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Effect of different levels of supplemental yeast on performance indices, serum enzymes and electrolytes of broiler chickens

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The aim of the study was to investigate the effect of supplemental probiotic preparation on performance indices, serum enzymes and electrolytes of broiler chickens. Two hundred (200) day-old Marshall broiler chicks were randomly selected and distributed based on the level of supplementation into four groups of 50 chicks each (Control, C; $E_1$0.5%, $E_2$1.5% and $E_3$2.0%). Chickens fed 2.0% probiotic had a significantly higher body weight when compared with the control group. Activity of alanine aminotransferase differed significantly in the group $E_1$0.5%, and especially ($p<0.01$) in the group $E_1$1.5%. Alkaline phosphatase activity decreased significantly ($p<0.05$), when compared with that of the control group. Serum calcium and phosphorus concentrations in experimental groups were significantly higher. Potassium concentration in experimental group rose significantly ($p<0.05$), when compared with that of the control broiler chickens. In conclusion, supplementing broiler feeds with 2.0% yeast probiotic improved performance indices, serum enzyme activities and enhanced the maintenance of electrolyte homeostasis in broiler chickens.

Key words: Body weight, feed conversion ratio, serum biochemistry, *Saccharomyces cerevisiae*, broiler chicken.

INTRODUCTION

One of the major challenges facing poultry industry in the developing world, including Nigeria, is the improvement of production efficiency. In an attempt to address this problem, concerted efforts have been made to incorporate antimicrobials and other natural products, such as yeasts in animal feeds (Muihead, 1992). Live yeast addition to livestock feed has been shown to improve the nutritive quality of feed and performance of animals (Martin et al., 1989; Glade and Sist, 1998). Non-antibiotic growth promoters, such as organic acids and probiotics are increasingly being established in animal nutrition (Windisch et al., 2008). Probiotics are viable single or mixed cultures of microorganisms that, when given to animals or humans, beneficially affect the host by improving the properties of the indigenous microflora (Kyriakis et al., 1999; Lee et al., 2008). They have been used as an alternative to antibiotics in animals and humans; their efficiency in animals has been widely discussed (O'Sullivan, 2001; Siriken et al.,

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Abbreviations: ALT, Alanine aminotransferase; **ALP,** alkaline phosphatase; BW, body weight; **FCR,** feed conversion ratio; **FI,** feed intake; **AST,** aminotransferase.

Table 1. Dietary composition of starter and finisher feeds.

Ingredient	Starter	Finisher
Crude protein (%)	20	19
Fat (%)	10	10
Crude fibre (%)	9	10
Calcium (%)	1.0	1.0
Available phosphorus (%)	0.45	0.40
Metabolisable Energy (Kcal/Kg)	2800	2900

Table 2. Proximate analysis of starter, finisher and probiotic.

Ingredient	Starter	Finisher	Probiotic
Dry matter (%)	92.92	93.55	93.82
Crude protein (%)	18.83	15.81	34.87
Crude fibre (%)	7.42	9.92	9.13
Oil (%)	6.08	5.29	8.56
Ash (%)	5.40	8.33	8.03
NFE (%)	62.27	60.65	39.41

2003; Park et al., 2005). The use of probiotics is aimed at stabilizing the microbial communities and the health-promoting effects have an important position in the medical health care of different age groups (Mikelsaar and Zilmer, 2009). *Saccharomyces cerevisiae* yeast is rich in biologically important proteins, B-complex vitamins, trace minerals and several unique 'plus factors'. Other identified beneficial factors include the enhancement of phosphorus availability (Glade and Biesik, 1986; Brake, 1991; Moore et al., 1994) and nutrient utilisation by animals (Thayer et al., 1978; Erdman, 1989; Pagan, 1990), reduction in cases of disease infection (Line et al., 1997) and improvement of feed efficiency (Onifade and Babatunde, 1996). However, there are still conflicting reports on the beneficial effect of yeast inclusion in poultry diets. Hayat et al. (1993) suggested that the beneficial effects of *Saccharomyces* dried yeast in feeds may be influenced by the birds' genome. In rats, *Lactobacillus plantarum* and *Bifidobacterium infantis* added to the feed caused a decrease in alanine aminotransferase (ALT) activity (Osman et al., 2007), but addition of *S. cerevisiae* significantly increased the activities of serum ALT and alkaline phosphatase (ALP) (Mannaa et al., 2005). Some researchers (Bradley and Savage, 1985; Hayat et al., 1993) have attributed the increase in mineral retention and better bone mineralization of broilers to supplementation with mannan-oligosaccharide probiotic. Also, *S. cerevisiae* cells are engineered to produce active 1, 25 (OH)-2D3 receptor (Donald et al., 1989). Ross et al. (1978), Bowes et al. (1989), Krasnodebska-Debta and Koncicki (2000), and Silva et al. (2007) reported no significant differences observed in inorganic phosphorus (P), sodium (Na), potassium (K), chloride (Cl) and iron (Fe) concentrations in the blood of experimental birds, and the levels of all analyzed minerals remained in a wide range of physiological values defined for 6-week-old meat type chickens. We hypothesized that different levels of supplemental yeast have effect on the performance indices, serum enzymes and electrolytes of broiler chickens. The aim of the study was to investigate the effect of supplemental probiotic preparation on performance indices, serum enzymes and electrolytes of broiler chickens.

