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Research Article
Evaluation of the Antibiotic Properties of Probiotics and their Efficacy on Performance and Immune Response in Broiler Chicken

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Abstract

Objective: The study aimed to evaluate the extent of use of probiotics in the poultry feed industry and their efficacy in broiler diets. **Methodology:** Two surveys involving 100 agro vets and 36 poultry farmers were carried out. Fifteen brands of probiotics 0were found in agro vets with Product 1, 2, 3 and Product 4 being common. A total of 74.4% of the farmers used probiotics and Product 1, 7, 2 and Product 4 were common. Three hundred and seven, day-old broiler chicks were randomly assigned to dietary treatments; Control diet, Diet 2 (Product 1), Diet 3 (Control+Product 4), Diet 4 (Control+Product 7) and Diet 5 (Product 2). Disk diffusion test was used to test the inhibitory effect of probiotics on bacteria cultures; *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus* and *Candida albicans*. **Results:** Probiotics had no significant effect ($p>0.05$) on daily weight gain, feed intake and feed conversion ratio during starter phase. Performance was not significantly affected by probiotics during the finisher phase except for Product 2 which depressed growth. Blood samples were collected to test the effects of probiotics on antibody response to Infectious Bursal Disease virus and they had no significant effect ($p = 0.6868$). Product 4 and 7 had an inhibitory effect while Product 1 and 2 did not. **Conclusion:** Performance of broilers was not affected by the inclusion of probiotics in the diet.

Key word: Antibody response, broilers, growth performance, inhibitory effect, probiotics

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Competing Interest: The authors have declared that no competing interest exists.

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

INTRODUCTION

The worldwide increase in population growth and urbanization has created increased demand for livestock products hence, a major reason for the rapid changes in livestock production systems in order to increase production[1]. This has resulted in intensification of livestock production[2]. Poultry production has shown the highest increase in intensity compared to other livestock, with high growth rate and feed efficiency being the two main targets to ensure high production over the years[3]. The use of antibiotics increased concurrently with the intensification of the livestock industry to improve animal welfare and obtain economic benefits in terms of improved animal performance and reduced medical costs. However, there was an increasing risk of prevalence of antimicrobial-resistant bacteria in both humans and livestock as a result.

This led to the ban on the use of antibiotics as growth promoters in animal feed in 2006 by the European Union[4]. The ban on antibiotic use as growth stimulants for farm animals and concerns regarding the side-effects from their use as therapeutic agents, has produced a climate in which both the consumer and manufacturer are looking for alternatives to antibiotics. With the increasing demand for quality animal products, as well as a vast awareness about the effects of these products on human health in Kenya, animal production systems have not only been focusing on increased production but also on their effects on the environment and health of the consumers.

Probiotics have stood out as alternatives with the ability to maintain high productivity and to be economically feasible, as well as safe to human and animal health, thereby meeting the requirements of consumers and foreign markets. Palamidi *et al.*[5] concluded that probiotics have the potential to replace antibiotics as growth promoters since dietary inclusion of probiotics positively enhanced broiler performance in a similar manner to avilamycin supplementation. Results on Kenyan indigenous chicken suggested that supplementation with probiotics (Mola plus) in drinking water significantly improved weight gain[6].

The main objective of the study was to evaluate the extent to which probiotics are used in the Kenyan feed industry and their efficacy in broiler diets in terms of performance, antibody production against Infectious Bursal Disease (Gumboro) and their antimicrobial effects.

MATERIALS AND METHODS

Experiment 1

The use of probiotics in poultry production in Kenya (Kiambu county): Two surveys involving 100 agro vets and 36 poultry farmers were carried out using semi-structured questionnaires in Kiambu County, Kenya. The questionnaires were administered to the owners of the agro vets stockiest and farmers with the aim being to evaluate which products were sold in the market as probiotics, their use and effect on performance in layer/broiler production according to the farmers. The sample size was calculated with the method of Cochran[7] as indicated below;

$$n = pqz^2/e^2$$

Where

n = Sample size
z = Confidence level ($\alpha = 0.01$)
p = Proportion of the population containing the variables of interest

All data was entered into Microsoft Excel and analyzed in Statistical Package for Social Sciences (SPSS) version 22. The common probiotics found from the survey were tested in a controlled experiment (Experiment 2) to evaluate their effectiveness in broiler performance.

Experiment 2

Effects of common probiotics on broiler chicken performance in Kenya

Study site: The study was carried out at Kenya Agricultural and Livestock Research Organization (KALRO) station in Naivasha, Nakuru County.

Birds and experimental facility: Three hundred and seven, day-old Cobb 700 broiler chicks (of mixed sexes), weighed individually on arrival from the hatchery and randomly placed into individual experimental cages. Vaccination against New Castle disease (NCD) was given at days 7 and 21 while that against Infectious Bursal Disease (Gumboro) was given at days 14 and 28 via drinking water as per the hatchery/breeder recommendations. The experiment and feeding trial lasted for six weeks (42 days). The first experimental phase was the growing phase (day old to 21) followed by finishing phase (day 22-42).

