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Gut microbial population as affected by probiotic (*Saccharomyces cerevisiae*) inclusion in broiler chicken diet

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Abstract

The gut of the chicken is a fundamental organ which plays an important role in digestion and host defence. In this study, the gut microbial population as affected by probiotic Saccharomyces cerevisiae (SC) inclusion in broiler chicken diet was investigated. A total of 255 broiler chicks (White Ross breed) were used for this study. The chicks were allocated to five treatment groups in a completely randomized design with each treatment having three replicates of 17 birds each. The experimental diets (T_1, T_2, T_3, T_4 and T_5) were formulated with varying levels of inclusion of probiotic (SC) T_1 served as the control while T_2, T_3, T_4 and T_5 had 0.5, 1.0, 1.5 and 2.0% level of inclusion of probiotic (SC), respectively. The parameters taken were the gut content from the jejunum, ileum and caecum which were collected by dissecting the segments. The gut content was then placed in a plane sterile tube and taken to the laboratory for microbial count of Lactose fermenting bacteria (LFB) and non-lactose fermenting bacteria (NLFB). The results from the study showed that there was significant ($P<0.05$) difference in the population of Lactose fermenting bacteria (LFB) and non-lactose fermenting bacteria (NLFB) in the jejunum, caecum and ileum of the dietary treatment groups. Treatment group fed 0.5% SC had the highest population ($9.30x10^8$ cells/ml) of LFB in the jejunum while treatment group fed 1.0% SC had the highest population of $1.73x10^9$ and $1.08x10^9$ cells/mlof LFB in the ceacum and ileum respectively. The population of NLFB was higher in the control group compared to other treatment groups. This study concludes that dietary inclusion of SC in broiler chickens can influence the gut status thereby benefiting the animal through production of digestive enzymes, synthesis of vitamin B and immune stimulation.It is suggested that broiler chicken farmers should be encouraged to include Probiotic (SC) in the diet of broiler chickens for improved gut health.

Keywords: Probiotic, gut, microbes, diet, broiler chickens.

Introduction

The gut of the chicken is a fundamental organ which plays an important role in digestion and host defence (Agboola *et al*., 2015). To have a well-functioning and healthy gut, the balance of gut ecosystem is of importance. The microbial populations in the gastrointestinal tracts of poultry play a key role in normal digestive processes and in maintaining animal health (Falaki *et al.,* 2011). Disease, changes in the gastrointestinal tract or simple changes in feed management practices can significantly influence the microbial populations and their effects on animal performance and health (Falaki *et al.,* 2011). A wide range of factors related to diets and infectiousdisease agents seem to affect this balance, and subsequently affect

the health status and productionperformance of the chicken. It has been reported that the population of useful bacteria like lactobacillus in the ileum increased the pH of the gastro intestinal tract (GIT) due to increased production of volatile fatly acids; therefore the environment of the GIT became unsuitable for the activity and multiplication of pathogens (Ziggers, 2000). Probiotic have been reported to help in gut microbial balance through production of digestive enzymes, synthesis of vitamin B and immunity stimulation (Fuller, 1989; Rolfe, 2000). Therefore, the aim of this study is to investigate the gut microbial population as affected by dietary probiotic inclusion in broiler chickens diet.

Materials and methods

Experimental site

The study was conducted at the Poultry Unit of Skill Acquisition and entrepreneurship Centre , National Agricultural Extension and Research Liaison Services, Ahmadu Bello University, Zaria, located within the northern guinea savannah zone of Nigeria at latitude 11° 09' 06" N and longitude 7° 38' 55" E, at an altitude of 706m above sea level (GPS, 2017). The area falls within the Northern-Guinea Savannah Zone having an average annual rainfall of 1100mm, which starts from late April or early May to mid-October. The peak of rainy season is between June and September, followed by the harmattan period of cool and dry weather which last from November to January. This is then followed by hot – dry weather from February to April. The mean maximum temperature varies from 26°C to 35°C depending on the season, while the mean relative humidity during harmattan period and wet season are 21% and 72% respectively (Akpa *et al.,* 2002)

Source of experimental birds

A total of two hundred and fifty five (255) commercial broiler chicks of Ross White breed were purchased from a reputable hatchery for these studies. The Probiotic (SC) was purchased in Samaru market in Zaria, Kaduna State, Nigeria.

