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Research Article
Gut Morphometric Characteristic and Ecological Response of Broiler Starter Fed Varied Levels of Protein

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Abstract

Background and Objective: Meeting nutrient requirement of broiler chicks to supply animal protein requirement cannot be overemphasized. Therefore research was designed to determine gut morphometric and ecological response of broiler starter fed varied levels of protein. **Materials and Methods:** A total of 144 days-old-broiler chicks were used for the study. Broiler standard starter mash with varied protein percentages of 20, 23 and 26%, respectively for treatments 1, 2 and 3 were used. Data were analyzed using ANOVA at $\alpha_{0.05}$. **Results:** Except feed conversion ratio (FCR) other parameters measured were significantly different, 26% CP had most efficient FCR (2.85). Average feed intake, daily feed intake, cost per kg feed and cost per kg weight gain increase with increase in the levels of protein. Weight of gut sections of broiler differ significantly ($p<0.05$) across treatments except oesophagus, proventriculus, duodenum and large intestine. Coliform forming unit of total aerobic microbes (CFU g^{-1}) was higher than other identified microbes in the GIT of broiler chicks. Caecum total aerobic plate count and total anaerobic plate count were higher compare to other sections. *Enterobacter aerogenes* was found in all regions of broiler examined irrespective of dietary protein levels. *Salmonella* specie also present across the treatments and present in every section except intestinal of broilers fed 26% CP. *Lactobacillus* species was found common in caecum and intestine of broilers. **Conclusion:** There is need for broiler farmers to embark on phase feeding to allow fast growing of broilers and sound biosecurity to suppress the growth of the pathogenic microbes.

Key words: Gut ecology, *Enterobacter aerogenes*, varied protein, gut morphometry, pathogenic microbe and crude protein

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Broilers are fast growing birds that are common on farm today because of fast growth rate, efficient use of feed and quick return to the farmer. Formulation of balanced diets is fundamental to economical poultry production and this process depends on knowledge of nutrient requirements of broiler and the nutritional attributes of nutrient sources NRC[1]. Thus, a compilation of information on protein requirements and recommendable levels that can be used by feed formulators as a guideline is an important resource. Two phases feeding that were accustomed by many farmers to meet the recommendation according to NRC[1] needs to be reviewed base on the continuous effort of breeders all over the world to improve the growth of broiler that has yielded serious positive results, by reducing the number of days used before attaining the slaughtering weight of which feed has major effects.

Feed accounts for 50-70% of the total costs in animal production[2] and feed is the major factors that affect the rate of growth while the digestive tract is the engine required for proper digestion of feed in monogastric animals like broiler. Digestive tract also known as gastrointestinal tracts are tube line by specialized epithelial cells that are continuous with the epithelial layers covering the skin and according to Kogut and Arsenault[3], gut health is an increasingly important topic in animal nutrition. Digestive tract can be referred to as internal milieu. Effective functionality of the gastrointestinal tract (GIT) and its health are important factors in determining animal performance. Several, complex mechanisms are involved in the regulation of GIT functionality and health, therefore it is crucial to deepen our knowledge of these interactions so that strategies for the modulation of GIT functionality and health, in context of improved animal performance, can be developed[4]. Digestive tract has many sections that play different role in the digestive process of animal and throughout the entire life, the intestine changes, while some alterations in its form and function may be genetically determined and some are the result of adaptation to diet, temperature, or stress[5]. For example according to Gabrielle[6], protein affects villus height and cell turn over and abnormality of the intestine makes our valuable diet to end up in the faeces as a result of malabsorption in the gut. Challenges of gut make the gut to concentrate the valuable protein needed for growth on the recuperation of the gut, because healthy gut is vital to animal growth and development. According to Montagne *et al.*[7] maintenance of gut is a complex process and relies on a delicate balance between the diet and commensal microflora. This is so because the animal health begins in the gut. Gail[8] reported that all diseases begin in the gut and this as further established the important of gut to the animal. Gut is a major user of protein especially when challenge by disease and during microflora variation that is capable of altering the physiological activity of the gut.

The normal digestion and absorptions in the small intestine depend upon a self-renewing population of cells developed from intestinal crypts to the tips of villi. Villi are microscopic, fingerlike structures that increase the surface area of the gastrointestinal tracts and the lengths of gut determine its population. Therefore efforts need to re-examine the protein requirement with respect to the production performance, gut morphometry characteristics and gut microflora changes as affected by protein variation in the broiler.

