



COMPARATIVE STUDY OF BACTERIA IN THE DIGESTIVE TRACTS OF CHICKEN REARED AS FREE RANGERS AND THOSE REARED IN POULTRY

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ABSTRACT

The bacterial flora of the digestive tracts of chickens reared at home was compared with those of poultry chickens. Samples obtained from the crop, gizzard and intestine were cultured on nutrient agar. Bacterial population was estimated using plate count method. Poultry chickens had higher population of bacteria while a greater diversity occurred in the digestive tracts of chickens reared at home. Among the isolates, Lactobacillus sp. and Escherichia coli were found in all the compartments of the two categories of chickens studied. Enterobacter aerogenes, Bacillus subtilis and Bacillus cereus were also isolated from all the chickens. In addition, Proteus vulgaris, Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecium, Bacillus megaterium, and Streptococcus pyogenes occurred in the digestive tracts of the free rangers. The high diversity of bacteria obtained from the free rangers reflects the method of feeding which may predispose that category of chickens to attack by pathogenic organisms. A number of isolates hydrolyzed starch and cellulose. They may play important role in the digestion of the carbohydrate especially cellulose.

Keywords: Poultry Chickens, Free Rangers, Digestive Tracts, Bacteria.

INTRODUCTION

Chickens primarily are rich source of protein in terms of eggs and meat, but also provide minerals and vitamins such as calcium, phosphorus, iron, vitamin B₁₂ and thiamin (Philips, 1990). Chicks are widely used in scientific research and play important roles in the discovery of many vitamins and essential minerals. Eggs of chickens are used as growth medium in virology and production of vaccines.

The digestive tract of a newly hatched chick is usually sterile. Microorganisms however gain access within minutes from the mother and the environment. Mead and Adams (1975) isolated large numbers of anaerobic bacteria from chicks only 3 to 6 hours after hatching. The digestive tract of adult chicken is dominated by anaerobes in the genera *Bacteriodes*, *Bifidobacteria*, *Enterococcus*, *Lactobacillus*, and *Streptococcus* (Amit-Romach *et al.*, 2004). The microflora of the digestive tract of chickens play major roles in the animal's nutrition, in the detoxification of certain compounds, in growth performance as well in the protection of the animal against pathogenic bacteria (Gibson and Roberfroid, 1995; Mead, 1997; Vispo and Kanasor, 1997; van der Wielen *et al.*, 2002). In poultry, the absence of normal microflora in the cecum has been considered a major factor in the susceptibility of chicks to bacterial infection (Barrow, 1992).

Among the factors that may determine the microflora of the digestive tract is diet (Gabriel *et al.*, 2006). Chickens are usually reared in commercial poultry

facilities where the diet is enriched. Some are reared as free rangers that pick particles indiscriminately from the floor and are fed with diets that may not be as rich.

In this study, the microflora of the two categories of chickens were investigated in relation to their diet and as determined by the rearing facilities.

MATERIALS AND METHODS

Sample collection

Five chickens reared as free rangers (about 12 weeks old) and five others reared in poultry (12 weeks old) were purchased and sacrificed. The crop, gizzard and intestine of the chickens were removed, sliced and samples were taken from the content. The samples comprised of foods at different stages of digestion.

Isolation, enumeration and characterization of organisms

Each sample was treated by measuring 1g into sterile distilled water (9ml). The mixture was thoroughly shaken to dislodge the microorganisms. A serial dilution up to 10⁻⁵ was prepared. An aliquot (0.1ml) was inoculated on nutrient agar plates from each of 10⁻⁴ and 10⁻⁵ dilutions. Plates were allowed to dry and then incubated at 37°C for 18h. Cultures were removed from incubator and bacterial growth enumerated with the aid of a colony counter. Individual colonies were transferred to separate agar plates and subcultured thrice to obtain pure cultures. Cultures were maintained on agar slants.



Isolates were characterized in the microbiology laboratory of the University of Ilorin based on conventional laboratory methods (Fawole and Oso 2004).

Carbohydrate hydrolysis tests

Hydrolysis tests for cellulose were performed following a plate screening technique described by Ten *et al.* (2004). Organisms were grown on nutrient agar plates comprising of 1% (w/v) carboxyl methyl cellulose for 24hrs. Plates were flooded with congo red solution for 15 minutes, drained and flooded with 1N sodium chloride solution for another 15 minutes. Plates were observed for clearance around bacterial colonies. Diameter zone of clearance around each colony was measured in three planes and mean calculated.

