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EFFECTS OF PHYTASE PRODUCING YEAST IN POULTRY FEED MILLS

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ABSTRACT
Yeast in form of phytase has been implicated as having a buffering effect in the digestive tract and proper feed additives in layers diets with improvement in birds' digestive efficiency. Phytase breaks down phytate by hydrolyzing its phosphate groups and releasing the chelated minerals and proteins in them. The degradation of phytate is, therefore, necessary for the animals to assimilate the nutrients bound in their feed. This study evaluated phytase-producing potentials of yeast species isolated from wastewaters and deployed them in the production as additives in poultry feed. The isolated yeasts were screened for their ability to produce phytase. The highest yeast producing phytate was incorporated into layers feed. These led to an increase in the egg yield on daily basis, egg size, egg mass, feed intake and feed efficiency. It can be concluded that phytate possesses some functional properties which improved the feed.
KEY WORDS: *yeast, phytase, cereals, phytate, layers feed, wastewater*

INTRODUCTION

Most animal feeds are cereal/legume based; having a major limitation of containing phytate that has been shown to chelates micronutrients thereby reducing the bioavailability of those nutrients to the animals. Phytase are enzymes that break down phytate by hydrolyzing its phosphate group thereby releasing the chelated minerals and proteins. The degradation of phytate is important for monogastric animals to digest the nutrients bond in their feed (Rizzello *et al.*, 2010).Yeasts have a lot of potentials among which is phytase production. Phytase hydrolyzes phytate to liberate soluble and thus readily utilize inorganic phosphate. Phytase are produced by various groups of microbes, yeasts being simple eukaryotes and mostly non-pathogenic with proven probiotic benefits can serve as an ideal source for phytase research. Phytase production from yeasts has been fairly well investigated. Pable *et al.* (2014), screened yeast strains from chickpeas for their ability to hydrolyze phytate, and among

these, *Schwanniomyces castellii* (CBS 2863) exhibited high phytase activity. Several yeast species were investigated for extracellular phytase, *Pichia spartinae* (*P. spartinae*) and *P.rhodanensis* exhibited high levels of phytase with the optimum reaction temperature at 75–80°C and 70–75°C, at pH 3.6–5.5 and 4.5–5.0, respectively (Nakamura and Yabe 2000).

According to Pable *et al*. (2014), only twelve isolates were capable of manufacturing phytase from sixty–one samples screened for Phytase production. In another work Pables and his colleagues also investigated 600 yeast isolates and screened for intracellular and extracellular phytase production in which out of them stand out only five (5) isolates).

Therefore this work is designed to screen yeasts isolated from wastes for a probiotic potential and the highest producer of phytase.

MATERIAL AND METHODS

Collection of samples. Four different samples (whey wastewater, cassava wastewater, human urine, and rabbit dung) were used for this study. Whey wastewater was collected from a Fulani settlement at Aduramigba in Osogbo, cassava processing wastewater was collected from a local fufu producer at Ofatedo in Osogbo, human urine was collected from a student donor and rabbit dung was collected from an animal house at Mercy-land unit of Ladoke Akintola University of Technology campus, Osogbo.

Reagent Preparation. All the media and reagents were prepared according to the manufacturers' instructions.

Pre-treatment of Animal Dung. The animal dung was treated before use for this study. They were dried in an oven at 50ºC, pulverized in a mortar, and kept in an air-tight container.

Isolation of organisms (yeasts) from the various wastes. Isolation of yeast was done according to standard procedure (Benson, 2005). One (1) gram of the rabbit dung was weighed into a 10ml universal bottle, emulsified with 5ml physiological saline, and made up to 10ml with physiological saline to form the stock solution. Ten millilitres (10ml) human urine, cassava wastewater, and whey wastewater were put into different universal bottles and labeled for them to serve as stock solutions for each of these samples. The stock sample solutions were serially diluted to the power of 10^{-7} dilution and inoculated on potato dextrose agar using the spread plate method. Chloramphenicol was added to the medium to inhibit bacterial growth. The plates were incubated at 37ºC for 24 hours. Organisms were sub-cultured on separate plates to

obtain pure cultures. Isolated organisms were maintained on a PDA slant at 4 ºC until further use.

Characterization and identification of isolated organisms were done using phenotypic and molecular protocols.

Induction of phytase producing properties in isolated yeasts. Before the screening of the isolates for phytase production, they were induced for enzyme production. The yeast isolates were inoculated into a broth medium composed as follows; 2 g of yeast extract, 20 g of peptone, 10 g of glucose, 0.65 g sodium phytate in a litre of water. They were incubated at 37ºC for 24 hours and centrifuged at 12,000 rpm for 15 minutes. Cell pellets were harvested and inoculated into 20 ml of sterile normal saline and used as stock for further work.

