Comparing the Effects of Supplementary Antibiotic, Probiotic, and Prebiotic on Carcass Composition, *Salmonella* Counts and Serotypes in Droppings and Intestine of Broiler Chickens

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Abstract
The effects of antibiotic, probiotic, and prebiotic as dietary feed additives on carcass composition as well as *Salmonella* counts and its serotypes in droppings and intestine of broiler chickens on days 1, 28, and 56 of experiment were investigated. Dietary treatments were control diet (basal diet without additives), OXYT diet (basal diet with 600 ppm of the antibiotic oxytetracycline), GRO-UP diet (basal diet with 500 ppm probiotic), and MOS-500 or MOS-1000 diets (basal diet with 500 or 1000 ppm mannan oligosaccharide prebiotic, respectively). From an initial total of 190 day-old Arbor acres broiler chicks, two birds were randomly selected from each treatment and sampled for *Salmonella*. The remaining 180 birds were randomly allotted to the five dietary treatments with three replicates of 12 birds each. Feed and water were supplied *ad libitum*. The results indicated that breast, neck, drumsticks and liver yields significantly affected by the inclusion of feed additives ($P < 0.05$). Significant differences were observed in average Salmonella counts in both the intestines and droppings of broiler chickens at different days of experiment ($P < 0.05$). *Salmonella* Gallinarum and *Salmonella* Typhi were serotypes identified in the droppings and intestine of broiler chickens. Our findings reveal that the inclusion of prebiotic oligomanno or probiotic GRO-UP as antibiotic substitutes in diets of broiler chickens can rapidly reduce some Salmonella serotypes and aid to the control Salmonella organisms.

Introduction

The benefits of broiler production cannot be over emphasized in the face of rising demand for animal protein in many developing countries. To maximize production, broilers must be free from diseases and fed appropriate diets that meet their nutritional requirements for optimal production (Tannock, 1998). Antibiotics are widely used in animal feed to boost animal performance and productivity. Tetracyclines (oxytetracycline and chlortetracycline) are arguably the most commonly used therapeutic antibiotics in food animal production (Fairchild *et al*., 2005).

Oxytetracycline (OTC) is a broad spectrum antibiotic known as tetracyclines which were developed to enhance the control of bacterial infections (Alam, 2000). Stutz and Lawton (1984) showed that using 55 ppm OTC improved growth performance and reduced the ileum weight of chicks. Zulkifli *et al.* (2000) demonstrated that chicks given diets supplemented with 50 mg/kg OTC during 21-42 days of age increased body weight gain (BWG) but the antibody produced against Newcastle disease virus (NDV) was not affected. Talabi *et al.* (2013) also documented the use of OTC powder in feed of broiler chicks at 0.05 g/kg as a growth promoter. However, at low levels of antibiotic administration, resistant microbial cells survive and develop resistance (Huyghebaert *et al.*, 2011; Toghyani *et al.*, 2011).

As a result, other feed additives such as prebiotics and probiotics have been suggested for animal feeding. They are increasingly adopted as antibiotic substitutes to improve performance and gut health in poultry and pigs (Higgins *et al.*, 2008; Marković *et al.*, 2009). Probiotics are live microbial feed supplements that enhance intestinal health (Fuller, 1989). Prebiotics are indigestible food ingredients that selectively induce growth and/or activity of one or more bacterial population in the colon (Gibson and Roberfroid, 1995). Mohammadi Gheisar *et al.* (2016) concluded that the inclusion of prebiotic (e.g. lactulose) improves growth performance and alters excreta microbial populations with no adverse effect on broiler chickens. Nonetheless, more work is needed to establish useful relationships in prebiotic and probiotic supplementation and poultry performance. The objective of the current study is to compare the effects of prebiotic, probiotic, and antibiotic supplementation on carcass composition, and Salmonella counts and their serotypes in droppings and intestine of broiler chickens.

Materials and Methods
Experimental location
The experiments were conducted at the Poultry Unit of the Directorate of University Farms (DUFARMS) of the Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. The area lies within the rainforest zone of South-Western Nigeria (7°10'37"N, 3°26'58"E) at an altitude of 173 m above sea level. The climate is humid with a mean annual rainfall of 1037 mm. The mean annual temperature and humidity are 34.7°C and 82%, respectively (Google Earth, 2013).