MATERIALS AND METHODS

Experimental design and animal management

The experiment was carried out on hybrid broiler Marshall (n = 200). Two hundred (200) day-old chicks were randomly selected and distributed into four groups of 50-day-old chicks each (Control, C; $E_1$0.5%, $E_2$1.5% and $E_3$2.0% experimental groups). They were housed in an environmentally controlled poultry house with the floor covered with wood shavings and kept dry throughout the experimental period by routine replacement of the spoiled litter. The feeding lasted 42 days. Chicks were fed commercial broiler starter diet for the first 28 days of age, and pelleted finisher diet from 29 to 42 days of age. Ingredient and nutrient compositions of diets and proximate analysis of diets and probiotic are shown in Tables 1 and 2, respectively. Feed and water were provided *ad libitum*. Body weight (BW), feed conversion ratio (FCR), feed intake (FI) and mortality were recorded weekly for comparative evaluation and interaction effects of all treatment groups. The birds were weighed individually at weekly intervals and body weight gains were calculated from the values obtained. Feed conversion ratio was calculated by the standard formula using total feed intake in g/bird, divided by the total weight gain (g) for each period. Feed intake was calculated as the difference between the amount of feed supplied to the birds and the amount of feed that remained at the end of each feeding period. In order to determine mortality, daily observations were made every morning and evening to record the occurrence of deaths in different experimental groups.

Blood sampling and analyses

After 42 days of feeding, blood was collected from 10 randomly selected broiler chickens from each group through the brachial vein into polythene tubes. Serum was obtained by first allowing the blood to clot, followed by centrifugation at 2000 *x g* per minute. Colourimetric methods were used to determine the activity of serum aminotransferase (AST) (Reitman and Frankel, 1957), ALT and ALP (Kind and King, 1954), respectively. Blood albumin content was assayed using the dye binding method (Spencer and Price, 1977). Serum sodium (Na) and potassium (K) were determined by conventional flame photometry method (Model: Flame Photometer - Corning 410, Essex, UK), while serum calcium (Ca) and phosphorus (P) were determined using cresolpthalein complexone and ammonium molybdate methods by Baginski (1973) and Gomorri (1942), respectively.

Statistical analysis

Graph Pad Prism Software, version 4.03 for Windows (San Diego, California, USA) was used to analyze and the data obtained were expressed as mean ± standard error of the mean (Mean ± S.E.M.). Data were analyzed using repeated measures ANOVA. Dunnett's post-hoc test was used to compare all experimental groups with the control. Values of $p<0.05$ were considered significant.

Table 3. Effects of dietary yeast probiotic supplement on body weight of broilers.

Week	C	$E_1$0.5%	$E_2$1.5%	$E_3$2.0%
1	130.9±2.65	131.6±3.06	129.5±2.75	133.5±2.96
2	302.9±8.86	305.4±8.90	305.5±7.25	306.8±9.41
3	572.8±17.53	566.4±13.11	540.5±13.52	594.7±17.89
4	779.2±14.41	812.8±16.57	800.3±16.52	860.2±21.34[a]
5	1049.0±23.58	1162.0±31.99[b]	1121.0±30.87	1166.0± 31.67[b]
6	1342.0±47.17	1387.0±35.22	1376.0±40.32	1509.0±44.42[b]

[a]p<0.01 vs. control; [b]p<0.05 vs. control; †, the data are presented as mean ± SEM, (n = 40); ‡C, control group (without probiotic); E_1, first experimental group; E_2, second experimental group, and E_3, third experimental group.

Table 4. Weekly feed conversion ratios of broiler chickens supplemented with probiotic.

Week	C	$E_1$0.5%	$E_2$1.5%	$E_3$2.0%
1	1.14	1.51	1.47	1.37
2	1.45	1.44	1.46	1.41
3	1.31	1.33	1.29	1.23
4	1.24	1.24	1.24	1.19
5	1.16	1.21	1.14	1.26
6	1.21	1.56	1.19	1.18

C, Control group (without probiotic); E_1, first experimental group; E_2, second experimental group; E_3, third experimental group. Value of n = 40.

