

MICROBIOLOGY OF THE GASTROINTESTINAL TRACT OF TURKEY FED FERMENTED AND NON FERMENTED LIQUID FEED

¹Uchewa, E.N., ²Nwibo, S. U and ²Mbam, B.N

Department of Animal Science, Ebonyi State University, P.M.B 53 Abakaliki
Department of Agric. Economics, Ebonyi State University, P.M.B 53 Abakaliki

Abstract

The microbiology of the gastrointestinal tract of turkey fed fermented and non-fermented liquid feed was investigated in an experiment that lasted for 12 weeks. Sixty five day old poults were used for the experiment. In a Completely Randomized Design each treatment was replicated 3 times with 7 birds per replicate. Commercial poultry starter and growers diets were used such that T₁ is dry feed, T₂ diet was non fermented liquid diet while T₃ diet was fermented with lactobacillus acidilactici. Non fermented feed (T₂) was prepared daily by mixing the dry feed with water in a feed to water ratio of 1:2 while the fermented liquid feed (T₃) was prepared by the addition of lactobacillu acidlactici, a strain of lactic acid bacteria for a period of 24hrs at temperature of 3⁰C . The birds were given ad liitum access to experimental diets and water. Data was collected on the pH value of feed, Lactic Acid Bacteria: coliform ratio, coefficient of correlation. indicates; significant (p < 0.05) higher concentration of lactobacillus spp in T₃ (0.00021 CFU), significant (p<0.05) higher lactobacillus- coliform ratio in T₃ (7.04 CFU), higher concentration of salmonella spp in T₁ (0.000042) and higher concentration of sacharomyces spp in T₂ (0.00060 cells/ml). Cost benefit analysis indicated higher cost of production in T₂ and T₃ with mean values of ₦1743.42 and ₦1770.26 respectively. Similarly, higher revenue was generated in T₂ and T₃ turkeys with mean values of ₦696.58 and ₦1014.74 respectively. Results for coefficient of correlation of GIT bacteria to body weight of turkey shows that lactobacillus and lactobascillus- coliform ratio were positively correlated while coliform, salmonella and saccharomyces spp were negatively correlated to body weight of turkey. This study therefore concluded that turkeys fed T₃ diets fermented with lactic acid bacteria culture had a better microbial profile, highest Body weight, better cost benefit ratio and better result for correlation.

Introduction

The chicken gastrointestinal (GI) tract contains many bacteria. These bacteria are distributed throughout the GIT tract, but due to differences in morphology, functionality, metabolic interactions, a microenvironment, regional heterogeneity in community composition is observed along the different GIT segments (Urlings *et a* 1993). The composition of the bacterial communities is believed to be influenced mainly by age, diet, and gut location (Moran, 2001). However, host genetics, rearing environment, stress, immune status, and interactions within bacterial communities are also important factors. The bacterial communities play a significant role in broiler growth, modulating the development of the digestive tract, influencing the production of bile acids and digestive enzymes, and consequently influencing nutrient digestion and absorption (Mikkelson and Jensen 1997). Further, they stimulate gut immune functions and prevent the colonization of the GI tract with avian-pathogenic or zoonotic bacteria via competitive exclusion and the nonspecific small intestinal overgrowth of certain intestinal bacteria (Jensen, 1998)

