NOVEL PROBIOTICS PROMOTES OXIDATIVE STABILITY IN BROILER PRODUCTION: CASE REPORTS OF *Enterococcus spp*

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Abstract

In this research, three novel probiotic *Enterococcus hirae, Enterococcus faecium* and *Enterococcus durans* recently developed by our team were evaluated on the performance and oxidative status of 384 broiler chickens. One day old broiler chickens were randomly allotted to nine treatments in a 3x2x4 (probiotics vs route vs dosage) factorial arrangement. The dosage of probiotics formulated were 10^4cfu/L, 10^7cfu/L and 10^{11}cfu/L of each probiotic and a control without probiotics, administration routes were feed vs water. At the end of a 42day feeding trial, blood samples were collected and assayed for glutathione peroxidase, catalase, superoxide dismutase, total antioxidant activity and lipid peroxidation using standard procedures. Result obtained revealed that administration of the E*nterococcus durans* probiotic in water enhance oxidative stability better than in feed. Administration of *Enterococcus hirae* probiotics generally (feed and water) promoted better oxidative stability than control, while the higher the dosage consumed by the bird the better oxidative stability of the birds. Probiotics *Enterococcus faecium* enhanced total antioxidant activity of birds and reduced lipid peroxidation than the control. In conclusion, the three novel probiotics enhance antioxidant defense of broiler chicken and reduced lipid peroxidation, while inclusion of the three probiotics in water elicit better response than inclusion in feed.

Keywords: Antioxidant; peroxides, antimicrobial; oral supplements ; feed

Introduction

Large-scale broiler chicken production is blooming along with the rising demand for chicken, in order to meet the large demand for broilers in the market, leading to abuse of antibiotics growth promoters, bringing about drug resistance in animals and drug residues in livestock products. Consequently, alternative growth promoters as a replacement for antibiotics has become a hot topic in feed research (Zhang et al., 2021). Probiotics or direct-fed microbials (DFM) are live micro-organisms that can be incorporated in diets in order to; populate the intestine with beneficial bacteria and modulate the conditions within the gastro-intestinal tract. The probiotics are nutritional tools in poultry feeds for promotion of growth, modulation of intestinal microflora and pathogen inhibition, immunomodulation and promoting meat quality of poultry. Probiotics positively modulate the dynamics of oxidants and antioxidants in the body of chickens, Japanese quails, and rats (Sohail *et al.*, 2011). The development of probiotics in Nigeria has been extensively used for humans with little effort on animals. In Nigeria, high cost of probiotics owing to its importation has reduced patronage of probiotics by poultry farmers in favour of antibiotics leading to its abuse and increase resistance by microbes. It is now important to find sustainable, cheap, reliable and sensitive alternative probiotics for usage by Nigerian poultry farmers. With the global economic meltdown, inflating costs of poultry production might be mitigated with the development of local probiotics from our indigenous animal microbiota. The development of probiotics for Nigeria poultry sector will be pivotal in reducing the cost of production, promote local content in probiotic production and enhance the nation's GDP in the face of the current economic downturn. Hence, our research group explored rabbit gut for the development of viable probiotics candidates; *Enterococcus hirae, Enterococcus faecium* and *Enterococcus durans*. Hence, the effect of the three probiotics were assessed on oxidative stability of broilers administered the probiotic in water and feed.

Materials and Methods

This study was carried out in Teaching and Research Farm, The Federal Polytechnic Ado-Ekiti, Ekiti State in the Southwest of Nigeria. In this research, three novel probiotic *Enterococcus hirae, Enterococcus faecium* and *Enterococcus durans* were selected as the experimental strains. The