Dietary treatments: The chicks were randomly assigned to the five dietary treatments which were; Diet 1 (Control), Diet 2 (Product 1 as a powder), Diet 3 (Control diet+product 4 added in water), Diet 4 (Control diet+product 7 added in water) and Diet 5 (Product 2 as a powder). The chicks were fed on starter diet from day 1-21 and finisher diet from day 22-42 and all the diets were formulated to meet the National Research Council[8] requirements. The composition of the diets used in this experiment are shown in Table 1 and 2. Product 1 and 2, which were in powder form, were added in the feed in accordance to the manufacturer's specifications while Product 4 and 7 were added into drinking water at the rate of 5 mL of microbes/1 litre once a day. The diets and water were provided ad-libitum. No antibiotics were used during the entire experimental period.

Data collection: Weekly body weight gain measurements for each dietary treatment were determined by calculating the difference in weight between two consecutive weighing. Feed intake was recorded daily. Feed conversion ratio (FCR) was determined as the ratio between feed intake and body weight gain as shown below;

$$FCR = \frac{\text{Feed Intake (g day}^{-1})}{\text{Body weight gain (g day}^{-1})}$$

Experiment 3

Effect of common probiotics fed to broilers on antibody production when vaccinated against Infectious bursal disease

Sampling: Blood samples to determine serum titers of antibodies against Gumboro were collected from the wing vein of one chick per replicate on days 13 and 35. Enzyme-linked immunosorbent assay was used to determine antibody titres of the chickens against Infectious bursal disease. A total of 34 samples were analyzed for antibody production against Gumboro disease after vaccination.

Statistical analysis: The data were analyzed using the SAS version 9.00 (2007) with a Complete Randomized Design using the General Linear Models (GLM) procedure. Least Significant Difference (LSD) method at a level of $p<0.05$ was used to separate treatment means.

Experiment 4

Antimicrobial susceptibility Test of common probiotics in Kenya on *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus* and *Candida albicans*

The disk diffusion test was used where 16 disc plates had 4 probiotic treatments with 4 bacteria cultures of *E. coli*, *Staph. Aureus*, *Bacillus cereus* and *Candida albicans*. A standard

Table 1: Composition and nutrient content of starter (day 1-21) basal diets for broiler chicks (%)

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Maize	61.60	60.00	61.60	61.60	61.45
Soybean meal	21.95	22.00	21.95	21.95	21.97
Fishmeal	12.00	12.15	12.00	12.00	12.00
Oil	2.00	2.00	2.00	2.00	2.00
Dicalcium phosphate	1.20	1.20	1.20	1.20	1.20
Limestone	0.45	0.45	0.45	0.45	0.45
Vitamin/trace mineral premix*	0.50	0.50	0.50	0.50	0.50
Salt	0.30	0.30	0.30	0.30	0.30
Probiotics					
None (control)	-	-	-	-	-
Product 1	-	1.40	-	-	-
Product 4	-	-	+	-	-
Product 7	-	-	-	+	-
Product 2	-	-	-	-	0.13
Total	100.00	100.00	100.00	100.00	100.00
Calculated analysis					
Calculated crude protein (%)	21.90	21.90	21.90	21.90	21.90
Metabolizable energy (MJ kg^{-1})	15.72	15.50	15.72	15.72	15.72
Lysine (%)	1.30	1.30	1.30	1.30	1.30
Methionine+Cysteine (%)	0.80	0.80	0.80	0.80	0.80

+(added in water at 5 mL per 1 litre), *Composition of vitamin/trace mineral premix per kg diet; Vitamin A: 8×103IU, Vitamin D3: 2.0 IU, Vitamin E: 10.0 IU, Vitamin K3: 1.5 mg, Vitamin B2: 2×10 mg, Vitamin B12: 0.5 mg, Folic acid: 0.6 mg, Nicotinic acid: 5 mg, Calcium panthotenate: 4 mg, Choline: 0.078 mg, Trace elements: Mg (5×10 mg, Zn: 5×10 mg, Cu: 2.5 mg, Co: 0.5 mg, I: 2 mg, Se: 0.2 mg, Antioxidants: Butylated hydroxytoluene (0.625 mg), Carrier: Calcium carbonate q.s.p (0.25 kg)

Table 2: Composition and nutrient content of grower (day 22-42) basal diets for broiler chicks (%)

Ingredients	Diet 6	Diet 7	Diet 8	Diet 9	Diet 10
Maize	66.35	64.70	66.35	66.35	66.20
Soybean meal	19.20	19.45	19.20	19.20	19.22
Fishmeal	10.00	10.00	10.00	10.00	10.00
Oil	2.00	2.00	2.00	2.00	2.00
Dicalcium phosphate	1.20	1.20	1.20	1.20	1.20
Limestone	0.45	0.45	0.45	0.45	0.45
Vitamin/trace mineral premix*	0.50	0.50	0.50	0.50	0.50
Salt	0.30	0.30	0.30	0.30	0.30
Probiotics					
None (Control)	-	-	-	-	-
Product 1	-	1.40	-	-	-
Product 4	-	-	+	-	-
Product 7	-	-	-	+	-
Product 2	-	-	-	-	0.13
Total	100.00	100.00	100.00	100.00	100.00
Calculated analysis					
Calculated crude protein (%)	19.80	19.80	19.80	19.80	19.80
Metabolizable energy (MJ kg^{-1})	15.51	15.49	15.51	15.51	15.62
Lysine (%)	1.10	1.10	1.10	1.10	1.10
Methionine+cysteine (%)	0.70	0.70	0.70	0.70	0.70

