Scientia Africana, Vol. 23 (No. 5), December, 2024. Pp 213-230
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

BIOEFFICACY PERFORMANCE AND MEAT QUALITY OF A COMMERCIAL FEED SUPPLEMENTED WITH PROBIOTICS ON BROILERS

Ire, F.S.*[1], Njoku, T.T.[1], Ahaotu, I.[1] and Maduka, N.[2]
[1]Department of Microbiology, Faculty of Science, University of Port Harcourt, Port Harcourt Nigeria
[2]Department of Microbiology, Faculty of Science, Federal University Otuoke, Bayelsa State, Nigeria
*Corresponding author: francis.ire@uniport.edu.ng. Orcid ID: 0000-0001-5599-1292

Received: 02-11-2024
Accepted: 17-12-2024

https://dx.doi.org/10.4314/sa.v23i5.18

Journal Homepage: http://www.scientia-african.uniportjournal.info
Publisher: *Faculty of Science, University of Port Harcourt.*

ABSTRACT

Probiotics are live microorganisms which when administered in adequate amounts confer health benefit and used as an alternative to antibiotics in poultry farming. They have been receiving increasing attention due to its benefits. This study was designed to investigate the synergetic effect of selected probiotics on the immunity, intestinal microbiota and the meat quality of broilers when they are eviscerated. A total of sixty (60) broilers, 1-15 day old birds divided into group A, B, and C were fed with a commercial feed supplemented with Lactobacillus casei (4.7 x 10^3CFU/ml), Bacillus subtilis (4.5 x 10^5CFU/ml), mixed culture of L. casei + B. subtilis, respectively, while the group D received no probiotic (control) for seven weeks. Although the broiler's feed was contaminated with pathogens, the average weekly feed intake (FI) and weight gain (WG) steadily increased across the groups during the trial. At week 7, the highest FI (401g) and WG (1045g) involved the control. The broilers in group D had the highest mortality (40 %), while the least involved group A (0%).After 24 hours the broilers were slaughtered, the carcass from group C had the highest protein (22.96%) and least moisture (69.43%) content, while the group D had the least and highest values of 19.21% and 74.76%,respectively.The antimicrobial activity demonstrated by L. casei + B. subtilis against the pathogens isolated from the broiler's feaces was more effective than the single culture. Overall, itis a probiotic recommended for the broilers based on improved meat quality and growth parameters of the broilers. Therefore, poultry farmers are encouraged to use probiotics to reduce mortality and cost of feeding the birds in order to achieve high productivity,

Keywords: Poultry farming, antibiotic growth promoter, antibiotic resistance, intestinal microbiota

INTRODUCTION

It is a concern to farmers that the bulk of the expenses which is up to 70 %of the total cost of poultry farming is attributed to feed (Dham a*et al*., 2011). The profit margin of the business will become less attractive if this trend is not controlled. The use of feed additives mainly probiotics generally regarded as safe is aimed at reducing the cost of feeding poultry and promote their growth (Untoo *et al*., 2018; Youssaf *et al*., 2022; Aliyi, 2023). High demand for poultry meat and eggs due to increase in global population expected to reach 9.3 billion in 2050 is putting poultry flocks under stress and too much pressure. In the year 2050, it is estimated that there will be a 60 % increase in today's food production and consumption (Halder *et al*., 2024). Globally,

the consumption of poultry meat and egg in 2050 is expected to increase by 52% and 39%, respectively (Gul and Alsayeqh, 2022). Global total output of poultry meat which include chicken, goose, duck etc in 2023 is 139.68 million metric tons (Dong *et al*., 2024).

The use of antibiotics to increase efficiency in feed conversion, preventive measure against intestinal infections, and growth promoters in the poultry industry, used to be a common practice until few decades ago when strategic steps were taken in some countries to ban or drastically reduce the level of antibiotic usage (Abdel *et al*., 2022; Çapan and Bağdatli, 2022;Tsega*et al*., 2024).Excessive use of antibiotics in poultry birds could cause an imbalance in the intestinal microbiota (Abbas *et al*., 2024). Consequently, the weight gain, viability, and feed efficiency of the animals are affected. It becomes easier for pathogenic microorganisms to gain entry and colonize the intestine of the animals. There is a high risk of disease conditions in the poultry birds whose normal flora in the intestine has been utterly disrupted(Paz *et al*., 2019). The rapid spread of antibiotic resistant bacterial strains among human population is attributed to indiscriminate use of antibiotics in the poultry industry, among other factors. Due to the consequences associated with this ugly development, the use of probiotics in poultry feed is recommended as a good alternative to antibiotics (Maduka and Ire, 2018; Jeni *et al*., 2021).

The essence of using probiotic as a feed additive in agriculture is to promote protein utilization and growth of food animals, stimulate the immune system of the animals, increase feed efficiency, subdue the activities of pathogenic microorganisms in the gastrointestinal tract, and promote the growth of beneficial microorganisms (Devi *et al*., 2019; Jeni *et al*., 2021; Halder *et al*., 2024). Improvement in performance of broiler could be achieved using probiotics which include *Bifidobacterium* spp., *Lactobacillus casei, L. acidophilus, Pediococcus pentosaceus* (Untoo *et al*., 2018; Lokapirnasari *et al*., 2024). The effect of administering probiotic microorganisms as feed supplement in the quality of poultry meat and eggs have been reported by different researchers.

According to Maduka and Ire (2018), the use of animal feed mixed with microorganisms in the interest of the animals is known as direct fed microbial (DFM) supplementation. Some of the benefits of feeding poultry with probiotics include increase in tenderness, improvement in colour, flavour, and juiciness of fresh meat (Çapan and Bağdatli, 2022). Despite the successes recorded in producing probiotic additives using different bacterial strains, there is need to develop new and more effective products (Poberezhets *et al*., 2021). Therefore, this study was aimed at evaluating the synergetic effect of selected probiotics on the immunity, intestinal microbiota and the meat quality of broilers when they are eviscerated

MATERIALS AND METHODS

Source of broilers

A total of sixty chicks (1-15 days old) were purchased from a well-recognized poultry farm in Port Harcourt, Rivers state. A commercially available feed meant for broilers of one day old to 30 days old is called broilers starter or chicken mash was purchased from a reputable dealer in Port Harcourt, Rivers state.

Experimental design

The chicks were divided into four (4) groups including the control group following the procedure described by Devi *et al*. (2019) and Mogotlane *et al*. (2024) with slight modification.

Feeding groups

Group 1

A total of fifteen (15) chicks was placed in this group. They were used as control. The chicks were fed only with conventional feed meant for broilers without any dietary treatment with probiotics 1.5kg/ton diet all over the period of rearing.

Scientia Africana, Vol. 23 (No. 5), December, 2024. Pp 213-230
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

Group 2

The total number of chicks in this group is fifteen (15). They were feed conventional feed meant for broilers which is broiler mash. The birds received the probiotic (*Lactobacillus casei*,4.7 x10^3CFU/ml) through their drinking water at two days interval when the water was changed.

Group 3

The total number of chicks that constitute this group is fifteen (15). Broiler mash, a conventional mash was used to feed the chicks, while their drinking water was supplemented with probiotic 1 (*Bacillus subtilis*,4×10^5CFU/ml).

Group 4

The chicks in this group were given drinking water that was supplemented with probiotic 1 and 2 (*Bacillus subtilis* and *Lactobacillus casei).* The number of chicks in this group is fifteen (15). They were fed with a conventional feed, broiler mash.

Daily ration

Each group of chicks was administered a daily ration of 1.5kg/ton of commercial broiler feed for chicks.

Immunization

All the birds in Group 1-4 were vaccinated with Baby chick Ranikhet disease vaccine (BCRDV) through intraocular (i/o) route at day 3 and boosted at day 17 via intraocular route as recommended by the vaccine manufacturer. In addition to BCRDV, the experimental birds were vaccinated with Gamboro vaccine at day 10, followed by a booster dose at day 17through their drinking water. Also administered to the chicks is Newcastle disease vaccine on the 14^{th} day followed by a booster dose at 25^{th} day. The birds were reared in a hygienic environment throughout the period of experiment and carefully observed for unusual signs or behaviour.