Starch hydrolysis was tested by incorporating soluble starch into the nutrient agar used to cultivate the bacteria. Cultures, 24hr old were flooded with iodine solution for ten minutes and observed for zone of clearance around the isolates.

RESULTS

The results of the bacterial colony count obtained in samples from the different compartment of the digestive tracts of the chickens are shown in figure 1. The population of bacteria isolated from all the three compartments of the digestive tract of poultry chickens was higher compared to those obtained from

chickens reared as free rangers. In addition, the intestines of the two categories of chickens harbor more bacteria than the other parts of the digestive tract.

The diversity of bacteria in the digestive tracts of chickens reared as free rangers was greater than those of poultry chicken (Table 1). *Escherichia coli* and *Lactobacillus* sp. were obtained from all the compartments of the digestive tract of poultry chickens. *Bacillus cereus* occurred in the crop and gizzard while *Enterobacter aerogenes* was isolated only from the intestine. Bacteria from the intestine of free rangers were *Escherichia coli*, *Lactobacillus* sp., *Bacillus cereus*, *Enterobacter aerogenes*, *Bacillus subtilis*, *Staphylococcus epidermidis*, *Enterococcus faecium*, *Bacillus megaterium* and *Proteus vulgaris*. All the bacteria were also isolated from the crop except *Enterobacter aerogenes* and *Bacillus cereus*. *Staphylococcus aureus* was found only in the crop while *Streptococcus pyogenes* occurred in the gizzard of the free rangers only. In addition, *E. coli*, *Lactobacillus* sp. and *B. megaterium* were also isolated from the gizzard of the free rangers.

Eight of the isolates were cellulolytic while only four hydrolyzed starch. Highest cellulase activity represented by diameter zone of clearance was obtained with *Streptococcus pyogenes* while the least activity occurred with *Staphylococcus aureus* (Table 2).

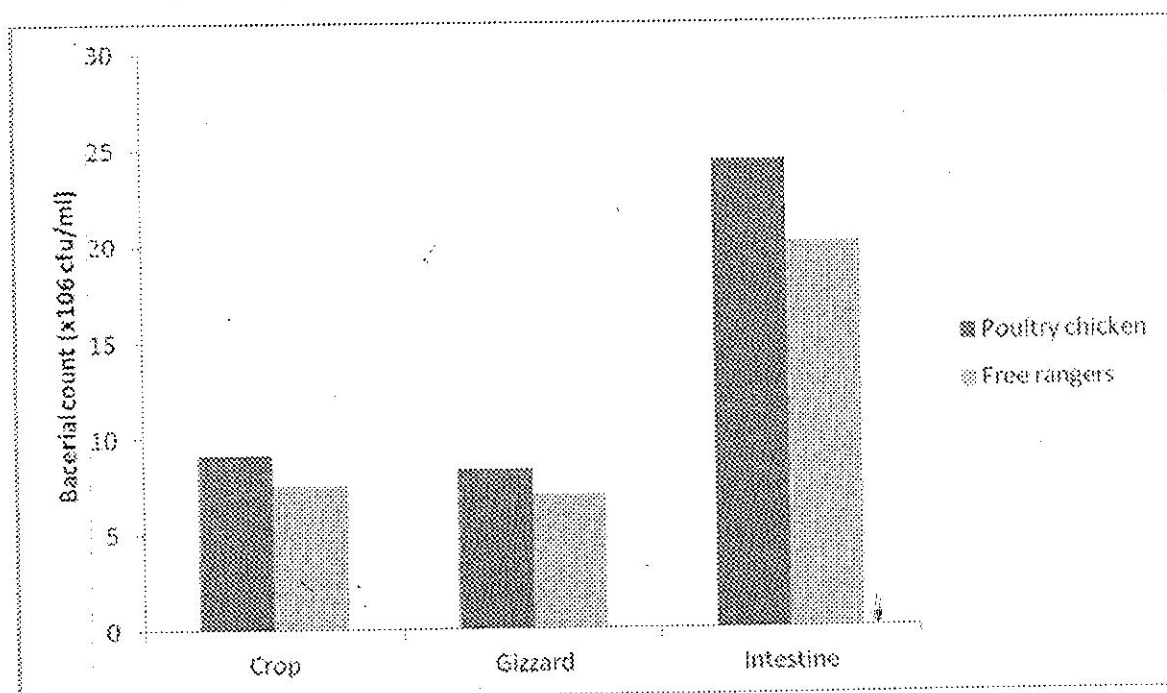


Fig. 1 Comparative distribution of bacteria in the digestive tract of chickens reared in a poultry and those reared as free rangers. Each value represents the mean ($P < 0.5$) obtained from five samples taken from different chickens in the category.