+(added in water at 5 mL per 1 litre), *Composition of vitamin/trace mineral premix per kg diet; Vitamin A: 8×103 IU, Vitamin D3: 2.0 IU, Vitamin E: 10.0 IU, Vitamin K3: 1.5 mg, Vitamin B2: 2×10 mg, Vitamin B12: 0.5 mg, Folic acid: 0.6 mg, Nicotinic acid: 5 mg, Calcium panthotenate: 4 mg, Choline: 0.078 mg, Trace elements; Mg: 5×10 mg, Zn: 5×10 mg, Cu: 2.5 mg, Co: 0.5 mg, I: 2 mg, Se: 0.2 mg, Antioxidants: Butylated hydroxytoluene (0.625 mg), Carrier: Calcium carbonate q.s.p (0.25 kg)

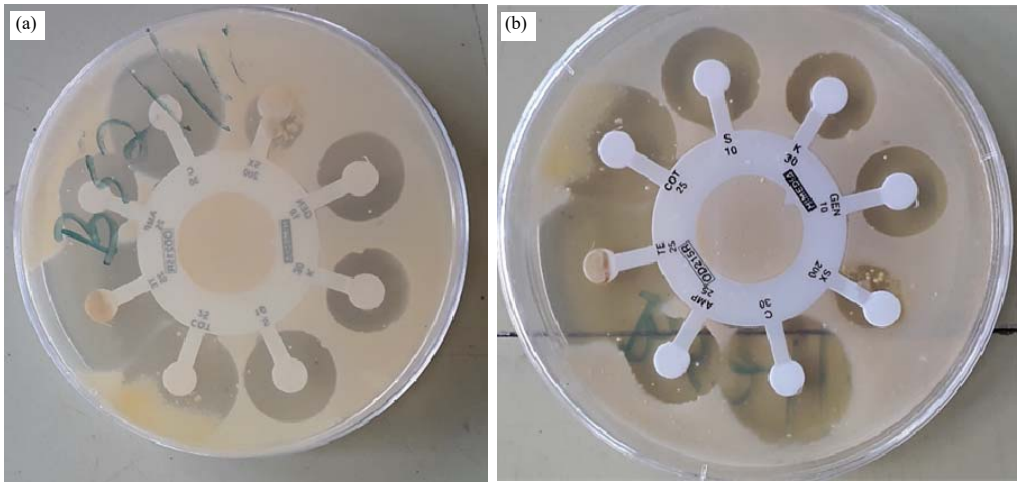

Fig. 1(a-b): Disc plate showing the inhibitory effect of 8 different antibiotics on *Staphylococcus aureus*

disc plate showing the inhibitory effect of 8 antibiotics [Tetracycline (TE), Streptomycin (S), Kanamycin (K) Gentamycin (GEN), Sulphamethoxazole (SX), Co-Trimoxazole (COT), Chloramphenicol (C) and Ampicilin (AMP)] on *Staphylococcus aureus* was used as control as shown in Fig. 1.

RESULTS

Experiment 1: The results from the agro vet stockiest survey showed that, a total of 15 types of probiotics were found in the market in Kiambu County as shown in Table 3. The most common probiotics were Product 1 (25.6%), Product 2 (14.3%), Product 3 (13.7%) and Product 4 (11.3%). A total of 74.4% of the poultry farmers were using probiotics as feed additives. According to the study, probiotics were mainly being used by the commercial poultry farmers while the household poultry farmers did not use probiotics. They had other alternatives which included antibiotics, poultry supplements and medicinal plants and trees (Table 4). Most of the respondents surveyed in this study administered the probiotics in the early growth stages of the chicken (day 1-4 weeks) to allegedly increase their appetite hence boost their growth

Table 3: Types of probiotics used in livestock production in Kiambu county and the frequency of occurrence

Products	Frequency	Percentage
Product 1	43	25.6
Product 2	24	14.3
Product 3	23	13.7
Product 4	19	11.3
Product 5	18	10.7
Product 6	16	9.5
Product 7	6	3.6
Product 8	4	2.4
Product 9	4	2.4
Product 10	3	1.8
Product 11	2	1.2
Product 12	2	1.2
Product 13	2	1.2
Product 14	1	0.6
Product 15	1	0.6
Total	168	100.0

Table 4: Summary of the probiotics brands used by poultry farmers in Kiambu county

Probiotics	Frequency	Percentage
Product 1	9	23.1
Product 4	8	20.5
Product 7	6	15.4
Product 2	5	12.8
Product 16	1	2.6
N/A	10	25.6
Total	39	100.0

N/A represents the number of non-respondents

Table 5: Summary of the mortality rate of broilers

Treatments	Mortalities	Percentage
Control	0	0.00
Product 1	2	3.33
Control+product 4	4	6.67
Control+product 7	1	1.67
Product 2	0	0.00
Total	7	2.28

rate. All the poultry farmers who used probiotics gave a positive feedback on its performance in poultry. The common probiotics from both surveys were; Product 1, Product 7 and Product 4

Experiment 2

Mortality: The total mortality of the birds was generally low at 7 out of 307 birds (2.28%) as shown in Table 5. There were no mortalities reported in the control diets and those containing Product 2.