RESULTS

Body weight of broiler chickens

Body weights from week 4 in $E_3$2.0% probiotic supplemented group differed significantly (p<0.01) when compared with control as shown in Table 3. On the 5th week, body weights differed significantly (p<0.05) in $E_1$0.5 and $E_3$2.0% probiotic-supplemented groups, respectively when compared with the control group. Starting from week 1 to 3, body weights of broiler chickens did not differ (p>0.05) when compared with that of the control group. Overall, the body weight obtained in $E_3$2.0% probiotic supplementation at the 6th week was higher (p < 0.05) than that of the control group (C).

Feed conversion ratio of broiler chickens

A significant improvement in feed conversion ratio was recorded in E_2 and E_3 probiotic supplementation. Feed conversion ratio, for Marshall broiler chickens supplemented with 1.5 and 2.0% dietary probiotic ranged from 1.14 to 1.19% and the experimental groups possessed the highest body weights (Table 4). At 6th week of age, there were significant (p < 0.05) changes in the feed conversion ratio of broiler chickens in experiment $E_2$1.5 and $E_3$2.0% probiotic supplementation when compared with the control.

Serum enzymatic profile of broiler chickens

Serum ALT activity decreased significantly (p < 0.05) in experimental group $E_1$0.5% when compared with control (C). Also, there was a highly significant difference (p < 0.01) in ALT activity in experimental group $E_2$1.5% when compared with control group. Serum ALP activity decreased significantly (p < 0.05) in the experimental groups $E_1$0.5 and $E_2$1.5%, when compared with the control group. There was no significant difference in the activity of AST (Table 5).

Mineral parameters and serum electrolytes of broiler chickens

As shown in Table 6, there was a significant (p < 0.05) difference in calcium concentration in the probiotic supplemental group $E_1$0.5% when compared with the control. Also, a highly significant difference (p < 0.01) in phosphorus concentration was observed between experimental group $E_1$0.5% and control. Sodium ion concentration decreased significantly (p < 0.05) in experiment $E_2$1.5% probiotic supplementation, when compared with control group. Highly significant (p < 0.01) difference in potassium concentration was observed in experimental groups $E_1$0.5, $E_2$1.5 and $E_3$2.0%, respectively.

DISCUSSION

Body weight of broiler chickens

The result showed that probiotic supplementation had no effect on body weight of broiler chickens from the 1st to 3rd week of life. The delay in the manifestation of the probiotic effect may be due to the time it takes for the

Table 5. Effect of probiotic supplementation on activity of serum enzymes of broiler chickens.

Activity of enzyme	C	$E_1$0.5%	$E_2$1.5%	$E_3$2.0%
AST (IU/L)	233.1±10.86	248.6±17.00	251.8±24.23	234.8±8.24
ALT(IU/L)	86.40±1.64	76.00±3.50[a]	75.00±2.92[b]	79.90±1.38
ALP (KAU/L)	172.7±2.31	148.1±9.57[d]	143.2±10.28[c]	157.6±1.88

C, Control group (without probiotic); E_1, first experimental group; E_2, second experimental group; E_3, third experimental group; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase. Mean ± S.E.M, (n = 10) [acd]$p< 0.05$ vs. control, [b]$p<0.01$ vs. control.

Table 6. Effect of probiotic supplementation on mineral parameters and serum electrolytes of broiler chickens.

Parameter	C	E10.5%	E21.5%	E32.0%
Calcium (mmol/L)	3.49±0.14	2.55±0.18*	3.15±0.37	3.38±0.10
Phosphorus (mmol/L)	2.07±0.03	1.88±0.05**	1.98±0.05	2.13±0.0 3
Sodium (mmol/L)	160.4±5.33	153.5±3.37	148.5±3.10*	153.2±1.98
Potassium (mmol/L)	4.10±0.25	3.24±0.19**	2.90±0.12**	2.97±0.15**

C, Control group (without probiotic); E_1, first experimental group; E_2, second experimental group, E_3, third experimental group. Mean ± S.E.M, (n = 10) *$p < 0.05$ vs. control, **$p < 0.01$ vs. control.

yeast to re-establish the conditions of eubiosis in the digestive tract. However, from the 4th to 6th week, body weight increased ($p<0.05$) between control and experimental groups E_2 and E_3, but not in group E_1 birds fed the lowest probiotic level of supplementation. This finding demonstrates that probiotic supplementation has more positive effect on body weight when administered in higher concentrations. Santin et al. (2001) showed that cell walls of *S. cerevisiae* improve nutrient absorption from the intestinal mucosa and suggested that this factor may be responsible for the improvement in performance of broilers supplemented with *S. cerevisiae*.