Shed design

The shed which is a poultry house for rearing the birds was divided into 4 pens (2.75 x1.4m). Each pen housed a total of 15 birds. The shed was an air tight enclosure, with a regulated heat source. The floor of the pen was filled with wood shavings of about 2cm height. The birds were reared under good hygiene conditions careful observation for unusual sighs or behaviour.

Source of probiotics

Probiotic microorganisms selected for this study are *Bacillus subtilis*(4.5×10^5CFU/g) and *Lactobacillus casei*(4.7×10^3CFU/g). *Lactobacillus casei* was isolated from broilers meat, while *Bacillus subtilis* was from tap water.

Administration of probiotics

Probiotics was administered through the birds' drinkers. One hundred milliliters (100 ml) of water was consumed each day by 15 birds in each group. The number of cells in each 100 ml of water is 4.7×10^3 CFU/ml. Each chicken consumed 6.667 ml of water containing 3.133×10^2CFU/ml probiotic cells.

Growth performance traits

All birds were weighed individually after transporting them from the hatchery to the experimental farm to obtain their initial weight. Daily weight gain for each dietary treatment was calculated. Feed consumption was recorded in the course of the whole experiment for each treatment, and the feed conversion rates were calculated subsequently (Devi *et al.*,2019; Abdel *et al*. 2022; Tsega *et al*., 2024).

Serial dilution of fecal samples

Fresh excreta were collected from the birds in Group 1-4, at one week interval. Each excreta sample was suspended in buffered 1% peptone water (1:9 w/v). Peptone water served as an enrichment for the test microorganisms. Then serial dilutions of the fecal samples were prepared for further microbiological analysis.

Serial dilution of carcasses

Fresh ileac and cecal samples (0.5 g) were diluted with 9.5 mL of sterilized distilled water and vortexed until a pH of 6.0 was obtained. One gram (1 g) of wet sample was diluted with 10 mL of distilled water of which 1 mL was transferred into 9 mL of sterilized distilled water. Subsequent transfers were made until dilution 10^{-7} was reached.

Microbiological analysis of carcasses

Determination of total viable aerobic counts

Appropriate dilutions of the homogenate samples were made and aerobic plate counts were determined using the pour plate method. One milliliter (1 ml) of the sample was inoculated onto plate-count agar (Unipath Ltd., Basingstoke, UK) in duplicate. The plates were incubated at 37ºC for 24 hours. Representative colonies that appeared in the culture plates were counted and the results expressed as colony forming units (Roy *et al.*, 2017).

Enumeration of *Escherichia coli* count

Homogenate samples serially diluted, inoculated into lactose broth, and incubated at 30 ºC for 24 hours was spread-plated onto Eosin Methylene Blue (EMB) agar plates in duplicate (Oxoid CM 69). The inoculated plates were incubated at 37 ºC for 24 hours. *Escherichia coli* colonies on the culture plates were counted. Appropriate dilutions were directly spread-plated onto EMB agar plates in duplicate and incubated at 37 ºC for 24 hours for the stored samples (Tsega *et al*., 2024)..

Enumeration of *Lactobacilli* sp.

A liquid media repair method was employed for enumeration of sub-lethally injured lactobacilli (Speck, 1984). Appropriate dilutions of the homogenate samples were inoculated into tubes of lactose broth. The tubes were incubated at 30ºC for 24hours to allow metabolic recovery of lactobacilli. Inoculums from the tubes were then spread-plated onto plates of MRS agar (Merck Ltd.) in duplicates and incubated at 37ºC for 72hours. For the stored samples, appropriate dilutions were directly spread-plated onto MRS agar plates in duplicate and incubated at 37 ºC for 72 hours (Tsega *et al*., 2024).

Enumeration of *Salmonella* sp.

A liquid media repair was adopted for stressed *Salmonella* spp. Appropriate dilution of the homogenate samples were inoculated into tubes of tetrathionate broth (culture A), incubated at 42-43ºC for 24hours and tubes of selenite broth (culture B**),** incubated at 37ºC for 24hours. After 24hours incubation in both tetrathionate broth (culture A) and selenite broth (culture B), samples (0.1ml) of each culture were then spread-plated onto Wilson-Blair medium and *Salmonella Shigella* agar, respectively. In order to isolate colonies of *Salmonella* sp., the inoculated plates were incubated at 37ºC for 48 hours (Hafez, 2001).

Enumeration of *Bacillus* sp.

Appropriate dilutions were heat-shocked in water bath at 80ºC for 10minutes, then 0.1ml of the dilutions were spread-plated onto Mossel agar (*Bacillus cereus* selective agar) supplemented with polymyxin-egg yolk-mannitol-bromothymol blue agar (PEMBA) (Unipath Ltd.). The plates in duplicate were incubated at 37ºC for 24hours.

Identification of bacterial isolates

The morphological tests performed on the bacterial isolates include Gram-staining and motility test, while the biochemical characteristics of the isolates involved the following tests: indole, citrate utilization, catalase, methyl red, Voges Proskauer, and triple sugar iron agar test (Shoaib *et al*., 2020).

Physicochemical analysis of carcasses

pH

The pH of the carcasses (breast muscle) was determined using a digital pH meter. The analysis was carried out after 24 hours of slaughtering (ultimate pH). The carcass was stored inside a refrigerator (4±2 ºC). The analysis was repeated at two (2) days interval

Scientia Africana, Vol. 23 (No. 5), December, 2024. Pp 213-230
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

until signs of spoilage were observed (Ire *et al*., 2020).

Proximate analysis

The percentage of crude protein, fiber, moisture and ash content of broilers feed and carcasses were determined using standard methods (AOAC, 2016).

Antibiotic susceptibility test

Standardization of the probiotics using 1.0 McFarland standard

A sterile test tube was weighed (W_1) in order to prepare the bacterial suspension. Then, a loopful of an eight (8) hour culture was taken with a sterile loop and placed inside the test tube. The test tube containing the culture was weighed (W_2). The weight of the bacterial suspension (W_3) was calculated by subtracting W_1 from W_2. To obtain a bacterial suspension of 1mg/ml, sterile distilled water equal to 1000 ml × bacterial weight was prepared. The suspension was shaken for 1 minute to obtain a homogenous suspension. To prepare the McFarland 1.0 standard, 1% barium chloride was added to 1% H_2SO_4 which resulted in a fine precipitate, barium sulfate. The McFarland standard was prepared in different concentrations ranging from 0.50-10.0 using ten different test tubes shaken very well. The turbidity of a McFarland standard was visually comparable to a bacterial suspension of known concentration as indicated in a standard McFarland 1.0 table which corresponds to approximately $3.0x10^8$cells/ml(Roy *et al.*, 2017; Gayathiri et al., 2018).

Antibacterial activity of isolates(*invitro* challenge test)

Agar well diffusion method was used to determine the antibacterial activity of bacterial isolates from the carcasses using the method described by Roy *et al.* (2017) with a slight modification. Exactly 2 ml of an 8 hour old nutrient broth culture of potentially pathogenic bacterial culture was spread on sterile nutrient agar plates. A sterile cork-borer was used to punch a well in the nutrient agar plates that is 3 mm in diameter. Exactly 0.1ml of an 18 hour culture of the potential probiotics isolates in a nutrient broth was pipetted into the wells. The inoculated plates were incubated for 24hours at 37^{o}C. The control involved using nutrient broth in the well in place of probiotics. The presence of antibacterial activity was noted as clear zones around the wells which was measured using a meter ruler.

Standardization of candidate probiotics

The probiotics were standardized as previously described. 0.5 McFarland standard which corresponds to approximately $1.5x10^8$cells/ml bacterial suspension was used to standardize the bacterial population in the broth culture by comparing the turbidity of the probiotics with McFarland standard. The isolates in the broth were also plated out in a nutrient agar to confirm the bacterial population (Amosun *et al.*, 2019).