MATERIALS AND METHODS

Experimental site: The experiment was carried out at the Poultry Unit of the Teaching and Research Farm, LAUTECH, Ogbomosho, Oyo state, Nigeria. This research was conducted from January, 2017 to March, 2017.

Pre-experimental management: Preparation before arrival of the birds include general cleaning of the pen which involved sweeping, washing, scrubbing, fumigation of the pen, removal of unused wood, repair and partitioning of damaged nets and also washing and disinfecting the feeder and watering trough, all these were done before the wood shavings was sourced for at Ogbomoso saw mill. Materials for brooding were made available while alternative provisions were put in place in case of electricity fluctuation.

Animal handling: A total of one hundred and forty-four days old broiler chicks (Arbor acre strain) were used for the study and broiler starter mash containing 3000 Metabolisable energy (kcal kg^{-1}) with varied protein percentage of 20, 23 and 26%, respectively for treatments 1, 2 and 3, tagged Diet 1, diet 2 and diet 3, respectively, were fed to the broiler chicks (Table 1). The chicks were weighed and randomly allotted to the three dietary treatments in triplicate of 16 chicks each in a completely randomized design.

Data collection: During the period of experiment, the following data were taken.

Table 1: Composition of starter diets (g/100 g DM)

	Diet		
Ingredients	1	2	3
Maize	58.25	52.05	41.95
Soybeans	30.20	34.20	44.30
Fish meal	2.00	2.00	2.00
Brewer's dried grain	4.20	4.20	4.20
Vegetable oil	3.00	3.00	3.00
Di calcium phosphate	1.50	1.50	1.50
Oystershell	1.25	1.25	1.25
Salt	0.25	0.25	0.25
Methionine	0.15	0.15	0.15
Lysine	0.15	1.15	1.15
**Premix	0.25	0.25	0.25
Total	100.00	100.00	100.00
Crude protein (%)	20.02	23.05	26.00
Metabolisable energy (kcal g^{-1})	3164.45	3131.02	3056.88
Crude fibre (%)	3.32	3.68	3.75

Diet 1: 20% CP, Diet 2: 23% CP, Diet 3: 26% CP, **Premix composition: Premix composition (per kg of diet) Vit (Vitamin) A: 1000 IU, Vitamin D_3: 2000 IU, Vit. E: 4000 mg, Vit K_3: 900 mg, Vit B_1: 500 mg, Vit B_2: 2200 mg, Vit B_3: 5500 mg, Vit B12: 4 mg, pp: 18000, Folic acid: 400 mg, Choline chloride: 150000 mg, Antioxidant BHT: 0.05%, Iron: 1.80%, Copper: 0.20%, Mn: 2.40%, Cobalt: 0.04%, Zn: 2.80%, Iodine: 0.04%, Selenium: 0.016%, Ca: 12.8570% in 2.5 kg

Weekly weight changes: Records of growth of the birds were taken by weighing on arrival on the farm at the beginning of experiment and on weekly basis throughout the research period. The growth rate was determined as the difference between the weight of the previous week and the present week. This is mathematically expressed as[9]:

Weekly weight gain = Present week weight-Previous week weight (g)

Feed intake calculation: The feed intake was calculated by measuring the amount of diet given and the left over diet. The deduction of the weight of unexpended feed from the weight of feed supplied gives the feed intake and this is mathematically expressed as[9]:

Feed intake = Feed supplied-Left over feed (g)

Feed to gain ratio: This is also known as the feed conversion ratio, calculated as total feed intake divided by the total weight gain[9].

Gut analysis: Gut relative weight and length were determined using sensitive scale and measuring tape/digital vernier caliper respectively.

Isolation and identification of bacteria: Two birds were randomly picked from each replicate making six birds per treatment and digesta were aseptically collected into sterile bottles, covered and kept in a cooler. Digesta were collected from the gizzard, small intestine and caecum. Isolation and identification of bacteria was done based on their morphological, staining, cultural, haemolytic and biochemical properties described by Chessbrough[10]. Laboratory procedure include serial dilution, inoculation of diluents in to a sterile nutrient agar for incubation at 37°C and catalase test and gram staining for characterization and identification were conducted.