Broiler performance: Table 6-8 show the effects of probiotics on the growth rate, feed intake and feed conversion during both the starter and finisher period. The initial body weight of the day old chicks ranged from 39.8-45.5 g. The results indicate that the addition of probiotics had no significant ($p>0.05$) effect on the daily weight gain in the chicks during the starter phase (day 1-21). Daily weight gain in the finisher stage tended to increase significantly ($p<0.05$) in the experimental groups and especially in diets 8 and 9 in comparison to the control. However, the diets 7 and 10 depressed the growth of the chicks in the finisher phase. Overall, diet 10 depressed growth rate of the broilers (Table 6). Feed intake of broilers did not differ significantly ($p>0.05$) between the dietary treatment groups in the starter phase (day 1-21) (Table 7). However, diets containing Product 1 and Product 2 depressed daily feed intake during the finisher period (22-42 days) ($p<0.05$). During the entire period of experiment, the diets containing Product 2 suppressed the feed intake by 22.3% ($p<0.05$) compared to the control diet.

In the present experiment, there was no significant differences in FCR ($p>0.05$) among the treatments during the

Table 6: Effects of treatments on average daily gain (ADG)

Growth rate	Diets 1 and 6	Diets 2 and 7	Diets 3 and 8	Diets 4 and 9	Diets 5 and 10	p-value
ADGs	22.59±0.91	22.16±0.96	19.05±0.91	20.43±0.91	20.32±0.96	0.0594
ADGf	47.38±2.31^{ba}	41.38±2.44^{b}	47.83±2.31^{ba}	48.75±2.31^{a}	28.44±2.44^{c}	0.0001
ADGo	34.98±1.44^{a}	31.77±1.51^{a}	33.44±1.44^{a}	34.59±1.44^{a}	24.65±1.51^{b}	0.0001

Diet 1-5 are starter phase diets and Diet 6-10 are finisher phase diets, $^{a-c}$Means in the same row with different superscripts differ significantly ($p<0.05$), The results are reported as Mean±SEM (standard error of means), ADGs: Average daily gain starter, ADGf: Average daily gain finisher, ADGo: Average daily gain overall

Table 7: Effects of treatments on average daily feed intake (ADFI)

Feed Intake	Diets 1 and 6	Diets 2 and 7	Diets 3 and 8	Diets 4 and 9	Diets 5 and 10	p-value
ADFIs	37.67±1.49	35.68±1.57	34.49±1.49	35.54±1.49	34.35±1.57	0.5439
ADFIf	118.16±3.55^{a}	104.09±3.74^{b}	111.91±3.55^{ba}	110.67±3.55^{ba}	86.10±3.74^{c}	0.0001
ADFIo	72.78±2.44^{a}	65.88±2.57^{a}	68.18±2.44^{a}	69.14±2.44^{a}	56.54±2.57^{b}	0.0001

Diet 1-5 are starter phase diets and Diet 6-10 are finisher phase diets, $^{a-c}$Means in the same row with different superscripts differ significantly ($p<0.05$), The results are reported as Mean±SEM (standard error of means), ADFIs: Average daily feed intake starter, ADFIf: Average daily feed intake finisher, ADFIo: Average daily feed intake overall

Table 8: Effects of treatments on feed conversion ratio (FCR)

Feed conversion	Diets 1 and 6	Diets 2 and 7	Diets 3 and 8	Diets 4 and 9	Diets 5 and 10	p-value
FCRs	1.67±0.06	1.61±0.06	1.84±0.06	1.75±0.06	1.70±0.06	0.1185
FCRf	2.52±0.15^{b}	2.57±0.16^{b}	2.45±0.15^{b}	2.29±0.15^{b}	3.04±0.16^{a}	0.0227
FCRo	2.09±0.09	2.09±0.09	2.07±0.09	2.02±0.09	2.33±0.09	0.1544

Diet 1-5 are starter phase diets and Diet 6-10 are finisher phase diets, $^{a-c}$Means in the same row with different superscripts differ significantly ($p<0.05$), The results are reported as Mean±SEM (standard error of means), FCRs: Feed conversion ratio starter, FCRf: Feed conversion ratio finisher, FCRo: Feed conversion ratio overall

Table 9: Antibody responses against Infectious bursal disease (expressed as Log_{10} Titre) of broilers fed on the dietary treatments

Treatments	Log_{10} Titre mean value
Control	2.61±0.11^{a}
Product 1	2.61±0.11^{a}
Control+product 4	2.67±0.11^{a}
Control+product 7	2.77±0.11^{a}
Product 2	2.85±0.11^{a}

$^{a-c}$Means in the same row with different superscripts differ significantly ($p<0.05$), The results are reported as Mean±SEM (standard error of means)

starter phase and overall period. However, in the finisher phase the feed efficiency was poorer ($p<0.05$) for the diet containing Product 2 (Table 8).

Experiment 3: The results indicated that the addition of probiotics had no significant ($p = 0.6868$) effect on the antibody response to Infectious Bursal Disease after vaccination as shown in Table 9.

