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Assessment of the effects of supplementing rabbit diets with a culture of *Saccharomyces cerevisiae* using growth performance, blood composition and clinical enzyme activities

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Abstract

The effects of dietary supplementation with a pure culture of *Saccharomyces cerevisiae* at 0.0, 1.5 and 3.0 g kg^{-1} on growth performance, blood composition and clinical enzyme activities in serum of rabbits were studied during a 56-day experiment. Rabbits fed 3.0 g kg^{-1} attained the heaviest ($P < 0.05$) body weight, consumed the highest ($P < 0.05$) quantity of feed and had the best ($P < 0.05$) feed conversion. Rabbits fed 1.5 g kg^{-1} yeast had higher ($P < 0.05$) body weight, feed intake and feed conversion efficiency than the unsupplemented group. The haematocrit, erythrocytes, haemoglobin, serum albumin : globulin ratio, and erythrocytic indices in rabbits fed 3.0 g kg^{-1} were superior ($P < 0.05$) to the unsupplemented group. Other haematological indices were similar ($P > 0.05$); but differential populations of lymphocytes were fewer ($P < 0.05$) and monocytes and eosinophils were larger ($P < 0.05$) in rabbits fed the basal group. Serum Ca^{2+}, globulin, cholesterol, aspartate and alanine aminotransferases, and alkaline phosphatase were higher ($P < 0.05$) in the basal group than with the yeast-supplemented diets. The data obtained suggest that yeast addition significantly improved growth performance, enhanced haematopoiesis, reduced serum cholesterol and maintained the serum enzymes at normal ranges. Furthermore, the activities of the clinical enzymes suggested liver- and bone-specific advantages from supplemental yeast. The effects of yeast were dose-dependent, and there is a need to determine the economically optimum dietary concentration. © 1999 Elsevier Science B.V.

Keywords: Rabbits; Yeast culture; Performance; Blood composition; Clinical enzymes

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1. Introduction

Rabbit production has been recognized as one of the ways by which animal protein intake can be sustainably increased among the populace of the developing countries, and especially tropical countries where there is an abundance of underutilized by-product feedstuffs (Cheeke, 1986; Onifade and Tewe, 1993; Onifade and Abu, 1998). The potential of rabbits derives from their highly prolific nature, relative preference for high-fibre diets, rapid growth rate, efficient feed utilization and economic management among other attributes.

Nevertheless, improved feed formulation and strategies for enhancing the productive potential of rabbits especially in tropical and sub-tropical regions of the world apparently are not fully exploited. Such nutritional strategies will ensure greater productivity of rabbits fed by-product feedstuffs, and will invariably assist in matching rabbit production with domestically available feed ingredients.

One such approach of potential benefit is dietary supplementation with yeast, *Saccharomyces cerevisiae*. Indeed, there is paucity of information on the use of direct-fed microbials like yeast in rabbit diets. Supporting evidence for nutritional feasibility and benefit of dietary yeast has been obtained from our studies with broiler chickens fed yeast (Onifade and Babatunde, 1996; Onifade, 1997).

Therefore, the expediency of promoting sustainable rabbit production, and the potential usefulness of information on the supplemental effects of yeast stimulated the current investigation of the growth performance, haematology and serum clinical chemistry in rabbits fed diets containing different levels of yeast.

2. Materials and methods

Thirty 5–6 weeks old New Zealand White (NZW) rabbits with a mean body weight of 602 g were randomly allocated on weight basis to three dietary treatments. The rabbits were housed individually in metal cages provided with separate facilities for feeding and watering, and thus each rabbit was treated as a replicate. A basal diet (Table 1) was formulated containing predominantly three by-products of considerable importance in West Africa. A commercial yeast culture, YeaSacc$^{1026®}$ containing pure culture of *Saccharomyces cerevisiae* was obtained from Alltech, Nicholasville, KY. The yeast culture was added at three concentrations viz. 0.0, 1.5 and 3.0 g kg^{-1} into the basal diet. This incremental inclusion of yeast gave rise to experimental diets 1, 2 and 3, respectively. The diet without (0.0 g kg^{-1}) yeast culture constituted the control treatment. The unpelleted feed and water were offered ad libitum to the rabbits throughout the 56-day trial. Feed intake, body weight, body weight gain, gain : feed, and protein intake : gain of each rabbit was determined on weekly basis.