Animal management and experimental diets
All procedures used in this study were approved by the Animal Management Committee of the Federal University of Agriculture, Abeokuta and in accordance with the guidelines for care and use of animals in research (FASS, 2010). A total of 190 day-old broiler chicks (Arbor acres strain) were sourced from a reputable hatchery in Abeokuta, Ogun state, Nigeria. The birds were weighed and randomly allotted to five dietary treatments including control diet (basal diet without additives), OXYT diet (basal diet with 600 ppm of the antibiotic oxytetracycline), GRO-UP diet (basal diet with 500 ppm probiotic), and MOS-500 or MOS-1000 diets (basal diet with 500 or 1000 ppm mannan oligosaccharide prebiotic, respectively, with three replicates of 12 broiler chickens. Two birds were randomly selected from each treatment group and sampled for Salmonella. The chicks were subjected to standard brooding in a deep litter system. At the starter phase (0-4 weeks), birds were fed with formulated broiler starter diet (23.01% CP and 2856 ME Kcal/kg) while at finisher phase (5-8 weeks), they were fed with broiler finisher diet (20.71% CP and 2911 ME Kcal/kg). Fresh water and feed were supplied *ad libitum*. Routine vaccination and medications were administered to the birds accordingly.

Probiotic GRO-UP™ was supplied by Bio Ingredients Ltd., Lagos, Nigeria with the composition of *Saccharomyces cerevisiae*: 1.5×10^{11} cfu/kg, *Lactobacillus sporogenes*: 3×10^7 cfu/kg and fortified with phytase phosphorus, proteins, calcium and carbohydrates. Prebiotic oligomanno® (Mannan oligosaccharide) was supplied by Yonichi Chemical Institute Co., Ltd. Machikita 9-25, Moriyama-Ku, Nagoya, Japan and composed of hydrolyzed Guar gum fiber. The composition of the experimental basal diet is presented in the Table 1.

Table 1. Ingredients and chemical composition of basal diet (as - fed basis)

Ingredients (%)	Starter (Weeks 0-4)	Finisher (Weeks 4-8)
Corn	50.66	55.00
Wheat offal	5.00	6.00
Fish meal (72% CP)	3.00	2.00
Soybean meal	24.24	18.00
Groundnut cake	10.00	13.00
Palm kernel cake	3.00	-
Bone meal	2.00	2.00
Oyster shell	1.00	2.75
Lysine	0.10	0.20
Methionine	0.25	0.30
Premix*	0.50	0.50
Salt (NaCl)	0.25	0.25
Calculated analysis		
Metabolizable energy (Kcal/kg)	2856	2911
Crude protein (%)	23.01	20.71
Lysine (%)	1.30	1.20
Methionine (%)	0.60	0.60
Methionine + Cystine (%)	1.00	0.97
Available phosphorus (%)	0.50	0.50
Calcium (%)	1.20	1.80

*Each kg premix contains Vit. A: 10000000 IU, Vit. D_3: 2500000 IU, Vit. E: 20000 mg, Vit. K_3: 3000 mg, Vit. B_1: 30000 mg, Vit. B_3: 3000 mg, Vit. B_2: 7000 mg, Vit. B_6: 5000 mg, Vit. B_{12}: 25 mg, Panthotenic acid: 10000mg, Folic acid: 800 mg, Biotin: 50 mg, Manganese: 80000 mg, Iron: 40000 mg, Zinc: 60000 mg, Copper: 8000 mg, Cobalt: 250 mg, Iodine: 1000 mg, Selenium (1%): 150 mg, Choline: 200000 mg and Antioxidant: 100000 mg.

Sample collection

Bird droppings were collected from two birds per replicate three times (on days 1, 28 and 56 of experiment) by collecting from the cloaca using a sterile glass rod. Intestines scrapings were also collected from two birds per replicate after slaughtering. Both droppings and intestinal scrapings were analyzed for Salmonella isolation/screening and population studies.

Salmonella count

2 g of each sample was homogenized in 20 mL of Selenite F broth and 1 mL of the homogenate was added to 9 mL of Selenite F broth. 9 mL of Selenite F broth was then placed in six sterile tubes and serial diluted to 10^{-6}-fold. 1 ml of the final diluent was spread on a dried Xylose Lysine Desoxycholate (XLD) Agar and incubated at $37 \pm 1°C$ for 24 hrs. Colonies identified as Salmonella were counted and estimated as follows:

Let **X** be total number of colonies counted.

Weight of sample ~ 2 g; Volume used for culture ~ 2/20 = 1/10; Dilution used for culture ~ 10^{-6}

Total colony forming unit (cfu) = **X** $\times 10^{-1} \times 10^6$ = **X** $\times 10^5$ cfu/mL

Since, 2g of sample was put into 20 mL of peptone water = 2/20 = 0.1g/mL.

Then, 0.1 g = **X** $\times 10^5$ cfu of isolates

$$1 \text{ g} = \frac{\textbf{X} \times 10^5 \times 1}{0.1} = \textbf{X} \times 10^6$$

Pre-enrichment, Salmonella isolation and screening

Salmonella isolation and screening were carried out at the Veterinary Microbiology Laboratory of the College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Nigeria. The representative samples (droppings and intestine) were transferred separately into a non-selective medium (Buffered Peptone Water, BPW agar) for pre-enrichment, then incubated at $37°C \pm 1°C$ for 16-20 hours (Hendriksen, 2003).

