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Effect of Dietary Inclusion of Cassava Yeast as Probiotic Source on Growth Performance, Small Intestine (Ileum) Morphology and Carcass Characteristic in Broilers

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Abstract: The study was conducted to investigate the effect of dietary inclusion of cassava yeast as a probiotic source on growth performance, ileum morphology and carcass characteristic. Two hundred and forty 1-day old broiler chicks of both sexes were used for 42-days. The chicks were randomly allocated to 24 pens containing 10 chicks each with 6 replicates and assigned to receive one of 4 dietary treatments (1. Control, 2. *S. cerevisae* $1x10^6$ organisms/kg, 3. *S. cerevisiae* $1x10^7$ organisms/kg, 4. *S. cerevisiae* $1x10^8$ organisms/kg) in a completely randomized design. There was no significant difference in the feed intake starter period, feed conversion ratio, average daily gain and dressing percentage. Significant differences were observed in feed intake finisher period, tight percentage and villus height. The results of the present experiment showed that dietary inclusion of cassava yeast as a probiotic to broiler seems to have minimal influence on broiler performance. Also, further research is required to determine the dose and form of the probiotic used.

Key words: Probiotic, cassava yeast, growth performance and broilers

Introduction

The use of antibiotics for growth promotion in poultry species has been banned in Europe and the United States (Ahmad, 2006). As a result, nutritionists and production managers have become interested in compounds that may serve as possible replacements. Probiotics are one of the approaches that have a potential to reduce chances of infection in poultry. There many probiotics used in poultry feed such as, lactobacillus and bifidobacteria (Ziggers, 2000), Lactobacillus strains (Lan *et al.*, 2003), protexin® (multi-strain probiotic) (Ayasan *et al.*, 2006; Ahmad, 2004; Gunal *et al.*, 2006), *Saccharomyces cerevisiae* (Lila *et al.*, 2004; Ghasemi *et al.*, 2006; Adams *et al.*, 1995), Thepax® (Yousefi and Karkoodi, 2007). The probiotics have been shown to improve feed conversion efficiency (Ayanwale *et al.*, 2006; Day, 1997), improve weight gain and reduce mortality (Jin *et al.*, 1997), reduce disease infection (Line *et al.*, 1997) and stimulate the immune system (Havenaar and Spanhaak, 1994).

Yeast products, such as *Saccharomyces cerevisiae,* have been used as supplements in animal feed for decades. Live yeast addition to animal feed has been known to improve the nutritive quality of feed and performance of animals (Matin *et al.*, 1989). In addition, mannan oligosaccharides and fructo-oligosacharide derived from the cell wall of the yeast *Saccharomyces cerevisiae*, has shown promise in suppressing enteric pathogens and modulating the immune in studies with poultry (Santin *et al.*, 2001; Spring *et al.*, 2000; Iji *et al.*, 2001).

Cassava yeast is a naturally produced live yeast culture of *Saccharomyces cerevisiae* together with its growth medium of cassava, soybean, sugar cane molasses, urea, $MgSO_4$ $2H_2O$, KH_2PO_4 and citric acid. Cassava yeast are composed of natural concentrate mixture of essential nutrients, live cell of yeast, mannan oligosacharide derived from cell wall of dead cell and vitamin B-complex. This work therefore, has the objective of evaluating the supplemental effect of cassava yeast (*Saccharomyces cerevisiae* plus the growth medium) on growth performance, small intestine (Ileum) morphology and carcass characteristics in broilers.

Materials and Methods

Preparation of cassava yeast: Cassava chip and soybean meal were ground using a hammer mill and both flours were subjected to fermentation. Pure strain of *S. cerevisiae* was sub-cultured into 730 mL nutrient solution (yeast (5 g), sugar cane molasses (24 g), urea (10 g), $MgSO_4$ $2H_2O$ (7 g), KH_2PO_4 (13 g) and citric acid (20 g)) and inoculated into 1 kg of flour (cassava (900 g) and soybean (100 g) and then allowed to ferment for 15 hr. The product obtained was subsequently dried at room temperature. The cassava yeast is composed of yeast (*Saccharomyces cerevisiae*) and the medium on which it was grown. Dried cassava yeast sample was chemically analyzed in the laboratory for proximate constituents (AOAC, 1990) Viable cell counts on the product was estimated using the methylene blue staining method (Lindegren, 1949). This product contains, dry matter 88.28%, ash 8.69%, crude protein

Table 1: Ingredients composition (%) of broiler starter and finisher diets different levels of cassava yeast