Although the mechanism of action of the probiotic was not investigated in the present study, Nilson et al. (2004) demonstrated that probiotics act by reducing the feed conversion ratio, resulting in increased daily live weight gain. This is achieved through improvement of digestion by a balanced composition of the resident gut microflora. Essentially, they help the animal to fulfil its genetic potential. The significant decrease in feed conversion ratio obtained in the present study agrees with the findings in the field studies conducted by Pradhan et al. (1998), Richter et al. (2000), Cmiljanic et al. (2001), Banday and Risam (2002) that probiotic supplementation improved performance in broiler chickens. According to Ashayerizadeh et al. (2009), prebiotics and probiotics are growth promoters that may be used as alternative non-antibiotic feed additives. This is because they improve growth indices of broiler chickens without side effects on the consumers. Similar findings on the positive effect of probiotics on growth performances have been well documented by Sieo et al. (2005), Apata (2008) and Yu et al. (2008).

Feed conversion ratio of broiler chickens

The result of the present study is in agreement with the finding of Jin et al. (1998), who reported a significant improvement in feed conversion ratio in probiotic-supplemented broilers, but with inconsistent results. Improvement in feed conversion ratio might be due to efficient ileal digestibility of nutrients (Sahane, 2001; and Pelicia et al., 2004). Bansal et al. (2011) reported significant and better weekly feed conversion efficiency on probiotic supplementation in the diet of commercial broiler chicks. The improvement in feed conversion ratio of the probiotic supplemented groups is an indication that they had better feed utilisation than the control group.

Serum enzymatic profile of broiler chickens

The decrease in activities of serum ALT and ALP of the broiler chickens were recorded in all the probiotic supplemented groups, when compared with the control. Also, serum ALP level decreased in all the experimental groups. The decrease in ALT activity obtained in the present study agrees with similar observations made in studies on rats in which addition of *L. plantarum* and *B. infantis* to diets fed to rats decreased ALT activity (Osman et al., 2007). In another study, Mannaa et al. (2005) showed that the addition of *S. cerevisiae* caused significant increase in serum ALT and ALP activities. The differences in the enzymatic activity may be due to animal species and probiotic interventions. In this study, no significant differences ($p > 0.05$) between control group and probiotic supplemented groups were observed in the activity of AST, and similar results were observed in broiler chickens fed probiotic supplemented diet (Baidya et al., 1994; Panda et al., 2000). Any abnormal increase in serum levels of AST, ALT and ALP may imply liver damage (Yalcin et al., 2012); therefore, the relatively stable levels of AST may be associated with hepato-protective effects of the yeast probiotic.

Mineral parameters and serum electrolytes of broiler chickens

Mineral and serum electrolyte indices are presented in Table 6. In the present study, the probiotic supplemented group $E_1$0.5% was able to maintain the calcium (Ca) and available phosphorus (aP) levels at a constant ratio of 2:1. This is in agreement with the result obtained in probiotic-supplemented rabbits in which a dietary relationship of Ca to available P of 2:1 to 1.5:1 was reported (Vandelli, 1995). The reason for the maintenance of the Ca available P (aP) levels at relatively constant ratio of 2:1 may be attributed to the yeast probiotic's role in maintaining Ca and P homeostasis in blood of birds; albeit, mineral homeostasis is regulated by both neural and humoural mechanisms. This finding is very important because rapid growth rate in broiler chickens rearing is often associated with skeletal abnormalities (Scott, 2002). Leg weakness in broiler chickens represents both an economic and an animal welfare concern, and it is often singled out as being of particular importance. Birds are unable to eat and drink because of the pain associated with the pathology of leg weakness (Garner et al., 2002). Also, Akhavan-Salamat et al. (2011) reported that addition of yeast in diets increases bone calcium values which improves bone force in broilers. Panda et al. (2006) reportted that addition of probiotic strain (*Lactobacillus sporogenes*) had a positive effect on bone breaking strength and bone ash content, attributed to the favourable environment in intestinal tract due to feeding of probiotic strains. The mineral content in the serum of birds is considerably dependent on its mineral concentration in feeds as well as factors influencing the degree of their absorption in the digestive tract (Monika et al., 2012).

Concentrations of serum sodium (Na) in probiotic supplemented groups were not significantly different, when compared between groups. However, significant difference was obtained when the concentration of Na in the experimental group $E_2$1.5% was compared with that of the control group (C). This result agrees with the finding of Silva et al. (2007) in which serum Na concentrations recorded fell within the range of 136.4 - 150.1 mmol/L for Hybro-PG broilers aged between 21 and 35 days. Highly significant difference was observed in serum potassium (K) concentrations in probiotic supplemented groups. Decreases in K concentrations tend to follow the different percentages of probiotic supplementation within groups. Both serum Na and K concentrations were within the normal range, indicating that supplementation of broiler chickens diets with yeast probiotic did not alter the ratio of electrolytes in the blood.

In conclusion, supplementing broiler feeds with yeast probiotics improved performance indices of broiler chickens, serum enzyme activities and maintenance of electrolyte homeostasis. Further investigation is required to determine the optimum dose for yeast probiotics in order to enhance production efficiency in broiler chickens.

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