Selective enrichment

0.1 mL of the pre-enriched sample was transferred into 10 mL of Rappaport-Vassiliadis R10 Broth with soya peptone (RVS) medium and incubated at $41.5 \pm 1°C$ for 24 ± 3 hrs. The sample was then inoculated with a sterile loop onto plates containing Xylose Lysine Desoxycholate (XLD) Agar and Brilliant Green Agar (BGA) so that colonies were well isolated. The inoculated petri dishes were inverted and transferred to an incubator at $37 \pm 1°C$ for 24 ± 3 hrs. Typical colonies of Salmonella grown on XLDA had a black centre and a lightly transparent zone of

reddish colour due to the colour change of the indicator. Typical colonies of Salmonella on BGA appeared pink measuring 1 mm to 2 mm in diameter and caused the colour to change to red. Suspected colonies were streaked onto pre-dried nutrient agar plates and incubated at $37 \pm 1°C$ for 24 ± 3 hrs and the cultures were further used for confirmatory tests (Rappaport *et al.*, 1956; Vassiliadis *et al.*, 1978; Peterz *et al.*, 1989).

Identification of Salmonella serotypes
Motility test
Motility test was carried out to determine the motility of the Salmonella organisms (i.e. motile or non-motile).

Biochemical test
Serotypes were biochemically identified using a kit (Oxoid microbat 24E). The Oxoid microbat 24E kit contains the following reagents:
H_2S (hydrogen sulphide gas), to determine the ability of the Salmonella organisms to produce H_2S; glucose, to determine the ability of the Salmonella organisms to ferment glucose and consequently producing acid and gas; mannitol, to determine the ability of the *Salmonella* organisms to ferment mannitol; O-Nitrophenyl-β-D-galactopryranosidase, to determine the presence of enzyme β-galactosidase by utilizing O-Nitrophenyl-β-D-galactopryranosidase to differentiate late lactose fermenting Salmonella organisms from non-lactose fermenting organisms; xylose, to determine the ability of the Salmonella organisms to ferment xylose sugar; indole test, to determine the ability of Salmonella organisms to split indole from tryptophan present in peptone water; and urease test, to determine the ability of Salmonella organisms to produce enzyme urease as this will split urea to form ammonia and CO_2 (Hendriksen, 2003; Collins *et al.*, 2004; Park *et al.*, 2009; Nataro *et al.*, 2011; PHE, 2015).

Carcass yield evaluation
At week eight of the experiment, 30 birds (2 birds per replicate) were randomly selected, weighed, and slaughtered. Scalding was done at $60°C$ following standard commercial procedures (Jensen, 1984), then evisceration. Dressed weight was determined. Parts such as head, neck, shank, thigh, drumstick, back, breast weight and visceral organs (gizzard, intestines, liver, heart and kidney) were weighed. The weights of the various parts were expressed as percentage of the live weights.

Statistical analysis
Data obtained were subjected to Analysis of Variance (ANOVA) in a Completely Randomized Design (CRD) using SAS (2003). Significant means among variables were separated using Duncan's Multiple Range Test.

Results
The effects of dietary feed additives on carcass composition of broiler chickens are presented in Table 2. There were significant ($P < 0.05$) differences in the weights of breast, neck, drumsticks, and liver. Breast and drumstick weights were similar between the control, OXYT, and GRO-UP treatments. Birds in both MOS-500 and MOS-1000 groups had the lowest ($P < 0.05$) breast weight and drumsticks. Birds fed OXYT and GRO-UP diets had the greatest neck weights while the lowest weight was found in birds fed MOS (500 ppm) diets. Birds fed OXYT diets had the highest ($P < 0.05$) liver weight while birds fed the control diet had the lowest. However, there was an insignificant difference between dressing percentage of treatments.

Salmonella counts in droppings of broiler chickens were significantly different between dietary treatments at different intervals ($P < 0.05$; Table 3). The initial count in all droppings ranged from 5.50 to 6.50 cfu $\times 10^6$/g though there was a reduction in colony counts in all treatments over time. The total colony counts of Salmonella in the droppings of birds fed the control diet were higher ($P < 0.05$) than other treatments. Salmonella counts in droppings of birds fed OXYT, GRO-UP and MOS (1000 ppm) diets were lower than other treatments during the first four weeks of rearing. The highest ($P < 0.05$) count in droppings and the lowest percentage reduction (15.38%) was recorded in birds fed control diet. During the second four weeks, the lowest ($P < 0.05$) count in droppings and highest percentage reduction (95.45%) was recorded for birds fed MOS (1000 ppm) diets while the lowest percentage reduction (18.18%) was recorded for birds fed control diet. Overall, birds fed diets with MOS (1000 ppm) had the highest percentage reduction in average Salmonella counts (98.18%), followed by MOS (500 ppm) (92.31%), while birds fed the control diet had the lowest percentage reduction in Salmonella counts (30.77%).