isolates of the three probiotics were culture in agar and prepared in broth to arrive at the required dosage. 384 a-day old broiler chickens were randomly allotted to nine treatments in a 3x2x3 (probiotics vs route vs dosage) factorial arrangement with a control. The birds were allotted 4 replicates, 4 birds per replicate. Treatments were consists of an effective live bacteria content 10^4cfu/L, 10^7cfu/L and 10^{11}cfu/L of each probiotic in water and 10^4cfu/kg, 10^7cfu/kg and 10^{11}cfu/kg of each probiotic in feed of all probiotics and a control (without probiotic in feed or water). To ensure the activity and the effect of the probiotic preparation, the individual strain was refrigerated at 4°C, and every single strain of probiotic was thoroughly mixed every morning before use. The probiotics were mixed thoroughly with water and feed daily before serving to the animals. Adjustments were made appropriately depending on quantity of feed and water to be offered daily, to arrive at the treatment dose. Standard experimental ration was formulated to contain 23% CP, 3031.27 kcal/kg ME, 3.03 CF, 3.94% fat, calcium 1.2%, phosphorus 0.32%, lysine 1.4% and methionine 0.65% for starter ration and 20% CP, 3086.45 kcal/kg ME, 3.32 CF, 3.9% fat, calcium 1.1%, phosphorus 0.25%, lysine 1.2% and methionine 0.56% for finisher ration. The birds were vaccinated and no medication offered throughout the 42-day experiment, performance indices were monitored. At the end of the feed trial, blood samples were collected and serum obtained to assess oxidative stress markers using methods outline in Jimoh *et al.* (2019). Data obtained were subjected to GLM procedure of ANOVA of IBM SPSS 20.

Results

The effect of E*nterococcus durans* on oxidative stability of broiler chicken as shown in Table 1 reveals that the probiotics enhanced total antioxidant activity of birds and reduced lipid peroxidation than the control. Administration of the E*nterococcus durans* probiotic in water enhance oxidative stability better than in feed. Effect of E*nterococcus hirae* on oxidative stability of broiler chicken shown in Table 2, revealed that the higher the dosage of the probiotics the better oxidative stability of the birds. Administration of probiotics in water promoted better oxidative stability in the birds than through feed. All dosage of probiotics (feed and water) promoted oxidative stability better than control. Effect of *Enterococcus faecium* on oxidative stability of broiler chicken shown in Table 3, reveals that the probiotics enhanced total antioxidant activity of birds and reduced lipid peroxidation than the control. High dosage of the probiotics in water enhance antioxidant profile, and inclusion of probiotics in water promote better oxidative stability in birds than in feed.

Discussion

The trend of result obtained in this study signifies that, the probiotics *E.faecium*, *E. hirae* and *E. durans* enhance antioxidant defense of broiler chicken and reduced lipid peroxidation. However, incorporating the probiotics in water tended to enhance mode of action of the probiotics in the birds. This could be due to the bacteria promote resistance of biological macromolecules oxidation and take off hydroxyl radicals, thereby increasing the body's antioxidant capacity (Zheng *et al*., 2016). Previously, Zheng et al . (2016) had reported that *Enterococcus faecium* promotes oxidative stability. Oral administration of Lactobacillus delbrueckii to suckling porcine showed tremendously improved antioxidant capacity (Li et al., 2019). Previous research efforts have reported that LAB based probiotics can affect the activity and content of antioxidant enzymes in the body and reduce oxidative stress damage in the intestine (Inatomi and Otomaru, 2018). The response could be associated with hypothesis of Abudabos et al. (2016) that beneficial intestinal bacteria produce certain factors that have the ability to chelate free radicals, capturing ROS and inhibiting their cytotoxic activity. Also, probiotics produce butyric acid, hydrogen, which may play a stimulating role in the production of antioxidants and free radical scavenging (Zheng et al., 2019).

Zhang *et al*. (2021) reported that L. casei and Bifidobacterium probiotics in water had increase serum CAT, GPx, SOD and TAC concentration and a significant decrease in MDA concentration, fortifying claims in this study that probiotics had an obvious antioxidant effect on broilers. Deraz *et al.* (2019) reported that administration of probiotic preparations of *Lact. lactis* and/or *L. plantarum* at concentrations of 10^9 or 10^{12} cfu/ml to chickens caused a reduction in MDA content and significant increase in TAC in serum. This was attributed to the significant reduction in lipid peroxidation due to the probiotic ability to confer sufficient antioxidant protection against lipid peroxidation. The result of this study, is in tandem with claims of Rajput *et al*. (2013) and Ognik and Krauze (2016) who stated

that probiotics increase the activities of antioxidant enzymes including superoxide dismutase, catalase, and glutathione peroxidase and reduce the concentration of MDA.

