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The Use of Probiotic Containing Lactic Acid Bacteria to the Rescue of Antibiotics in Broiler Production

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

The research was aimed to study the effect of *Lactobacillus fermentum*, *Lactobacillus plantenrun* and *Weissalla ciberia* on growth performance, feed conversion ratio and mortality of broiler chicken. This was design to find a possible alternative to antibiotics in broiler production. The study was carried out at the Department of microbiology, faculty of sciences Kaduna State University, Kaduna between January to April 2018. A total of ten raw milk samples were screened for the isolation of Lactic Acid Bacteria (LAB) and twenty day-old broiler chicks (initial body weight 41 ± 0.5 g) were administered probiotics (*Lactobacillus fermentum*, *Lactobacillus plantenrun* and *Weissalla ciberia*) in water at 10^8 cells/milliliters/isolates/birds/day for six weeks. Body Weight (BW), Weight Gain (WG) and Feed Intake (FI) were measured weekly just as feed conversion ratio was calculated and mortality was recorded throughout the duration of the experiment. The results showed the identification of *Lactobacillus fermentum*, *Lactobacillus plantenrun* and *Weissalla ciberia* that were used as probiotics. Significant differences was observe between treatment on BW at day 14 P=0.0292 WG, P=0.0004 and FI P=0.0176, day 21 P=0.0329, WG P=0.0004 and FI P=0.0176, day 28 P=0.0025, WG P=0.0053 and FI P=0.0189, day 42 WG P=0.0112 and FI P=0.0006 and day 49 BW P=0.011, WG P=0.5289 and FI P=0.0006. Probiotics group showed a better body weight and weight gain with a lower feed intake and highest feed conversion ratio compared with antibiotic and

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control group. There was a progressive increase in weight gain from the first to the fourth but decreases from week five and six. The LAB group recorded 5%, mortality rate, antibiotics group recorded 10% and the control group recorded 0%. Probiotic lactic acid bacteria showed promising tendencies to replace antibiotics in broiler production as illustrated in this research work.

Keywords: Lactobacillus; body; weight; feed; conversion; ratio; probiotics; antibiotics and broiler.

1. INTRODUCTION

Probiotics is a specific live or inactivated microbial culture that has documented targets in reducing the risk of human disease or in their nutritional management [1]. The underlying basis of in-feed antibiotics and probiotics is that they impact the composition of intestinal microflora in favour of the host [2]. Scientific studies have shown a beneficial effect of such products on the growth, feed consumption, and stabilisation of animal health. However, continuing use of antibiotics and chemical growth promoters increases the development of resistant pathogenic micro-organisms and reduces the efficacy of antibiotics and chemotherapeutics in the treatment of some diseases [2].

The Lactic Acid Bacteria (LAB) are a group of Gram-positive bacteria, non-respiring non-spore-forming, cocci or rods, which produce lactic acid as the major end product of the fermentation of carbohydrates [3,4]. Look at LAB as a group of Gram-positive bacteria that lack cytochromes and preferring anaerobic conditions, fastidious, acid-tolerant and strictly fermentative. They are catalase, oxidase, indole, methyl red, voges-proskauer and citrate negative [5]. Among different genera of LAB; Lactobacilli produce various organic acids like lactic acid, acetic acid and propionic acid exhibiting anti-microbial activity [6,7]. *Lactobacillus plantarum* isolated from soy milk also have strong antibacterial activity against *Escherichia coli (E. coli)* and other pathogenic bacteria [8]. *Lactobacillus fermentum* was reported to have improved the intestinal balance of the diverse microflora species in the rectum of broiler chickens [9]. They are also responsible for the production of bacteriocin [10] and diminished atopic dermatitis [11].

The European Food Safety Authority (ESFA) in April, 2007 published a survey on the levels of Salmonella detected in broiler flocks across the European Union in 2005-6. It was reported that one in four broiler flocks rose over the one year period, was Salmonella-positive. *Salmonella enteritidis* has been related to human salmonellosis, a common and widespread zoonosis worldwide [12]. Both the association of Salmonella infections with the consumption of poultry products and the fact that in the living bird Salmonella carriage is mainly asymptomatic have been led to a demand to find ways of preventing infection of commercially reared poultry and product contamination [13]. The probiotic properties of LAB have been widely studied, demonstrating that their capability of adhering to mucus and epithelial cells is one of the potential mechanisms of providing a competitive advantage in the intestinal microbiota [14] and consequently inhibiting the in vitro growth of *S. enteritidis* [15]. Studies on probiotics products incorporating *L. fermentum* and *Saccharomyces cerevisiae* indicated that they improved the intestinal balance of the diverse microflora species in the rectum of broiler chickens [9].

This research was aimed to study the effect of Lactic acid bacteria (*Lactobacillus fermentum*, *Lactobacillus plantenrun* and *Weissalla ciberia)* on growth performance, feed conversion ratio and mortality of broiler chicken.