Table 2. Effects of dietary feed additives on carcass composition of broiler chickens (% live body weight)

Parameters	Control	OXYT	GRO-UP	MOS (500 ppm)	MOS (1000 ppm)	SEM	P-Value
Live weight (g)	2234.67	2107.00	2184.93	2114.8	2079.87	27.16	0.11
Dressing	68.03	62.90	64.45	66.73	64.51	0.84	0.24
Head	2.49	2.47	2.49	2.70	2.44	0.04	0.71
Shank	4.97	4.24	4.85	4.64	4.71	0.11	0.16
Thigh	10.74	11.09	11.54	10.62	10.06	0.22	0.45
Breast	18.95[ab]	19.54[ab]	21.00[a]	18.15[b]	18.19[b]	0.41	<0.001
Back	17.35	16.20	15.99	17.05	17.12	0.31	0.72
Wings	8.83	9.18	8.98	8.46	8.87	0.12	0.33
Neck	3.16[bc]	4.74[a]	4.24[ab]	2.86[c]	3.52[bc]	0.24	0.01
Drumsticks	10.78[ab]	10.25[ab]	11.52[a]	10.10[b]	9.94[b]	0.21	0.03
Heart	0.44	0.53	0.44	0.54	0.48	0.02	0.58
Liver	1.97[c]	2.70[a]	2.46[ab]	2.04[bc]	2.00[bc]	0.09	<0.001
Gizzard	1.76	2.24	2.01	2.08	1.75	0.08	0.75
Spleen	0.15	0.15	0.11	0.14	0.16	0.01	0.41
Lungs	0.54	0.63	0.56	0.58	0.52	0.24	0.23
Kidney	0.35	0.58	0.48	0.48	0.39	0.36	0.22

[a-c] Means on the same row having different superscripts are significantly different (*P* < 0.05).
Control (No additive); OXYT: Oxytetracycline (antibiotics); GRO-UP (probiotics); MOS: Mannan oligosaccharide (prebiotics).

Table 3. Effects of dietary feed additives on average Salmonella counts (cfu/g × 10^{-6}) in droppings of broiler chickens at different intervals

Treatments	Control	OXYT	GRO-UP	MOS (500 ppm)	MOS (1000 ppm)	SEM	P-Value
Week 0	6.50[a]	6.25[b]	5.60[d]	5.85[c]	5.50[d]	0.10	0.01
Weeks 0-4	5.50[a] (15.38)	2.25[c] (64)	2.30[c] (58.93)	2.50[b] (57.26)	2.20[c] (60)	0.34	0.00
Weeks 4-8	4.50[a] (18.18)	0.70[c] (68.89)	1.10[b] (52.17)	0.45[d] (82)	0.10[e] (95.45)	0.43	0.04
Reduction in colonization (%) (Weeks 0-8)	30.77	88.89	80.35	92.31	98.18		

[a-e] Means on the same row having different superscripts are significantly different (P<0.05).
Values in brackets indicate percentage reduction.
Control (No additive); OXYT: Oxytetracycline (antibiotic); GRO-UP (probiotic); MOS: Mannan oligosaccharide (prebiotic).

Table 4 shows the effects of dietary feed additives on Salmonella serotypes present in the droppings of broiler chickens at different time intervals. The results revealed that *Salmonella* Gallinarum and *Salmonella* Typhi were identified across all treatments. In the droppings of birds fed the control diet, *Salmonella* Gallinarum was present at weeks 0 and 4 but absent at week 8 while *Salmonella* Typhi was present throughout the experiment. In the droppings of birds fed OXYT diets, *Salmonella* Gallinarum was present throughout the experiment while *Salmonella* Typhi was absent throughout the experiment. In the droppings of birds fed GRO-UP supplemented diet, *Salmonella* Gallinarum was present at weeks 0 and 8 but absent at week 4 while *Salmonella* Typhi was present at weeks 0 and 4 but absent at week 8 of the experiment. In

the droppings of birds fed MOS-500 and MOS-1000 diets, *Salmonella* Gallinarum was present throughout the experiment while *Salmonella* Typhi was present at week 0 but absent at weeks 4 and 8 of the experiment.

There were significant (*P* < 0.05) differences in average Salmonella counts between dietary treatments in the intestine of broiler chickens at different intervals (Table 5). The initial count across all treatments ranged from 6.65 to 7.80 cfu × 10^6/g. However, there was a reduction in colony counts in all treatments by weeks 4 and 8. The total colony counts of Salmonella in the intestine of birds fed OXYT diets was higher (*P* < 0.05) than other treatments. By the end of week 4, the Salmonella counts in the intestine of birds fed OXYT and MOS (500 ppm) diets were lower than other treatments (*P* < 0.05). The highest (*P* <

0.05) count in the intestine and the lowest percentage reduction (17.29%) was observed in birds fed control diet. By the end of week 8, Salmonella counts in the intestine of birds fed diets with feed additives were statistically similar and significantly lower than the control ($P < 0.05$). The highest percentage reduction was recorded for birds fed MOS (1000 ppm) diets

(60%) followed by MOS (500 ppm) (50%), while the lowest percentage reduction was recorded for birds fed control diet (14.55%). Overall, birds fed diets with MOS (1000 ppm) had the highest percentage reduction in the Salmonella counts (86.11%), followed by MOS (500 ppm) (83.01%), while the control diet had the lowest percentage reduction in Salmonella count (29.32%).