Experimental diets

Five experimental diets (broiler starter and broiler finisher)were formulated to meet the nutrient requirement of broilers as shown in Tables 1 and 2. Diet 1 serves as the control while diets 2, 3, 4, and 5 contained 0.5, 1.0, 1.5 and 2.0% of probiotic (SC) respectively.

Experimental design and management of birds

Two hundred and fifty five, day old broiler chicks were allotted to five treatments (0.0, 0.5, 1.0, 1.5 and 2.0) consisting of 17 chicks per replicate and three replicates per treatment during the starter phase. T_1 was the basal diet (0.0% SC) while T_2, T_3, T_4 and T_5 had probiotic (SC) included at 0.5, 1.0, 1.5 and 2.0% of the total diet. The design of the experiment was a completely randomized design. The birds were reared in deep litter system with feed and water provided *ad libitum* during the experimental period. The chemical composition of the probiotic (SC) was also determined and all the diets were formulated to meet the nutrient requirements of the broiler chickens (NRC, 1994). During the finisher phase, the birds were adjusted for seven days then randomized per treatment and adjusted for weight.

Microbial count

For each of the treatment groups, nine birds were selected randomly for gut microbial count. The birds were slaughtered and the intestine was removed, then using a surgical blade, the gut content from the jejunum, ileum and caecum were collected by dissecting the segments. The gut content

was then placed in a plane sterile tube and taken to the laboratory for microbial count.Serial dilution of the gut content was carried out by transferring (pipetting) 1mL from the gut solution into a 9mL of normal saline. This was mixed and 1ml was pipetted into the next sterile normal of 9mL. This was done to the dilution factor of 10^{-10}. Then 0.1mL was transferred from 10^{-6} dilution factor into the sterile macconkey agar plates and a sterile glass was used to spread the inoculum round the agar plate sceptically. Plates were incubated at 37°C for 24hrs then lactose and Non-lactose fermenters colonies were counted

Table 1: Composition of broiler starter diets containing varying levels of probiotic (SC)

	Levels of inclusion of probiotic SC (%)				
Ingredients	**0.0**	**0.5**	**1.0**	**1.5**	**2.0**
Maize	50.66	50.63	50.60	50.55	50.52
Groundnut cake	25.31	24.81	24.31	23.82	23.32
Soya cake	12.59	12.59	12.59	12.59	12.59
Fish meal	3.00	3.00	3.00	3.00	3.00
Palm oil	3.69	3.72	3.75	3.79	3.82
Limestone	0.90	0.90	0.90	0.90	0.90
Bone meal	2.75	2.75	2.75	2.75	2.75
Common Salt	0.30	0.30	0.30	0.30	0.30
Premix**	0.30	0.30	0.30	0.30	0.30
Lysine	0.30	0.30	0.30	0.30	0.30
Methionine	0.20	0.20	0.20	0.20	0.25
Probiotic (SC)	0.00	0.50	1.00	1.50	2.00
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Calculated Analysis					
ME (Kcal/kg)	3008	3008	3008	3008	3008
Crude protein (%)	23.31	23.31	23.31	23.31	23.31
Ether extract (%)	8.11	8.12	8.12	8.13	8.14
Crude fibre (%)	3.70	3.68	3.66	3.65	3.63
Calcium (%)	1.28	1.28	1.28	1.28	1.28
Lysine (%)	1.24	1.23	1.22	1.22	1.21
Methionine (%)	0.35	0.34	0.34	0.34	0.34
Available P (%)	0.57	0.57	0.58	0.58	0.59
Feed cost/kg(₦)	100.84	103.28	105.71	108.18	110.48