Identification of fungal isolates

Test for fungi isolates include staining with lactophenol cotton blue as previously described by Ahaotu *et al*. (2022). A portion of the fungal mycelium was teased out in a drop of lactophenol cotton blue on a grease-free microscope slide and examined microscopically. The cultural and morphological characteristics of the fungus were observed and compared with earlier descriptions (Barnett and Hunter, 1972).

RESULTS

The results presented in Table 1 represents the various sources of microorganisms associated with broilers. They include poultry feed, broiler's feaces, and carcasses. *Salmonella* spp. is common among the three sources of microorganisms.

Table 1: Potential pathogens isolated from the broilers.

Source of microorganism	**Microorganisms isolated**
Poultry feed	*Pseudomonas* spp., *Escherichia coli, Salmonella* spp. *and Bacillus* spp. *Aspergillus* spp., *Fusarium* spp., *Penicillium* spp., and *Rhizopus* spp.
Broiler's feaces	*Vibrio cholerae, Enterobacter* sp., *E. coli, Staphylococcus aureus, Lactobacillus casei, Bacillus subtilis,* and *Salmonella* spp.
Broiler's carcasses	*Salmonella* sp.,*Lactobacillus casei,* and *Bacillus subtilis*

Figure 1 shows the *Salmonella* count in the feaces of broilers fed with potential probiotic microorganisms incorporated into commercial feed. The feaces of all the broilers that consumed the commercial feed supplemented with potential probiotic microorganisms and the control had an average *Salmonella* count of 1.5 x 10^5CFU/ml at week 1. The broilers that consumed the commercial feed without the probiotic added to it had the lowest *Salmonella* count of 2.2 x 10^5, 2.0 x 10^5, 1.9 x 10^5, 1.8 x 10^5CFU/ml at week 3, 4, 5, and 6, respectively. On the contrary, the highest *Salmonella* count (4.5 x 10^5CFU/ml) in the feaces of the broilers was reported at week 2. The broiler's feaces that had the highest *Salmonella* count (6.9 x 10^5CFU/ml) at week 7 consumed a commercial feed supplemented with *Lactobacillus casei* and *Bacillus subtilis*. The highest *Salmonella* count in the feaces was also obtained from the broilers that consumed a commercial feed supplemented with *Lactobacillus casei* at week 4, 5, and 6 reported as 3.2 x 10^5,3 x 10^5, and 3.2 x 10^5CFU/ml, respectively.

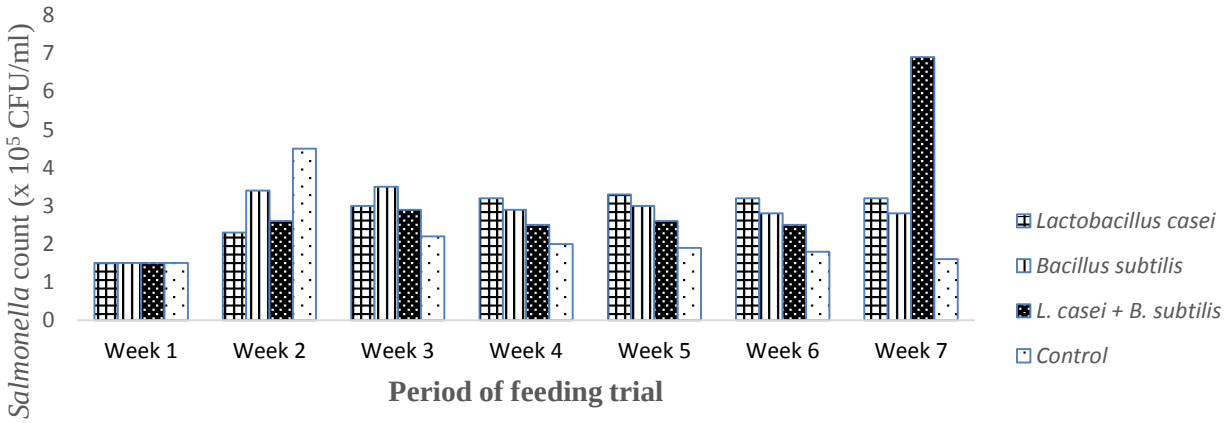

Figure 1: *Salmonella* count in the feaces of broilers that consumed feed supplemented with potential probiotic microorganisms

In Figure 2 is the pH of broiler breast muscles monitored at 2 days interval until visible signs of spoilage was observed. The broilers that consumed the commercial feed without probiotic microorganisms added to it maintained the highest pH during the feeding trial that range from 4.8-6.1, while the broilers that consumed the feed supplemented with probiotic microorganisms had a lower pH (4.6-5.9).

Scientia Africana, Vol. 23 (No. 5), December, 2024. Pp 213-230
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

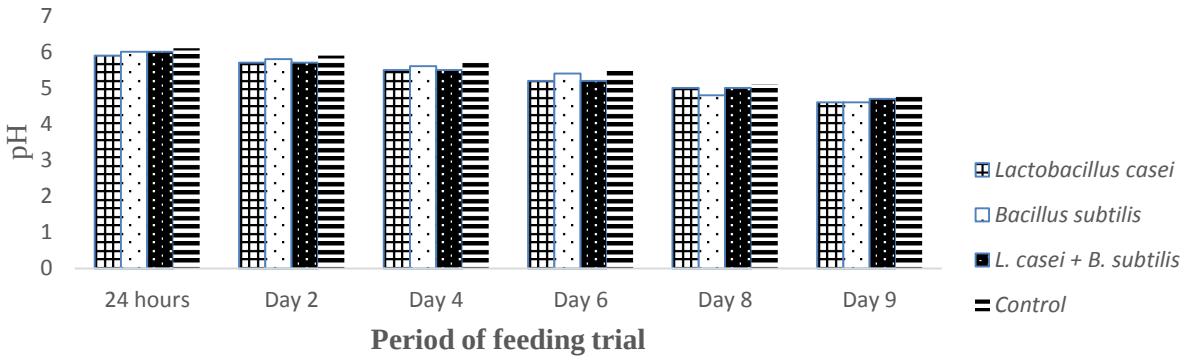

Figure 2: pH of broiler's breast muscles stored until spoilage set in

Figure 3 shows the average feed intake of the broilers divided into four groups that consumed feed incorporated with potential probiotic microorganisms and the control. During the trial (week 2 - 7), the broilers that consumed feed without probiotic microorganisms added to it maintained the highest feed intake that ranged from 44.6-401 g, while the broilers that consumed feed supplemented with probiotic microorganisms had values from42-378 g.

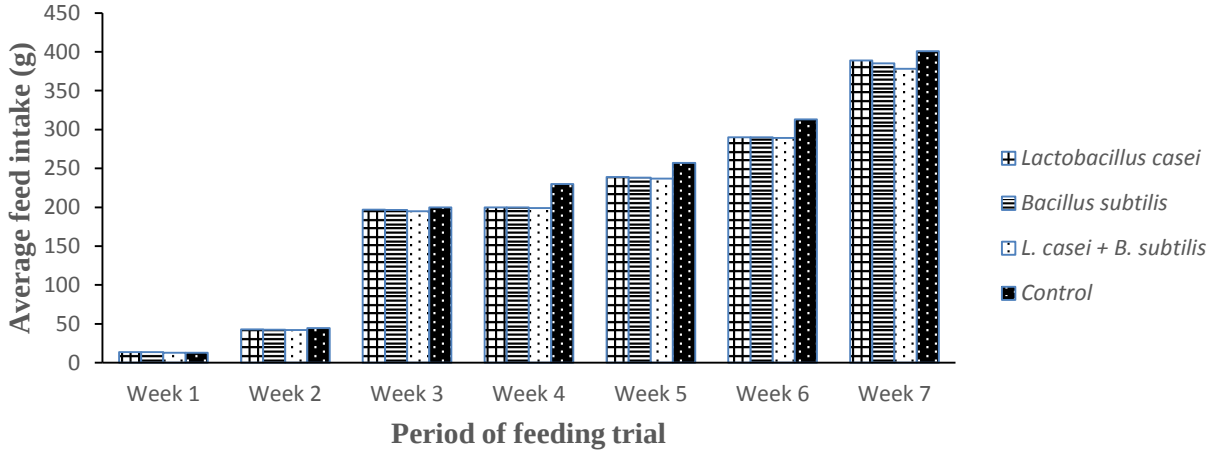

Figure 3: Average feed intake of the broilers during the period of feeding trial.