Table 4. Effects of dietary feed additives on Salmonella serotypes present in the droppings of broiler chickens at different intervals

Treatments	Week 0		Week 4		Week 8	
	S. Gallinarum	*S.* Typhi	*S.* Gallinarum	*S.* Typhi	*S.* Gallinarum	*S.* Typhi
Control	+	+	+	+	-	+
OXYT	+	-	+	-	+	-
GRO-UP	+	+	-	+	+	-
MOS (500 ppm)	+	+	+	-	+	-
MOS (1000 ppm)	+	+	+	-	+	-

+: present;   -: absent;
Control (No additive); OXYT: Oxytetracycline (antibiotics); GRO-UP (probiotics); MOS: Mannan oligosaccharide (prebiotics).

Table 5. Effects of dietary feed additives on average Salmonella counts (cfu/g × 10^{-6}) in the intestine of broiler chickens at different intervals

Treatments	Control	OXYT	GRO-UP	MOS (500 ppm)	MOS (1000 ppm)	*SEM*	*P*-Value
Week 0	6.65[d]	7.80[a]	7.65[b]	6.80[d]	7.20[c]	0.13	0.04
Weeks 0-4	5.50[a] (17.29%)	2.25[d] (71.15%)	2.80[b] (63.40%)	2.30[d] (66.18%)	2.50[c] (65.28%)	0.34	0.00
Weeks 4-8	4.70[a] (14.55%)	1.35[b] (40%)	1.60[b] (42.86%)	1.15[b] (50%)	1.00[b] (60%)	0.38	0.01
%reduction in colonization (Weeks 0-8)	29.32	82.69	79.08	83.09	86.11		

[a-d] Means on the same row having different superscripts are significantly different ($P < 0.05$).
Values in brackets indicate percentage reduction.
Control (No additive); OXYT: Oxytetracycline (antibiotics); GRO-UP (probiotics); MOS: Mannan oligosaccharide (prebiotics).

Table 6. Effects of dietary feed additives on Salmonella serotypes present in the intestine of broiler chickens at different intervals

Treatments	Week 0		Week 4		Week 8	
	S. Gallinarum	*S.* Typhi	*S.* Gallinarum	*S.* Typhi	*S.* Gallinarum	*S.* Typhi
Control	+	+	+	+	-	+
OXYT	+	+	-	+	-	+
GRO-UP	+	+	-	+	-	+
MOS (500 ppm)	+	-	+	-	-	-
MOS (1000 ppm)	+	-	+	-	-	-

+: present -: absent
Control (No additive); OXYT: Oxytetracycline (antibiotics); GRO-UP (probiotics); MOS: Mannan oligosaccharide (prebiotics).

Table 6 shows the effects of dietary feed additives on Salmonella serotypes present in the intestine of broiler chickens at different time intervals. *Salmonella* Gallinarum and *Salmonella* Typhi were identified across all treatments. In the intestine of birds fed control diet, *Salmonella* Gallinarum was present at week 0 and 4 but absent at week 8 of the experiment, while

Salmonella Typhi was present throughout the experiment. In the intestine of birds fed OXYT diets, *Salmonella* Gallinarum was present at week 0 but absent at weeks 4 and 8 of the experiment, while *Salmonella* Typhi was present throughout the experiment. In the intestine of birds fed GRO-UP diets, *Salmonella* Gallinarum was present at week 0 but absent at weeks 4 and 8 of

the experiment, while *Salmonella* Typhi was present throughout the experiment. In the intestine of birds fed MOS (500 ppm) and MOS (1000 ppm) diets, *Salmonella* Gallinarum was present at weeks 0 and 4 but absent at week 8 of the experiment, while *Salmonella* Typhi was absent throughout the experiment.