2. MATERIALS AND METHODS

2.1 Isolation and Biochemical Identification of LAB

A total of ten raw cow milk samples were collected directly from the hawkers within Kaduna metropolis, Kaduna state, northern Nigerian. The samples were collected aseptically in sterile bottles and kept cool in an ice bag and transported to Department of Microbiology laboratory, Kaduna State University for isolation of lactic acid bacteria. Ten millilitres of the milk samples was aseptically measured and homogenised to obtain a uniform sample. From each sample, 1:10 (one millilitre of sample into ten millilitres of sterile peptone water) dilution was subsequently made using peptone water followed by making a tenfold serial dilution. Then 0.1 millilitre (ml) from each dilution was inoculated in duplicate into De Man, Rogosa and Sharpe (MRS) agar used for isolating LAB. To prevent the growth of yeasts, the media was

supplemented with 100 mgL^{-1} of cycloheximide [16] before incubation. The MRS agar plates were incubated aerobically and anaerobically using the Gas Pack system at 37°C for 48hrs. Colonies were randomly selected and then streaked on MRS agar severally to purify the strains and subsequently stored at 37°C for further identification [17].

All the purified strains were initially tested for gram's reaction, catalase production and spore formation [16]. The strains were further tested for Indole, Methyl red, Voges proskauar Citrate utilisation (IMVC) using the method of Monica [18].

2.2 Molecular Identification of Lactic Acid Bacteria

2.2.1 DNA extraction and storage

The DNA extraction was achieved according to the manual method described by Akhmetsadykova et al. [19]. The extracted DNA was stored at -20°C. The purity of DNA was verified by electrophoresis in 0.8% (w/v) agarose gel (Merck KGaA Germany) in TAE 1X buffer under UV light after staining with ethidium bromide.

2.2.2 Amplification of extracted genetic material

The Polymerase Chain Reaction (PCR) reaction mixture consisted of 5 µl of 10X buffer (100 mM HCl pH 8.3) 20 mM MgCl2, 500 mM KCl, 1% gelatin, 200 µM concentrations each of deoxyribonucleotide triphosphates (dATP, dTTP, dGTP and dCTP), 0.5 µl of each primer (GGACTACAGGGTATCTAAT 16S for primer RIBOS-1 Forward and AGAGTTTGATCCTGG 16S for primer RIBOS-2 Reverse), template genomic DNA, 200 ng and 1.5 units of Taq polymerase. The PCR was run in a programmable thermocycler (Bulldog bio Inc, USA) having an initial delay at 95°C for 10 min and final delay at 72°C for 10 min followed by 30 cycles of denaturation at 95°C for 1min, annealing at 55°C for 1 min followed by extension at 72°C for 1 min. PCR amplified product was resolved in a 1.5% agarose gel by electrophoresis.

2.2.3 Purification and sequencing of PCR products

The PCR purification was done using kit (QIAquick USA). The Purified PCR products were sent to GATC (Accegen Biotech USA) for sequencing. Sequence annotation and database searches for similar sequences were done using Basic Local Alignment Search Tool (BLAST) at National Center for Biotechnology Information to determine the closest known relative species.

2.3 Standardisation of Pure Isolates of LAB

The standardisation was achieved by 0.5 McFarland turbidity standards. Preparation of 0.5 McFarland turbidity standard was done as described in microbesoline website. One millilitre (ml) of concentrated H_2SO_4 was added to 99 ml of distilled water in a conical flask and mix well. A 1% v/v solution of H_2SO_4 is prepared. Then 0.5 grams (g) of dihydrate barium Chloride salt ($BaCl_2$. $2H_2O$) was dissolved in 50 ml of distilled water. In this way, a 1% w/v of $BaCl_2$ was prepared. This is followed by adding 0.6 ml of $BaCl_2$ solution to 99.4 ml of H_2SO_4 solution to make up to 100 ml. The solution was then mixed well. This is the stock solution of the 0.5 McFarland turbidity standards. Exactly 2 ml of the solution was transferred into capped tubes and store at room temperature until ready for use.

2.4 Experimental Design

A total of sixty, one day old broiler chicks were used in this research work. Out of which 20 birds were fed with probiotic LAB, 20 were administered one table spoon in four litres of water of broad spectrum antibiotic: oxytetracycline (Sam pharmaceuticals Ltd Nigeria) for prevention of bacterial infection and 20 were used as control without antibiotic or probiotic administration. The standardised LAB (10^8 cells/ milliliters/isolates/birds/ day) was administered in 200ml of drinking water at days 6, 7, 8, 21, 22, and 23 of age [20]. The birds were administered live vaccines against Gumboro virus disease at week 1 and 3, La sota vaccine (NewCastle virus disease) at week 2 and 4 of age. Hybrid feed (from Nigeria) was used to feed the birds which were provided in mash form in two phases (starter phase 0 to 3 weeks and finisher phase 4 to 6 weeks old). Ethical approval was sought and obtained from Kaduna State Ministry of Agriculture, Kaduna.