RESULTS AND DISCUSSION

Growth performance of broiler starter fed varied level of protein is presented in Table 2. Except the feed conversion ratio all other parameters measured were significantly different and 26% crude protein (CP) had the most efficient feed to gain ratio (FCR). The average feed intake, daily feed intake, cost per kg feed and cost per kg weight gain increase with increase in the level of protein. The birds placed on high protein level consumed the highest quantity of feed and recorded significantly ($p<0.05$) higher weight (878.76 g/bird) gain compare to the other birds on lower protein levels (816.70 and 872.70 g/bird for 20 and 23% CP, respectively). Results of growth of broiler chicks as shown in Table 2 agreed with the report of Teteh *et al.*[11] who found that 28 days old chicks fed low-protein diet (16.00 vs. 20.00% CP) had a lower weight gain than the control group, due to a lower feed intake. Aletor *et al.*[12] associated the discrepancies to several factors such as the degree of CP reduction, the amino acids supplementation, the level of metabolisable energy, the class and age of the chickens. Increase in the feed intake may be as a result of dietary composition. Ferket and Gernat[13] established that dietary nutrient composition is one of the major factors that affect feed intake. The high feed intake that led to increase in the weight gain during starter phase can only be attributed to the protein level as suggested by Ferket and Gernat[13], that feed intake is greatly influenced by the diet. The high level of protein might have encouraged the distention of gut to hasting its motility, this according to Ferket and Gernat[13] observed that gut distension and gut motility most likely influence feed intake. Higher protein level in this study enables fast growth during starter phase this agreed with the report of Adeyemo *et al.*[14] that deficient protein affected growth development in young birds and the overall vitality in adult birds can be affected. Carlomagno *et al.*[15] and Malik *et al.*[16] reported that protein deficiency inhibited antibody production and development of antibody production cells in response to T-dependent antigens.

Table 2: Growth performance of broiler starter fed varied levels of protein

	Crude protein (%)			
Parameters	20	23	26	SEM
Avg initial weight (g)	42.58	42.58	42.33	-
Avg total feed intake (g)	1648.02^{c}	1674.94^{b}	1736.83^{a}	3.51
Avg daily feed intake (g)	58.85^{c}	59.81^{b}	62.02^{a}	0.30
Avg weight gain (g) (28 days)	816.70^{b}	872.70^{a}	878.72^{a}	1.38
FCR	3.04	2.94	2.85	0.04
Cost/kg feed (₦/kg)	108.21^{c}	115.67^{b}	122.78a	1.16
Cost/kg weight (₦)	329.14^{c}	340.60^{b}	349.34^{a}	4.73

$^{a-c}$Mean in the same row with different superscripts are significantly ($p<0.05$) different, SEM: Standard error of mean

Table 3: Relative gut weight of broiler fed varied level of protein (starter)

	Crude protein (%)			
Parameters	20	23	26	SEM
Oesophagus	0.33	0.42	0.47	0.50
Proventriculus	0.53	0.49	0.57	0.01
WGIT	13.26^{a}	11.95^{a}	10.77^{b}	0.29
Gizzard	4.12^{a}	3.86^{a}	3.11^{b}	0.10
Duodenum	1.26	1.29	1.25	0.04
Small intestine	7.42^{a}	6.87^{ab}	6.22^{b}	0.17
Jejunum	3.67^{a}	2.75^{b}	2.80^{b}	0.15
Ileum	2.48^{ab}	2.82^{a}	2.16^{b}	0.10
Caecum	1.31^{a}	1.33^{a}	0.83^{b}	0.00
Large intestine	0.61	0.49	0.46	0.05

$^{a-b}$Mean in the same row with different superscripts are significantly ($p<0.05$) different, SEM: Standard error of mean

Table 4: Relative gut length of broiler fed varied level of protein (starter)

	Crude protein (%)			
Parameters	20	23	26	SEM
Oesophagus	1.72	2.04	2.04	0.08
Proventriculus	0.97^{a}	0.70^{b}	0.71^{b}	0.03
WGIT	45.91^{ab}	48.11^{a}	46.62^{b}	0.09
Duodenum	5.09^{ab}	5.97^{a}	4.65^{b}	0.24
Small intestine	35.23^{ab}	37.57^{a}	32.59^{ab}	0.79
Jejunum	14.93	15.39	14.00	0.29
Ileum	79.33^{c}	92.00^{a}	85.00^{b}	1.47
Caecum	2.98^{ab}	3.29^{b}	2.83^{b}	0.08
Large intestine	1.70	1.88	1.47	0.34

$^{a-b}$Connote means in the same row with dissimilar superscripts are significant ($p<0.05$), SEM: Standard error of mean, WGIT: Whole gastrointestinal

Higher protein levels may be required at the first few day of broiler life to improve the growth rate. This was in line with the report Abdelrahman and Aljumaah[17], that sufficient supply of protein and well balanced amino acids, especially the most essential amino acids, methionine and lysine, is a very crucial factor for proper growth. Thus the concept of phase feeding has to be encouraged which according to Hong *et al.*[18] divides the growth periods into several phases and provide feed designated to each phase.