In order to control GI problems, different dietary interventions have been suggested; among these, fermented feed has gained increasing attention in animal nutrition (Ref). Fermented feed has a low pH (3.5 to 4.5) and contains numerous lactic acid bacteria (LAB) (10^8 to 10^9 CFU/g feed) that have been shown to improve chicken gut health(Ref). The low pH of the fermented feed results in acidification of the upper digestive tract, supporting its function as a barrier against acid-sensitive pathogenic bacteria such as *Escherichia coli*, *salmonella* spp., and *campylobacter* spp. According to Brooks (2008) about 68% of the energy value of fermented carbohydrates can be metabolized into SCFA. All SCFA can contribute to the energy supply of the host (Brooks *et al.* 1996). Therefore, energy availability to the animal fed low energy diets containing potentially fermentable substrates could be increased by microbial fermentation. It is now realized that one of the principal functions of the GI tract includes the salvage of energy and nutrients through its symbiotic relationship with gut microflora (Brooks, 2008). The most important part of hydrolysis by enzymes takes place in the small intestines. However, a large proportion of digestion, which takes place by microbial fermentation in non-ruminants, occurs in the large intestine (Brooks *et al.* 1996, Brooks, 2008). Furthermore, fermentation in non-ruminant animals occurs to the largest extent in the large intestines (caecum and colon), mainly due to the longer transit time of the diet in this part of the GI tract (Brooks, 2008) in chicken particularly, a major portion of microbial fermentation is concentrated in the caeca Jensen, (1998). Therefore, improvements in fermentative activities within the gut will depend on the inclusion of ingredients that can escape the hosts' digestive enzymes in the small intestines and be available for fermentation by microflora in the large intestines. The objectives of the study therefore are to determine the ratio of microbial load (LAB: Coli form) in the gastrointestinal tract of turkey fed fermented and non-fermented liquid feed, to evaluate the cost benefit analysis of turkey fed fermented and non-fermented liquid feed and to determine the correlation between bacterial and body weight of turkey fed experimental diets.

Materials and Methods

Experimental site

The experiment was carried out at the poultry unit of the Department of Animal science, Faculty of Agriculture and Natural Resource Management of Ebonyi State University, Abakaliki. Abakaliki is within latitude $06^{\circ} 4'$ N, and $08^{\circ} 65'$ E. It has a mean elevation of 400m above sea level. The annual rainfall is about 1700mm to 2000mm spread between April and November(Ref).

Experimental Animals/Design

Sixty three day old turkeys were used for the experiment. The birds were allowed to adjust to the environment for complete seven days. On the 8th day, the birds were randomly selected and divided into 3 treatment groups in a Completely Randomized Design (Steel and Torrie 1980). Then each treatment was replicated 3 times with 7 birds per replicate.

Experimental diet

Commercial poultry starter and growers diets were used for the experiment. Non fermented feed was prepared daily by mixing the dry feed with water in a feed to water ratio of 1:2, while the fermented liquid feed was prepared by the addition of *lactobacillus acidilacti*, *lactobacillus bulgricus*, *streptococcus thermophilus*, strains of lactic acid bacteria for a period of 24hrs at a temperature of 30°C . The birds were given *ad libitum* access to experimental diets and water.

Feeding

The turkey poultts were given *ad libitum* access to experimental diets from rubber feeding trough , water was made available for consumption on watering troughs.

Laboratory Analysis

Samples (approx. 20ml) of the liquid feeds were removed from each batch of the formulation for pH measurement and for microbial analysis. The pH was measured using a digital electric pH meter (W.G.PYE and CO, Cambridge, UK), microbial analysis of the feed samples was conducted at the end by a serial dilution of representative sample in 10 folds using maximum recovery Diluent (MRD). Coliform was enumerated on De Man Rogosa and Sharpe (MRS) Agar using the doubled layered pour plate technique and was incubated aerobically for maximum of 24hours at 30⁰C.

Culture from Gastro- intestinal tract samples

Fresh samples from different part of the GIT (the small intestines, colon, ileum) were collected and homogenized immediately after slaughter and taken to the laboratory under the protection of ice blocks for microbial load analysis as was described by Derneckova *et al.* (2002)

Economics of production

This shows the profitability of the test diet. The economic benefit of feeding fermented and non-fermented liquid feed to turkeys was assessed by obtaining the cost (₦), Revenue, Benefit and cost benefit ratio for each treatment diet using the following formula.

Cost (₦)/kg feed intake= feed cost (₦)/kg x total feed intake

Cost (₦)/kg weight gain = Total feed cost (₦)

Kg Weight gain/turkey

Total cost = cost of feed intake + cost of turkey + miscellaneous

Revenue = Final Body Weight (kg) x cost (₦)/kg Live Weight Benefit

= total revenue – total cost

Cost benefit ratio = $\frac{\text{total cost}}{\text{Benefit (gain)}}$

Statistical analysis

The bacteria count per gram of gastro- intestinal tract samples was also transformed, tabulated and statistically analyzed. All other data collected was subjected to one- way ANOVA using SPSS package and the treatment means compared using F-LSD

The statistical model used is shown below

$$X_{ij} = \mu + T_i + E_{ij}$$

Where

X_{ij} = Any observation U= Population

mean T_i = Treatment effect

E_{ij} = (Epsilon) experimental error.