Blood samples were collected terminally by ear venipuncture on the 56th day from overnight-fasted rabbits, using sterile needles and syringes. Haematological samples were collected in EDTA-treated tubes, while samples for serum clinical parameters were collected without anticoagulant. Our laboratory clinical procedures for haematological measurements and use of Sigma assay kits for protein and albumin consistent with

Table 1
Composition of the basal diet fed to weanling rabbits[a]

Ingredients	g kg^{-1}
Corn	390.0
Groundnut meal	120.0
Palm kernel meal	160.0
Brewers dried grains	200.0
Maize bran	80.0
Blood meal	24.0
Bone meal	8.0
Oyster shell	12.0
Iodized salt	4.0
Vitamin–mineral premix[b]	2.0
Calculated analysis (g kg^{-1})	
Dry matter	903.8
Crude protein	175.4
Crude fibre	109.4
Ether extractives	26.8
Ash	83.8

[a]Yeasacc$^{1026®}$ containing 10^8 cfu g^{-1} of *Saccharomyces cerevisiae* was added to separate batches of the basal diet at 0.0, 1.50 and 3.0 g kg^{-1} to provide dietary treatments 1, 2 and 3, respectively.
[b]Provided per kilogram of diet: vitamin A, 10 000 IU (retinyl acetate); cholecalciferol, 3000 IU; vitamin E, 8.0 IU (DL-α-tocopheryl acetate); K, 2.0 mg; thiamine, 2.0 mg; pyridoxine, 1.2 mg; cyanocobalamin, 0.12 μg; niacin, 1.0 mg; pantothenic acid, 7.0 mg; folic acid, 0.6 mg; choline chloride, 500 mg; Fe, 60 mg; Mn, 100 mg; Cu, 8.0 mg; Zn, 50 mg; Co, 0.45 μg; I, 2.0 mg; Se, 0.1 mg.

conventional practice have been summarized elsewhere (Onifade and Tewe, 1993). The erythrocytic indices were computed. Serum aspartate and alanine aminotransferases (Reitman and Frankel, 1957), alkaline phosphatase (Wright et al., 1972), cholesterol (Roschlan et al., 1974), creatinine (Slot, 1965), and calcium were determined using atomic absorption spectrophotometry.

The data collected were subjected to *t*-test using analysis of variance (ANOVA) of paired comparisons of all data obtained and means were considered different at $P < 0.05$ using Duncan's Multiple Range Test (SPSS, 1988; Daniel, 1995).

Linear regression analysis for all the parameters indicated positive responses of all parameters to increasing concentrations of the yeast supplement; however, we did not show the equations.

3. Results and discussion

For all the performance indices (Table 2) there were linearly increasing responses to dietary concentrations of *S. cerevisiae*. Body weight and body weight gain at 56 days of age were heaviest ($P < 0.05$) with 3.0 g kg^{-1}, followed by 1.5 g kg^{-1} and least ($P < 0.05$) on the unsupplemented diet. Feed and protein intake pattern of rabbits were identical with body weight and body weight gain. It was therefore obvious that the increasing feed and protein intakes due to the addition of yeast predicate an increase in body weight and body

Table 2
Performance characteristics of rabbits fed basal or yeast-supplemented diets

Characteristics	Yeast (g kg^{-1})						
	0.0	1.5	3.0	SEM	Paired comparison		
	Diet 1	Diet 2	Diet 3		1 vs. 2	1 vs. 3	2 vs.3
Final body weight (g rabbit^{-1})	1489^{c}	1540^{b}	1660^{a}	16.25	**	***	*
Body weight gain (g rabbit^{-1})	887^{c}	942^{b}	1054^{a}	15.60	**	***	*
Feed intake (g rabbit^{-1})	3430^{c}	3610^{b}	3850^{a}	45.45	*	**	*
Gain : feed (g kg^{-1})	256^{b}	261^{b}	274^{a}	3.69	NS	**	*
Protein intake (g rabbit^{-1})	602^{c}	635^{b}	679^{a}	7.55	**	***	**
Protein intake : gain (g kg^{-1})	679^{b}	674^{b}	644^{a}	3.65	NS	**	**

