an important socio-economic, food and nutrition security roles in most households and especially in rural areas. Kenyan poultry population is estimated at 42.4 million birds (FAO, 2014) out of which 75% are local indigenous chicken. The remaining 22% is broilers while the rest is breeding stock and spent layers. Meat quality and palatability is affected by several factors. A major one is the fatty acid composition in the muscles and adipose tissue (Hoffman *et al*., 2005). The amino acid profile is also responsible for nutritional and functional quality of chicken meat. The most common rearing method for indigenous chicken is through the free range backyard system. According to (Pavlovski *et al*., 2013) chicken meat from intensive broiler production differs in quality from native breeds and those in a free range system. Intensification of native/indigenous chicken rearing has been on the increase for the past 20 years. Rearing native chicken under an intensive system in Serbia has been studied by (Bogosavljevic-Boskovic *et al*.., 2010; Milošević *et al*., 2003; and Blagojević *et al*., 2009); Pavlovski *et al*., 2013). Because of their work it is now considered that to develop an intense meat flavour, broilers should be kept under free range production system, a practice that is currently becoming common in Europe and the USA. This concept is also supported by the strong animal welfare groups (Dawkins, 2003). The demand for poultry meat has been estimated to triple in Africa, a trend that is likely to be replicated in other parts of the world by the year 2030 (Zootecnica, 2016; Heinz *et al*., 2007).

Food nutrition data is important to international

Food Science and Nutrition Studies
ISSN 2573-1661 (Print) ISSN 2573-167X (Online)
Vol. 2, No. 3, 2018
www.scholink.org/ojs/index.php/fsns

Original Paper

Quantity and Functionality of Protein Fractions Isolated from 3 Ecotypes of Indigenous Chicken in Kenya

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Received: September 19, 2018 Accepted: October 5, 2018 Online Published: November 21, 2018

doi:10.22158/fsns.v2n3p70 URL: http://dx.doi.org/10.22158/fsns.v2n3p70

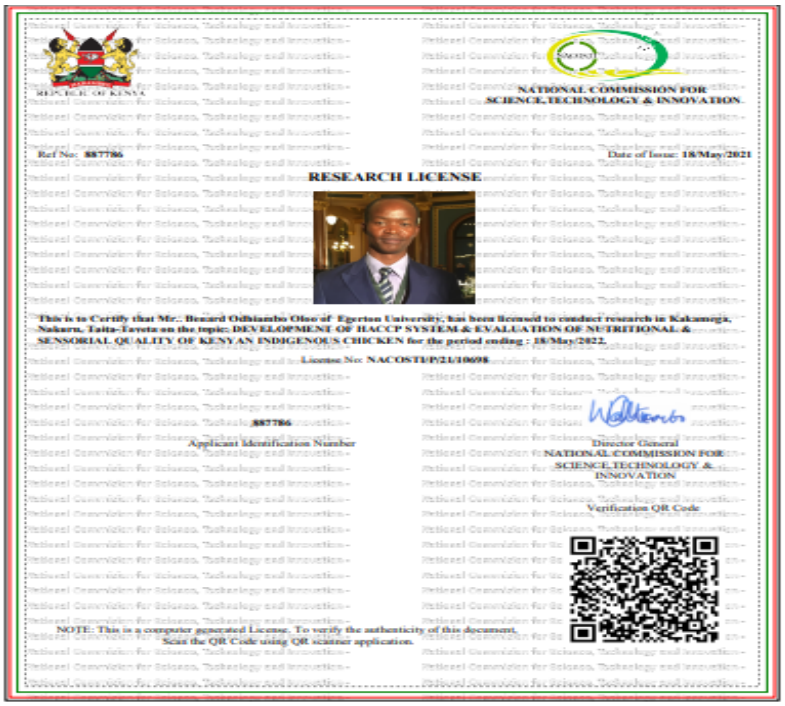
Abstract

The aim of this study was to evaluate the effect of the cluster ecotype and the part of chicken on nutritional composition, and functionality of sarcoplasmic and myofibrillar proteins that are most relevant to the technological features of chicken meat. Over 50 chickens from each ecotype cluster purchased, slaughtered and the meat stored under refrigeration at -20°C and later on transferred in cooler box on ice and flown to South Africa, at the Durban University of Technology. Protein fractions were extracted with a cocktail of Sodium Chloride buffer (50mM NaCl, 50mM Tris HCl; 75mM DTT and 1mM EDTA at pH 7) and quantified by Bradford method. One dimensional Sodium Dodecyl Polyacrylamide Gel Electrophoresis (SDS PAGE) was applied to separate protein fractions. Emulsifying capacity, emulsifying stability, solubility, and in vitro digestibility were determined on the total protein isolates. Significant differences in band expressions were recorded for the myofibrillar and the sarcoplasmic proteins. The three ecotypes had high quality proteins with all the limiting and essential amino acids at concentrations higher than FAO/WHO recommended daily allowance for adults and children. Distinct protein bands at larger molecular weight proteins >100 kDa, corresponding to Myosin Heavy Chain, medium fractions 75 kDa and 45 kDa and even lower molecular weight fraction <25 kDa were present in the chicken breast and the thighs. It concludes that Indigenous chicken protein isolates' nutritional and functional properties are affected by part of chicken and ecotype clusters.

Keywords

Indigenous chicken, protein isolate, functionality, sarcoplasmic, myofibrillar

Appendix L: Research Permit



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