Table 1: Distribution of bacteria among compartments of chickens reared in poultry and those reared as free rangers

ISOLATES	POULTRY CHICKENS			FREE RANGERS		
	CROP	GIZZARD	INTESTINE	CROP	GIZZARD	INTESTINE
<i>Escherichia coli</i>	+	+	+	+	+	+
<i>Bacillus cereus</i>	+	+	-	-	-	+
<i>Bacillus subtilis</i>	-	+	-	+	-	+
<i>Ent. aerogenes</i>	-	-	+	-	-	+
<i>Lactobacillus sp</i>	+	+	+	+	+	+
<i>Strept. pyogenes</i>	-	-	-	-	+	-
<i>Staph. epidermidis</i>	-	-	-	+	-	+
<i>Staph. aureus</i>	-	-	-	+	-	-
<i>Ent. faecium</i>	-	-	-	+	-	+
<i>Bacillus megaterium</i>	-	-	-	+	+	+
<i>Proteus vulgaris</i>	-	-	-	+	-	+

Key + denote occurrence of bacteria in the sample
- denote that the bacteria species was not found in the sample

Table 2: Hydrolysis of Starch and Cellulose

Probable Organisms	Starch Hydrolysis	Cellulose Hydrolysis	Zones of Clearance (mm) (cellulose hydrolysis)
<i>Escherichia coli</i>	-	-	Nil
<i>Bacillus cereus</i>	+	-	Nil
<i>Enterobacter aerogenes</i>	+	+	7mm
<i>Lactobacillus sp</i>	+	+	14mm
<i>Bacillus subtilis</i>	-	+	13mm
<i>Proteus vulgaris</i>	-	+	15mm
<i>Staphylococcus aureus</i>	-	+	6mm
<i>S. epidermidis</i>	-	+	9mm
<i>Enterococcus faecium</i>	-	+	7mm
<i>Bacillus megaterium</i>	-	-	Nil
<i>Streptococcus pyogenes</i>	+	+	18mm

KEY: + denote hydrolysis of the carbohydrate, - denote carbohydrates not hydrolyzed

DISCUSSION

Poultry chickens are fed with diets that have been enriched for improved productivity in terms of quality of eggs and meat. Bacteria take advantage of the nutrients to multiply in the digestive tract. Hence the comparative high population observed in the gut of poultry chickens. A similar high bacterial load had been reported (Gabriel *et al.*, 2006). On the other hand, free rangers pick their food indiscriminately and are therefore prone to picking wide varieties of organisms including the beneficial and pathogenic bacteria. Gabriel *et al.*, (2006) suggested that beneficial bacteria found in the gut of chickens may serve to protect them against pathogens through a competitive exclusion process. The occurrence of *Escherichia coli* in all the compartments of the chicken's digestive system is in not unexpected since the organism is one of the normal flora of the digestive tract of most animals (Amit-Romach *et al.*, 2004). *E. coli* is however an adaptive species that is both a commensal resident of the intestine and a versatile pathogen of humans and other animals causing enteric infections (Dozois *et al.*, 2003). Therefore, its role as a potential pathogen in the

intestinal tract cannot be overemphasized. *Lactobacillus sp.* is a probiotic and also a normal flora of the digestive system (Barrow, 1992; Amit-Romach *et al.*, 2004; Lucchini *et al.*, 1998; Mikkelsen *et al.*, 2003). Most of the other bacteria found in the digestive tracts of the poultry chickens are also normal flora of chicken's digestive tracts (Owings *et al.*, 1990) and may not constitute any threat except under immuno-compromised situation. The digestive tracts of the free rangers however harbor some bacteria such as *Staphylococcus aureus* and *Staphylococcus epidermidis* which may be pathogenic on the birds and may affect productivity in terms of the quality of eggs and meat. Apajalahti *et al.*, (2004) reported that the level of hygiene in the environment where chickens grow is important in determining their microflora and may be responsible for the high diversity obtained. The ability of an organism to utilize wide variety of substrates may give it a competitive advantage (Apajalahti *et al.*, 2004). This explains the occurrence of the bacteria that have cellulolytic activities majorly in the digestive tracts of the free rangers whose diets are likely to contain high level of cellulosic materials.