2.5 Evaluation of Body Weight, Weight Gain, Feed Intake, Feed Conversion Ratio and Mortality of Broilers

The body weights of birds were recorded per treatment from week one to the 6^{th} week of the

experiment. Growth performance parameters were measured as describe by Mountzouris et al. [21]. Parameters such as body weight (BW) weight gain (WG), feed intake (FI), Feed conversion ratio (FCR), defined as FI:WG (g:g) were determine on weekly basis. Overall WG, FI and FCR were calculated for the whole duration of the experiment. The mortality was recorded throughout the period of the experiment.

2.6 Data Analysis

The data were analysed using One Way Analysis of Variance with the aid of graph pad prism (USA) version 6. Statistically significant effects were further analyzed and means were compared using Duncan's multiple range test. Statistical significance was obtained at $P \leq 0.05$.

3. RESULTS AND DISCUSSION

The results of the biochemical identification of the isolates were shown in Table 1. The colonies for 2AN appear Convex, 3AN appear flat, circular and non-pigmented and 4AN appear convex and dispersed. Their morphology shows that they are all rod shaped bacilli. All the isolates were positive to Gram's reaction, catalase positive and lack spores which might confirm the isolates were *Lactobacillus* spp. These results are in agreement with the work of Tharmaraj and Shah Shah [22], Gebreselassie et al. [23]. All the isolates were found to be negative to indole, methyl red, voges-proskauer and citrate utilisation tests which further confirm the isolates to be *Lactobacillus* spp. This is parallel with the works of Dhanasekaran et al. [5], Kamrun et al. [24], Kostinek et al. [25], Baccigalupi et al. [26]. The primer GGACTACAGGGTATCTAAT 16S for primer RIBOS-1 Forward and AGAGTTTGATCCTGG 16S REV primer RIBOS-2 Reverse with Amplicon size of 789bp were used to amplify the LAB DNA Sequence which were shown in Table 2. The BLAST on National Center for Biotechnology Information (NCBI) website confirm sample 2AN to be *Lactobacillus fermentum* with accession number NC010610.1 and 99% identification, sample 3AN to be *Lactobacillus plantenrun* with accession number MF428738.1 and 99% identification and sample 4AN to be *Weissalla ciberia* with accession number N2CP012873.1 with 98% identification.

The effects of probiotic LAB on body weight, weight gain, feed intake and feed conversion ratio was shown in Tables 3&4 and Fig. 2 & 3. At the grower stage, it was observed that there was no significant differences between the mean of the treatment on body weight, weight gain and feed intake at the end of first week (day 7 of age) with P>0.05. At 14 days old, there was a significant differences between the mean of each treatment on body weight P<0.05 (P=0.0292), but there was no significant differences between

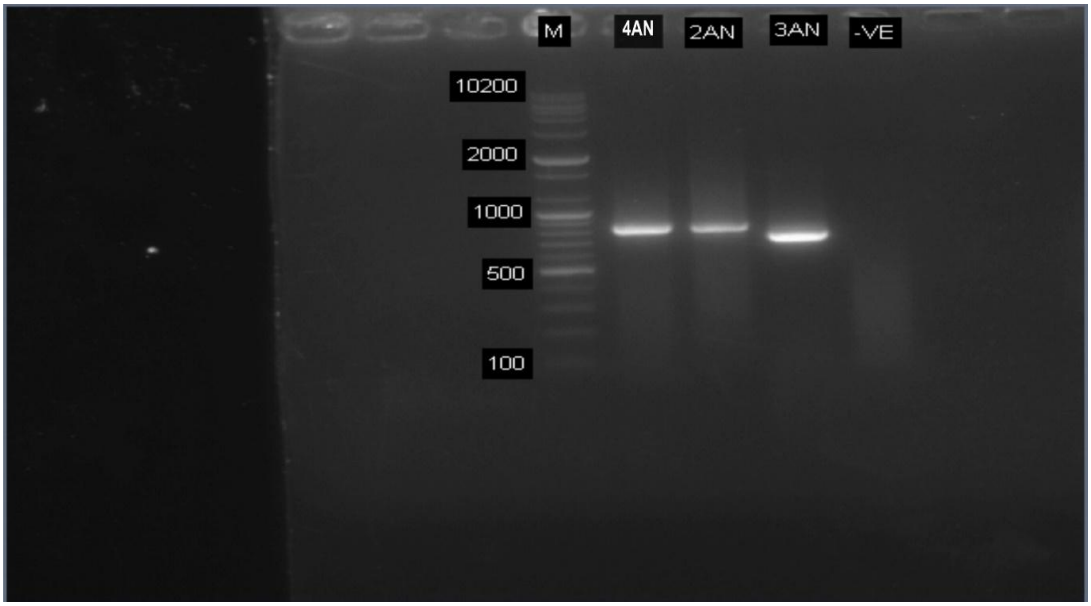

Fig. 1. DNA band on agarose gel

Key: M= positive control 4AN= Sample, 2AN= Sample, 3AN= Sample and -Ve = negative control

