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METAGENOMIC ANALYSIS OF POULTRY GUT MICROBIOTA OF DIALLEL CROSSES BETWEEN NOILER AND NIGERIAN HEAVY ECOTYPE CHICKENS

By

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Abstract

A novel, unbiased approach of next generation sequencing – Roche 454 GS – FLX technology was used to study the metagenomic analysis of poultry gut microbes. The metagenomic analysis of the cecal microbial genome in 4 genotype chickens (NxN, HxH, NxH and HxN) of the offsprings were carried out. Birds from the 4 genotypes were sacrificed and the cecum collected and stored in EDTA for laboratory analysis. The composition and function of these Nigerian's poultry cecal microbiota were analyzed using high-through put sequencing of 16SrRNA genes (V1-V9) hyper variable regions. The cecal microbiota of four Nigerian indigenous chicken genotypes were described and compared. The taxonomic composition of the cecal microbiota was determined by blasting the unigenes to the NCBI Ref Sequence protein database. The results showed that the relative abundance of the dominant genera in the cecal of the 4 genotypes were mainly Bacteroides, Prevotella, Allistrips, Lactobacillus and Rikenellaceal, while the relative abundance of the dominant organisms in the phyla are: Bacteroides, Firmicutes, and Actinobacteria. Microbiological diversity was found in the community, with Noiler chicken having the most eveness, followed by main cross (Noiler x Ecotype Chicken), Heavy Ecotype x Heavy Ecotype while the reciprocal cross (Heavy Ecotype x Noiler Chicken) had the least figure. There were a lot of similarities between the four genotypes where it come to genetic diversity between them and composition. More understanding is needed on the Gut microbiota of the poultry birds.

***Keys**: Microbiota, Metagenomic, Noiler and Heavy Ecotype Chicken.*

Introduction

Metagenomics has emerged as a powerful tool for analyzing the complex microbial communities in the chicken Gastro Intestinal Tract (GIT). Metagenomics allows researchers to study the diversity and functions of microbes that are difficult to culture in the laboratory (Choi et al., 2015). Recent advances in next-generation sequencing have enabled extensive research into the chicken gut microbiome, revealing its crucial roles in nutrient absorption and immune functions as well as chicken meat safety.

The chicken gut microbiome represents gut microorganism and genomes as well as gene belonging to those microorganisms. Due to the increase of the human population, the daily protein requirement and meat intake is increasing. Low production costs, high feed conversion ratios and low product prices have contributed to making poultry meat of choice, both for producers and consumers.

In recent years, due to metagenomic approaches based on high-through put sequencing methods, the research of the gut microbiota has made rapid progress (Wu et al., 2020). The gastrointestinal tract (GIT) of Chicken is the major site for nutrient absorption and food digestion and has a highly diverse and dynamic microbial ecosystem (Yan et al., 2017). Changes of microbial diversity in the GIT have been associated with the differences of breeds (Pandit et al., 2018), sex (Siegersteller et al., 2017) growth stages (Yan et al., 2017) and intestinal segments (Al-marzoogi et al., 2020) in chicken as a result of various environmental and genetic factor.

Poultry gut contains millions of diverse micro-organism. The majority of these microbes are uncharacterized and represented an enormous reserviour of genetic and metabolic diversity. The cecum, which is the main functional section in the distal intestine plays important roles not only in vitamin and amino acid production but also in the prevention of pathogen colonization, detoxifying harmful substances, absorbing additional nutrients, nitrogen recycling from uric acid, producing essential amino acids, and digestion of non-starch polysaleharides (NSPs). The cecal microbiota was found to be highly related to the feed efficiency, which suggested an important role in chicken feed efficiency (Yan et al., 2017). Some indigenous chickens like Noiler and heavy ecotype chickens breeds have a higher product quality, productivity and pathogen resistance which have been widely reported (Duah et al., 2020). For example, Noiler chicken breed, was characterized by the fast growth rate. Strong survivability, large size, and tender meat and have been enjoying a high reputation in the commercial market of Nigeria.

Studies, mostly in chickens, have found that intestinal microbiota are dynamic and complex and play an important role in nutrition immunity (Choi et al., 2015) and detoxification in avian hosts have an enormous impact on the maintenance of health.