Discussion

The relative weights of breast, neck, drumsticks and liver (but not dressing) were significantly affected by dietary supplementation of feed additives. The addition of MOS had no effect on body weight at any age. Mathis (2000) found that the combination of certain antibiotics with MOS could lead to additive or synergistic benefits on broiler performance, compared to using antibiotics alone. The lowest drumsticks and breast meat yield recorded were in birds fed MOS at 500 ppm and 1000 ppm contrasts the report of Clementino Dos Santos *et al.* (2002) who stated that dietary MOS (0.1%) significantly increased breast yield as a percentage of dressed carcass. Mohammed *et al.* (2008) did not observed any significant effects of prebiotic and probiotic supplements on the relative weights of dressing, liver, heart, and gizzard. Similarly, Midilli *et al.* (2008) did not observe any impact of MOS on carcass traits and relative weights of internal organs in broiler chickens. Dosage, method of preparation, and animal condition may be responsible for such inconsistencies. Growth stimulants as feed additives are added to poultry diet to enhance growth rate and economic meat production (Bunyan *et al.*, 1997). The observed effects of feed additives on some of the carcass traits could point to the growth-promoting effects of these dietary additives in relation to improved gut environment and a stable intestinal flora (Eltazi, 2014).

Previous studies demonstrated that prebiotics can moderate the gut environment by increasing the number of beneficial microbes and hindering rapid multiplication of intestinal pathogens (Patterson and Burkholder, 2003; Higgins *et al.*, 2008). Dietary prebiotics reduced Salmonella population in the intestine of chickens (Bailey *et al.*, 1991; Pascual *et al.*, 1999; Stern *et al.*, 2001) and supported competitive exclusion and immune modulation (Jin *et al.*, 1997; Simon *et al.*, 2001). The observation that feeding broiler chickens diets supplemented with Mannan oligosaccharide (MOS at 500 ppm

and 1000 ppm) reduced Salmonella counts in the droppings and intestine support the argument of the active prebiotic function that mannan oligosaccharide (MOS) performs in the gut of broiler chickens. A similar trend was observed by Mohammadi Gheisar *et al.* (2016) with the prebiotic lactulose. Spring *et al.* (2000) noted a significant reduction in caecal Salmonella counts in chickens that received 4000 ppm of dietary MOS. Oyofo *et al.* (1989) found that dietary inclusion of 2.5% mannose reduced Salmonella colonization and 0.1% mannose decreased shedding, and colonization of caecal and liver after infection with 2×10^7 cfu of *Salmonella enteritidis* in 2-week old chickens (Fernandez *et al.*, 2000; Agunos *et al.*, 2007).

The beneficial activity of GRO-UP and MOS against *Salmonella* spp observed in the present study was supported by Courtin *et al.* (2008) who found that feed additives increase the number of bifidobacteria in the caecum of chickens. *Salmonella* Gallinarium and *Salmonella* Typhi were identified in the droppings and intestine of broiler chickens at different time intervals. This result agrees with Davies and Wray (1993) who reported that Salmonella bacteria are more closely associated with poultry than their ubiquitous distribution deserves. Salmonella bacteria may not exist as single entities but in a huge range of serotypes, including poultry-specific pathogens like *Salmonella* Gallinarium and *Salmonella* Typhi. The serotype distribution at different time intervals in droppings and intestine of broiler chickens is of high economic interest.

Conclusion

Carcass composition was not adversely affected by the inclusion of feed additives in broiler diets. There was a higher percentage reduction in Salmonella colonization in birds fed diets with prebiotics and probiotics. At the end of week eight, broiler chickens fed diets with mannan oligosaccharide (1000 ppm MOS) had the highest percentage reduction in overall Salmonella count, followed by birds fed 500 ppm MOS. Birds fed the control diet had the lowest percentage reduction in Salmonella counts in the droppings and intestine. The inclusion of MOS (both 500 or 1000 ppm) or GRO-UP as antibiotic substitutes in diets of broiler chickens can control and eradicate some Salmonella serotypes.

References

Agunos A, Ibuki M & Mine Y. 2007. Effect of dietary βl-4 mannobiose in the prevention of *Salmonella enteritidis* infection in broilers. British Poultry Science, 48: 331-341. DOI: 10.1080/00071660701370442

Alam S. 2000. Handbook of poultry disease and treatment. 9th Ed. Anglo Egyptian Library Press, Egypt in Arabic. Pages, 28, 34 & 40.

Bailey JS, Blankenship LC & Cox NA. 1991. Effect of fructooligosaccharide on Salmonella colonization of the chicken intestine. Poultry Science, 70: 2433–2438. DOI : 10.3382/ps.0702433

Bunyan J, Jeferies L, Sayers JR, Gulliver AL & Colemon K. 1997. Antimicrobial substances and chick growth promotion: The growth-promoting activities of antimicrobial substances, including fifty-two used either in therapy or as dietary additives. British Poultry Science, 18: 283-294. DOI: 10.1080/00071667708416364

Clementino dos Santos E, Soares Teixeira A, Gilberto Bertechini R, Tadeu Fonseca de Freitas P, Borges Rodregues E, Souza Dias DM, Torres DM, Vierra Santos A & Giacometi RA. 2002. Effects of growth beneficial additives on broiler carcass yield. Federal Agrotech. School of Inconfidentes and Federal University of Lavras, Minas Gerais, Brazil. In: Proceedings of Brazilian Society of Animal Production (Sociedade Brasileria de Zootecnia). Page, 5.