CONCLUSION

Results obtained from this study have demonstrated the effects of diet and the conditions under which chickens are reared, on their microflora which may

have a consequential effect on the health of the chickens. The microflora also affects the metabolic activities of the chickens particularly regarding digestion of cellulose.

REFERENCES

- Amit-Romach E., D. Sklan and Z. Uni. 2004. Microflora Ecology of the Chicken Intestine Using 16S DNA primers. *Poultry Science* 83:1093-1098.
- Apajalahti, J., A. Kettunen and H. Graham (2004). Characteristics of the gastrointestinal microbial communities with special reference to the chickens. *World Poultry Science Journal*. Pp 60.
- Barrow, P. 1992. Probiotic for chickens. Pages 225–257 In: Probiotics, the Scientific Basis. R. Fuller, ed. Chapman and Hall, London.
- Dozois, C. M., F. Daigle, and R. Curtiss, III. 2003. Identification of pathogen-specific and conserved genes expressed *in-vivo* by an avian pathogenic *Escherichia coli* strain. *Proceedings of the National Academy of Science, USA* 100:247–252.
- Fawole, M. O. and Oso, B. A. (2004). *Characterization of Bacteria*. In: *Laboratory Manual of Microbiology*. Spectrum Books Limited, Nigeria. pp 22-33
- Gabriel, I. M., Lessire, S. Mallet and J.F. Guillot (2006). Microflora of the digestive tract: critical factors and consequences for poultry. *World's Poultry Science Journal* 62:499-511.
- Gibson, G. R. and M. B. Roberfroid (1995). Dietary modulation of the human colonic microbiota: Introducing the concept of probiotics. *Journal of Nutrition* 125:1401-1412
- Lucchini, F., V. Kmet, C. Cesena, L. Coppi, V. Bottazzi, and L. Morelli. 1998. Specific detection of a probiotic lactobacillus strain in faecal samples by using multiplex PCR. *FEMS Microbiology Letters* 158:273–278.
- Mead, G. C. (1997). *Bacteria in the gastrointestinal tract of birds*. In: *Gastrointestinal Microbiology. 2. Gastrointestinal Microbes and Host Interactions*. R. J. Mackie, B. A. White, and R. E. Isaacson (eds.) Chapman and Hall, New York. Pp 216–240
- Mead, G. C., and B. W. Adams. 1975. Some observations on the caecal microflora of the chick during the first two weeks of life. *British Poultry Science* 16:169–176.
- Mikkelsen, L., C. Bendixen, M. Jakobsen, and B. Jensen. 2003. Enumeration of bifidobacteria in gastrointestinal sample from piglets. *Appl. Environ. Microbiol.* 69:654–658.
- Owings, W. J., D. L. Reynolds, R. J. Hasia and P. R. Ferket (1990). Influence of dietary supplementation with *Streptococcus faecium* M-74 on broiler body weight, feed conversion, carcass characteristics in the intestinal microbial colonization. *Poultry Science* 69:1257-1264
- Phillips, R.W. (1990). *Microlivestock, National Research Council*. Academic press, Washington. Pp. 91-99.
- Ten L. N., W. T. Im, M. K. Kim, M. S. Kang and S. T. Lee. (2004). Development of a plate technique for screening of polysaccharide-degrading microorganisms by using a mixture of insoluble chromogenic substrates. *Journal of Microbiological Methods* 56: 375-382
- van der Wielen, P. W., D. A. Keuzenkamp, L. J. Lipman, F. van Knapen, and S. Biesterveld (2002). Spatial and temporal variation of the intestinal bacterial community in commercially raised broiler chickens during growth. *Microb. Ecol.* 44:286–293.
- Vispo, C. and W. H. Karasov (1997). *The interaction of avian gut microbes and their host: An elusive symbiosis*. Pages 116–155. In: *Gastrointestinal Microbiology 1 - Gastrointestinal Ecosystem and Fermentations*. R. J. Mackie, and B. A. White, ed. Chapman and Hall, New York.