Table 1. Biochemical identification of lactic acid bacteria

Tests	Sample		
	2AN	3AN	4AN
Colony characteristics	**Convex colonies**	**Flat circular non-pigmented colonies**	**Convex dispersed colonies**
Morphology	Rod	Rod	Elongated Rod
Gram staining	+	+	+
Spore staining	-	-	-
Catalase	+	+	+
Indole	-	-	-
Methyl red	-	-	-
Voges-proskauer	-	-	-
Citrate	-	-	-
Possible organism	*Lactobacillus spp*	*Lactobacillus spp*	*Lactobacillus spp*

Key: 2AN, 3AN and 4AN are different Samples

Table 2. Molecular Identification of Lactic Acid Bacteria

Samples	Max score	Query cover	E-value	Accession no.	Identification	Organism
2AN	1413	100%	0.0	NC010610.1	99%	*L. fermentum*
3AN	1282	100%	0.0	MF428738.1	99%	*L. planterun*
4AN	1373	100%	0.0	N2CP012873.1	98%	*Weissalla ciberia*

Table 3. Effects of probiotic lactic acid bacteria on body weight, weight gain, feed intake and feed conversion ratio at grower stage

	Experimental treatment			
Components	**A**	**B**	**C**	**P value**
Week 1				
BW (g)	110	106	109	0.13
WG (g)	67	63	66	0.44
FI (g)	107	107	106	0.97
FCR (FI/WG)	1.6	1.7	1.6	
Week II				
BW (g)	288	280	275	0.03
WG (g)	178	174	166	0.65
FI (g)	250	250	253	0.97
FCR (FI/WG)	1.4	1.4	1.5	
Week III				
BW (g)	625	560	550	0.03
WG (g)	337	280	275	0.0004
FI (g)	395	368	363	0.02
FCR (FI/WG)	1.2	1.3	1.3	

*Key: A = Probiotics group; B = Antibiotics Control Group; C = Negative Control Group; a, b & c are mean of the treatment; Significant value, * P < 0.05*

the mean of each treatment on weight gain and feed intake P>0.05. At the end of second week of age (day 21), there was a significant differences between the mean of the treatment on body weight P=0.0329, weight gain P=0.0004 and feed intake P=0.0176. The probiotics group recorded the highest mean of body weight; weight gain feed intake and lowest feed conversion ratio. This was closely followed by antibiotics group and then controls which share the same mean for feed conversion ratio that is higher than that of the probiotics group. At the end of third week (day 28) there was a significant difference between mean of the treatment on body weight P=0.0025, weight gain P=0.0053 and feed intake P=0.0189 with probiotics group having the highest mean of weight, weight gain and feed intake, this was followed by control

group while the antibiotics group recorded the lowest mean of weight, weight gain and feed conversion ratio at this day 28. These results were supported by the works of Mountzouris et al. [21], Bai et al. [27], Bostami et al. [28].

By the end of fourth week (day 35), no significant differences was observed between the mean of the treatment on body weight even though the probiotics group recorded the highest mean followed by control group and then antibiotics group. Significant differences was observed between the mean of the treatment on weight gain P=0.0089 and feed intake P=0.0017. The probiotics group recorded the lowest mean of weight gain at this 35th day of age as the antibiotic and control group shared the same mean which of cause is higher than the

Table 4. Effects of probiotic lactic acid bacteria on body weight, weight gain, feed intake and feed conversion ratio at finisher stage

Components	Experimental treatment			
	A	B	C	P value
		Week IV		
BW (g)	1100	975	1025	0.003
WG (g)	475	415	475	0.005
FI (g)	665	630	663	0.02
FCR (FI/WG)	1.4	1.5	1.4	
	Week V			
BW (g)	1425	1350	1400	0.10
WG (g)	325	375	375	0.009
FI (g)	714	783	753	0.002
FCR (FI/WG)	2.2	2.1	2.0	
	Week VI			
BW (g)	1775	1650	1650	0.01
WG (g)	350	300	250	0.53
FI (g)	879	783	760	0.001
FCR (FI/WG)	2.5	2.6	3.0	

Key: A = Probiotics group; B = Antibiotics Control Group; C = Negative Control Group
a, b & c are mean of the treatment
*Significant value, * P < 0.05*