Collins CH, Lyne PM, Grange JM & Falkinham JO. 2004. Identification methods. In: Collins CH, Lyne PM, Grange JM & Falkinham JO. (Eds). 8th Ed. Collins and Lyne's Microbiological Methods. Arnold. Hodder Headline Group. London. 512 Pages.

Courtin CM, Broekaert WF, Swennen K, Lescroart O, Onagbesan O, Buyse J, Decuypere E, Van de Wiele T, Marzorati M, Verstraete W, Huyghebaert G & Delcour JA. 2008. Dietary inclusion of wheat bran arabinoxylooligosaccharides induces beneficial nutrirional effects on chickens. Cereal Chemistry, 85: 607-613. DOI: 10.1094/ CCHEM-85-5-0607

Davies RH & Wray C. 1993. Salmonella pollution in poultry units and associated enterprises; Pollution in livestock production system. Dew A, Axford R & Marai FM. (Eds). International, Wallingford, UK. 463 Pages.

Eltazi SMA. 2014. Response of broiler chicks to diets containing different mixture levels of garlic and ginger powder as natural feed additives. International Journal of Pharmaceutical Research & Allied Sciences, 3: 27-35.

Fairchild AS, Smith JL, Idris U, Lu J, Sanchez S, Purvis LB, Hofacre C & Lee MD. 2005. Effects of orally administered tetracycline on the intestinal community structure of chickens and on *tet* determinant carriage by commensal bacteria and *Campylobacter jejuni*. Applied and Environmental Microbiology, 71: 5865–5872. DOI: 10.1128/AEM.71.10.5865-5872.2005

FASS (Federation of Animal Science Societies). 2010. Guide for the Care and Use of Agricultural Animals in Research and Teaching, 3rd Ed. Federation of Animal Science Societies, Champaign-Urbana, Illinois, IL.

Fernandez F, Hinton M & Van Gils B. 2000. Evaluation of the effect of mannan-oligosaccharides on the competitive exclusion of *Salmonella enteritidis* colonization in broiler chicks. Avian Pathology, 29: 575-581. DOI: 10.1080/03079450020016823

Fuller R. 1989. Probiotics in man and animals. Journal of Applied Microbiology, 66: 365-378. DOI: 10.1111/j.1365-2672.1989.tb05105.x

Gibson GR & Roberfroid MB. 1995. Dietary modulation of human colonic microbiota: Introducing the concept of prebiotic. Journal of Nutrition, 125: 1401- 1412.

Google Earth. 2013. Google location map; Google earth imagery date; December 3rd, 2013.

Hendriksen RS. 2003. Identification of *Salmonella*. Global Salm-Surv. A global *Salmonella* surveillance and laboratory support project of the World Health Organization. Pages, 1-6.

Higgins SE, Higgins JP, Wolfenden AD, Henderson SN, Torres-Rodriguez A, Tellez G & Hargis B. 2008. Evaluation of a *lactobacillus*-based probiotic culture for the reduction of *Salmonella* enteritidis in neonatal broiler chicks. Poultry Science, 87: 27–31. DOI: 10.3382/ps.2007-00210

Huyghebaert G, Ducatelle R & Immerseel FV. 2011. An update on alternatives to antimicrobial growth promoters for broilers.

The Veterinary Journal, 187(2):182–188. DOI: 10.1016/j.tvjl.2010.03.003

Jensen JF. 1984. Method of dissection of broiler carcasses and description of parts. World's Poultry Science Association European Federation Working Group V, Copenhagen. p. 32.

Jin LZ, Ho YW, Abdullah N & Jalaludin S. 1997. Probiotics in poultry: Modes of action. World's Poultry Science Journal, 53: 351–368. DOI: 10.1079/WPS19970028

Marković R, Šefer D, Krstić M & Petrujkić B. 2009. Effect of different growth promoters on broiler performance and gut morphology. Archivos de Medicina Veterinaria, 41: 163–169.

Mathis GF. 2000. Effect of MOS on growth and feed efficiency in commercial broiler chickens. Southern Poultry Research, Inc., Athens, Georgia USA.

Midilli M, Alp M, Kocabach N, Muglah ÖH, Turan N, Yilmaz H & Çakir S. 2008. Effects of dietary probiotic and prebiotic supplementation on growth performance and serum IgG concentration of broilers. South African Journal of Animal Science, 38: 21-27.

Mohammed MA, Hassan HMA & El-Barkouky EMA. 2008. Effect of mannan oligosaccharide on performance and carcass characteristics of broiler chicks. Journal of Agriculture and Social Sciences, 4: 7-13.