Table 3 and 4 showed gut morphometric characteristics of broiler fed varied levels of protein. Weight of the gut components of broiler differ significantly ($p<0.05$) across the treatment except oesophagus, proventriculus, duodenum and large intestine. Birds fed lower dietary protein had significantly higher gut weight compared to other treatments (Table 3). This shows that lower dietary protein did not affect the muscle turnover of the gut. The length of the different segment of the gut of broiler chicks as affected by dietary treatment is presented in Table 4, except proventriculus, other length of the guts that were affected by the dietary treatment, treatment 1 (20% CP) had lowest value. There was a significant reduction in the length of the ileum, jejunum, caecum and small intestine in response to different dietary protein levels. The reduction in the length of ileum as recorded in the Table 4 may have a significant reduction in the absorptive surface area of the gut. The reduction may be as a result of insufficient protein intake by the birds. The result of this study indicated that insufficient dietary protein affects the gut length and similar results has been reported that antibiotics increased body weight and decrease intestinal length and weight at all-time compare with control bird[19].

Table 5: *Faecal microbial load of broiler chicks fed varied levels of protein (starter phase)

	Crude protein (%)			
Parameter site	20	23	26	SEM
Total coliform (CFU M^{-2})				
Gizzard	14.40^{b}	16.80^{a}	15.00^{b}	4.59
Intestine	24.00^{a}	19.60^{b}	19.00^{b}	5.57
Caecum	17.00^{a}	13.00^{b}	13.00^{b}	0.62
Total aerobic plate count (×104 CFU g^{-1})				
Gizzard	101.00^{c}	127.80^{a}	121.00^{b}	0.38
Intestine	118.00^{c}	136.00^{b}	160.20^{a}	3.05
Caecum	132.00^{c}	145.00^{b}	173.00^{a}	1.20
Total anaerobic plate count (CFU g^{-1})				
Gizzard	22.00	22.00	22.00	1.43
Intestine	22.00	23.00	21.80	0.65
Caecum	51.00^{a}	32.00^{b}	23.00^{c}	0.33
Fungi count (×102 CUF mL^{-1})				
Gizzard	2.00^{c}	12.00^{b}	14.00^{a}	0.34
Intestine	6.00^{b}	15.00^{a}	6.80^{b}	1.13
Caecum	8.00^{c}	10.00^{b}	18.00^{a}	5.09

*Faeces collected in the intestine, $^{a-c}$Connote means in the same row with dissimilar superscripts are significant ($p<0.05$)

Table 5 shows the faecal microbial load of broiler chicks fed varied levels of protein at starter phase. Dietary protein levels affect the value of microbial load in the gut. Table 5 establishes that the broiler GIT consist bacteria and other microflora. Total coliform (CFU M^{-2}) was higher under lower CP while there were similar coliform count at both 23% and 26% CP. Total aerobic plate count ($\times 10^4$ CFU g^{-1}) and total anaerobic plate count (CFU g^{-1}) increase along the GIT. Coliform forming unit of total aerobic microbes (CFU g^{-1}) was higher than anaerobic plate count. Coliform forming unit of total aerobic microbes (CFU g^{-1}) was higher than other

Table 6: Microbial isolates from the different gut sections of the broiler chicks fed varied protein level (starter phase)

Treatments	Site	Isolated microorganisms
20% crude protein	Gizzard	Ea, Sp, Ag, As
	Intestine	Ea, Sp, Ec, Lb, Ml, As
	Caecum	Ea, Sp, Ec, Ml, Cl, Lb
23% crude protein	Gizzard	Ea, Sp, Sa, Ag, An
	Intestine	Ea, Sp, Ec, Ml
	Caecum	Ea, Sp, Ec, Cl, Ml, Lb
26% crude protein	Gizzard	Ea, Sp, Sa, As, Ag
	Intestine	Ea, Ec, Ml, Lb, As
	Caecum	Ea, Sp, Ec, Cl, Ml, Lb,

Ea: *Enterobacter aerogenes*, Ml: *Micrococcus luteus*, Ec: *Escherichia coli*, As: *Alternaria* species, Sp: *Salmonella* specie, Cl: *Clostridium welchii*, Sa: *Staphylococcus aureus*, Ag: *Aspergillus glaucus*, An: *Aspergillus niger*, Lb: *Lactobacillus* species

identified microbes in the GIT of broiler chicks (Table 5) this is not in tandem with the finding of Pan and Yu[20] which concluded that gastrointestinal (GI) tract of poultry comes into contact with exogenous microorganisms shortly after hatch and steadily becomes a warm section for a complex microbiome consisting primarily anaerobic bacteria. Fungi count is smaller compare to other microbes found in the GIT of the broiler chicks. This may be an indication of presence of sufficient oxygen along the gastrointestinal tract of broilers therefore; GIT can be referring to external milieu. Variation in the population of the different types of microbes may be as a result of varied requirement for survival by different microbes and the environment. The microflora population that lives in the GIT is a mixture of bacteria and other microorganism such as fungi and protozoa although bacteria make up the largest portion of the population[21].

