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Effect of Oral Administration of Three Probiotic Lactobacillus strains on Alkaline Phosphatase Activity in Albino Rats

Okafor, Arthur C.
Department of Microbiology, Renaissance University ,P.M.B. 01183, Ugbawka, Enugu State, Nigeria

*Corresponding Author: okafor_edu@yahoo.com

Abstract: The response of serum alkaline phosphatase (ALP) activity in albino rats following oral administration of three Nigerian strains of Lactobacillus was investigated. Twenty albino rats weighing between 50g – 65g were grouped into five (groups BD, BM, BL1, BL2 and BL3) without bias. Three groups were orally dosed with the three strains of Lactobacillus each, while two groups received basal diet and sterile skimmed milk, respectively for six days. After which a post treatment period of ten days was allowed before blood samples were collected and analyzed for levels of ALP activity. The weights of the albino rats were also monitored throughout this period. Results indicated that groups BL1 and BL2 had higher levels of ALP activity than other groups, while the third test group (BL3) had the lowest level of ALP. Since the strains had been found to improve liver function in a previous study, the findings of the present study may not connote liver dysfunction given the fact that albino rats in their bone-forming stage of growth always have increased levels of ALP. The rate of increase in weights of albino rats within groups BL1 and BL2 was also highest throughout the study period which supports this explanation. The conclusion is that the use of albino rats in their bone-forming stage of growth could mask the effect of these strains on ALP.

Keywords: Probiotics, Lactobacillus, Albino rats, Serum Alkaline Phosphatase.

INTRODUCTION

An organism is designated “probiotic” (i.e. health promoting) if it is able to survive the low pH and bile salts of the stomach, adheres to intestinal cells and antagonizes pathogenic bacteria [1, 2]. Lactobacilli, which represent an important part of the intestinal micro flora in both humans and animals, have been intensively studied as probiotics [1]. Lactobacilli isolated from sources other than the intestine have also been explored recently as probiotics [3, 4, 5]. The health benefits of probiotics include: alleviation of lactose intolerance [6], cholesterol lowering effect, enhancement of the immune system [7] and improvement of liver function [3, 4].

In the literature, several methods have been formulated to elucidate the various health benefits of lactic acid bacteria, with conflicting findings that are not well explained to aide future studies adopt better methods. It is important to note that there is no universal strain of bacteria that would provide all proposed benefits, not even strains of the same species [8]. This explains the high interest being shown towards determining the probiotic potentials of bacteria from

several sources in order to improve human health. An FAO/WHO[9] working group has suggested that negative findings, from probiotic studies, should be published as such findings add to the totality of evidence available for validation of claims.

The objective of this work was to investigate the effect of oral administration of three probiotic strains of Lactobacillus on ALP activity in albino rats.

MATERIAL AND METHODS

Source of Lactobacillus: Three strains of Lactobacillus, previously isolated from local raw cow milk, identified and found to possess some probiotic features by Okafor and Umeh[4], were used for this study. The strains were coded Lactobacillus AC, Lactobacillus AD and Lactobacillus AE.

Preparation of fermented Skimmed Milk: Prior to oral administration, the fermented skimmed milk was prepared daily by inoculating sterile skimmed milk (10% w/v and autoclaved at 110^0C for 10 minutes) with a strain of Lactobacillus and incubating micro-aerophilically for 18 hours at 37^0C. The bacterial number in the fermented milk was between 10^8 – 10^{10} cfu/ml.

Experimental design: Twenty albino rats of 3 - 4 weeks old were adopted from the Faculty of Veterinary Medicine, University of Nigeria, and Nsukka. The rats were weighed and grouped into five according to treatments to be given. They were fed ad libitum on basal diet and water for fifteen days before treatment. The rats were grouped during the treatment period as follows:

Group BD fed on basal diet alone throughout the period (Control 1), group BM fed on basal diet and administered orally with 0.5 ml of sterile skimmed milk daily (Control 2), group BL1 were fed daily with the basal diet and orally dosed with 0.5 ml of skimmed milk fermented by Lactobacillus AC, group BL2 were nourished daily with the basal diet and 0.5 ml skimmed milk fermented by Lactobacillus AD, group BL3 fed daily on the basal diet and administered orally with 0.5 ml of skimmed milk fermented by Lactobacillus AE. These treatments were carried out for six days, and a post-feeding period of ten days was observed. Individual weight of the rats was monitored once a week, and the mean weight per week was calculated. At the end of the ten-day post-feeding period, the rats were anaesthetized by mild chloroform inhalation, and blood samples were taken from the heart. The blood samples were collected into plain plastic bottles and EDTA bottles for analysis of Alkaline Phosphatase activity.