Results and Discussion

Microbiology of the gastro intestinal tract

The results for the GIT of turkey fed fermented and non- fermented liquid feed is presented in Table 1.

Table 1: Microbiology of the Gastro intestinal tract of turkey fed fermented and non-fermented liquid feed (LAB: coliform)

Parameters	T1	T2	T3	₦ SEM
PH	5.13	4.00	3.77	0.22
Lactobacillus spp (cells/ml)	0.0000020 ^a	0.000018 ^b	0.00021 ^a	0.000034
Coliforms (cells/ml)	0.000085 ^a	0.000005 ^c	0.000038 ^b	0.000012
Lactobacillus- coliform <u>ratio</u>	0.020 ^b	3.86 ^{ab}	7.04 ^b	0.000012
Salmonella spp (cells/ml)	0.000042 ^a	0.0000092 ^b	0.0000080 ^b	0.0000007
Sccharomyces spp (cells/ml)	0.000050 ^b	0.00060 ^a	0.000026 ^b	0.000095

abc: means with the same superscript in the same row are not statistically significant ($p < 0.05$).

The result shows significant ($p < 0.05$) differences in the mean values for all parameters analyzed. T2 and T3 had non-significant ($p < 0.05$) difference and reduced P^H value as against the control feed (T1) with a significant increased P^H value. The inclusion of LAB in the liquid fermented feed increased the *lactobacillus spp* in T3 and was not significantly different, while T1 with lesser *lactobacillus spp* load was significantly different to T2 and T3. Mean values for coliforms were significantly different across all treatment as T1 recorded the highest values followed by T1 and T2. The ratio of lactobacillus to coliform was significantly different ($p < 0.05$) as T1 and T2 were not significant ($p < 0.05$) in mean values, same with T2 and T3. Mean values for salmonella spp were not significant ($p < 0.05$) for T2 and T3, but T1 with the highest salmonella spp load was significantly ($p < 0.05$) different to other treatments. Mean values for *saccharomyces spp* were not significant ($p < 0.05$) in T1 and T3, while T2 with the highest value was significantly ($p < 0.05$) different to T1 and T3.

Cost benefit analysis

The results for the cost benefit analysis of turkey fed fermented and non-fermented liquid feed is presented in Table 2. The cost benefit analysis of Turkeys fed fermented and non-fermented liquid feed is presented in table 2. Significant differences were observed were observed in mean values for final body weight, body weight gain and total feed intake per bird as T3 recorded the highest significant ($p < 0.05$) value respectively. Furthermore, uniform cost/kg feed were observed with mean value of ₦ 136 per kg. the cost/kg intake per bird was highest in T3 (₦ 1049.66) followed by T2 and T1. On the other hand, T recorded the highest cost/kg weight gain with mean value of ₦ 1173.66. The cost of purchase was uniform at ₦=700, while cost of LAB (Lactic acid bacteria culture) was higher in T3. The total cost of production was higher in T2 and T3 with mean values of ₦ 1743.42, T3 with mean values of ₦2440.00 and ₦2785.00. Same trend was observed in net return as T3 and T2 recorded the highest values respectively. The cost benefit ratio was better in T3 with mean value of 1.74 followed by T1 (2.19) and lastly T2 (2.50).

Table 2: Cost benefit analysis of turkey fed fermented and non-fermented liquid feed

Parameters	T1	T2	T3	₦ SEM
	0.686^c	0.976^b	1.114	0.22
Body weight gain (kg)	0.615 ^c	0.880 ^a	1.067 ^a	0.22
Total feed intake/bird (kg)	3.425 ^b	7.594 ^a	7.718 ^a	0.24
Cost/kg feed (₦)	136.00	136.00	136.00	0.00
Cost/kg feed intake/birds (₦)	465.83	1032.82	1049.66	191.84
Cost/kg weight gain(₦)	757.45	1173.66	988.38	120.39
Cost of procurement/bird(₦)	700.00	700.00	700.00	0.00
Cost of LAB & Misc (₦)	10.60	10.60	20.60	3.33
Total cost of production (₦)	1176.43	1743.42	1770.26	193.62
Revenue/bird(₦)	1714.25	2440.00	2785.00	315.54
Net return/bird (₦)	537.82	696.58	1014.74	140.21
Cost benefit ratio	2.19	2.50	1.74	0.22

Abc: means with the same superscript in the same row are not statistically significant (p<0.05).