Ingredients	Diets* T1	T2	T3	T4
Broiler starter				
Yellow maize	50.20	50.10	49.30	42.50
Vegetable oil	5.20	5.20	5.20	5.00
Soybean meal (42% CP)	35.20	35.30	35.30	35.00
Fish meal (60% CP)	7.00	7.00	7.00	7.00
Cassava yeast	0.00	0.08	0.80	8.10
Dicalcium phosphate	1.00	1.00	1.00	1.00
Limestone	0.70	0.70	0.70	0.70
Vitamin premix	0.50	0.50	0.50	0.50
DL-Methionine	0.25	0.25	0.25	0.25
Broiler finisher				
Yellow maize	55.70	55.70	55.00	47.50
Vegetable oil	5.00	5.00	5.00	5.00
Soybean meal (42% CP)	31.00	31.00	31.00	31.00
Fish meal (60% CP)	5.00	5.00	5.00	5.00
Cassava yeast	0.00	0.08	0.80	8.10
Dicalcium phosphate	2.00	2.00	2.00	2.00
Limestone	0.70	0.70	0.70	0.70
Vitamin premix	0.50	0.50	0.50	0.50
DL-Methionine	0.25	0.25	0.25	0.25

*T1: 0% cassava yeast, T2: 0.08% cassava yeast, *S. cerevisiae* $1x10^6$ organisms/kg, T3: 0.8 % cassava yeast, *S. cerevisiae* $1x10^7$ organisms/kg, T4: 8.1% cassava yeast, *S. cerevisiae* $1x10^8$ organisms/kg

9.94 %, crude fiber 5.61 %, ether extract 0.42 %, nitrogen free extract 75.34 % and living cell of yeast 1.23 x 10^6 organisms/g.

Experimental birds: A total of 240 one-day old broiler chickens, having 51 g average body weight, were randomly divided into 24 separate floor pens (each 3x1.5 m) each comprising 10 chicks and six pens (replicates) per treatment group following a completely randomized design. The experimental house was thoroughly cleaned and disinfected before the arrival of chicks. Experimental chickens were maintained under standard management conditions for 42 days on deep litter system. The brooder temperature was maintained at about 95°F up to 7 days and gradually decreased to 85°F by 14th day of age, after which the chickens were kept at room temperature.

The experimental chicks were fed broiler starter form one-day old to three weeks and broiler finisher rations in the four to sixth week *ad libitum*. The birds had free access to feed and clean drinking water. All chicks were vaccinated against new castle disease and infectious bronchitis disease.

Experimental diets: Four isonitrogenous and isocaloric broiler starter and finisher diets in the form of mash were prepared (NRC, 1994). The different cassava yeast such as 0, 0.08, 0.8 and 8.1 % were used in both the broiler starter and finisher diets (Table 1 and Table 2) fed to the experimental groups T1, T2, T3 and T4, respectively. The control diets had no *Saccharomyces cerevisiae* while diets T2, T3 and T4 contained *S. cerevisiae* at $1x10^6$, $1x10^7$, $1x10^8$ organisms/ kg. Yellow maize, vegetable oil, fishmeal and soybean meal served as main energy and protein sources. Each diet was analyzed as described methods in AOAC (1990).

Table 2: Percent nutrient composition of broiler starter and finisher diets with different levels of cassava yeast

Nutrient composition	Diets* T1	T2	T3	T4
Broiler starter				
Dry matter, %	94.52	93.73	94.17	94.84
Ash, %	7.14	6.91	6.78	7.21
Crude protein, %	25.72	25.57	24.45	24.01
Crude fiber, %	4.97	4.52	2.76	5.54
Ether extract, %	8.00	7.48	6.82	7.10
Calcium, %	0.61	0.72	0.63	0.67
Phosphorus, %	0.53	0.46	0.49	0.52
Lysine#	1.51	1.52	1.52	1.52
Methionine and cystine#	1.02	1.02	1.02	1.00
Tryptophan#	0.29	0.29	0.29	0.29
Treonine#	0.93	0.93	0.93	0.92
Metabolizable energy (kcal/kg) #	3,200.00	3,200.00	3,201.00	3,200.00
Broiler finisher				
Dry matter, %	89.64	90.41	89.56	93.31
Ash, %	8.30	7.03	7.15	8.60
Crude protein, %	22.51	24.04	22.60	21.09
Crude fiber, %	5.23	2.85	3.59	3.25
Ether extract, %	6.52	7.43	6.30	7.62
Calcium, %	0.73	0.70	0.79	0.80
Phosphorus,%	0.37	0.32	0.36	0.33
Lysine#	1.30	1.30	1.30	1.31
Methionine and cystine#	0.95	0.95	0.95	0.93
Tryptophan#	0.26	0.26	0.26	0.26
Threonine#	0.83	0.83	0.83	0.82
Metabolizable energy (kcal/kg) #	3,200.00	3,203.00	3,205.00	3,206.00

*T1: 0% cassava yeast, T2: 0.08 % cassava yeast, S. cerevisiae $1x10^6$ organisms/kg, T3: 0.8% cassava yeast, S. cerevisiae $1x10^7$ organisms/kg, T4: 8.1 % cassava yeast, S. cerevisiae $1x10^8$ organisms/kg # Calculated values.