Determination of Alkaline Phosphatase (ALP) level: One ml of alkaline buffer was added to test tubes according to the number of serum samples (20). Phenyl phosphate substrate (1 ml) was added and left at 37^0C for 5 minutes. Serum samples (0.1 ml) was added, mixed well and allowed to stand in the incubator for 30 minutes at 37^0C. At the end of 30 minutes, the tubes were removed from the incubator, and 0.8 ml of 0.5N NaOH, 1.2 ml of 0.5N sodium

bicarbonate, 1.0 ml of 4-amino antipyrine, and 1.0 ml of potassium ferric cyanide were added to the tubes. The standard was prepared by adding 1.2 ml of buffer, 1.0 ml of phenol and treated as with the test samples outlined earlier. "Blank" tube was added 1.1 ml of buffer, 1.0 ml of water, and was treated like test samples after incubation. All samples were read at 510 nm wavelength spectrophotometrically and the ALP activity was calculated in iu/l [10].

Statistical analysis: Results were expressed as mean ± standard deviation (SD) for each group. The data were processed using one-way analysis of variance (ANOVA). The level of significance was set at $P < 0.05$; difference between means was checked using a two tailed student's t-test.

RESULTS

Abnormal behaviours were not found among the albino rats throughout the period of this study. The composition of the basal diet served ad libitum to the rats is shown in Table 1 which is similar to the diet Oyetayo and Osho[3] used in their study. The composition of the skimmed milk, which served as the carrier of the strains, is presented in Table 2.

Table 1. Composition of basal diet

Ingredients	Levels in diet
Crude protein	19
Fat	8.6
Crude fibre	5.4
Calcium	1.2
Phosphorus	0.4
Lysine	0.8
Methionine	0.3
Metabolisable energy, Kcal/kg	2,900

Manufactured by Vital feed, Plateau state, Nigeria.

Table 2. Composition of Skimmed Milk

Formula/Nutrition	Per 100g of dry powder
Energy	348 kcal
Protein	36.1g
Carbohydrate	52.9g
Fat	0.6g
Sodium	0.5g
Calcium	1280 mg 16% RDA

Product: Premier International Foods (UK) Ltd, Republic of Ireland.
RDA: Recommended Daily Amount.

The mean weights of the rats per group (Figure 1) showed that the weights of the three test groups were significantly higher ($p < 0.05$) than control group

BD. The rate of weight gains with time was highest in groups BL1 and BL2 throughout the 28 days.

The mean levels of ALP activity after the treatment period is presented in Table 3. The result appeared to be surprising because groups BL1 and BL2 had higher mean values than the two control groups. Only group BL3 had lower mean level which was not significantly different ($p < 0.05$) from control groups. This corroborates with the findings of Babazadeh et al.[11], which recorded increased ALP activity among male birds fed synbiotics. However, Zavisic et al.[12] observed reduced ALP activity in all lactobacilli-treated rats. This does not agree with the result of the present study.