ME=Metabolizable Energy; **Biomix premix supplied per kg of diet: Vit. A, 10,000I.U; Vit.D $_3$, 2000 I.U; Vit E, 23 mg;Vit. K, 2mg: Vit.B_1,1.8;Vit. B_2, 5.5mg; Niacin, 27.5mg; Pantothenic acid, 7.5mg; Vit. B_{12}, 0.015mg: Folic acid, 0.75mg;Biotin, 0.06mg; Choline chloride, 300mg; Cobalt, 0.2mg; Copper, 3mg; Iodine, 1 mg; Iron, 20 mg; Manganese, 40 mg; Selenium, 0.2 mg; Zinc, 30mg; Antioxidant, 1.25mg; P=Phosphorus.

Statistical analysis

Data obtained from all the experiments were subjected to the analysis of variance using the general linear model procedure of (SAS, 2001). Significant differences among treatment means were separated using Dunnetts.

Results

The result of the effect of dietary inclusion of probiotic (SC) in the diet of broilers on the population of lactose fermenting bacteria in various segments of the gut is presented in Table 3. Dietary inclusion of SC significantly ($P<0.05$) affected the population of lactose fermenting bacteria in the jejunum and ileum with treatment

Table 2: Composition of broiler finisher diets containing varying levels of probiotic (SC)

Ingredients	Levels of inclusion of Probiotic SC (%)				
	0.0	**0.5**	**1.0**	**1.5**	**2.0**
Maize	57.52	57.52	57.52	57.52	57.52
Groundnut cake	11.67	11.17	10.67	10.17	9.67
Soya cake	20.00	20.00	20.00	20.00	20.00
Fish meal	3.00	3.00	3.00	3.00	3.00
Palm oil	2.81	2.81	2.81	2.81	2.81
Limestone	1.10	1.10	1.10	1.10	1.10
Bone meal	2.80	2.80	2.80	2.80	2.80
Common Salt	0.30	0.30	0.30	0.30	0.30
Premix**	0.30	0.30	0.30	0.30	0.30
Lysine	0.30	0.30	0.30	0.30	0.30
Methionine	0.20	0.20	0.20	0.20	0.20
Probiotic (SC)	0.00	0.50	1.00	1.50	2.00
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Calculated Analysis					
ME (Kcal/kg)	3045	3044	3042	3041	3039
Crude protein (%)	21.00	21.00	21.00	21.00	21.00
Ether extract (%)	7.21	7.18	7.16	7.13	7.11
Crude fibre (%)	3.66	3.65	3.63	3.62	3.60
Calcium (%)	1.35	1.35	1.35	1.35	1.35
Lysine (%)	1.25	1.24	1.23	1.22	1.21
Methionine (%)	0.35	0.35	0.35	0.35	0.35
Available P (%)	0.57	0.57	0.58	0.59	0.59
Feed cost/kg(₦)	94.51	96.86	99.21	101.56	103.91

ME=Metabolizable Energy; **Biomix premix supplied per kg of diet: Vit. A, 10,000I.U; Vit.D $_3$, 2000 I.U; Vit E, 23 mg;Vit. K, 2mg: Vit.B_1,1.8;Vit. B_2, 5.5mg; Niacin, 27.5mg; Pantothenic acid, 7.5mg; Vit. B_{12}, 0.015mg: Folic acid, 0.75mg; Biotin, 0.06mg; Cholire chloride, 300mg; Cobalt, 0.2mg; Copper, 3mg; Iodine, 1 mg; Iron, 20 mg; Manganese, 40 mg; Selenium, 0.2 mg; Zinc, 30mg; Antioxidant, 1.25mg; P=Phosphorus.

groups supplemented with 0.5% SC having the highest population ($9.30x10^8$ cells/mL) of LFB in the jejunum. No significant (P>0.05) difference observed within the treatment groups in terms of LFB population in the caecum. Treatment group fed 1.5% SC had the highest ($1.08x10^9$ cells/mL) population of LFB in the ileum compared to other treatment groups.