The weekly weight gain of the broilers divided into four groups is presented in Figure 3. The result shows that broilers that consumed commercial feed without the probiotic added to it had the highest feed intake (44.6-401 g) compared with broilers in other groups, except at week 1. On the contrary, broilers that consumed feed incorporated with *Lactobacillus casei* + *Bacillus subtilis* had the lowest feed intake (12.8-401 g), compared with broilers in other groups.

Analysis result shows that the proximate value of moisture, protein, ash, and fat content of the broiler feed (vital feed) is 71.0%, 21.0%, 2.30% and 5.10%, respectively. Figure 5 shows the percentage mortality of broilers fed with commercial feed supplemented with potential probiotic microorganisms. The result shows that the percentage mortality of broilers that consumed feed supplemented with *Lactobacillus casei*, *Bacillus subtilis*, *L. casei* + *B. bacillus*, and the control is 0%, 6.67%, 20%, and 40%, respectively.

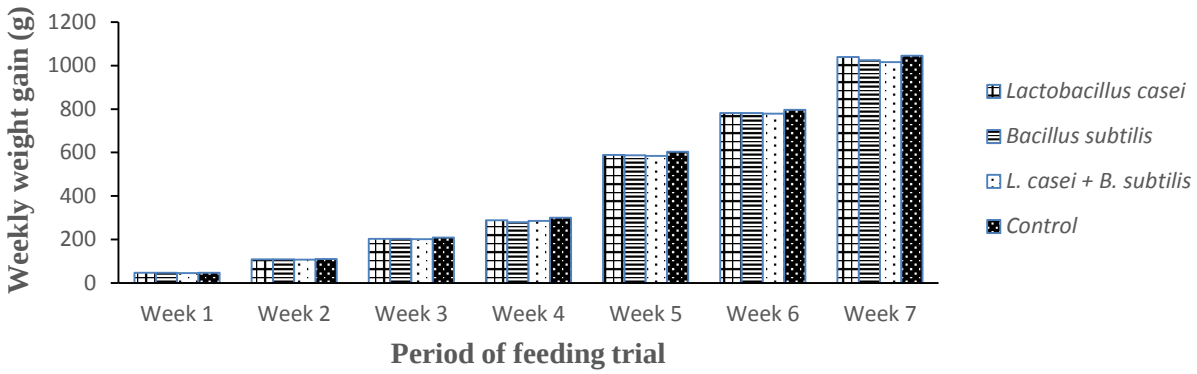

Figure 4: Weekly weight gain of broilers during the period of feeding trial.

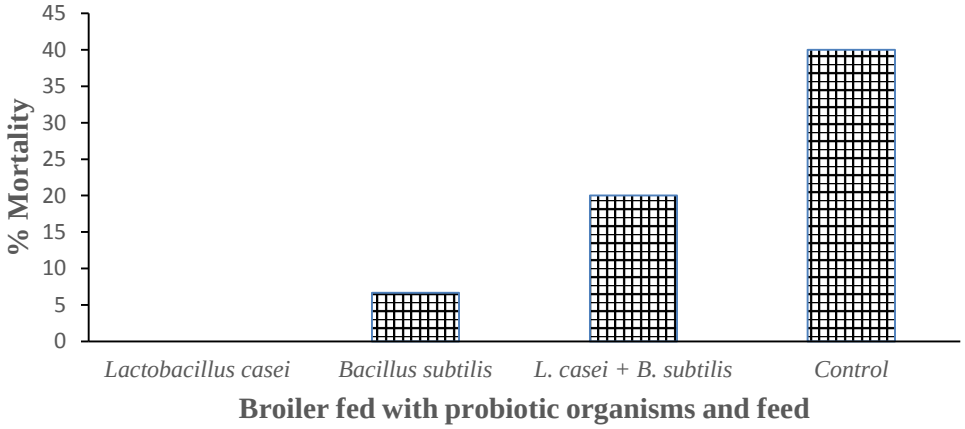

Figure 5: Percentage mortality of broilers fed with commercial feed supplemented with potential probiotic microorganisms

Table 2 shows the percentage occurrence of *Salmonella* sp., *Lactobacillus* sp., and *Bacillus* sp. isolated from broilers' carcasses of broiler's carcasses that consumed fed supplemented with potential probiotic microorganisms and the control. The broilers that consumed feed without probiotic microorganisms (i.e. the control) had the highest percentage of occurrence of *Salmonella* sp. in their ileum (23%) and cecum (24%), compared with broilers in other groups. On the contrary, the control also had the lowest percentage occurrence of *Lactobacillus* sp. (12%)in both the ileum and cecum. The broilers that consumed feed supplemented with *Lactobacillus casei* had the highest percentage occurrence of *Lactobacillus* sp. The broilers that consumed feed supplemented with *Bacillus subtilis*had the highest percentage of occurrence of *Bacillus*sp in the ileum (30%) and cecum (31%), whereas the least percentage of occurrence in the ileum (14%) and cecum (16 %) involved broilers that consumed feed supplemented with *Lactobacillus casei*.

Scientia Africana, Vol. 23 (No. 5), December, 2024. Pp 213-230
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

Table 2. Percentage occurrenceof *Salmonella* sp., *Lactobacillus* sp., and *Bacillus* sp.isolated from broilers' carcasses

Samples	Groups	% Occurrence of *Salmonella* sp.	% Occurrence of *Lactobacillus* sp.	% Occurrence of *Bacillus* sp.
Ileum	*Lactobacillus casei*	12	31	14
	Bacillus subtilis	13	16	30
	L. casei + B. subtilis	14	27	20
	Control	23	12	15
Cecum	*L. casei*	11	34	16
	B. subtilis	12	15	31
	L. casei +B. subtilis	10	25	20
	Control	24	12	17

Figure 6 shows the proximate composition of broilers' carcasses 24 hours after slaughter. The control had the highest moisture content (74.76%) and lowest protein content (19.21%) compared with the carcasses of broilers that consumed commercial feed supplemented with potential probiotic microorganisms. The moisture and protein content of carcasses of broilers that consumed feed supplemented with probiotic microorganisms is within the range of 69.425-72.95% and 20.01-22.96%, respectively. The lowest proximate values that involved ash content of broilers' carcasses in the four groups is within the range of 1.069-1.095%.

The proximate composition of broilers' carcasses stored for twenty one (21) days after slaughter is depicted in Figure 7. The group of broilers that had the highest moisture (75.81%) and lowest protein (19.20%) content carcasses is the control. The moisture and protein content of carcasses of broilers that consumed feed supplemented with probiotic microorganisms is within the range of 69.50-72.87% and 20.21-22.94%, respectively. The ash content of the broilers' carcasses is reported as the lowest proximate values in all the groups. It is within the range of 1.01-1.069%.

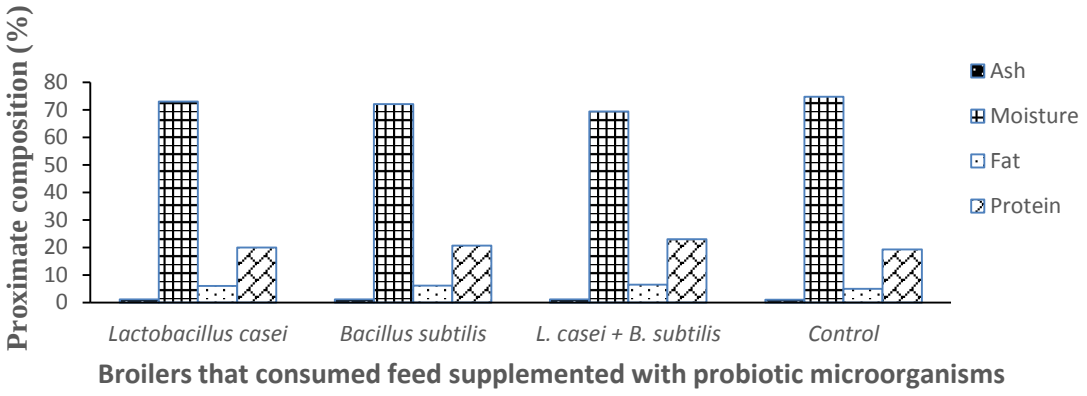

Figure 6: Proximate composition of broiler's carcasses 24 hours after slaughter.