At present, our understanding of the intestinal microbial community of indigenous chicken in Nigeria remains limited and scarce. Although research on the gut microbiota of poultry (especially cecum) is increasing. Most of the current information about the intestinal microbiota is still limited to humans (WU et al., 2020).

Materials and Methods

Location of the Study

The experimental birds were purchased and housed in the Poultry Unit of Department of Animal Science Farm, University of Nigeria, Nsukka (UNN). Nsukka is located in latitude 86 ^{0}84. North and longitude 7^0 37’ East with annual rainfall ranging from 100mm to 300mm/2023, world weather online.com). The natural day length for Nsukka is between 12 to 13 hours and average annual maximum and minimum temperature are 35^{0}c to 21^{0}c respectively. The relative humidity ranges from 34 to 78% (Momoh et al., 2010).

Base Population of the Study

The base population comprised 42 Noiler chickens (36 females and 6 males) and 42 local heavy ecotype chickens 36 females and 6 males). These Noiler chickens and local heavy ecotype chickens were assigned into different breeding groups with a mating ratio of 1:6. The cocks were allowed some periods with the hens before commencement of artificial insemination. The semen of the cocks were collected from each group and inseminated with a gap of 3 days. After successful insemination the birds were monitored till they started laying eggs. Eggs were collected once a day between the hours of 11.00am and 12.00 noon each day. Fertile eggs for hatching were stored in a room before incubation. At the end of seven days of eggs collective, the total eggs collected for the week were set in the incubation. The chicks were vaccinated and all medication regimes were properly carried out as well as hygienic measures.

Chicken Gut Morphology

At 28 weeks of age of the birds, two chickens were randomly selected from each genotype group and slaughtered be cervical dislocation. The cecum of each slaughtered chicken was harvested, and this was preserved in frozen liquid nitrogen under aseptic conditions. All samples were transferred to 80^{0}c refrigerator for preservation until DNA extraction and 16S rRNA Gene sequencing and Analysis.

To minimize the influence of genetics, diet and external factors, all experimental chickens were selected with similar genetic backgrounds and the same management, environmental and dietary conditions. The taxonomic composition of cecal microbiota was determined by blasting the unigenes to the MCBI RefSeq non redundant protein database.

Results and Discussion

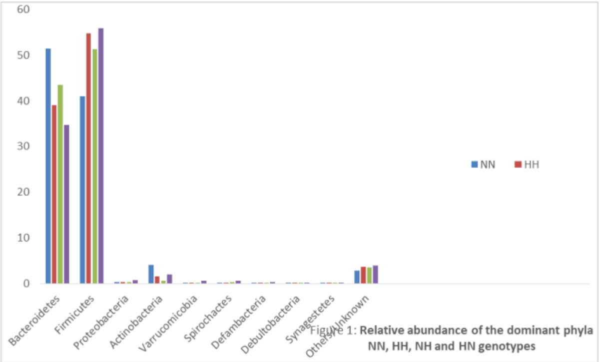

Figure 1: **Relative abundance of the dominant phyla NN, HH, NH and HN genotypes**

From the figure 1 above, the phylum classification for NN indicates Bacteroidata, Firmicutes and Achinobacteriota as the prominate microbiome composition with over (<90%), NH had Firmcutes and Bacteroidata as the major composition, also HH genotype had Firmicutes and Bacteroidata as the major composition while HN had Firmicutes and Bacteroidata (<80%) as the major microbiome composition.

In all the genotypes, NN had the highest composition of Bacteroidata with over (<50%) concentration while the other 3 genotypes had at (<30%) concentration.

The genera classification of NH genotypes had the highest amount of lactobacillus composition with 13.72% concentration also NN with 1.68%, HH having the least with 1.04%.

In the species level, HH genotype had the highest concentration composition of rickenellaceal RC9(7.02%) gut group followed by NN with (4>174%), HN (4.56%) while NH had the least rickenellace are concentration with (2.77%).

The genera levels of the genotypes had prominent compositions of lactobacilli, Bacteriods and rickenellace are RC9 gut group in higher amount.