Experiment 4: After incubation, the plates were examined for zones of inhibition, which are the areas wherein there is a prominent reduction (80%) in growth. Product 4 (Sample B) and Product 7 (Sample C) showed inhibitory effects on all the cultured microorganisms; (*E. coli*, *Staph. aureus, Bacillus cereus* and *Candida albicans*) as shown in Fig. 2-5 respectively. Product 1 (Sample A) and Product 2 (Sample D) showed no inhibitory effect on the growth of any microorganism cultures; (*E. coli*, *Staph. aureus, Bacillus cereus* and *Candida albicans*) as shown in Fig. 6-8 and 9, respectively.

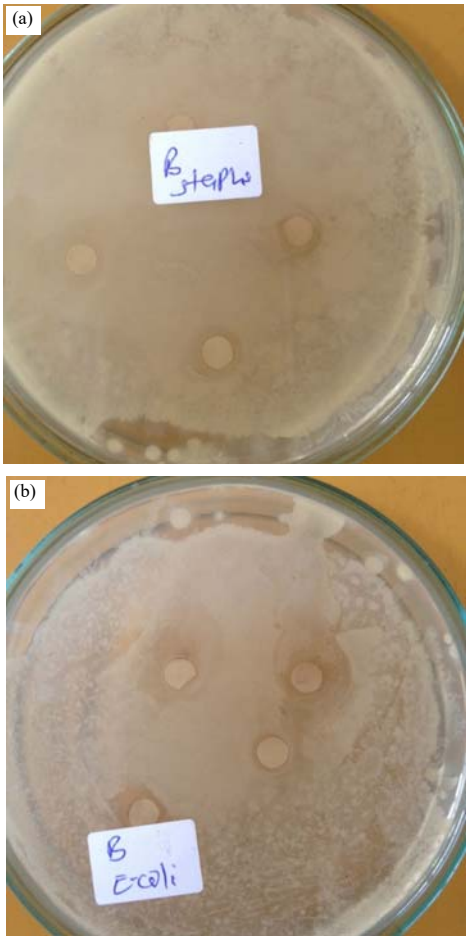

Fig. 2(a-b): Showing the inhibitory effect of sample B on (a) *Staphylococcus aureus* and (b) *Escherichia coli*

DISCUSSION

Experiment 1: After using the probiotics, poultry farmers noted an increase in feed intake and faster growth rate. Similar results were obtained by Samanya and Yamauchi[9] who indicated that the birds fed on probiotics had a tendency to display prominent villi height which increase the rate of absorption of available nutrients, resulting in increased growth rate and weight gain. Other studies have shown that supplementation of broiler diets with probiotics increased the villus height: crypt depth ratio in the ileum significantly[10].

Experiment 2: According to the study, probiotics were of no benefit at all in the starter phase (1-21 days). The performance of the birds supplemented with probiotics was as good as that of the birds in control group. Similar results were obtained by Fernandes *et al.*[11] who reported that birds fed on probiotic, prebiotic, synbiotic and organic acids in the starter period were similar in weight gain to those in control group. Pelicano *et al.*[12] observed that there were no differences in weight gain for birds receiving probiotics and control group in the starter phase. During the finisher period (22-42 days), broilers fed diets containing Product 2

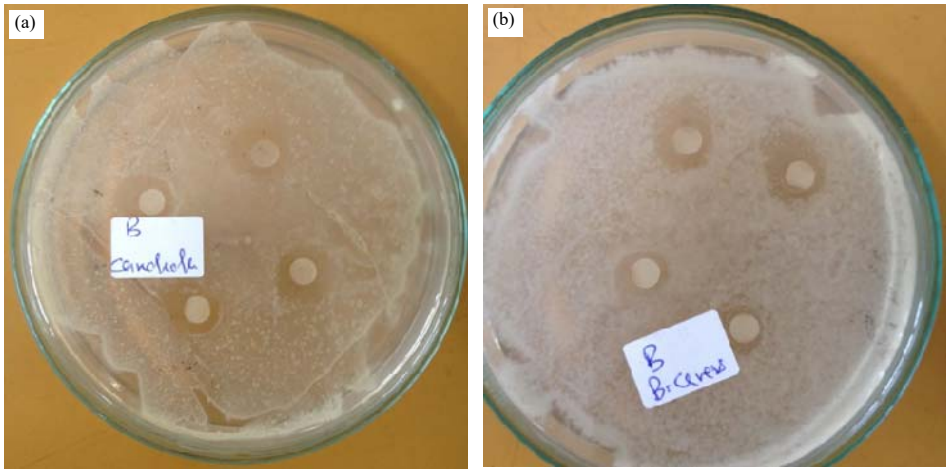

Fig. 3(a-b): Showing the inhibitory effect of sample B on (a) *Candida albicans* and (b) *Bacillus cereus*