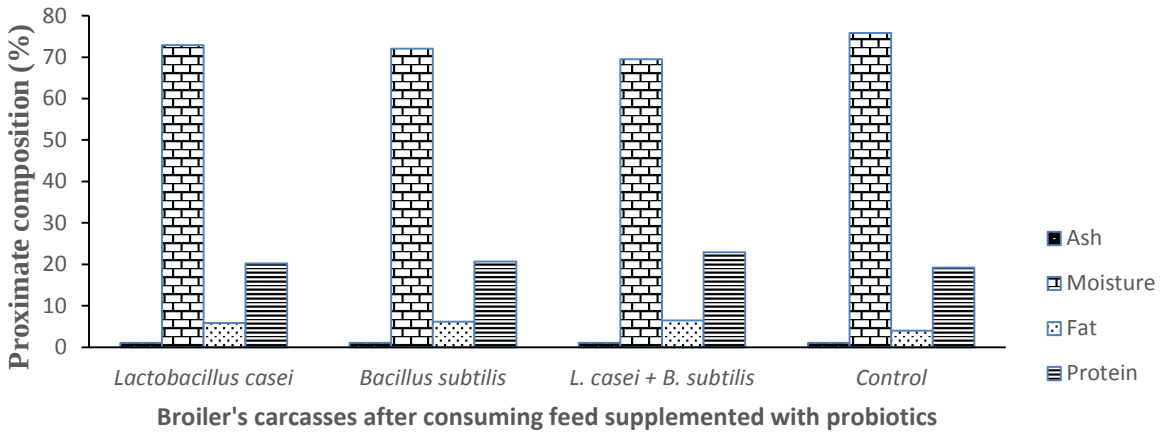

Figure 7: Proximate composition of broiler's carcasses after 21 days of storage.

Figure 8 depicts the percentage occurrence of bacteria isolated from the commercial feed which include *Bacillus* spp. (33%), *Escherichia coli* (26%), *Pseudomonas* spp. (26%), and *Salmonella* spp. (15%), while the fungi isolates involved (Fig. 9) are *Penicillium* spp. (26%), *Fusarium* spp. (26%), *Rhizopus* spp. (25%), and *Aspergillus* spp. (23%).

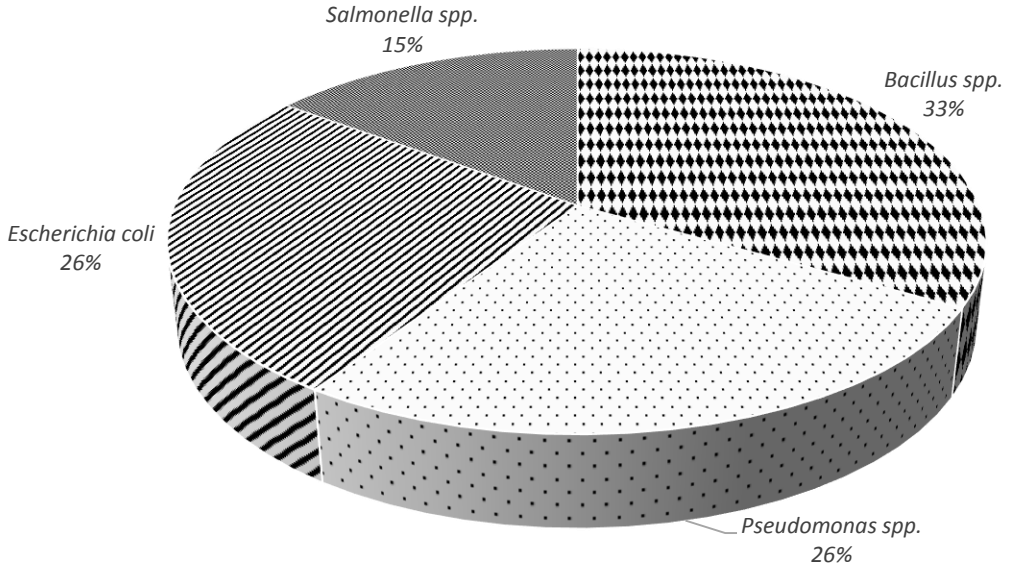

Figure 8: Percentage occurrence of bacterial isolates in the commercial feed.

Scientia Africana, Vol. 23 (No. 5), December, 2024. Pp 213-230
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

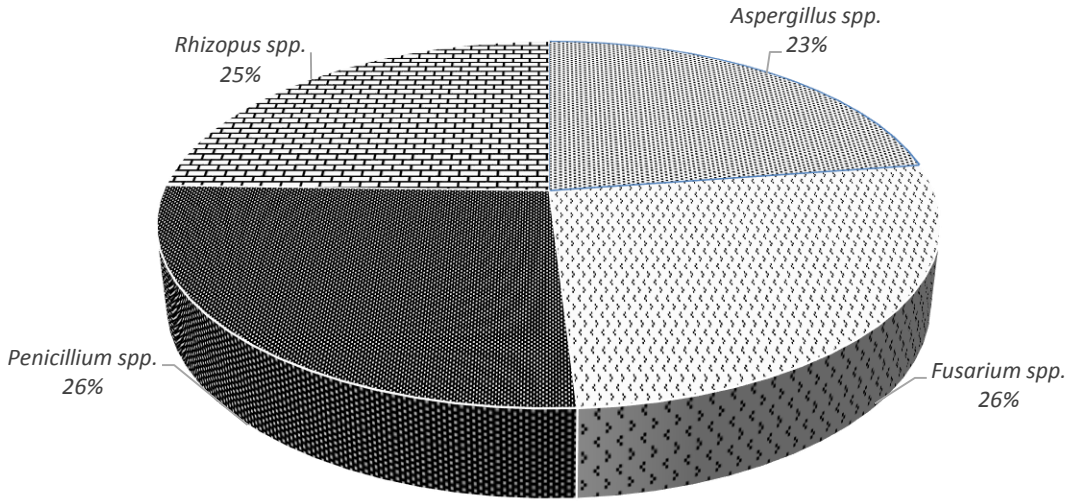

Figure 9: Percentage occurrence of fungal isolates in the commercial feed.

Figure 10 shows the *invitro* antimicrobial activity of potential probiotic microorganisms against the isolated pathogens. The result clearly shows that a combination of two potential probiotic microorganisms, *Lactobacillus casei + Bacillus subtilis* had the highest zone of inhibition against *Escherichia coli* (13 mm), *Staphylococcus aureus* (15 mm), *Enterobacter* sp. (12 mm), *Vibrio cholerae* (11 mm), and *Salmonella* sp. (12 mm). However, *L. casei* demonstrated the lowest zone of inhibition (4-8 mm) against the isolated pathogens, with the exception of *Staphylococcus* sp.

The antibiotic sensitivity profile of the isolated pathogens is presented in Figure 11. All the isolates exhibited multiple resistance to at least three antibiotics. Notably, all the isolated pathogens were resistant to Augmentin. Two antibiotics which include Septrin and gentamicin demonstrated varying levels of zone of inhibition against all the isolated pathogens. A total of 7 out of 8 of the antibiotics demonstrated varying levels of zone of inhibition against *Enterobacter* sp.