The dominant phyla in the cecal microbiome of chickens are typically firmicutes, bacteroidetes, and proteobacteria.

Firmicute is consistently one of the two most abundant phyla in the chicken cecum, often accounts for 20-30% of the total microbiome (h-3) (Shen et al., 2024). In commercial yellow broilers, firmicute was confirmed as the dominant phylum (Yi et al., 2023). Bacteroidetes is also a very abundant phylum sometimes surpassing firmicutes in relative abundance (Shen

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et al., 2024). In one study, Bacteriodetes made up 41-66% of the cecals seen on day 3 after hatch (Xue et al., 2022).

Proteobacteria is the third major phylum, typically comprising 10-25% of the cecal microbiome in young chicks (Yan et al., 2017). The relative abundance of Proteobacteria decreases with age, dropping from around 27% on day 1 to less than 10% by day 28 (1) (Gong et al., 2019). Other phyla like cyanobacteria, Actinobacteria and Tenericutes are also sometimes detected in smaller amounts (Gong et al., 2018). The composition can vary based on factors like age, diet and health status but in general, diet and health status. But in general, Firmicutes, Bacteroides and Proteobacteria make up the core of the chicken cecal microbiome (Du et al., 2020)

At the phylum level, Bacteriodata, Firmicutes and Proteobacteria were the most abundant microbial population phyla in cecal microbiota of the Chicken which was the report from some authors which were consistent with previous reports (Lee et al., 2017; Dong et al., 2019; Shi et al., 2019). The gut microbiota of Noiler and Nigerian Heavy Ecotype Chickens differs significantly due to genetic and environmental factors.

Research indicates that Noiler Chickens, bred for meat production, exhibit higher abundance of Firmicutes compared to Nigerian Heavy ecotypes, which may have a more diverse microbial community with varying proportions of Proteobacteria and other phyla (Kers et al., 2018),

Additionally, differences in diet, age, and rearing conditions further influence microbial composition, affecting responses in these breeds (Chen et al., 2023). Overall, the distinct gut microbiota profiles reflect their breeding purposes and environmental adaptions.

In the gut microbiota of Noiler Chicken, specific bacteria such as Lactobacillus, Clostridium Ruminococcus are more abundant compared to Nigerian Heavy Ecotype chickens Notably, Lactobaccillus is prevalent in the crop and gizzard, while clostridium dominates in the cecum, contributing to fermentation processes. The diversity of these bacteria can vary significantly due to factors like diet, and environmental conditions, which influence microbial composition and functionality in these chicken breeds.

The gut microbiota of Noiler Chickens positively influences their growth rates compared to Nigerian Heavy Ecotype Chickens and their crosses through several mechanisms:

1. Nutritional bacteria, such as Lactobacillus enhances nutrient absorption and conversion ratios (FCR) in Noiler Chickens.
2. Short-Chain Fatty Acid Production. Gut microbiota fermentation produces Short Chain Fatty Acids (SCFAs) which promotes intestinal health and growth by stimulating enterocyte proliferation and improved gut morphology.
3. Immune Function: A diverse microbiota can enhance immune responses, reducing disease incidence and promoting better growth performance under varying environmental conditions.

In all, these factors above contribute to the superior growth rates observed in Noiler Chickens compared to their Nigerian Heavy ecotype counterpart and the crosses (Kers et al., 2018)

The gut morphology of Noiler and ecotype chickens differs in several key aspects.

1. Intestinal Length: Noiler Chickens generally have a longer intestinal tract, which enhances nutrient absorption and digestive efficiency compared to the heavy ecotype chickens.
2. Cecal development: the ceca in Noiler chicken are after more developed, supporting a greater microbial population that aids in fermentation and nutrients breakdown.
3. Villus Height: Chickens exhibit taller intestinal villi, which increases absorption, contributes to better growth rates. These morphological differences can significantly impact the overall health and growth performance of these chickens. Studies have shown that different structures and compositions of the gut microbiota can affect the nutritional metabolism and sensitivity to external pathogen infection of the host (Qing et al., 2020).