Coefficient of correlation of GIT bacterial to body weight of Turkeys

The result in Table 3 shows that body weight of turkey is positively correlated and significant for lactobacillus spp and lactobacillus- coliform ratio with mean values of 0.759 and 0.733 respectively. Furthermore, coliforms, salmonella spp and saccharomyces spp are negatively correlated to the body weight of turkey with mean values of -0.612, -0.717 and -0.925. in terms of significances, saccharomyces spp were highly significant, salmonella spp were significant while coliforms were not significant to the body weights respectively.

Table 3: coefficient of correlation of GIT bacterial to body weight of turkey

Weight	Body spp	lactobacillus coliforms	lactobacillus coliform ratio	salmonella	saccharomyces spp	
Body weight	1					
Lactobacillus	0.759*	1				
Coliforms	-0.612	-0.172	1			
Lactob-coliform ratio	0.743*	-0.664	1	0.733*		
Salmonella	-0.717*	-0.439	0.762*	-0.621	1	
Saccharomyces spp	-0.925**	-0.565	0.815**	-0.746*	0.788*	1

*correlation is significant at the 0.05 level (1 tailed)

** correlation is significant at the 0.01 level (1 tailed).

Microbiology of the gastro intestinal tract

The p^H contents in both fermented- feed groups was significantly lower (feed effect, P <0.05) (table 1) compared with the pH contents of the dry feed groups. The reduction in p^H thus influences the GIT by increasing the proliferation of beneficial micro organisms, which when populated, dominates harmful bacterial in the GIT. This finding is in line with the report of Russell *et al.*, (1998). A significantly higher pH in turkey fed fermented feed compared with those fed dry feed was observed, which was in agreement with Urlings *et al.* and Fransen *et al.* Urlings *et al.* hypothesized that the reduced available substrates in the large

intestine resulted in less microbial growth and therefore in reduced volatile fatty acid concentrations in the lower part of the GIT. Furthermore, lactic acid in fermented feed reduces the PH of the gut and thereby inhibits the growth of other bacteria including the enteropathogens. Hence it has positive association with animal health (Rowland; 1992 McDonald *et al.*, 1995). Significant increase in the *lactobacillus spp* across the treatments was as a result of fermentation. This corresponds to the findings of Urlings *et al.* (1993). *Lactobacillus spp* of bacteria can produce bacteriocins to selectively inhibit the growth of other bacteria. Bacteriocins are a group of antimicrobial peptides produced by bacteria and archaea (Dobson *et al.*, 2012). Various strains of *Lactobacillus salivarius* isolated from chicken GI tract can produce bacteriocins which are inhibitory to some Gram- negative and Gram- positive bacteria such as salmonella Enteritidis and *C. jejuni*. The inhibitory effect on various adverse bacteria and pathogens makes bacteriocin production a frequently considered trait in selection of probiotics. Nevertheless, it is worth noting that a variety of pathogenic bacteria (e.g., *staphylococcus aureus*) also produce bacteriocin effective against competing bacteria. Coliforms in the GIT were also significantly different, but increased across the treatments. It is evident that wet feed thus provides suitable medium for the growth of this bacteria. The population of coliforms affected by lactobacillus is shown in the ratio. Higher lactobacillus to coliform ratio was observed in T3. This was as a result of fermentation which thus produced more lactic bacterial that dominated the coliforms in the GIT. This is justifiable by the presence of high numbers of LAB in fermented liquid feed which contributes to the health status of the turkeys. Recent studies strongly support the hypothesis that orally administered LAB stimulate the immune system, both at the local and systemic level (Von der Weid *et al.*, 2001; Madsen *et al.*, 2001; Gill and Rutherford, 2001; Ggrangette *et al.*, 2001). Furthermore, feeding fermented liquid feed could reduce vertical transmission of enteropathogens. Taken together, these results indicate that fermented liquid feed can reduce the potential for enteropathogen transfer via the food, can beneficially influence the ecophysiology of the gut and can stimulate immune system of various animals