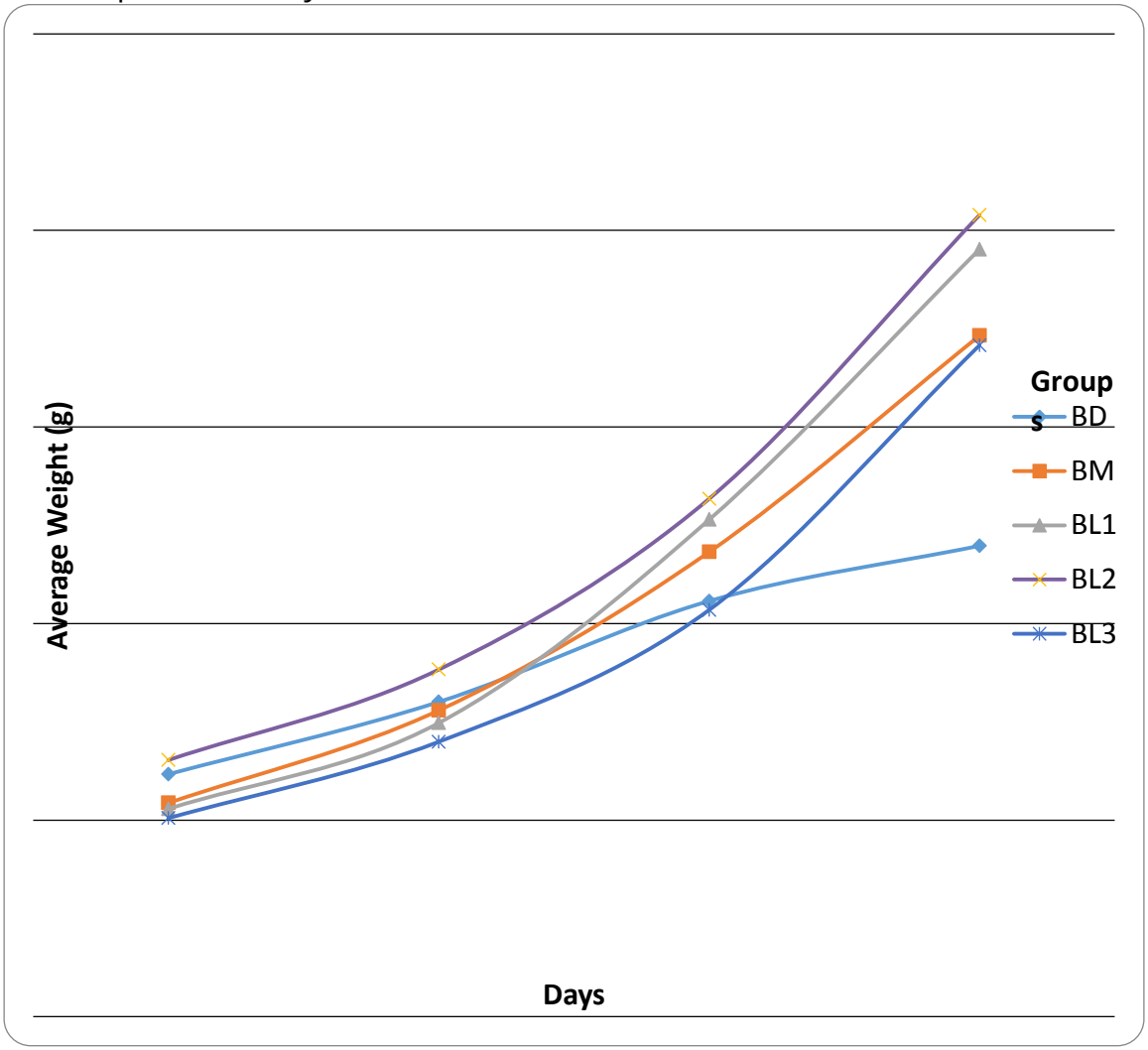

Fig 1. Average weights of the rats expressed per group at various periods

BD: rats placed on basal diet alone. BM: rats placed on basal diet and sterile milk. BL1: rats placed on basal diet and Lactobacillus AC. BL2: rats placed on basal diet and Lactobacillus AD. BL3: rats on basal diet and Lactobacillus AE. Days 0-15: Acclimatization period, Days 16-21: Feeding or treatment period, Days 22-28: Post feeding period.

ALP is an enzyme whose increase is commonly associated with lack of bile flow i.e. cholestasis [13] as a result of obstructed bile duct which is concomitant

with liver disease. Another major cause of increased levels of ALP is rapid growth rate of the animal since it is produced by bone forming cells[14].

Table 3. Mean levels of serum Alkaline Phosphatase (ALP) activity after the treatment period

Groups	ALP (iu/l)
BD	63±7.35[a]
BM	76±7.62[a]
BL1	135±31.53[b]
BL2	151±48.85[b]
BL3	59±4.40[a]

Two means marked by different superscripts along the rows are significantly different P < 0.05

It is fair to note that in a previous study, Lactobacillus AC and AD were found to improve liver function in albino rats[14], which excludes the possibility of liver dysfunction. Thus a cursory look at Figure 1 indicates that these albino (groups BL1 and BL2) were in their rapid stage of growth as evidenced by their high mean weights and could be responsible for the hike in ALP activity compared to control groups. This is further supported by the presence of calcium in the basal diet and skimmed milk served these rats (Tables 1 and 2). Calcium is necessary for bone formation [14].

The findings in this study could serve as supplementary information for assessing the probiotic features of Lactobacillus species.

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