The main differences between Noiler and the crosses (main and reciprocal crosses)

There is always reduced diversity between the Noiler and the main cross which can impact immune function and nutrient absorptions especially in broiler and commercial broilers breeds such as Ven Cobb line (Qing et al., 2020). Specific phylotypes and biomarkers are associated with each breed, indicating unique microbial communities that influence immune responses and nutrients absorption.

Main cross show lower diversity due to selective breeding for growth efficiency, leading to a less complex microbiome. This reduced diversity can negatively affect immune function and absorption. Main cross genotypes often display increased/ higher levels of potentially harmful bacteria like Escherichia and Clostridium, which are linked to reduced microbial diversity and potential health issues.

Microbial composition evolves with age, with distinct phases of dominance for different phyla for example, Firmicutes are prominent early on, while Bacteriodetes gain dominance as chicken nature (3PS) (Yi et al., 2023), (Yan et al., 2017).

Recent study has shown that the abundance of Bacteriodetes in the intestinal microbiota of the high pigs was lower than that of the low FE Pigs (Quan et al., 2020). In the research by Du et al., (2020) the same result was found using Chicken. Turnbauch et al. (2006) pointed out that that firmicutes play important role in energy extraction in mice and Yang et al. (2016) argued that the fatness might be improved when firmicutes increase in pigs, intestine. Du et al. (2009) point that the predominant phyla of Bacteriodetes, Firmicutes and the stability of gut function can be ensured. However, compared with previous results, the composition and relative abundance of cecal dominant microbes were different at the genus level (Pandit et al., 2018; Shi et al. 2019).

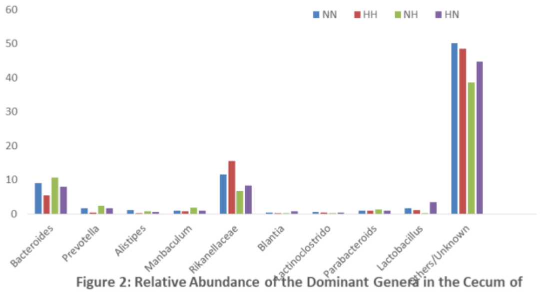

Figure 2: Relative Abundance of the Dominant Genera in the Cecum of NN, HH, NH and HN Genotypes

The presence of lactobacillus can effectively increase some beneficial bacteria to maintain the stability of microbial microbiota in the GIT (Forte et al., 2016). Wang et al. (2017) point out that lactobacillus Johnsonil BS15 reduces fat deposition and promoted the growth performance of Chicken.

Besides, lactobacelli are often used in the formulation of prebiotics and probiotics that enhance the intestinal health for improved colonization resistance to gut pathogens such as campylobacter and salmonella and the host performance (Khan et al., 2020).

Conclusion

The metagenomic analysis of poultry gut microbiota, particularly in diallel crosses between Nioler and Nigeria heavy ectotype chickens, reveals significant insights into microbial diversity and functionality. This study utilizes advanced sequencing technologies to explore the gut microbiota's role in nutrient absorption and overall health. Findings indicate a predominance of Firmicutes and Bacteroidetes, with implication for improving feed efficiency and resistance to diseases. This research underscores the importance of understanding gut microbiota dynamics to enhance poultry productivity and health outcomes (Choi et al., 2015; Yan et al., 2017).

During data analysis bioinformatics tool for metagenomic assembly and annotation are used, focusing on microbial taxonomic classification and functional gene identification, particularly, carbohydrate – active enzyme (Azymes) and short-chain fatty acid (SCFA) production pathways.

Recommendation

From the results of this study it is recommended that the Noiler Chicken should be genotype of choice for genetic upgrading in Nsukka region followed by main cross genotype (NH) because of its higher stability. It is vital to utilize genome-resolved metagenomics to identity and characterize microbial genomes in chicken gut samples, as demonstrated in some extensive studies on Chicken microbes.

Antibiotic resistance genes are investigated using metagenomic approaches to understand their prevalence and potential impact on poultry health. Different production systems (e.g barn verse free-range) should be explored on how they affect gut microbiota composition overtime, which can influence overall health and productivity.

There should be focus on functional genes related to nutrient metabolism and immune response, as these are critical for understanding the microbiota's role in host health.

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