Table 4 shows the effect of dietary inclusion of SC in the diet of broilers on population of non-lactose fermenting bacteria. Dietary inclusion of SC significantly (P<0.05) affected the population of non lactose fermenting bacteria in the jejunum, ileum and caecum. The control group (0.0% SC) had the highest ($5.47x10^8$, $1.60x10^8$ and $1.11x10^9$ cells/mL) value of non-lactose fermenting bacteria in the jejunum, ileum and ceacum respectively compared to other treatment groups.

Table 3: Effect of dietary inclusion of probiotic (SC) in the diet of broilers on the population of lactose fermenting bacteria in various segments of the gut

LFB in gut (cells/mL)	Levels of SC inclusion (%)					SEM
	0.00	**0.5**	**1.0**	**1.5**	**2.0**	
Jejunum	$2.67x10^{7b}$	$9.30x10^{8a}$	$7.20x10^{8a}$	$7.87x10^{8a}$	$3.23x10^{8ab}$	$3.05x10^8$
Caecum	$1.19x10^{9a}$	$1.67x10^{9a}$	$1.73x10^{9a}$	$1.22x10^{9a}$	$1.20x10^{9a}$	$3.09x10^8$
Ileum	$3.00x10^{8b}$	$6.57x10^{8ab}$	$1.08x10^{9a}$	$5.00x10^{8ab}$	$5.90x10^{8ab}$	$3.48x10^8$

[ab]: Means with different superscripts along same rows show significant differences (P<0.05)
SEM: Standard Error of Means
LFB: lactose fermenting bacteria

Table 4: Effect of dietary inclusion of probiotic (SC) in the diet of broilers on the population of Non-lactose fermenting bacteria in various segments of the gut

NLFB in gut (cells/mL)	Levels of SC inclusion (%)					SEM
	0.00	0.5	1.0	1.5	2.0	
Jejunum	$5.47x10^{8a}$	$1.50x10^{8b}$	$1.33x10^{8b}$	$2.00x10^{7b}$	$2.33x10^{7b}$	$1.95x10^{8}$
Caecum	$1.60x10^{8a}$	$2.00x10^{7b}$	$1.00x10^{7b}$	$3.00x10^{7b}$	$3.00x10^{7b}$	$5.53x10^{7}$
Ileum	$1.11x10^{9a}$	$2.00x10^{7b}$	$1.00x10^{7b}$	$1.13x10^{8b}$	$2.00x10^{7b}$	$3.03x10^{8}$

[ab]: Means with different superscripts along same rows show significant differences (P<0.05)
SEM: Standard Error of Means
LFB: lactose fermenting bacteria

Discussion

The dietary inclusion of probiotic (SC) in the diet increased the population of lactose fermenting bacteria within the digestive tract and this could have a beneficial barrier against pathogen as reported by Linge (2005). In the absence of glucose, the LFB have an enzyme called β galactosidase which breaks lactose into galactose and glucose which helps them to utilize the glucose for growth or as source of energy. Falaki *et al*. (2011) reported that supplementing diet with different levels of probiotic slightly increased the lactobacillus population in the ileum of chickens. Ziggers (2000) reported that the population of useful bacteria like lactobacillus in the ileum increased the pH of the GIT due to increasing production of volatile fatty acids hence the environment of the GIT becomes unsuitable for the activity and multiplication of pathogens. Nurmi and Rantala (1973) stated that pathogens would be expelled out of the gut by useful bacteria if it already occupied the gut sites. Colonisation of useful micro flora in the gut of young birds could inhibit further colonization of pathogens (Nurmi and Rantala, 1973).