Conclusion

This study reveals that probiotics *E.faecium, E. hirae* and *E. durans* enhance antioxidant defense of broiler chicken and reduced lipid peroxidation in addition to its growth promoting effects. The inclusion of the three probiotics in water elicit better response than inclusion in feed. Hence, the three novel probiotics can be used to mitigate oxidative stress induced pathological conditions in broiler chickens.

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Table 1: Effect of E*nterococcus durans* probiotics inclusion on oxidative status of broiler chicken

	WATER			FEED				
	A 10^4cfu/L	B 10^7cfu/L	C 10^{11}cfu/L	A 10^4cfu/kg	B 10^7cfu/kg	C 10^{11}cfu/kg	Control	SEM
Total antioxidant capacity (mM)	2.31^{b}	1.32^{c}	2.69^{a}	1.26^{c}	1.08^{c}	1.01^{c}	0.48^{d}	0.14
Lipid Peroxidation (MDA uM)	0.90^{d}	1.02^{d}	1.05^{d}	1.99^{c}	2.35^{bc}	2.47^{b}	4.36^{a}	0.23
Superoxide dismutase (U/mL)	7.91^{b}	11.95^{a}	10.61^{b}	4.28^{c}	3.68^{c}	3.33^{cd}	1.19^{d}	0.79
Catalase (umol/ml mins)	12.87^{a}	9.47^{b}	9.25^{bc}	9.05^{bc}	7.88^{cd}	7.40^{d}	4.30^{e}	0.47
Glutathione peroxidase (U/L)	117.76^{a}	102.62^{b}	116.82^{a}	97.57^{b}	88.10^{c}	84.59^{c}	46.55^{d}	4.48

Table 2: Oxidative status of broiler chicken administered *Enterococcus hirae* Probiotics

	WATER			FEED				
	A 10^4cfu/L	B 10^7cfu/L	C 10^{11}cfu/L	A 10^4cfu/kg	B 10^7cfu/kg	C 10^{11}cfu/kg	Control	SEM
Total antioxidant capacity (mM)	1.36^{a}	1.22^{b}	1.16^{c}	1.14^{c}	1.04^{d}	0.96^{e}	0.48^{f}	0.05
Lipid Peroxidation (MDA uM)	1.84^{e}	2.05^{de}	2.17^{cd}	2.23^{bcd}	2.41^{bc}	2.53^{b}	4.36^{a}	0.16
Superoxide dismutase (U/mL)	4.51^{a}	4.19^{b}	3.99^{c}	3.89^{d}	3.55^{e}	3.09^{f}	1.19^{g}	0.20
Catalase (umol/ml mins)	9.68^{a}	8.85^{b}	8.46^{bc}	8.25^{c}	7.60^{d}	7.09^{d}	4.30^{e}	0.32
Glutathione peroxidase (U/L)	105.39^{a}	95.07^{b}	91.27^{c}	90.33^{c}	86.72^{d}	82.19^{e}	46.55^{f}	3.36

Table 3: Oral administration of *Enterococcus faecium* probiotics on oxidative status of Broiler chicken

	WATER			FEED				
	A 10^4cfu/L	B 10^7cfu/L	C 10^{11}cfu/L	A 10^4cfu/kg	B 10^7cfu/kg	C 10^{11}cfu/kg	control	SEM
Total antioxidant capacity (mM)	1.36^{a}	3.25^{a}	3.25^{a}	1.11^{b}	1.19^{b}	0.93^{b}	0.48^{c}	0.23
Lipid Peroxidation (MDA uM)	0.58^{e}	0.69^{d}	0.74^{d}	2.29^{b}	1.16^{c}	2.59^{b}	4.36^{a}	0.27
Superoxide dismutase (U/mL)	11.59^{b}	12.23^{ab}	13.97^{a}	3.79^{c}	4.10^{c}	2.88^{cd}	1.19^{d}	0.98
Catalase (umol/ml mins)	18.00^{a}	16.99^{a}	18.46^{a}	8.08^{b}	8.61^{b}	6.91^{b}	4.30^{c}	1.13
Glutathione peroxidase (U/L)	137.95^{c}	165.01^{b}	191.82^{a}	89.15^{d}	92.73^{d}	80.92^{d}	46.55^{e}	9.44