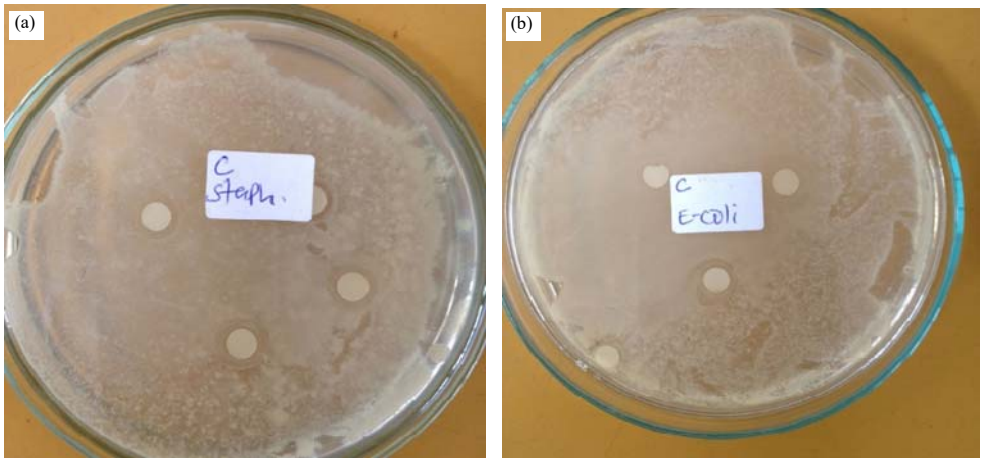

Fig. 4(a-b): P: Showing the inhibitory effect of sample C on (a) *Staphylococcus aureus* and (b) *Escherichia coli*

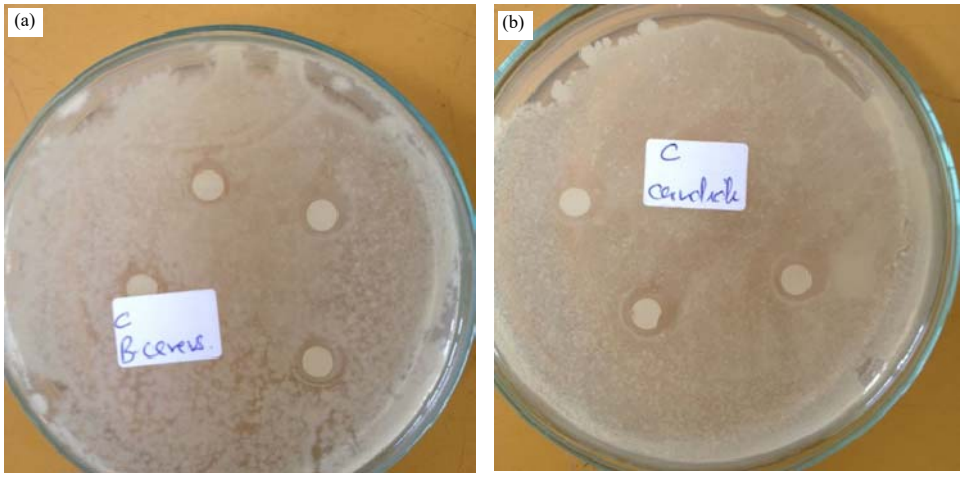

Fig. 5(a-b) Showing the inhibitory effect of sample C on (a) *Bacillus cereus* and (b) *Candida albicans*

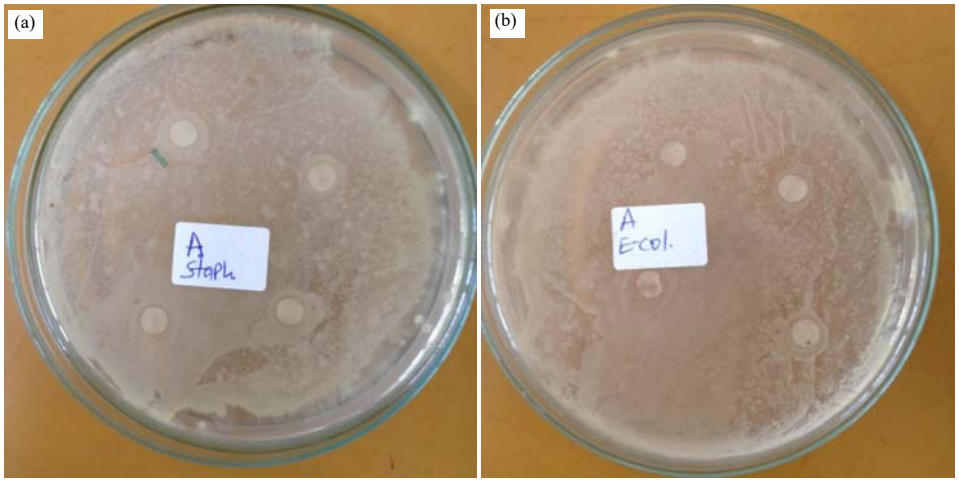

Fig. 6(a-b): Showing the inhibitory effect of sample A on (a) *Staphylococcus aureus* and (b) *Escherichia coli*