$^{a-c}$Means in the same row without similar supercripts are significantly (P <0.05) different.
*: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$
NS: Not significant.

weight gain of the rabbits. This observation agrees with one of the most consistent mechanisms of growth promotion by yeast culture in turkey poults and broiler chickens (Bradley and Savage, 1994; Onifade and Babatunde, 1996; Onifade, 1997). Furthermore, the addition of 3.0 g kg^{-1} yeast elicited significantly ($P < 0.05$) higher feed and protein conversions than either the 1.5 g kg^{-1} yeast or the basal groups both of which were similar ($P > 0.05$). These observations evidenced a direct relationship between yeast levels and performance of the rabbits as recently reported for broilers by Onifade and Babatunde (1996).

Relating the present experimental results with our previous study (Onifade and Tewe, 1993) in which diets of similar composition and same breed of rabbit were used, we observed that though the average feed intake of rabbits was similar (65.61 vs. 64.82 g rabbit^{-1} day^{-1}), the average daily gain was considerably higher (13.4 vs. 17.4 g rabbit^{-1} day^{-1}). The explanation for this could be the addition of blood meal as animal protein source and yeast in the current study, unlike the previous experiment. Blood meal was supplemented in this experiment because the lack of animal protein source contributed to the low growth rate obtained in our previous study. There is an erroneous belief that rabbits being a herbivorous non-ruminant do not need an animal protein supplement. It is apparent from the current results that the growth performance of rabbits can be significantly improved with improved diet quality employing a cheap animal protein supplement such as blood meal.

For indirect nutritional assessment, an examination of the haematological indices (Table 3) was conducted, and the magnitude of the parameters paralleled the growth performance characteristics. Rabbits fed 3.0 g kg^{-1} yeast had superior ($P < 0.05$) haematological indices, namely, haematocrit (PCV), erythrocytes (RBC), and haemoglobin (Hb) compared to other rabbits fed diets 1 and 2, both of which were similar ($P > 0.05$). Both higher magnitudes of mean corpuscular volume ($P < 0.01$) and mean corpuscular haemoglobin ($P < 0.05$) in rabbits fed 3.0 g kg^{-1} yeast indicated improved haemoglobin synthesis above the other groups since they are within normal ranges. The positive correlations between PCV, RBC, and Hb with performance evinced in this study

Table 3
Blood composition in rabbits fed basal or yeast-supplemented diets

Haematological indices	Yeast (g kg^{-1})						
	0.0	1.5	3.0	SEM	Paired comparison		
	Diet 1	Diet 2	Diet 3		1 vs. 2	1 vs. 3	2 vs.3
Haematocrit (%)	31.00^{c}	34.00^{b}	40.00^{a}	0.85	*	***	*
Haemoglobin (%)	9.71^{c}	10.50^{b}	12.40^{a}	0.44	*	**	*
Erythrocytes (10^{6} ul^{-1})	5.02^{b}	5.51^{b}	6.01^{a}	0.15	NS	**	*
MCV[1] (FL)	61.75^{b}	61.71^{b}	66.56^{a}	0.59	NS	**	**
MCH (pg)	19.34^{b}	19.06^{b}	20.63^{a}	0.25	NS	*	*
MCHC (%)	31.32	30.88	31.00	0.04	NS	NS	NS
Leucocytes (10^{3} ul^{-1})	4.95	4.40	4.90	0.06	NS	NS	NS
Lymphocytes[2]	47^{b}	51^{a}	52^{a}	0.44	*	*	NS
Neutrophils	46	47	47	0.10	NS	NS	NS
Monocytes	4^{a}	2^{a,b}	1^{b}	0.25	NS	*	NS
Eosinophils	3^{a}	–	–	0.30	*	*	NS

[1]MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration.
[2]Leucocyte differentials are expressed as percentage of the total cells count.
$^{a–c}$: Means in the same row without similar supercripts are significantly (P<0.05) different.
*: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.
NS: Not significant.

agreed with our previous results in rabbits and broiler chickens (Onifade and Tewe, 1993; Onifade, 1997; Onifade and Abu, 1998). Eggum (1989) also indicated that haematocrits, erythrocytes and haemoglobin tend to be positively correlated with protein quality and level, thus there is a likelihood of improved feed quality owing to yeast addition in rabbits' diets. This may be an additional mechanism of growth promotion by supplemental yeast. Reasonably, the enhanced haematological profiles in yeast-fed rabbits should not be unexpected since nutrients supportive of maximum performance will invariably elicit optimum haematopoiesis.