Qualitative Screening of yeasts isolates for phytase production. The induced isolates were screened for phytase production using the agar well diffusion method. The agar medium for the experiment was composed as follows: 15 g of glucose, 5 g of ammonium nitrate, 2 g of calcium chloride, 0.5 g of magnesium sulphate, 0.5 g of potassium chloride, 0.01 g iron sulphate, 0.01 g manganese sulphate, 2 g of sodium phytate, and 15 g of agar in 1 litre of water. The negative control medium had no sodium phytate in it while the positive control contained KH_2PO_4 in the place of sodium phytate. Media preparation and procedures for the experiment were according to standard microbiological protocols as described by (Collins *et al*. 1995). Inoculated plates were incubated at 37ºC for 48 hours. A clear zone around each isolate indicated positive phytase production.

Application of Phytase producing Yeast on feeds mills. *Candida tropicalis* (strain B02) was selected for use in the production of poultry feed based on the results of various tests conducted earlier. The selected yeast strain was cultured on YPD broth at 37ºC overnight. The culture was centrifuged at 4000 rpm for 30 minutes; pellets were collected and washed, and used for the production of poultry feed. The whole grain of clean cereals; white and red sorghum (*Sorghum bicolor*), pearl millet (*Pennisetum glaucum*), and wheat (*Triticum aestivan*) were drily milled separately using a hammer mill. The slurry was prepared by mixing an equal proportion of each of the grains (in ratio 1:1:1:1) in distilled water (10% w/v). A modified method of Ogunremi *et al*. (2015) was followed to add a different combination of sweeteners and spices to the slurry as follows: 1 % salt, 0.2 % black pepper, and 1% red chili powder. The slurry was sterilized at 121ºC for 15 minutes, allowed to cool, the yeast cells were added, and allowed to ferment at 37ºC for 24 h. Control without the yeast cell was produced for comparison. A total of 15 laying hens (ISA Brown) at 50 weeks old was

selected and provided with the experimental diets till 52 weeks old. These experimental birds were divided following a completely randomized design into 5 treatment units (A-type commercial cage) with 3 birds per experimental unit. These birds were offered a standard feed holding with 16 % crude protein and 2,600 k cal/ kg energy. The experiment lasted for 6 weeks.

Statistical analysis. The data generated in this study were expressed as means of two values. The data were analyzed with one-way ANOVA using IBM SPSS 20. The student's t-test was used for comparisons between groups. $P < 0.05$ was considered statistically significant at a 95 % confidence level.

RESULTS AND DISCUSSION

Qualitative screening for production of phytase. The isolated yeast species were qualitatively screened for their ability to produce phytase. Result obtained reveals that *Candida tropicalis* exhibited the highest potential for the enzyme production, while *Geotrichum candidum* showed a very minimal ability to produce the enzyme. This was indicated by the clear zone around each isolate (Plate 1).

Plate 1: Screening for the production of phytase enzyme among four different strains of Yeast
1- represent the zone created by *Candida tropicalis;* 2- represent the zone created by *Candida albicans;* 3- represent the zone created *by Geotrichum candidum; 4*-represent the Zone created by *Saccharomyces cerevisiae*

Effect of yeast supplementation on the laying hens. The effect of yeast supplementation on the digestibility of crude protein of laying hen 50-56 weeks as shown in Table 1. There was an increase in digestibility of feeds as the supplements increased (from 0.1 to 0.25). The effect of yeast supplement on layer birds was shown in Table 2. Increase in the yeast supplement led to increment in the feed intake, egg (yield, size and mass). Table 3 showed the effect of the yeast supplement on the egg quality of laying birds at $P < 0.0001$; the eggs experienced increase in height, width, egg grading and shell thickness.

Table 1: The effect of yeast supplementation on the digestibility of crude protein of laying hen 50-56 weeks

	Yeast Level %					
Age (Week)	Control	0.10	0.15	0.20	0.25	P-Value
50-52	65.55 ±0.62	66.06±0.44	68.22± 0.45	71.42±4.10	72.54±5.4	< 0.02
53-54	62.76±0.12	64.24±0.51	65.11±0.24	70.11±1.2	71.46±2.2	< 0.02
55-56	67.01±0.46	65.45±2.16	68.12±4.2	71.36±2.2	72.52±4.6	< 0.02
50-56	65.11±0.4	65.25±1.04	67.15±1.63	70.96±2.5	73.17±4.1	< 0.02