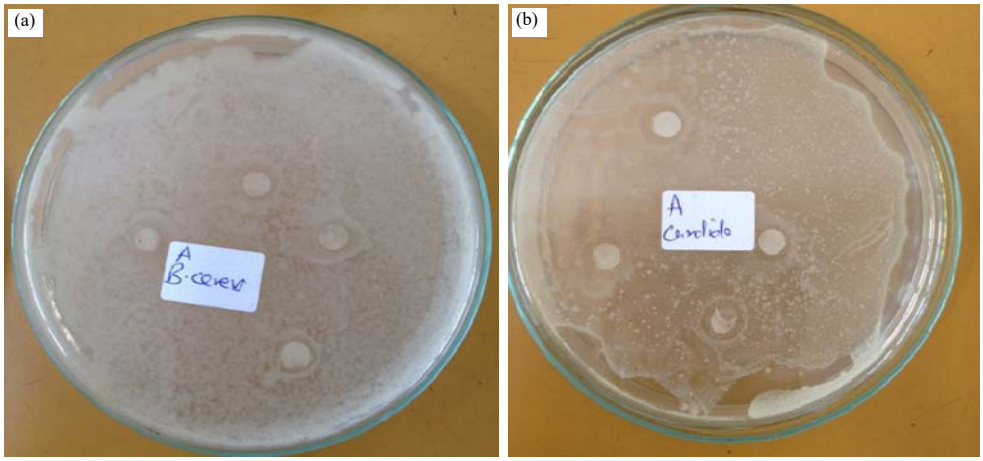

Fig. 7(a-b): Showing the inhibitory effect of sample A on (a) *Bacillus cereus* and (b) *Candida albicans*

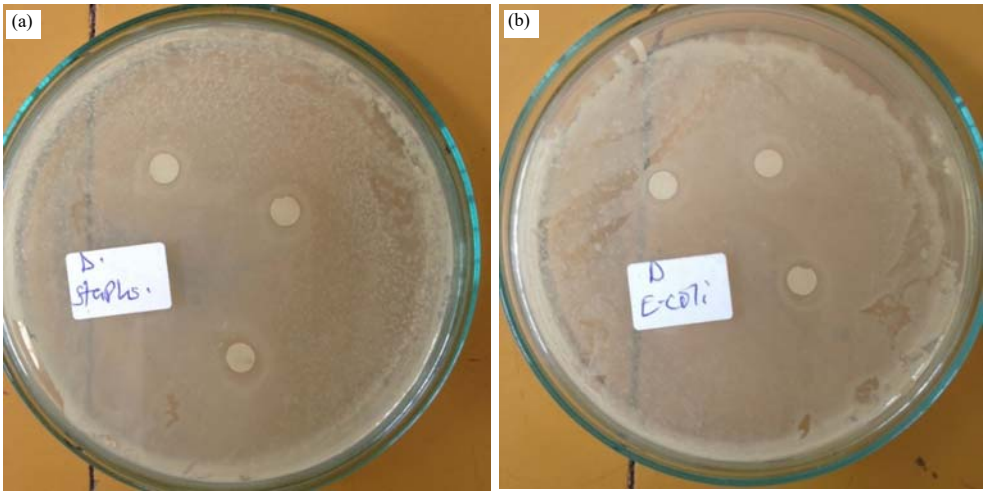

Fig. 8(a-b): Showing the inhibitory effect of sample D on (a) *Staphylococcus aureus* and (b) *Escherichia coli*

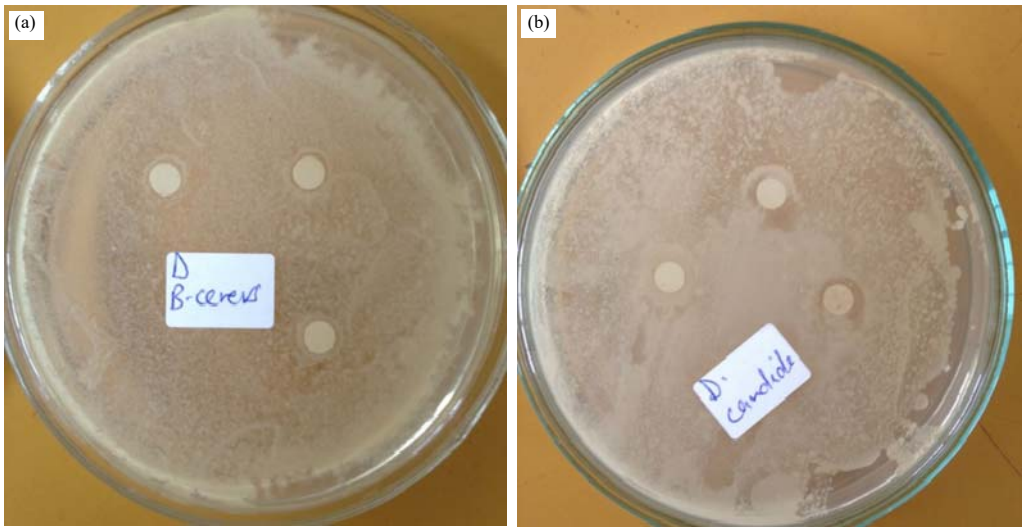

Fig. 9(a-b): Showing the inhibitory effect of sample D on (a) *Bacillus cereus* and (b) *Candida albicans*

performed poorer than those birds on the other diets. Broilers offered diets contained Product 1 also tended to perform poorly. These two probiotics suppressed the feed intake and feed efficiency. The reason for the poorer performance is not apparent.

Negative effects could also occur when high levels of probiotics are administered to chickens. Using probiotics at levels of the 1000 and 2000 g t^{-1} in dietary treatments caused serious damages to absorptive area of digestive system[13]. This caused a reduction in feed intake and negatively affected the FCR of the birds since probiotic supplementation at these levels had almost damaged the apical cells significantly ($p<0.05$). In the current experiment a dose response of Product 2 on broiler performance was not tested.