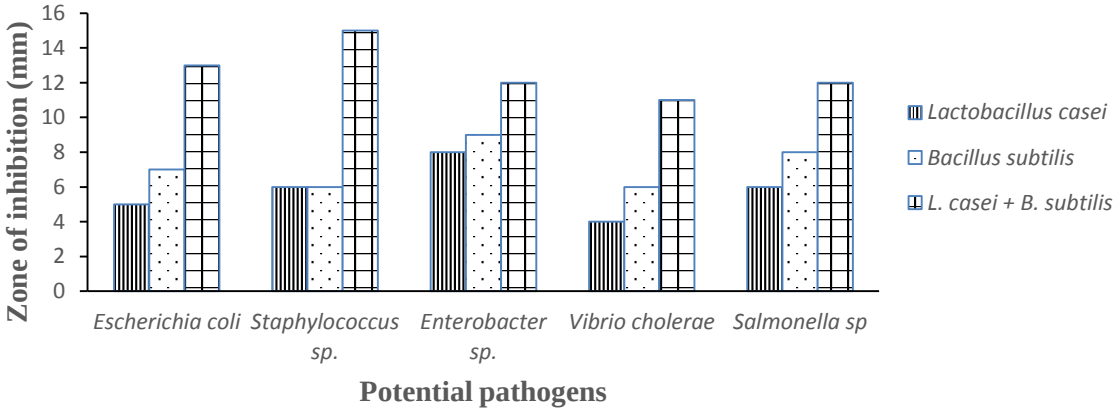

Figure 10: Potential pathogens isolated from carcasses of broilers

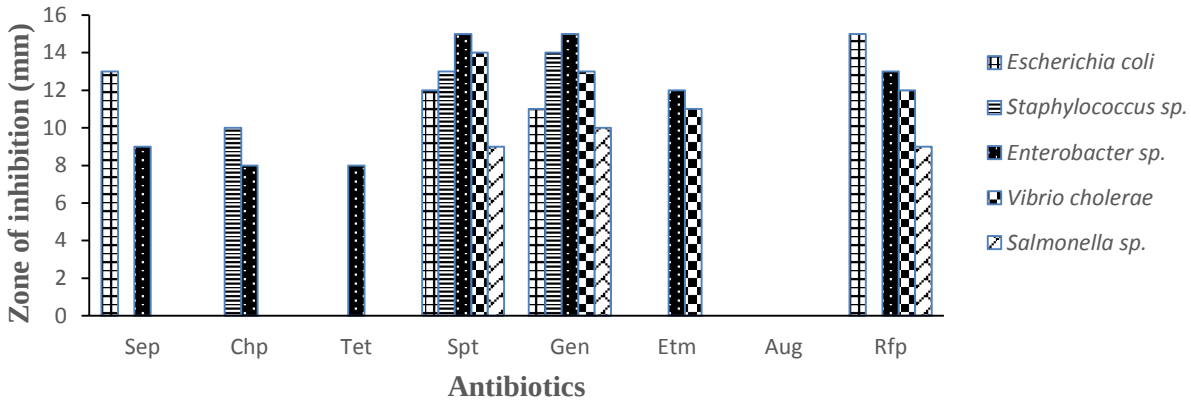

Figure 11: Antibiotic sensitivity profiles of potential pathogens isolated from feed, feaces, and carcasses.

Key: Sep-Streptomycin; Spt-Spectinomycin; Rfp-Rifampine; Tet-Tetracycline; Gen-Gentamicin; Chp-Chloramphenicol; Aug-Augmentin; Etm-Erythromycin.

DISCUSSION

This study revealed that *Salmonella* spp. is commonest microorganism associated with poultry. Boonprasert *et al.* (2014) reported a similar result from a related study. According to Mgbeahuruike *et al.* (2023), the consumption of poultry feed contaminated with pathogenic microorganisms could have negative effects in terms of weight gain, feed intake, feed conversion ratio, and functions of the organ. The presence of pathogens in the broiler's carcasses could be transferred to humans if the meat is poorly cooked and consumed. The *Salmonella* count of broilers that consumed feed supplemented with potential probiotic microorganisms and the control increased during the period of the feeding trial. The highest *Salmonella* count (6.9×10^5CFU/ml) was reported in week 7 in the broilers that consumed a combination of *Lactobacillus casei* and *Bacillus subtilis*. On average, the broilers that consumed feed without probiotic microorganisms i.e. the control had a lower *Salmonella* count during the trial which ranged from 1.45-4.5×10^5CFU/ml, compared with the broilers fed with probiotic bacteria. This result is an indication that the probiotic microorganisms demonstrated a level of *in vivo* antimicrobial activity against *Salmonella* sp. This result is partly in agreement with the findings from a related study by Dina *et al*. (2016).

Broilers that consumed feed supplemented with probiotic microorganisms and the control experienced a slight decrease in the pH of their breast muscles. Notably, the pH values of the broilers breast muscles that consumed probiotic diets were lower than the control. This result suggests that the potential probiotic microorganisms consumed by the broilers had a significant effect in decreasing the meat oxidative stability, compared with the control. The report from a related study by Haščik *et al.* (2015) is in agreement with our research findings.

The percentage occurrence of bacteria encountered in the broiler's feed commercially available in the market include *Bacillus* spp. (33%), *Escherichia coli* (26%), *Pseudomonas* spp. (26%), and *Salmonella* spp. (15%), while the fungi isolates involved are *Penicillium* spp. (26%), *Fusarium* spp. (26%), *Rhizopus* spp. (25%), and *Aspergillus* spp. (23%). A similar result was reported by Alabi *et al.* (2018) from a related study which involved selected brands of commercially available proprietary broiler feeds. Poultry farmers who purchase broilers feed contaminated with pathogenic microorganisms are endangering the health of

Scientia Africana, Vol. 23 (No. 5), December, 2024. Pp 213-230
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

their birds. They are likely to experience lower profitability from the poultry business. The proximate composition of vital brand of broiler's feed shows that the moisture, protein, ash, and fat content is 71.00%, 21.00%, 2.30%, ad 5.10%, respectively.

During the period of the feeding trial, there was an increase in the average feed intake of broilers in all the groups. The broilers that consumed a commercial feed which was not supplemented with probiotic microorganisms maintained the highest feed intake compared with the broilers in other groups that received probiotic microorganisms, except at week 1. This result is in agreement with the findings by Amerah *et al*. (2013) in a related study. The microorganisms incorporated into the feed as probiotics could be responsible for lower feed intake of the broilers. The total feed intake was approximately 7.8% lower for broilers fed probiotics than broilers that consumed basal diet without probiotics. The average daily feed intake was 401g for the control group and 389g for the experimental group. The findings by Anjum *et al.* (2005) correlate with the result obtained in this study as it relates to the feed intake and weight gain of broilers administered with probiotic microorganisms. The researchers reported that feed supplemented with *Lactobacillus* spp. produced a modest improvement in the weight gain and feed intake. A significant improvement in the feed conversion rate by 5% during 0-6 week was reported.

All the broilers experienced an increase in weekly weight gain during the feeding experiment (Fig. 4). The broilers that consumed a commercial feed without supplementing it with a probiotic maintained a higher weekly weight gain compared with the broilers that consumed feed supplemented with probiotic bacteria, except in week 1. Some researchers have reported that the addition of probiotic microorganisms to poultry feed increased the weight gain of the birds (Nam *et al*., 2022). According to Sari and Akbar (2019), the dosage of the probiotic product which is a function of the probiotic microorganism population could influence the weight gain of the poultry animals.

It is worthy to note that the broilers that consumed commercial feed without probiotic microorganisms had the highest mortality rate (40%). The broilers fed with probiotic microorganisms incorporated into the feed had lower mortality rate. Interestingly, the broilers that consumed feed supplemented with *Lactobacillus casei* had 0% mortality rate. The result from a related study carried out by Alam and Ferdaushi (2018) is in agreement with our findings. Continuous feeding of the poultry birds with probiotic microorganisms which probably suppressed the growth and multiplication of potentially pathogenic microorganisms in the birds reduced its mortality. Therefore, the broilers experienced an improved health status (build-up resistance), improved growth, and overall performance. Fuller (2001), Patterson and Burkholder (2003) explained the mechanisms used by probiotics to inhibit pathogens. They include inhibition by competition for nutrients, production of toxic condition and compounds (volatile fatty acids, low pH, and bacteriocins), competition for binding sites on the intestinal epithelium and stimulation of the immune system.