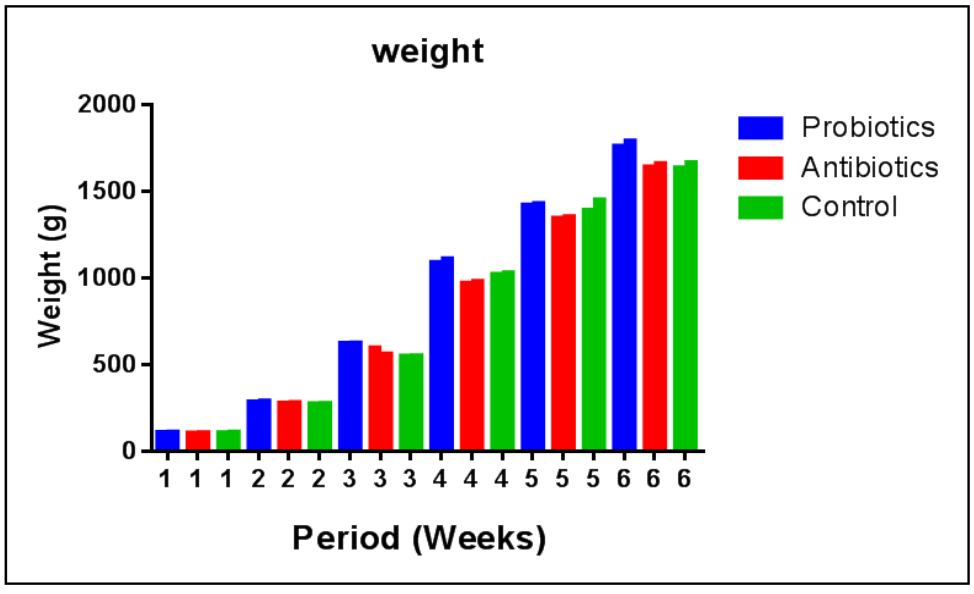

Fig. 2. Effect of probiotic LAB bacteria on body weight

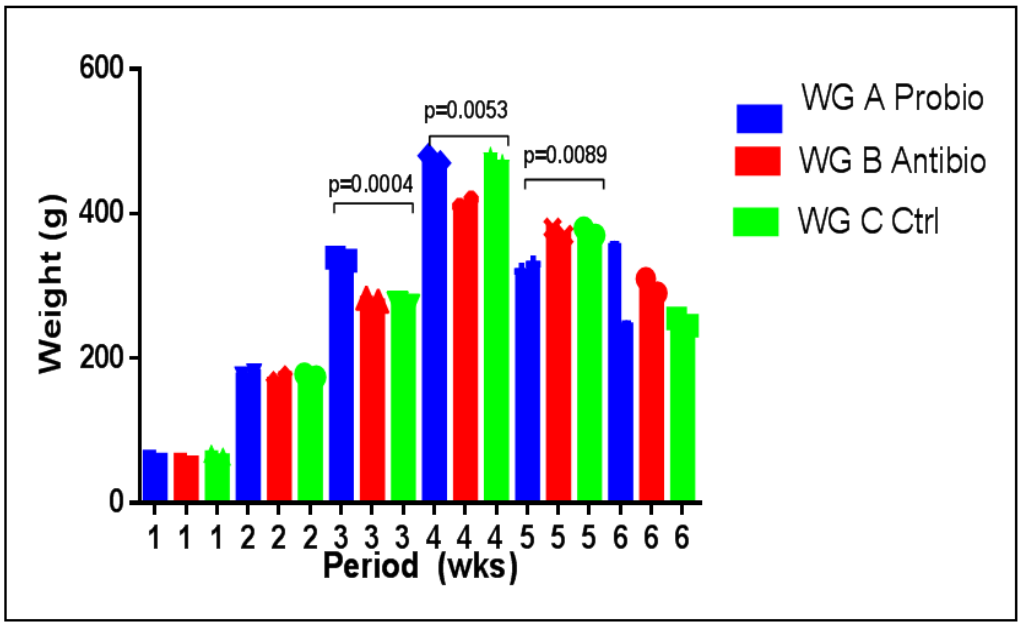

Fig. 3. Effects of probiotic LAB bacteria on weight gain

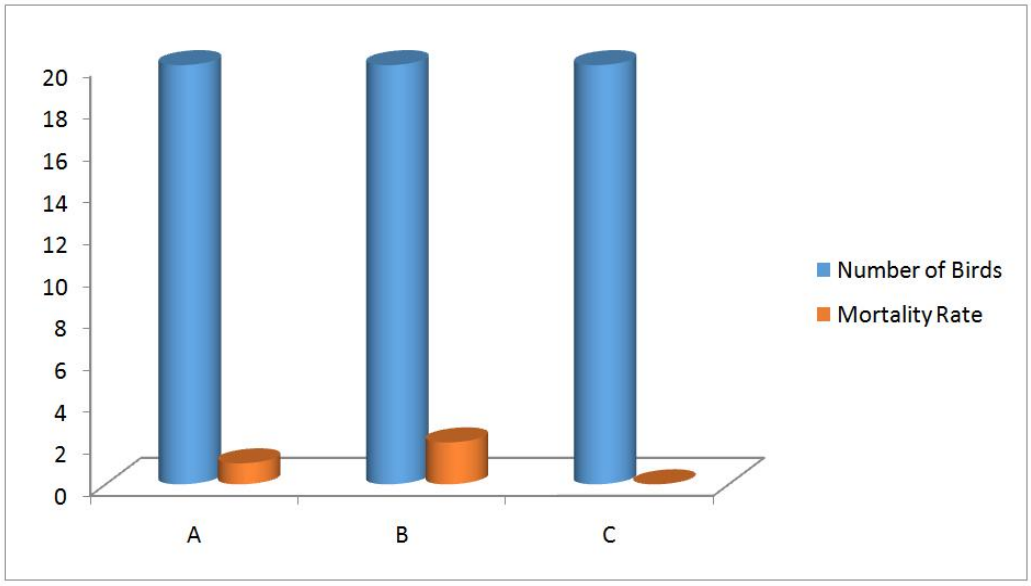

Fig. 4. Effect of probiotic LAB bacteria on mortality
KEY: A= Probiotics, B= Antibiotics, C= Control

probiotics group. The antibiotics group recorded the highest feed intake followed by the control group and the probiotics group recorded the least mean which is responsible for the probiotics group to have the highest feed conversion ratio followed by antibiotics group and then the control recorded the least. By the end of fifth week (day 42), a significant difference was also observed between the mean of the treatment on body weight (P=0.0112) with probiotics group having the highest mean while antibiotics and control group shared the same mean. There was no significant difference between mean of the treatment on weight gain even though the probiotics group recorded the highest mean followed by the antibiotics group as the control

group having the least mean. There was a significant difference between the mean of the treatment on feed intake (P=0.0006) with probiotics group consuming more feed which was followed by the antibiotics group and then, the control consuming the least feed. This resulted in probiotics having the least feed conversion ratio followed by the antibiotics group and the control with the highest feed conversion ratio. At the sixth week of this research, significant difference between the means was observed for body weight P=0.011 and feed intake P=0.0006, but there was no significant difference between the mean of weight gain P=0.5289. The Beneficial effects of supplementation of lactic acid bacteria, A (combination of *Lactobacillus fermentum*, *Lactobacillus plantenrun* and *Weissalla ciberia*) and B (antibiotic) on growth performance was supported by the works of [21,28]; where they reported growth promoting effects among birds fed with antibiotic and birds administered a multi-species probiotic product (comprising *Lactobacillus reuteri*, *Enterococcus faecium*, *Bifidobacterium animalis, Pediococcus acidilactici, Lactobacillus salivarius*) in feed and water. The Improved body weight gain as observed in Fig. 1 in this study could be induced by the synergistic