There are reports in the literature showing that probiotics have a positive effect on growth and feed efficiency. Administration of probiotics in diets of broilers displayed a growth-promoting effect and significantly improved the daily weight gain and feed efficiency[14,15].

However, in the current experiment, broilers receiving probiotics did not perform better than the control ($p>0.05$). The supplementation of probiotic EM.1 had no significant effect on weight gain, mortality and FCR in the Fayoumi and Horro chicken breeds[16]. Similar results were also observed by Fatufe and Matanmi[17] who concluded that probiotics generally have no effect on the growth performance of two strains of cockerels. Growth performance and FCR obtained in birds that were fed on a diet supplemented with a probiotic, "primalac", did not significantly improve compared with the control group[18]. It is possible that the rearing environment for the broilers in this experiment presented a low stress situation where all factors of management were handled well, hence presenting a low challenge.

Landy and Kavyani[19] demonstrated that supplementation with the probiotic "primalac" to broilers reared under heat stress conditions had a favorable effect on performance, immune responses and cecal microflora. Fox[20] concluded that efficiency of probiotic in performance of birds may be insignificant in conditions of minimum stress. It is therefore possible that the positive effect of probiotics in the present study could not be seen because of the good rearing conditions of the broilers. The other possible reasons for the lack of consistent results are low or variable viability of microbial cultures, strain differences in cultures selected, dose level and frequency of product feeding, antimicrobial and feed ingredient interactions which reduce/neutralize viable colonies before feeding and composition of diet. It is therefore important to control the factors causing the variations for more consistent results[21].

Experiment 3: According to this study, the addition of probiotics had no significant effect on the antibody responses to Infectious Bursal Disease vaccination. Our results agree with Balevi *et al.*[22] who reported that probiotic supplementation did not affect specific antibody synthesis to Newcastle Disease vaccine (NDV) antigen administered via drinking

water. Another study showed that use of probiotic *B. longum* PCB133 in turkeys had no significant effect on immune response to NDV antibody production[23].

So far, studies dealing with probiotic effects on vaccination efficiency on antibody production in poultry has shown mixed results. Khalifa *et al.* [24] reported that, the use of probiotic routinely in broiler diets improves the immune status and humeral immune response against New Castle Disease (ND) and Infectious Bursal Disease as well as treatment of *E. coli* infection in chicks. The exact mechanisms of stimulation of immune response by probiotics have not been fully explained but several studies have shown that they may stimulate different subsets of immune system cells. A study on oral administration of probiotics in broilers has shown significant effects on both the systemic and mucosa-associated immune responses, resulting in disease prevention[25]. According to the results of our study, it is uncertain if probiotics stimulate mucosal, cellular or humoral immunity response in broilers since we only focused on systemic immunity.

Experiment 4: It is essential for probiotic strains to show antagonism against pathogenic bacteria through antimicrobial substance production or competitive exclusion, to have an impact on the digestive system flora. Research has shown that different species produce different antimicrobial substances like: *Lactobacillus reuterii* produce a low molecular weight antimicrobial substance called reuterin and the subspecies of *Lactococcus lactis* produce a class I bacteriocin, known as nisin A. *Enterococcus feacalis* DS16 produces a class I bacteriocin cytolysin and *Lactobacillus plantarum* produces a class II bacteriocins plantaricin S. Lastly, *Lactobacillus acidophilus* produces a class III bacteriocin acidophilucin A[26].

The presence of antimicrobial effects was reported in probiotics isolated from different bio yoghurts; *Lactobacillus* sp., *Streptococcus* sp. and *Bifidobacterium* sp. against some common bacterial pathogens; *Staphylococcus aureus, Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa*[27]. Viable *S. cerevisiae* cells not only physically inhibited the *C. albicans* colonization of epithelia but also directly inhibited the elaboration of several key pathogenicity factors[28].

However, results from a different study showed that treatment of *E. coli* with probiotic suspension was not effective on inhibition of the plasmid carrying hypothetical ampicillin resistant gene[29]. Despite the variability in results, probiotics still provide the best alternative for prevention and treatment of various pathogenic microorganisms without causing harmful side effects to both animals and humans.

CONCLUSION

The Kenyan farmer in Kiambu has accepted to use probiotics in poultry production with the most common being, Product 1, Product 7, Product 2 and Product 4. However, the results demonstrated that the inclusion of feed additives marketed as "probiotics" were of no value on the performance and the antibody response of the probiotic treatments to Infectious Bursal Disease. Product 4 and Product 7 showed positive results in the experiments on the antimicrobial susceptibility test. It is unknown whether antibiotics were actually added to these products or the probiotic strains in the products have antimicrobial effects which enhanced immunity response in the chicken. Therefore, further studies are recommended to determine the ingredients and contents of the probiotics sold in Kenyan market.

SIGNIFICANCE STATEMENT

This study discovered that the inclusion of probiotics were of no value on weight gain, feed intake and feed efficiency of broilers. This study will help the researchers to uncover the critical areas of immunity in poultry production in Kenya. Thus a new theory on the effect of probiotics on mucosal, cellular and humoral immunities in poultry, may be arrived at.

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