There were variations in the type and population of microflora in the broiler GIT, this is in line with the finding of Poultry Health Today[22] that different bacteria have different food preferences, so the microbial population of GIT is largely affected and determined by what the birds eats. There was significant variation in bacterial population of the GIT and the population increase along the gastrointestinal tract, this is in accordance with the finding of Richards *et al*.[23], that there is diversity in bacterial population at various locations along the GIT and the populations tend to increase from the front to the back of the tract.

Caecum total aerobic plate count and total anaerobic plate count were higher compare to other sections; this is in tandem with the report of Albazaz and Buyukunal Bal[21], who recorded higher microbes in the caecum than any other regions of the GIT during the review work on the gut microbes of poultry. It has been reported that the microbial communities present in the gastrointestinal tract of poultry are influenced by a number of factors including stocking density, diet, feeding practices, housing conditions, age of the birds and pathogens[24].

The list of microorganisms isolated from the three regions of the gastrointestinal tract of broilers is shown in Table 6. There was a very wide range of microorganisms isolated from the alimentary tract of broilers fed different levels of protein at starter phase. Each of the regions of GIT has its own unique microbes. However, there were some bacteria species that were found to be common irrespective of dietary treatments and the region. *Enterobacter aerogenes* was found in all the regions of broiler examined irrespective of the dietary protein levels. *Salmonella* species also present across the treatments and present in every region except intestinal region of broilers fed 26% CP. *Lactobacillus* species was found common in caecum and intestine alone of the broilers. *Escherichia coli* were present in all the regions of the gut examined, except gizzard irrespective of the protein in the diet. Each of the regions of GIT has its own unique microbes as shown in table 6 and according to Richards *et al*.[23] becomes complicated as the birds ages. This is in line with the report of Sherfi *et al*.[25], which stated that gut flora can change in response to an altered environment and can vary qualitatively and quantitatively depending on the diet. Availability of *Enterobacter aerogenes* in the entire sections examined shows its ability to survive in different environment. It is the first most common species isolate. *Enterobacter* is a genus of gram-negative, rod shaped, facultative anaerobic and non-spore forming microbes of family *Enterobacteriaceae*. *Enterobacter aerogenes* (*E. aerogenes*) is well known opportunistic bacteria pathogen[26], which can cause respiratory tract infections. That means *E. aerogenes* has high resistant to many antibiotics making their availability easy in various sections of the broiler gut. Research has established increasingly resistance of *E. aerogenes* against different antimicrobials which has led to emergence of multidrug resistant (MDR) isolates[26]. According to Jacoby[27] the last 10 years, clinical isolates of this species have shown natural resistant against aminopenicillins. Enterobacter species are responsible for high morbidity and mortality rate in recent years due to nosocomial infections and other health issues[28], due to extended resistance of gram-negative bacteria against almost all antibiotics[29,30]. *Escherichia coli* is a rod shape bacteria which has ability to form capsule and able to produce both heat labile and heat stable toxins. Some serotypes can cause disease such as watery diarrhea, peritonitis, gallbladder infections, gastroenteritis etc. which

can be treated using sulfa drug or quinolone (which inhibit the replication of bacterial DNA). Although, Sherfi *et al.*[25] had earlier said *Escherichia coli* is inhabitant of the intestinal tract of humans and animals, this study has established the exception of the gizzard of broiler gut not habitable by *E. coli*.

CONCLUSION

Enterobacter aerogenes and *Salmonella* species were the most common bacteria in the GIT of broiler chickens and farmer must ensure good biosecurity to suppress the growth of the pathogenic microbes to a tolerable level by the animals.

SIGNIFICANCE STATEMENT

This study discovers the important of protein in the early development of broiler chicks, indicates that there were varied microorganisms in the sections examined and these can be beneficial to nutritionist, practicing farmer to practice phase feeding in the raising of broiler chicks. Also veterinary doctors and drug producers (pharmacists) will be accustoms with the likely available microbes in the gastrointestinal tracts of broiler chicks.

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