effect of probiotic action including the improvement of FI and nutrient digestibility, maintenance of beneficial gut microflora and increased digestive enzyme activity [28]. An important function of probiotic bacteria or A is to provide defense to the host gastrointestinal tract from pathogens [29,30]. Reported significant improvements in broiler performance in response to Bacillus, Lactobacillus and Clostridium based diets, which supports the present findings of combination of lactic acid bacteria. In this current research, it was compared with the three lactic acid bacteria (*Lactobacillus fermentum*, *Lactobacillus plantenrun* and *Weissalla ciberia*), the antibiotics and control (without LAB and antibiotics) but the LAB group was found to be the best. It have been suggested that LAB can stimulate broiler's performance by improving digestive function, increasing the bioavailability of dietary micronutrients, modulating intestinal microflora, enhancing immuno-modulation and better the health of the broiler [31,32,33]. In Fig. 1 of this research, it was observed that, there was a progressive increase in body weight throughout the duration of the treatment, with the lactic acid bacteria group recorded the highest body weight throughout the duration of the research wok. This is in agreement with the work of Brzoska and Stecka [34] and Brzoska et al. [20] in which probiotic bacteria significantly increased chickens' body weight. Fig. 3 showed the effect of experimental treatment on weight gain. It was observe that there was a steady increase in weight gain from week 1 to week 4 and the weight gain started declining from week 5 to week 6 with the lactic acid bacteria group recorded the highest weight gain throughout the duration of the research which is in total agreement with the work of Mountzouris et al. [21] in which Probiotic treatment performed well in terms of overall body weight gain and feed conversion ratio. The decline in the weight gain at week 5 and 6 could be as a result of a sudden rise in environmental temperature at this stage of the experiment which is backed by the work of Mountzouris et al. [21] which reported that environmental stress factors (e.g., temperature, stocking density) affects the efficacy of probiotics and in this present study imped performance (body weight gain) of broiler at week 5 and 6.