Parameter measured: Body weight gain and feed intake per pen were recorded at weekly intervals. The efficiency of feed utilization was calculated as feed intake per unit body weight gain. Mortality was recorded daily. At the termination of the experiment, two birds from each replicate were randomly selected, slaughtered and eviscerated to record carcass, breast, tights, wing, gizzard and liver yield. The small intestine was opened immediately after killing and ileum was sampling defined as extending form Meckel's diverticulum to point 4 cm to distal. They were fixed immediately in formalin for measurement of villus height, width and crypt depth. Samples were fixed in formalin, dehydrated with an alcohol - xylene sequence and embedded in paffin.

Table 3: Performance of broiler fed diets containing different levels of cassava yeast

Criteria	Diets* T1	T2	T3	T4	SEM
Initial body weight, g	50.68	50.95	51.22	51.15	-
Final body weight, g	1,597.97	1,554.36	1,548.25	1,537.25	-
Body weight gain, g/h/d					
0 to 21 d	22.56	23.25	23.92	23.12	0.40
22 to 42 d	52.46	48.35	45.72	49.35	1.20
Feed intake, g/h/d					
0 to 21 d	40.91	40.80	40.97	40.95	0.29
22 to 42 d	81.04^{a}	71.17^{b}	73.24^{b}	80.76^{a}	1.28
Feed conversion ratio					
0 to 21 d	1.81	1.74	1.70	1.75	0.02
22 to 42 d	1.75	1.57	1.67	1.71	0.05

*T1: 0% cassava yeast, T2: 0.08 % cassava yeast, *S. cerevisiae* 1x10^6 organisms/kg, T3: 0.8 % cassava yeast, *S. cerevisiae* 1x10^7 organisms/kg, T4: 8.1 % cassava yeast, *S. cerevisiae* 1x10^8 organisms/kg.

Three pieces of 5 µm slices were prepared and strained with hematotoxylineosin. Villus height, width and crypt depth were determined at a magnification of 10x.

Statistical analyses: All data obtained from the trials were subjected to the analysis of variance procedure according to a completely randomized design. Means were separated by Duncan New's Multiple Range Test. The level of significance was determined at P<0.05.

Results and Discussion

Chemical composition of dietary treatment: The feed ingredients used in the experiment are shown in Table 1. The ingredients of all treatment are the same, but different in levels of cassava yeast. A chemical composition analysis of the dietary treatments is presented in Table 2. All treatments had a similar chemical composition.

Effects of cassava yeast level on feed intake and feed conversion ratio: The effects of cassava yeast level on feed intake and feed conversion ratio are presented in Table 3. The results show that dietary inclusion of cassava yeast did not affect (P>0.05) the feed conversion ration and feed intake of boiler starter. During finisher period, feed intake was significantly (P<0.05) affected by cassava yeast levels. Comparatively lower feed intake was observed in chicken fed diet with *S. cerevisiae* 1x10^6 organisms/kg and 1x10^7 organisms/kg compared to other diets. However, feed conversion ratio was not affected (P>0.05) by dietary treatment. These results are in an agreement with those of Panda *et al.* (1999); Ergun *et al.* (2000); Mutus *et al.* (2006) who reported that the supplement of a probiotic did not have any effect on feed conversion ratio. Yousefi and Karkoodi (2007) also reported that feed consumption and feed conversion ratio were not affected by the dietary probiotic and yeast supplementation In addition Ahmad (2004) could not detect any difference in the feed conversion ratio of the

Table 4: Carcass characteristic and small intestine (ileum) morphology of broiler fed diets different levels of cassava yeast