Although leucocytes (WBC) were similar ($P > 0.05$) on the treatments, the differential counts were different ($P < 0.05$) as exemplified by the lower populations of ($P < 0.05$) lymphocytes and the higher number of phagocytic monocytes and eosinophils in rabbits fed the basal diet. This was unlike those fed on supplemental yeast that had higher ($P < 0.05$) populations of lymphocytes and lower ($P < 0.05$) monocytes and eosinophils. The trend in rabbits fed unsupplemented diet may be indicative of subclinical infection or just a demonstration of immune competence by rabbits. In a way, the latter reasoning is more likely because the polymorphonuclear leucocytes were similar ($P < 0.05$) in rabbits indicating lack of acute infection, and there is no indication of chronic infection by the lymphocyte populations either.

The results of clinical chemistry as a further measure of the response of rabbits to nutritional regimens are depicted in Table 4. Serum protein, albumin, and globulin were superior ($P < 0.05$) both on 1.5 and 3.0 g kg^{-1} yeast-fed rabbits to the basal group, though a higher total protein was observed on 3.0 g kg^{-1} yeast. Serum total protein and albumin

Table 4
Serum clinical chemistry of rabbits fed basal or yeast-supplemented diets

Biochemical indices	Yeast (g kg^{-1})						
	0.0	1.5	3.0	SEM	Paired comparison ($P <$)		
	Diet 1	Diet 2	Diet 3		1 vs. 2	1 vs. 3	2 vs.3
Total protein (g dl^{-1})	6.1^{c}	6.5^{b}	6.9^{a}	0.09	*	**	*
Albumin (g dl^{-1})	2.9^{c}	3.9^{b}	4.5^{a}	0.15	*	**	*
Globulin (g dl^{-1})	3.2^{a}	2.6^{b}	2.4^{b}	0.08	*	*	NS
Albumin / Globulin	0.91^{c}	1.5^{b}	1.87^{a}	0.09	*	**	*
Cholesterol (mg dl^{-1})	130^{a}	110^{b}	89^{c}	3.75	*	**	*
Creatinine (mg dl^{-1})	0.9	1.0	1.0	0.01	NS	NS	NS
Ca^{2+} (mg dl^{-1})	9.7^{a}	8.3^{b}	8.4^{b}	0.14	*	*	NS
Ca^{2+} / Creatinine	10.78^{a}	8.3^{b}	8.4^{b}	0.27	*	*	NS
Clinical enzymes							
AST[1] IU l^{-1}	315^{a}	155^{b}	157^{b}	16.75	**	**	NS
ALT[1] IU l^{-1}	128^{a}	78^{b}	66^{c}	6.12	**	**	*
ALP[1] IU l^{-1}	321^{a}	117^{c}	189^{b}	18.89	***	**	*

[1]AST, Aspartate aminotransferase; ALT, Alanine aminotranferase; ALP, Alkaline phosphatase.
[a–c]Means in the same row without similar superscripts are significantly ($P < 0.05$) different.
*: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.
NS: Not significant.

have been reported to be directly responsive to protein intake and quality (Eggum, 1989; Onifade and Tewe, 1993; Onifade and Abu, 1998). Serum albumin concentration was significantly lower ($P < 0.05$) in rabbits fed without yeast, probably because of reduced liver synthesis (Eggum, 1989; Walmsley and White, 1994; Rosenthal, 1997).