Fig. 4 showed the effect of the experimental treatment on mortality. The result indicates that one mortality was recorded at lactic acid bacteria goup representing 5%, two mortality was recorded for antibiotics group representing 10% and non was recorded for the control group representing 0%. This is in total variance with the work of Bostami et al. [28], Timmerman et al. [35] in which beneficial microorganism reduced mortality because of their synergistic and biotherapeutic effects which remarkably decrease mortality as observed in broiler after probiotic administration [36].

4. CONCLUSION

Probiotic containing lactic acid bacteria showed a significant potential to replace antibiotics in broiler production because of the edge over antibiotics as manifested in this reseach. Hence lactic acid bactera can come to rescue antibiotics and further help to curtail antibiotic resistance.

ETHICAL APPROVAL

Ethical approval was sought and obtained from Kaduna State Ministry of Agriculture, Kaduna.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Isolauri E, Rautava S, Kalliomaki M. Role of pro-biotics in food hypersensitivity. Current Opinion in Immunological Clinical Allergy. 2002;2:263-271.
2. Sabatkova J, Kumprecht I, Zobač P, Suchý P, Čermák B. The probiotic bioplus 2B as an alternative to antibiotics in diets for broiler chickens. Acta Veterinaria Brno. 2008;77(4):569-574.
3. Khalid K. An overview of lactic acid bacteria. International Journal of Bio-sciences. 2011;1(3):1-13.
4. Gatesoupe J, Lésel R. Flore digestive des poissons: Approche environnementale. Cahiers Agricultures. 1998;7:29-35.
5. Dhanasekaran D, Saha S, Thajuddin N, Rajalakshmi M, Panneerselvam A. Probiotic effect of Lactobacillus isolates against bacterial pathogens in fresh water fish. J. Coastal Dev. 2010;13:103-112.
6. Oluwafemi F, Adetunji AF. Antimicrobial activities of lactic acid bacteria isolated from traditionally- fermented maize (ogi) against Candida albicans. J Appl Biosci. 2011;41:2820–283.
7. Arokiyamary A, Sivakumar PK. Anti-bacterial activity of Bacterocin producing *Lactobacillus* sp., isolated from traditional milk products. Curr Bot. 2011;2(3):05–08.
8. Srinivasan P, Khan KA, Perumal UR, Kumar RV, Suganya K, Rajalakshmi M. *In vitro* anti-bacterial activity of *Lactobacillus plantarum* isolated from soy milk. Int J Pharm Bio Sci. 2012;3(3):209-219.
9. Lei Y, Zhang KY, Ding XM, Bai SP, Jung SC. Effect of probiotics on growth performance, development of small intestinal tract and microbial populations in broilers. J. Agric. Sci. Technol. 2009;3:24–31.
10. Lash BW, Mysliwiec TH, Gourama H. Detection and partial characterization of a broad-range bacteriocin produced by *Lactobacillus plantarum* (ATCC 8014). Food Microbiology. 2005;22(2-3):199-204.
11. Ouwehand AC, Nermes M, Collado MC, Rautonen N, Salminen S, Isolauri E. Specific probiotics alleviate allergic rhinitis during the birch pollen season. World Journal of Gastroenterology: WJG. 2009; 15(26):32-61.
12. Nava GM, Bielke LR, Callaway TR, Castañeda MP. Probiotic alternatives to reduce gastrointestinal infections: The poultry experience. Animal Health Research Reviews. 2005;6:105-118.
13. Revolledo L, Ferreira AJP, Mead GC. Prospects in salmonella control: Competitive exclusion, probiotics, and enhancement of avian intestinal immunity. Journal of Applied Poultry Research. 2006;15:341-351.
14. Ghadban GS. Probiotics in broiler production: A review. Arch. Geflugelk. 2002;22:49-58.
15. Kos B, Suskovic J, Vukovic S, Simpraga M, Frece J, Matosic S. Adhesion and aggregation ability of probiotic strain *Lactobacillus acidophilus* M92. Journal of Applied Microbiology. 2003;94:981-987.
16. Ali AA. Isolation and identification of lactic acid bacteria isolated from traditional drinking yoghurt in Khartoum State, Sudan. Current Research in Bacteriology. 2011;4(1):16-22.
17. Azadnia PKNA, Khan Nazer AH. Identification of lactic acid bacteria isolated from traditional drinking yoghurt in tribes of Fars province. Iranian Journal of Veterinary Research. 2009;10(3):235-240.
18. Monica Cheesbrough. District laboratory practice in tropical countries part 2: Second Edition, Cambridge University Press Cambridge, New York. 2006;64.
19. Akhmetsadykova SH, Baubekova A, Konuspayeva G, Akhmetsadykova N, Faye B, Loiseau G. Lactic acid bacteria biodiversity in raw and fermented camel milk. African Journal of Food Science and Technology. 2015;6(3):84-88.
20. Brzoska F, Śliwiński B, Stecka K. Effect of *Lactococcus lactis* vs. *Lactobacillus* Spp. bacteria on chicken body weight, mortality, feed conversion and carcass quality. Annals of Animal Science. 2012;12(4):549-559.
21. Mountzouris KC, Tsirtsikos P, Kalamara E, Nitsch S, Schatzmayr G, Fegeros K. Evaluation of the efficacy of a probiotic containing *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, and *Pediococcus* strains in promoting broiler performance and modulating cecal microflora composition and metabolic activities. Poultry Science. 2007;86(2):309-317.
22. Tharmaraj N, Shah NP. Selective enumeration of *Lactobacillus delbrueckii* ssp. bulgaricus, *Streptococcus thermophilus*, *Lactobacillus acidophilus*, bifidobacteria, *Lactobacillus casei*, *Lactobacillus rhamnosus*, and