The percentage occurrence of *Salmonella* sp. in the carcasses (ileum and cecum) of broilers that consumed feed supplemented with probiotic microorganisms is lower than the control. This observation is in agreement with the research findings from a related study by Torturo *et al.* (1973), and Dilworth and Day (1978). The researchers reported that the presence of direct fed microbes in the intestines of broilers could help control the spread of *Salmonella dysentrea*. Kyungwoo *et al*. (2010) reported that *Bacillus subtilis* exhibited *in vitro* inhibitory effect against *Escherichia coli*. The percentage occurrence of *Lactobacillus casei* in the cecum and ileum of the control is lower than the broilers that consumed feed supplemented with probiotic microorganisms. This result could be as a result of absence of *L. casei* in the diet

consumed by the control. Meanwhile, the broilers that consumed feed supplemented with *L. casei* had the lowest percentage of occurrence of *Bacillus* spp. in both the ileum and cecum. This result suggests that *L. casei* incorporated into the broiler's feed as a potential probiotic bacterium had a greater antimicrobial effect against *Bacillus* sp. (a potential pathogen), compared with other probiotic microorganisms.

The proximate composition of the broiler's carcasses 24 hours after the poultry animals were harvested, and the values reported after the carcasses was stored inside a freezer for 21 days had minimal variations. The carcasses were rich in moisture and protein content, but contain a low quantity of ash. This result is substantially in agreement with the findings by Begdildayeva *et al*. (2024) from a related study. Notably, the moisture content of the broiler's carcasses that consumed commercial feed supplemented with probiotic microorganisms were lower than the control. This result is an indication that feeding the broilers with a ration supplemented with probiotic microorganisms could improve the shelf life of the broiler carcasses by reducing its moisture content.

According to the Clinical and Laboratory Standards Institute (CLSI), the zone of inhibition 0-12mm, 13-17 mm, and ≥17mm of bacterial isolates is interpreted as resistant, intermediate, and sensitive, respectively (Elenwo *et al*., 2019). Based on the CLSI guideline, *Escherichia coli, Staphylococcus* sp., *Enterobacter* sp. *Vibrio cholerae*, and *Salmonella* sp. isolated from the feces of the broilers were resistant to Augmentin. This result is substantially in agreement with a related study by Amosun *et al*. (2019). Meanwhile, all the bacterial isolates demonstrated intermediate sensitivity to spectinomycin and gentamicin, with the exception of *Salmonella* sp. However, *Vibrio cholerae*, *Salmonella* sp., and *Staphylococcus* sp. were resistant to rifampine. A similar result was reported by Roy *et al*. (2017) from a related study. All the pathogens isolated from the feaces of the broilers were resistant to the potential probiotic microorganisms incorporated into the commercial feed, with the exception of *Lactobacillus casei + Bacillus* sp. which demonstrated intermediate sensitivity against *Escherichia coli* and *Staphylococcus* sp. The synergistic effect of *Lactobacillus casei + Bacillus* sp. could be responsible for demonstrating a better antimicrobial activity against the selected pathogens compared with the result obtained when a monoculture of the probiotic bacterium was used. This is in agreement with the research finding by Mirsalami and Mirsalami (2024) from a related study.

CONCLUSION

During the feeding trial that involved supplementing broiler feed with probiotic microorganisms, increase in the feed intake and weight gain of the birds occurred throughout the period, despite the fact that the commercial feed was contaminated with pathogens. The potential probiotic microorganisms incorporated into the feed contributed in reducing the *Salmonella* population in the broilers. The percentage mortality of the birds that fed on the probiotic microorganisms drastically reduced. In terms of protein and moisture content, the meat quality of the broilers that consumed feed supplemented with probiotic bacteria is better than the control that consumed 100% commercial feed. The probiotic microorganisms demonstrated varying levels of antimicrobial activity against the pathogens isolated from the broiler's feaces. All the pathogens were resistant to the conventional antibiotics tested with few exceptions. Therefore, the use of probiotics as animal growth promoters in rearing broilers instead of antibiotics is advisable.

Conflict of Interests

The authors declare that no competing interests exist.

Scientia Africana, Vol. 23 (No. 5), December, 2024. Pp 213-230
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

REFERENCES

Abbas, W., Ruichen, B., Hussain, M. D., Tajdar, A., Guo, F., Guo, Y. and Wang, Z. (2024). Antibiotic cocktail effects on intestinal microbial community, barrier function, and immune function in early broiler chickens. Antibiotics, 13, 413. https://doi.org/10.3390/antibiotics13050413

Abdel, M. Y. A., Eissa, N. M., Abouelezz, K. F. M. and Younis, M. (2022). Effect of dietary supplementation of probiotics, enzymes and their combination on growth performance, meat yield, intestinal microbiota and plasma analysis of broiler chicks. Archives of Agriculture Sciences Journal, 5(2):136-152. DOI: https://dx.doi.org/aasj.2022.144587.1121

Ahaotu, I., Ichendu, M. O. and Maduka, N. (2022). Microbiological, nutritional and sensory evaluation of snack bars developed using Bambara groundnut (*Vigna subterranean* L.) and maize (*Zea mays*). African Journal of Microbiology Research, 16(1): 8-23. DOI: 10.5897/AJMR2021.9583

Alabi, J. O., Fafiolu, A. O., Akande, F. A., Oluwatosin, O. O., Sogunle, O. M., Dada, I. D., Adeleye, O. O. and Olagoke, K. O. (2018). Assessment of microbial profile of selected proprietary broiler chicken feeds sold in Abeokuta, South-West, Nigeria. Nigerian Journal of Animal Science, 20(1): 183-190.

Alam, M. J. and Ferdaushi, Z. (2018). Use of probiotics instead of antibiotics in broiler production. Progressive Agriculture 29(4): 359-370.

Aliyi, M.B. (2023). Review on application of probiotics in poultry farm. International Journal of Probiotics and Dietetics, 3(2): 58-65.

Amerah, A. M., Quiles, A., Medel, P., Sánchez, J., Lehtinen, M. J., Gracia, M. I. (2013). Effect of pelleting temperature and probiotic supplementation on growth performance and immune function of broilers fed maize/soy-based diets. Animal Feed Science and Technology, 180: 55-63. http://dx.doi.org/10.1016/j.anifeedsci.2013.01.002

Amosun, E. A., Adepoju, B. C., Ogunbadewa, A. J. and Abatan, M. O. (2019). Antibiotic profiling of bacterial isolates obtained from turkey and chicken in selected farms in Ibadan, Nigeria. African Journal of Biomedical Research, 22: 281-286.

AOAC (2016). Official methods of analysis of AOAC International, 20th edition (AOAC: Gaithersburg).

Begdildayeva, N., Karahan, A. G., Kondybayev, A., Kudaibergenova, A., Nurgazina, A., Akhmetsadykov, N., Ospanova, A. and Akhmetsadykova, S. (2024). Probiotics effects on the growth performance and meat quality of broiler chickens. Animal Production Science, 64: 1-10. Doi: 10.1071/AN23424

Barnett, E. O. I. and Hunter, B. B. (1972). Illustrated General of Imperfect Fungi. 3rd edition, Mmessota Burgles Publishing Company, Minneapolis, 241-252p.

Boonprasert, N., Nuanualsuwan, S., Pulsrikarn, C., Pornaem, S. and Chokesajjawatee, N. (2014). Sources and disseminations of Salmonella spp. in an integrated broiler meat production. Thailand Journal of Veterinary Medicine, 44(1): 117-124.

Çapan, B. and Bağdatli, A. (2022). Usage of probiotics in the poultry industry and effects on meat quality. Selcuk Journal of Agriculture and Food Sciences, 36(1): 120-126. DOI: 10.15316/SJAFS.2022.017

Devi, N. D., Nizamuddin and Vidyarthi, V. K. (2019). Effect of dietary supplementation of probiotic on the performance of broiler chicken. *Livestock Research International* 7(02):62-67.

Dhama, K., Verma, V., Sawant, P. M., Tiwari, R., Vaid, R. K. and Chauhan, R. S. (2011). Applications of probiotics in poultry: enhancing immunity and beneficial effects on production performances and health-a review. Journal of Immunology and Immunopathology, 13(1): 1-19.