The population of *salmonella spp* was higher in T1 and reduced as a result of fermentation in T3. It is worthy of note that despite the fact that birds and its intestinal inhabitants both benefit from the host- microbe nutrient exchange, some of the intestinal bacteria are sometimes found to compete with the host for nutrients. Gut microbiome has evolved with the host towards a symbiotic relationship, and in healthy birds direct competition for nutrients is limited, as most digestible nutrients are absorbed by the host in the small intestine, where bacterial density is low and bacterial utilization of nutrient is suppressed due to the low pH and short retention time (Fransen 1995). However, when bacteria overgrow in the small intestine under certain circumstance, nutrients are captured and utilized by bacteria before normal absorption by host can take place. Surveillance studies by Geary *et al.* (1996) and Jensen and Mikkelsen. (1998) also showed that feeding pigs liquid diets and particularly fermented liquid diets reduces the incidence of salmonella positive herds. The concentration of 70 mmol kg⁻¹ lactic acid is bacteriostatic to salmonella and those concentrations > 100 mmol kg⁻¹ are bactericidal. FLF has beneficial effects on the gut architecture and significantly reduces coliform number in the lower gut. The population of saccharomyes spp was significantly higher in T2 and could be as a result of wetness of the feed which might have cultured a prolific environment for the growth of the organism which might have emanated from feed stuff.

Cost benefit ratio

Feed intake and weight gain were significantly higher ($p < 0.05$) in birds fed with fermented feed than those fed with non-fermented liquid diets. These findings were in agreement with that reported by *Yalda and Forbes (1995)* and *Awojobi and Meshioye (2001)*. The higher feed intake of birds fed liquid feeds may be attributed to the gastro intestinal tract. Interestingly, the higher feed intake resulted to higher body weight gain, thus indicating more efficient utilization of the feed by the birds. Total cost of production, revenue and net return was higher in birds fed fermented feed compared to other treatments. The findings here compares favourably with the report of *Marais et al., (1970)* that turkeys have greater efficiencies and up to the 5 weeks of age poults use approximately 2.0 pounds (0.9kg) of feed per pound/kg live- weight increase, while at the age of 12 weeks, they require approximately 2-3 pounds. The benefit cost ratio in this study was better in T3, probably because of the presence of lactic acid bacteria which must have facilitated fermentation and increased feed efficiency.

Coefficient of correlation

Positive correlation with body weight was observed for *Lactobacillus spp* and *Lactobacillus* to coliform ratio in the GIT of turkeys. Coliform to body weight and *Lactobacillus spp* concentration, were negatively correlated respectively. Salmonella with body weight and *Lactobacillus spp* concentration were negatively correlated respectively. Saccharomyces with body weight, *Lactobacillus spp* concentration and Lactobacillus- coliform ratio were negatively correlated respectively. Results for coefficient of correlation in this study is in line with the report of *Rene et al. (2001)* on the effect of fermented feed on the microbial population of the gastrointestinal tracts of pigs, in which case positive correlation exist in Lactobacillus to body weight ratio. Positive correlation indicates that as the concentration of specified bacteria increases, the body weight of turkey increase while the reverse is the case for negative correlation.

Conclusion

Gut microbiology is now recognized as an essential component of the intestinal ecosystem and is referred to as a forgotten organ, which contributes to the wellbeing of animal host in a range of aspects, especially nutrition and disease resistance. This study therefore has justified this as turkeys fed T3 diets fermented with lactic acid bacteria culture had the highest body weight, better cost benefit ratio and better results for coefficient of correlation. The prolificacy in using wet diet in animal nutrition cannot be overemphasized as it reduces dustiness of feed, improves palatability and generally increases consumption. The presence of beneficial bacteria e.g Lactobacillus in wet feed, thus improves feed efficiency in the GIT of poults. Therefore, feed fermentation and use of wet feed is recommended for commercial poultry farmers for efficient production.