propionibacteria. Journal of Dairy Science. 2003;86(7):2288-2296.
23. Gebreselassie N, Abay F, Beyene F. Biochemical and molecular identification and characterization of lactic acid bacteria and yeasts isolated from Ethiopian naturally fermented buttermilk. Journal of Food Science and Technology. 2016; 53(1):184.
24. Kamrun NI, Touaha A, Fahmida A, Nazneen NI. Characterization and confirmation of *Lactobacillus* spp. from selective regional yoghurts for probiotic and interference with pathogenic bacterial growth. Asian Journal of Biological Sciences. 2016;9:1-9.
25. Kostinek M, Specht I, Edward VA, Pinto C, Egounlety M, Sossa C, Mengu M. Characterisation and biochemical properties of predominant lactic acid bacteria from fermenting cassava for selection as starter cultures. International Journal of Food Microbiology. 2007;114(3): 342.
26. Baccigalupi L, Di Donato A, Parlato M, Luongo D, Carbone V, Rossi M, De Felice M. Small surface-associated factors mediate adhesion of a food-isolated strain of *Lactobacillus fermentum* to Caco-2 cells. Research in Microbiology. 2005;156(7): 830-836.
27. Bai SP, Wu AM, Ding XM, Lei Y, Bai J, Zhang KY, Chio JS. Effects of probiotic-supplemented diets on growth performance and intestinal immune characteristics of broiler chickens. Poultry Science. 2013;92(3):663-670.
28. Bostami ABMR, Islam MM, Ahmed ST, Mun HS, Hong SB, Yang CJ. Effect of beneficial microorganisms on growth performance, mortality and intestinal micro flora in broilers. Global Journal of Microbiology Research. 2015;3(2):126-133.
29. Gill HS, Cross ML. 13 probiotics and immune function. Nutrition and Immune Function. 2002;251.
30. Zhang ZF, Kim IH. Effects of multistrain probiotics on growth performance, apparent ileal nutrient digestibility, blood characteristics, cecal microbial shedding, and excreta odor contents in broilers. Poultry Science. 2014;93(2):364-370.
31. Gao J, Zhang HJ, Yu SH, Wu SG, Yoon I, Quigley J, Gao YP, Qi GH. Effects of yeast culture in broiler diets on performance and immunomodulatory functions. Poult. Sci. 2008;87:1377–1384.
32. Higgins SE, Higgins JP, Wolfenden AD, Henderson SN, Torres-Rodriguez A, Tellez G, Hargis B. Evaluation of a Lactobacillus-based probiotic culture for the reduction of Salmonella Enteritidis in neonatal broiler chicks. Poult. Sci. 2008;87:27–31.
33. Yang CM, Cao GT, Ferket PR, Liu TT, Zhou L, Zhang L, Chen AG. Effects of probiotic, *Clostridium butyricum*, on growth performance, immune function, and cecal microflora in broiler chickens. Poultry Science. 2012;91(9):2121-2129.
34. Brzoska F, Stecka K. Effect of probiotic, prebiotic and acidifier on the body weight of broiler chickens, feed conversion, and carcass and meat composition. Annals of Animal Science. 2007;2(07).
35. Timmerman H, Koning C, Mulder L, Rombouts F, Beynen A. Monostrain, multistrain and multispecies probiotics—a comparison of functionality and efficacy. Int'l J. Food Microbial. 2004;96(3):219-233.
36. Timmerman HM, Veldman A, van den Elsen E, Rombouts FM, Beynen AC. Mortality and growth performance of broilers given drinking water supplemented with chicken-specific probiotics. Poultry Science. 2006;85(8):1383-1388.

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