The low level of non lactose fermenting bacteria observed in this study across the various treatment groups as compared to the control showed that probiotic organisms inhibited some non beneficial pathogens by occupying intestinal wall space. This is in agreement with the work of Kabir *et al.* (2005) who evaluated the effect of probiotics with regard to clearing bacterial infections and regulating intestinal flora by determining the total viable count and total lactobacillus count of the crop and cecum samples of probiotics and non-probiotics fed group. Moutzouris *et al.* (2007) also reported that probiotics have a potential effect on modulation of intestinal microflora and pathogen inhibition. Saadia and Nagla (2010) reported that lactobacilli bacteria increased due to the addition of yeast at various levels into laying hens diet. Kabir (2009) reported an increase in viable count of ileal lactobacilli due to addition of live yeast in broiler diet. Lactobacilli bacteria secrete lactic acid which reduced pH of digesta. The reduction in pH value was due to direct action of intestinal bacilli bacteria (Saadia and Nagla, 2010).

Conclusion

The study showed that dietary inclusion of probiotic (SC) affected the gut microbial population of LFB and NLFB in the jejunum ileum and caecum of broiler chickens with the probiotic treatment groups having higher population of LFB in the different segments of the GIT.

References

Agboola, A. F., Omidiwura, B. R. O., Odu, O., Odupitan, F. T. and Iyayi, E. A. 2015. Effect of

Probiotic and Toxin Binder on Performance, Intestinal microbiota and gut morphology in broiler chickens. *Journal of Animal Science Advances.* 5(7):1369-1379.

Akpa, G. N., Ifut, O. J. and Mohammed, F. 2002. Indigenous management of Dystocia in ruminant livestock of northern guinea savannah of Nigeria. *Nigeria Journal Animal Production*29(2):264-70

Falaki, M., Shargh, M. S., Daster, B., Zerehdaran, S. and Khomairi, M. 2011. The investigation of intestinal microflora and growth response of young broilers given feed supplemented with different levels of probiotic and prebiotic. *Journal of Animal and Veterinary Advances*, 10(3):385-390.

Fuller R 1989. Probiotics in man and animals. A review. *Journal of Applied Bacteriology.* 66: 365-378.

GPS (Global Positioning System), 2017.

Kabir, S. M. L. 2009. The role of probiotics in poultry industry: A review. *International Journal of Molecular Science*, 10(8):3531-3546

Kabir, S. M. L., Rahman, M. M. and Rahman, M. B. 2005. Potentiating of probiotics in promoting microbiological meat quality of broilers.*Journal of Bangladesh Society of Agricultural Science Technology*, 2:93-96.

Linge, P. 2005. *The use of probiotics and yeast derivates in India. World's Poultry Science*, 21:12-15.

Mountzouris, K. C., Tsirtsikos, P., kalamara, E., Nitsch, E., Schatzmayr, G. and Fegeros, K. 2007. Evaluation of the efficacy of probiotic containing *Lactobacillus, Bifidobacterium, Enterococcus and Pediococcus* strains in promoting broiler performance and modulating cecalmicroflora composition and metabolic activities. *Poultry Science*, 86:309-317.

National Research Council, 1994. *Nutrient requirements of Poultry*.9th ed. National Acadamy Press, Washington. DC.

Nurmi, E. and Rantala, M. 1973. New aspects of *Salmonella* infection in broiler production. *Nature*, 241:210-211.

Rolfe, R. E. 2000. The role of probiotic cultures in the control of gastrointestinal health. *Journal of Nutrition.*130: 396-402.

Saadia, M. H. and Nagla, K. S. 2010. Effect of probiotic *(saccharomyces cerevisiae)* adding to diets on intestinal microflora and performance of Hy-line layers hens. *Journal of American Science*, 6(11):159-169.

SAS, 2001. SAS User's Guide.Version 6.11. SAS Institute, Inc. Cary, NC.

Ziggers, D. 2000. TOS, a new prebiotic from whey. *Animal Feed Science and Technology*, 5: 34-36.

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