References

- Awojobi H.A., Meshioye O.O (2001). A comparison of wet and dry mash feeding for broiler finisher during wet season in the tropics. *Nigerian J. Anim. Prod.*, 2, 143- 146.
- Brooks, P.H. (2008). Fermented Liquid Feed for Pigs. In *CAB reviews: perspectives in Agriculture, Veterinary Science, nutrition and natural Resources* pp. 1-18. <http://www.cababstracts.org/cabreviews/>.
- Brooks, P.H., Beal, I. D., Niven, S.J. and Demeckova, V. (2003b). Liquid feeding of pigs: 11 potential for improving pig health and food safety. In Review paper presented at the

- conference on Effect O.I genetic and non- genetic factors on carcass and meat qualizf O (pigs. Pp. 23– 40. Siedlce, Poland.
- Brooks P.H., Geary, T.M., Morgan D.T., Campbell, A., (1996). New developments in liquid feeding . *Pig Vet J.* 36, 43 – 64.
- Canibe, N., Hojberg, O., Badsberg, I. H. and Jensen, B.B. (2007a). Effect of feeding fermented liquid feed and fermented grain on gastrointestinal ecology and growth performance in piglets. *Journal of animal science* 85 (11): 2959-2971.
- Dobson, A., Cotter, P.D., Ross, R.P. and Hill, C. (2012). Bacteriocin production: a probiotic trait? *Appl Environ Microbiol* 78: 1-6
- Demeckova, V., Kelly, D., Coutts, A.G.P., Brooks, P.H and Campbell, A (2000). The Effect of liquid feed on the faecal Microbiology and Colostrum quality of farrowing Sows. *International Journal of Food Microbiology*, 79: 85-97
- Geary, T.M., Brooks, P.H.M., Morgan, D.T., Campbell, A.C., Russell, P.J., (1996). Performance of Weaner pigs fed ad libitum with V. Demeckova et al./ *International Journal of Food microbiology* 79 (2002) 85- 979 liquid feed at different dry matter concentrations. *J. Sci. foodagric.*72:17-24.
- Jensen, B.B (1998). The impact of feed additives on the microbial ecology of the gut in young pigs. *J. Anim. Feed Sci.* 7:45-64.
- Jensen, B.B., Mikkelsen, L.L., (1998). Feeding liquid diets to pigs. In: Garnsworthy, P.C., Wiseman, J. (Eds). *Recent advance in animal nutrition*. Nottingham Univ. Press, Nottingham, PP. 107-126.
- Mikkelsen, L.L., Jensen, B.B., (1997). Effect of fermented liquid feed (FLF) on growth performance and microbial activity in the gastrointestinal tract of weaned piglets. In: Laplace, J- p., fevrier, C., Barbeau, A. (Eds). *Digestive Physiology in pigs*, Vol. 88. EAAP Publication INRA, Paris, PP.639-642.
- Moore, R.W., Park, S.Y., Kubena, L.F., McReynolds, J.L. and Burnham, M.R (2004). Comparison of zinc acetate and propionate addition on gastrointestinal tract fermentation and susceptibility of laying hens to salmonella enteritidis during forced molt. *Polt. Sci.* 83, 1276-1286. Doi: 10.1093/ps/83.8.1276
- Moran, C.A., (2001). Developments and benefits of liquid feeding for the post- weaned pig. PhD Thesis, University of Plymouth, Plymouth, UK.
- Russell, J.B., and F. Diez- Gonzalez. (1998). The effects of fermentation acids on bacterial growth. *Adv. Microb. Physiol.* 39: 205-234.
- Steel, R.O.D and J. H. Torrie (1980) *Principles and Procedure of Statistics, A Biometric Approach* 2nd Edition McGraw Hill Books Co Inc- New York USA.
- Uurlings, H.A., A.J.Mul, A.T.van't Klooster, P.G.Bijker, J.G. van logtestijn, and L.G. van Gils. (1993). Microbial and nutritional aspects of feeding fermented feed (poultry by- products) to pigs. *Vet. Q.*15:146-151.
- Yalda A.Y. and Forbes J.M. (1995): Effect of wet feeding on the growth of ducks. *Br. Poult. Sci.*, 36: 878-879.