Furthermore, it was observed that serum cholesterol was significantly lower ($P < 0.05$) in rabbits fed yeast (1.5 and 3.0 g kg^{-1}) than in those fed the basal diet. Reduction in circulating cholesterol with supplemental yeast was remarkable and confirms recent information that the additions of innocuous microorganisms including yeast to diets of rats and broiler chickens decrease serum cholesterol, triglycerides, phospholipids and abdominal fat (Nakano, 1996; Onifade, 1997). Also, in rabbits, dietary vitamin E was found to have a hypocholesterolemic effect (Oriani et al., 1997). However, the mechanism(s) of action remains to be elucidated.

The results of the assessment of three clinically important enzymes are contained in Table 4. From the data obtained, both serum alanine aminotransferase (ALT, EC 2.6.1.2) and aspartate aminotransferase (AST, EC 2.6.1.1) were significantly ($P < 0.05$) lower in rabbits fed yeast (1.5 and 3.0 g kg^{-1}) than in the group fed the unsupplemented diet. This suggests that a low background rate of both enzymes was released into the serum from the liver. The implication is a normal functioning of the hepatic tissues (Walmsley and White, 1994; Rosenthal, 1997). On the contrary, the significantly higher ($P < 0.05$) AST in rabbits fed the diet without yeast suggests considerable leakage of the hepatic enzymes into the blood arising from a certain degree of damage of the hepatocyte and / or other tissues (Walmsley and White, 1994; Rosenthal, 1997). However, such a definitive conclusion was difficult in the present study because there was a lack of morbidity and

mortality associated with the marked increase in AST and ALT. Other non-hepatic conditions, macro- and micro-nutrient deficiencies, might have caused the observed elevated activities (Walmsley and White, 1994; Adisa and Odutuga, 1998). Asanuma et al. (1997) have indicated that in the absence of organ-specific disease, a high serum concentration of the aminotransferases may be a resultant of formation of aminotransferase–immunoglobulin complex.

We observed further that the alkaline phosphatase (ALP, EC 3.1.3.1) activity was highest ($P < 0.05$) in rabbits fed the unsupplemented diet. Also, there was a moderate increase in rabbits fed 3.0 g kg^{-1} yeast above the group fed 1.5 g kg^{-1} yeast. Rosenthal (1997) indicated that growth and bone activity make interpretation of increased serum ALP concentrations problematic, though the enzyme is an important indicator of bone formation and tissue disorder (Walmsley and White, 1994; Woitge et al., 1996). Improved bone mineralization elevates ALP in serum to 1.5–2.5 times the activity present in a normal subject (Walmsley and White, 1994; Woitge et al., 1996; Rosenthal, 1997). Enhanced bone mineralization due to yeast addition is suggested by the similarity in serum Ca^{2+} / Creatinine ratio because a high Ca^{2+} / Creatinine ratio (also in the urine) can be an indicator of osteoporosis or bone resorption. Furthermore, Bradley and Savage (1994) reported increased mineral retention in turkey poults fed supplemental yeast.

On the other hand, the highest ALP, serum Ca^{2+} and Ca^{2+} / Creatinine ratio in rabbits fed the unsupplemented diet may jointly suggest high turnover in bone metabolism in this group, from clinical patterns in human beings (Eastell et al., 1988; Woitge et al., 1996). However, the reason was not obvious and no symptom was presented. The measurement of bone-derived isoenzyme of ALP would improve the diagnostic accuracy of the present clinical judgement. In fact, Woitge et al. (1996) remarked that the clinical sensitivity and specificity of a marker enzyme are highly dependent on the definition of cut-off points or the normal reference interval.

Overall, a decisive diagnostic judgment may be difficult to make however, by integrating the results of the performance indices, it can be confirmed clinically that rabbits fed yeast-supplemented diets have biochemically determinable advantages above their counterparts fed no yeast. Analysis of the clinical chemistry substantially demonstrates liver-specific increased protein (albumin) synthesis; reduced cholesterol synthesis, normal intermediary metabolism and hepatic function as shown by AST and ALT activities. Other advantages are suggestive of bone-specific increased mineralization indicated by augmented ALP activity, and enhanced haematopoiesis.

It can be concluded from this study that the addition of a culture of *Saccharomyces cerevisiae* had a growth stimulating effect and the responses were generally linearly related to the concentration of yeast. However, more studies would be necessary to obtain definitive evidence on organ-specific advantages of supplementing yeast, and determining the economically optimum dietary concentration.

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