Criteria	Diets* T1	T2	T3	T4	SEM
Carcass characteristic					
Dressing percentage	81.18	81.96	81.15	80.92	0.72
Wing, %	9.34	9.49	9.62	10.23	0.17
Tights, %	24.42^{ab}	23.92^{b}	25.71^{a}	24.33^{ab}	0.28
Breast, %	19.85	20.28	20.24	18.75	0.50
Liver, %	3.37	3.42	3.53	3.25	0.08
Gizzard, %	1.94	2.19	2.06	2.06	0.07
Ileum morphology					
Villus height, µm	372.32^{a}	333.72^{ab}	289.67^{b}	326.02^{ab}	13.21
Villus width, µm	53.86	61.73	59.20	58.28	1.97
Crypt depth, µm	104.48	106.75	104.70	118.52	5.95

*T1: 0% cassava yeast, T2: 0.08 % cassava yeast, S. cerevisiae 1x10^6 organisms/kg, T3: 0.8 % cassava yeast, S. cerevisiae 1x10^7 organisms/kg, T4: 8.1 % cassava yeast, S. cerevisiae 1x10^8 organisms/kg

broilers as compared to the control. Some studies show that probiotics supplementation in the feed of chickens improve the feed conversion ratio (Ayanwale *et al.*, 2006; Silva *et al.*, 2000; Day, 1997). The reason for the variable effect of biological additives may be confounded by variations in gut flora and environmental condition (Mahdavi *et al.*, 2005). Several researchers reported that when chicks were housed in a clean environment a probiotic was un affective on performance (Gunal *et al.*, 2006; Anderson *et al.*, 1999).

Effects of cassava yeast level on average daily gain: The average daily gain of broilers fed the diet with different levels of cassava yeast is presented in Table 3. There was no significant difference for boiler starter and boiler finisher. These results are in agreement with Ayanwale *et al.* (2006) who found that yeast (*Sacharomyces cerevisiae*) supplementation in the diets of pullets did not have any effect of body weight gain. Onifade *et al.* (1999) supplementation of yeast to broiler diets did not improve growth rate. However, Santin *et al.* (2001) found that supplementation of feed with *S. cerevisiae* cell wall (0.2%) improved broiler body weight. In contrast, supplementation of feed with *S. cerevisiae* fermented cassava flour had negative effect on the body weight of albino rat (Oboh and Akindahunsi, 2005). But, there are also some reports which disagree with our findings (Ghasemi *et al.,* 2006), the broiler fed the diet supplement *S. cerevisiae* Sc47 improved body weight gain compared with an un supplemented diet. The reason of variation might be related to the strain of yeast, concentration and form of yeast used.

Effects of cassava yeast level on carcass characteristic: The effect of cassava yeast on dressing percentage and cut yields are shown in Table 4. There was no significant difference in dressing percentage, wing, breast, liver and gizzard (P>0.05). These results are in agreement with Karaoglu and Durdag (2005) who found that yeast (*Sacharomyces cerevisiae*) supplementation in the diets of broiler did not have any effect on hot carcass and cold carcass percentage. Other researchers found differences in carcass yield between birds that were fed probiotic and those not fed (Pelicano *et al.,* 2003; Vargas *et al.,* 2002). Tights percentage in broiler fed diet with *S. cerevisiae* (1x 10^7 organisms/kg) were significantly higher than others groups (P<0.05). Miazzo *et al.* (2005) also reported the broiler fed yeast (0.3%) had higher leg muscle than the other group. Loddi *et al.* (2000), on the other hand, observed no differences in leg yield between control and those receive.

Effects of cassava yeast level on ileum morphology: The effect of cassava yeast levels on ileum morphology are presented in Table 4. Villus height was greater in control, *S. cerevisiae* 1 x10^6 organisms/kg, and 1x 10^8 organisms/kg fed birds (P<0.05) than 1x10^7 organisms/kg fed birds. No significant differences were found in crypt depth and villus width among 4 treatments. Generally, probiotics will have increased villus height (Jin *et al.,* 1998; Zhang *et al.,* 2005) but will not effect crypt depth and villus width (Santin *et al.,* 2001). Ahmad (2004) also reported an increase of crypt cell proliferation of the small intestine with the use of probiotics as compared to control. In contrast, Bradley *et al.* (1994) reported that crypt depth in the ileal mucosa was reduced when the broiler diet was supplemented with *S. cerevisiae*. Our findings contrast with the results obtained from mentioned studies. The reason for the variable effect of *S. cerevisiae* additives may be confounded by the strain of yeast, concentration and form of yeast used.

Conclusions: The results of present study showed that dietary inclusion of cassava yeast as probiotic source have no beneficial effect on growth performance and carcass characteristics of broilers. However, dietary inclusions of cassava yeast have negative effect of villus height. Probiotic supplement of broilers maybe more helpful during periods of nutritional and other stress, but under normal environmental and management condition it seems to have minimal influence on performance and carcass characteristics.

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