Table 2: The effect of yeast supplement on layer birds

	Yeast Level %						
	Control	0.10	0.15	0.20	0.25	P-Value	Control
Feed intake(g)/week	149.45±0.41	148.21±0.11	149.75±0.07	149.75±0.07	148.98±0.71	149.45±0.41	> 0.651
Egg yield/week Basis %	1.59±0.79	2.02±0.75	2.07±0.78	2.28±0.86	2.38±1.06	1.59±0.79	< 0.0001
Egg mass (g)/ week	21.89±1.49	21.73±2.14	24.08±1.97	24.11±2.14	25.05±2.12	21.89±1.49	< 0.0001
Egg size / wt (g)	0.66±0.04	0.67±0.06	0.72±0.06	0.73±0.67	0.75±0.64	0.66±0.04	< 0.0001

Table 3: Effect of Yeast supplement on the egg quality of layer birds

Items	**Yeast Level %**					
-	Control	0.10	0.15	0.20	0.25	P-Value
Height (mm)	5.16±0.48	5.37±0.49	5.46±0.39	5.45±0.78	5.43±0.27	< 0.0001
Width	0.66±0.04	0.65±0.06	0.72±0.06	0.71±0.06	0.75±0.06	<0.0001
Albumen Height	6.45±0.61	6.68±0.52	6.75±0.51	6.73±0.4	6.83±0.39	<0.0001
Haugh Unit	105.29±1.81	106.12±1.85	106.27±1.54	106.27±0.71	106.06±1.49	< 0.012
Egg grading	AA	AA	AA	AA	AA	
Shell Thickness	0.44±0.07	0.43±0.07	0.41±0.08	0.41±0.08	0.38±0.09	< 0.017

When phenotypic and molecular tools were used, the isolated yeast species identified were *Candida tropicalis, Saccharomyces cerevisiae, Candida albicans*, and *Geotrichum candidum*. Similar isolates (*Candida tropicalis, Saccharomyces cerevisiae*, and *Candida albicans*) were isolated by Omemu *et al.* (2007) from wastewater of fermented maize. Phytase hydrolyzes phytate during fermentation processes thereby increasing the bio-availability of nutrients (Lopez *et al.* 1983). These four isolated yeast species were evaluated for their ability to produce phytase. Result obtained reveals that *Candida tropicalis* exhibited the highest potential for the enzyme production, while *Geotrichum candidum* showed a very minimal ability to produce the enzyme. Daniel *et al*., (2019) asserted that phytase was produced by various groups of microorganisms including yeasts. Meanwhile, Liu and Yoon (2002) indicated that feed efficiency was enhanced by the addition of yeast into the feed of layers. Also, they assumed that this enhancement might have improved the layers to get better nutrient preservation. Crude protein (CP) digestibility was increased by mounting yeast concentrations. Maximum CP digestibility was noted in hens provided 0.2%, and 0. 25% yeast supplement and minimum in control feed. The increase in CP digestibility due to the addition of yeast to the feed explained the advantage of the yeast diets over feed without yeast diets (Table 1). Phytase supplementation improves nitrogen retention in broiler chickens and improves the digestibility of nitrogen and amino acids. Phytase had also been reported to increase the utilization of methionine, lysine, valine, isoleucine and total amino acids in broiler diets (Biehl and Baker, 1997). Phytase supplementation improves the protein utilization in poultry by countering the anti-nutritive properties of phytic acid (Selle *et al*. 2007). The improvement in growth performance in chickens fed with phytase may be due to increased bioavailability of phosphorus by phytase, increase in feed intake and feed efficiency, enhanced utilization of inositol (Simons *et al*. 1990), improvement in starch digestibility (Knuckles and Betschart, 1987), improved utilization of protein and amino acids and overall utilization of nutrients.

In layers, supplementation of phytase from fungi to a low phosphorus diet was very effective as a replacement for in organic phosphorus. Phytase supplementation in a low- phosphorus diet improves the bioavailability of phytate phosphorus resulting in an increase in feed intake, egg production, egg weight and egg shell thickness. Moreover, the addition of phytase increases body weight gain, feed intake, feed efficiency and overall growth performance in broiler chickens (Singh and Khatta, 2002; Singh *et al.* 2003).

CONCLUSIONS

This study has demonstrated that the isolated yeast species, particularly *Candida tropicalis* has the potential to produce extracellular phytase. This enzyme has been established to be able to hydrolyze phytate, release the bond minerals for assimilation by monogastric animals. Therefore, *Candida tropicalis* can be effectively deployed as a probiotic agent in the production of poultry feed.

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