Mohammadi Gheisar M, Nyachoti CM, Hancock JD & Kim IH. 2016. Effects of lactulose on growth, carcass characteristics, faecal microbiota, and blood constituents in broilers. Veterinarni Medicina, 61: 90-96. DOI: 10.17221/8722-VETMED

Nataro JP, Bopp CA, Fields PI, Kaper JB & Strockbine NA. 2011. *Escherichia, Shigella,* and *Salmonella*. In: Versalovic J, Carroll K, Funke G, Jorgensen J, Landry ML & Warnock EW. (Eds). Manual of Clinical Microbiology. 10th Ed. Vol 1. Washington DC: ASM Press. 2630 Pages. DOI: 10.1128/9781555816728.ch35

Oyofo BA, DeLoach JR, Corrier DE, Norman JO, Ziprin RL & Molenhauer HH. 1989. Prevention of *Salmonella typhimurium* colonization of broilers with D-mannose. Poultry Science, 68: 1357-1360. DOI: 10.3382/ps.0681357

Park SH, Kim HJ, Cho WH, Kim JH, Oh MH, Kim SH, Lee BK, Ricke SC & Kim HY. 2009. Identification of *Salmonella enterica* subspecies I, *Salmonella enterica* serovars Typhimurium,

Enteritidis and Typhi using multiplex PCR. FEMS Microbiology Letters, 301: 137-146. DOI: 10.1111/j.1574-6968.2009.01809.x

Pascual M, Hugas M, Badiola JI, Monfort JM & Garriga M. 1999. *Lactobacillus salivarius* CTC2197 prevents *Salmonella enteritidis* colonization in chickens. Applied and Environmental Microbiology, 65: 4981–4986.

Patterson JA & Burkholder KM. 2003. Application of prebiotics and probiotics in poultry production. Poultry Science, 82: 627–631. DOI: 10.1093/ps/82.4.627

Peterz M, Wiberg C & Norberg P. 1989. The effect of incubation temperature and magnesium chloride concentration on growth of Salmonella in home-made and in commercially available dehydrated Rappaport-Vassiliadis broths. Journal of Applied Microbiology, 66: 523-528. DOI: 10.1111/j.1365-2672.1989.tb02535.x

Public Health England (PHE). 2015. Bacteriology – Identification of *Salmonella* species. UK Standards for Microbiology Investigations. Standards Unit, Microbiology Services, Public Health England. Issue no 3. Pages, 1-23.

Rappaport F, Konforti N & Navon B. 1956. A new enrichment medium for certain Salmonellae. Journal of Clinical Pathology, 9: 261-266.

SAS (Statistical Analysis System). 2003. SAS/STAT[(R)] User's Guide. SAS Institute Inc., Cary, North Carolina, USA.

Simon O, Jadamus A & Vahjen W. 2001. Probiotic feed additives – effectiveness and expected modes of action. Journal of Animal and Feed Sciences, 10: 51–67.

Spring P, Wenk C, Dawson KA & Newman KE. 2000. The effects of dietary mannaoligosaccharides on cecal parameters and the concentrations of enteric bacteria in the ceca of Salmonella-challenged broiler chicks. Poultry Science, 79: 205–211. DOI: 10.1093/ps/79.2.205

Stern NJ, Cox NA, Bailey JS, Berrang ME & Musgrove MT. 2001. Comparison of mucosal competitive exclusion and competitive exclusion treatment to reduce *Salmonella* and *Campylobacter spp* colonization in broiler chickens. Poultry Science, 80: 156–160. DOI: 10.1093/ps/80.2.156

Stutz MW & Lawton GC. 1984. Effects of diet and antimicrobials on growth, feed efficiency, intestinal *Clostridium perfringes,* and ileal

weight of broiler chicks. Poultry Science, 63: 2036-2042. DOI: 10.3382/ps.0632036

Talabi AO, Oyekunle MA, Adebayo LA & Apata SE. 2013. Effect of regimes of dietary oxytetracycline on the performance of broiler chicken. African Journal of Livestock Extension, 11: 26-30.

Tannock GW. 1998. The normal microflora: new concepts in health promotion. Microbiological Science, 5: 4-8.

Toghyani M, Toghyani M & Tabeidian SA. 2011. Effect of probiotic and prebiotic as antibiotic growth promoter substitutions on productive and carcass traits of broiler chicks. 2011 International Conference on Food Engineering and Biotechnology (IPCBEE), 9: 82-86.

Vassiliadis P, Trichopoulos D, Kalandidi A & Xirouchaki E. 1978. Isolation of *Salmonellae* from sewage with a new procedure of enrichment. Journal of Applied Microbiology, 44: 233-239. DOI: 10.1111/j.1365-2672.1978.tb00795.x

Zulkifli I, Abdullah N, Azrin NM & Ho YM. 2000. Growth performance and immune response of two commercial broiler strains fed diets containing Lactobacillus cultures and oxytetracycline under heat stress conditions. British Poultry Science, 41: 593-597. DOI: 10.1080/713654979