Dina, M. W., El-hamd, S., Hams, M. A. (2016). Effect of probiotic on *Salmonella enteritidis* infection on broiler chickens. Egyptian Journal of Chemistry and Environmental Health, 2(2): 298-314

Dong, S., Li, L., Hao, F., Fang Z., Zhong, R., Wu, J. and Fang, X. (2024). Improving quality of poultry and its meat products with probiotics, prebiotics, and phytoextracts. Poultry Science 103: 1-18. https://doi.org/10.1016/j.psj.2023.103287

Elenwo, M., Maduka, N. and Odu, N. N. (2019). Antibiogram testing of bacterial isolates from cassava, yam and plantain flours and shelf life studies of the products sold in some markets in Port Harcourt, Nigeria. International Journal of Pathogen Research, 2(4):1-11.

Fuller, R., Jayne-Williams, D. J. (1968). The origin of bacteria recovered from the peritoneum and yolk sac of healthy chickens. British Poultry Science, 9: 159-163.

Gayathiri, E., Bharathi, B., Priya, K. (2018). Study of the enumeration of twelve clinical important bacterial populations at 0.5 McFarland standard. International Journal of Creative Research Thoughts 6(2):880-893.

Gul, S. T. and Alsayeqh, A. F. (2022). Probiotics as an alternative approach to antibiotics for safe poultry meat production. Pakistan Veterinary Journal, 42(3): 285-291. http://dx.doi.org/10.29261/pakvetj/2022.061

Hafez, H. M. (2001). Salmonella infections in poultry: diagnosis and control. Periodicum Biologorum, 103: 103-113.

Halder, N., Sunder, J., De, A. K., Bhattacharya, D. and Joardar, S. N. (2024). Probiotics in poultry: a comprehensive review. The Journal of Basic and Applied Zoology, 85(23): 1-17. https://doi.org/10.1186/s41936-024-00379-5

Haščik, P., Elimam, I. O. E., Bobko, M., Kačániova, M., Ćuboň, J., Tkáčova, J. and Trembecká, L. (2015). The pH value of broiler breast and thigh muscles after addition probiotic, bee pollen and propolis into their feed mixture. Journal of Microbiology, Biotechnology, and Food Sciences, 4(3): 52-54.

Ire, F. S., Eze, O. M. and Maduka, N. (2020). Influence of different wrapping materials on microbiological, physicochemical and sensory properties of condiment product 'ogiri-egusi'. Journal of Life and Bio-Sciences Research, 1(02): 34-43.

Jeni, R. E., Dittoe, D. K. Olson, E. G., Lourenco, J., Corcionivoschi, N., Ricke, S. C. and Callaway, T. R. (2021). Probiotics and potential applications for alternative poultry production systems. Poultry Science 100: 1-14. https://doi.org/10.1016/j.psj.2021.101156

Kyungwoo, L., Hyun, L. S. and Gregory, S. R. (2010). Direct-fed microbials and their impact on the intestinal microflora and immune system of chickens. Journal of Poultry Sciences, 47: 106-114.Doi: 10.2141/jpsa.009096

Lokapirnasari, W. P., Al-Arif, M. A., Hidayatik, N., Safiranisa, A., Arumdani, D. F., Zahirah, A. I., Yulianto, A. B., Lamidi, M., Marun, T. D., Lisnanti, E. F., Baihaq, Z. A., Khairullah, A. R., Kurniawan, S. C., Pelawi, E. B. S. and Hasib, A. (2024). Effect of probiotics and acidifiers on feed intake, egg mass, production and egg yolk chemical composition in late-laying quails. Veterinary World, 17(2): 462-469. Doi: www.doi.org/10.14202/vetworld.2024.462-469

Maduka, N. and Ire, F. S. (2018). Studies on tigernut-ogi incorporated into basal fed as a potential animal growth enhancer using Wistar albino rats as experimental animal. Journal of Advances in Microbiology, 8(2): 1-15.

Mgbeahuruike, A. C., Agina, O. A., Anyanwu, M. U., Emejuo, N. T., Ekere, S. O., Ugwu, P. C., Uju, C. N. and Andong, F. A. (2023). Microbial contamination of poultry feed and the effects on birds' performance. Animal Research International, 20(1): 4834-4861.

Scientia Africana, Vol. 23 (No. 5), December, 2024. Pp 213-230
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

Mirsalami, S. M. and Mirsalami, M. (2024). Effects of duo-strain probiotics on growth, digestion, and gut health in broiler chickens. Veterinary and Animal Science, 24: 1-13. https://doi.org/10.1016/j.vas.2024.100343

Mogotlane, P. M., Ng'ambi, J. W., Nyazema, N. Z. and Chitura, T. (2024). Effect of feed supplementation with probiotics and antimicrobial agents on meat quality of broiler chickens. Agricultural Science Digest, 44(3): 530-535.

Nam, T. V. B., Anh, L. H., Loc, H. T., Trang, C. T. H., Thiet, N., Lan, L. T. T., Diep, T. H., Xuan, N. H. and Ngu, N. T. (2022). Effects of probiotic (*Lactobacillus plantarum* and *Bacillus subtilis* supplementation on mortality, growth performance, and carcass characteristics of native Vietnamese broilers challenged with *Salmonella* typhimurium. Veterinary World, 15(9): 2302-2308. Doi: www.doi.org/10.14202/vetworld.2022.2302-2308

Patterson, J. T. and Burkholder, K. M. (2003). Application of prebiotics and probiotics in poultry production. Poultry Science, 82: 627-631.

Paz, I. C. L. A., Almeida, I. C. L., Vega, L. T. L., Milbradt, E. L., Borges, M. R., Chaves, G. H. C., Ouros, C. C., Silva, M. I. L., Caldara, F. R. and Filho, R. L. A. (2019). Productivity and well-being of broiler chickens supplemented with probiotic. Journal of Applied Poultry and Research, 28: 930-942. http://dx.doi.org/10.3382/japr/pfz054

Poberezhets, J., Chudak, R., Kupchuk, I., Yaropud, V. and Rutkevych, V. (2021). Effect of probiotic supplement on nutrient digestibility and production traits on broiler chicken. Journal of Agricultural Science, 2(32): 296-302. DOI: 10.15159/jas.21.28

Roy, C. R., Ahmed, T. and Uddin, M. A. (2017). Microbiological analysis of poultry feeds along with the demonstration of the antibiotic susceptibility of the isolates and the antibacterial activity of the feeds. Bangladesh Journal of Microbiology, 34(2): 103-107. DOI: 10.3329/bjm.v3412.39620

Sari, R. M. and Akbar, S. A. (2019). The influence of lactic acid bacteria *Lactobacillus casei* for performance of broiler. IOP Conference: Earth and Environmental Science, 347: 1-4.doi: 10.1088/1755-1315/347/1/01209

Shoaib, M., Muzammil, I., Hammad, M., Bhutta, Z. A. and Yaseen, I. (2020). A mini-review on commonly used biochemical tests for identification of bacteria. International Journal of Research Publications 54(1):1-7.

Tsega, K. T., Kagira, J. M., Tessema, N. B. and Mekuria, S. A. (2024). Effects of *Lactobacillus* probiotics supplemented with concentrate feed on growth performance, carcass characteristics, and caecalmicroflora of RIR chickens. Cogent Food and Agriculture, 10:1-8.https://doi.org/10.1080/23311932.2024.2311959

Untoo, M., Banday, M. T., Afzal, I., Adil, S., Baba, I. A. and Khurshid, A. (2018). Review: potential of probiotics in poultry production. Journal of Entomology and Zoology Studies, 6(3): 1293-1300.

Youssaf, S., Nouman, H. M., Ahmed, I., Husain, S., Waseem, M., Nadeem, S., Tariq, M., Sizmaz, O. and Chudhry, M. F. Z. (2022). Postępy Mikrobiologii-Advancements of Microbiology, 61(3): 115-123. DOI: 10.2478/am-2022.010