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# Evaluation of probiotic *Lactobacillus plantarum* CS fermented culture and its crude enzymes as chicken growth enhancers

Ujunwa Felicia Nwachukwu* and Uzoamaka Ogechi George-Okafor

*Department of Applied Microbiology and Brewing, Faculty of Applied Natural Sciences, Enugu State University of Science and Technology, P.M.B 01660, Agbani. Enugu,Nigeria.*

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## Abstract

Studies have shown that supplementation of important enzymes in poultry feed can improve the growth of birds. However, there were little or no available information on the utilization of such enzymes or the probiotic fermented culture in local feed production in Nigeria. Therefore, the aim of the study was focused on the evaluation of probiotic *Lactobacillus plantarum* fermented culture and its crude enzymes as chicken growth enhancers. The organism was first screened for amylase and protease producing potential using starch agar and skim milk agar respectively. Thereafter, confirmation of the produced amylase and protease enzymes via shake-flask fermentation at 30°C for 3 days was by 3,5-dinitro salicylic acid and Folin ciocalteu reagents respectively. The produced fermented culture and its recovered crude enzymes (2 %v/v) were respectively supplemented in the locally produced chicken feed for 10weeks. The chicken fed without supplement served as control and all their growths were determined every week. The screening results revealed that *L. plantarum* CS produced more amylase(29 mm) than protease(19 mm) with a total enzyme yield of 22.50 U/ml and 20.92 U/ml respectively, The chicken fed with supplements containing either the enzymes or the fermented culture grew faster and significantly higher (5 kg /10 wk) than those without the supplement (3.0 kg/10 wk)at $p>0.05$. The growth enhanced by the fermented culture ((5 kg/10 wk) was more than that stimulated by either the amylase (3.52 kg/10 wk) or the protease (3.86 kg/10 wk), although not significant. Supplementation with protease enzyme resulted in significant robust and faster growth (3.57 kg/ 7 wk) than that with amylase enzyme (3.37 kg/7 wk). These results indicated the great potentials of both *L. plantarum* fermented culture and its crude enzymes especially protease as chicken growth stimulants.

**Keywords:** Chicken; Enzyme; Fermented Culture; Growth; *Lactobacillus plantarum* CS; Local feed; Screening

## 1. Introduction

Enzymes form part of living organisms that control specific cell metabolic processes through series of biochemical reactions [1]. They are among the most vital product gained for human requirement through plants, animals and microbial at the present time [2].

Although enzymes can be derived from these several sources, the enzyme from microbial sources generally meet industrial demands [2]. The major importance of using microorganisms for enzyme production are; its ability to produce in bulk and the ease at which it can be manipulated for desired products [3].

*Corresponding author: Ujunwa Felicia Nwachukwu
Enugu State University of Science and Technology, Faculty of Applied Natural Sciences, Department of Applied Microbiology and Brewing, P.M.B 01660, Agbani. Enugu,Nigeria.

Most industrial enzymes are secreted by *Bacillus* [4] and *Aspergillus* [5] species. However, recent studies have implicated some *Lactobacillus* species as amylase and protease producers [6, 7]. Amylases and proteases are the most widely in-use industrial enzymes [8].

Amylases as described by [9]; are known starch hydrolases that split it into less complex moieties such as maltose. Their applications in various industries such as pharmaceutical, detergent, food textile, baking and starch conversion industries have been on the increase [10]. Similarly, proteases are also utilized in food, detergent and pharmaceutical industries among others [11, 12]. These enzymes can be applied exogenously in poultry industry as recognized that the addition of enzymes in poultry feed as supplements greatly influenced growth [13]. It was found that gastrointestinal tract of a bird naturally produces enzymes that facilitate the digestion of its nutrients but does not have enough enzymes to completely digest fibre, hence the need for exogenous enzymes in their feeds for proper digestion [13].

Considering the high cost of commercial exogenous enzymes in Nigeria, coupled with the unawareness on their efficiency in poultry by some poultry feed producers, some locally formulated feeds as observed by their contents were neither fortified nor supplemented with such. Hence, the need to assess the efficacy of probiotic *L. plantarum* and its crude enzymes in promoting the growth of our local chickens. The interest on probiotic *L. plantarum* CS was based on the advantages also associated with probiotics in poultry [14].

## 2. Material and methods

### 2.1. Source of the test organism

The test organism *L. plantarum* CS was from stock culture previously isolated and characterized by [15, 14].

### 2.2. Source of chicken and their feeds

The two grades of chicken feeds (starter and finisher) used for this study were purchased at New market Enugu, Nigeria. The feed which comprised of maize, soybean meal, wheat offal, soy oil, salt, sodium bicarbonate, limestone, Di-calcium phosphate, bone meal, Di-methionine, L-lysine, L-threonine, choline chloride, vitamin/mineral, premix, pigment premix, natural growth promoters, toxin binder, carbohydrate and phytase enzymes(Crown Flour Mill LTD, Tin-Can Island, Apapa-Lagos Nigeria). Fifteen- day-old chickens were used.

### 2.3. Primary screening on agar surface culture

The organism was subjected to amylase and protease screening as described by [17, 18]. The active cells of *L. plantarum* CS were inoculated into starch agar (for amylase) and skim milk agar (for protease) and incubated for 48h at $30^0$C.The presence of amylase and protease shown by zones of hydrolysis was observed and measured with metre rule.

### 2.4. Secondary screening of *Lactobacillus plantarum* Cs by submerged fermentation

The full enzyme production for both amylase and protease was carried out via shake-flask fermentation with 2 %v/v inoculum at 30 $^0$C for 72 h [6, 19]. The fermentation medium for amylase contained peptone(1.0 %), beef extract(1.0 %),yeast extract(0.4%),glucose(2.0%),sodium acetate trihydrate(0.5 %),tween 80(0.1 %),Dipotassium hydrogen phosphate(0.2 %),triammonium citrate (0.2 %), magnesium citrate(0.02 %) andmanganese sulphate tetrahydrate(0.0005 %). The same fermentation medium was used for protease except that skim milk(1 %)replaced peptone as nitrogen source. A section of the fermented culture was centrifuged at 10,000rpm for 10min to recover the cell free supernatants as the crude enzymes while the other was left un-centrifuged to serve as the inoculum fermented culture. The amylase activity of the crude enzyme was determined by DNS method [20] while Lowry *et al*. (1951) method as described by [21] was utilized for protease assay. The amount of enzyme produced was read spectrophotometrically at 540 nm and 660 nm for amylase and protease respectively. A unit of amylase was described as the amount of amylase that hydrolyzed starchy compounds to release one microgram of glucose in a minute while the amount of protease that produced one microgram of tyrosine per minute under experimental conditions served as one unit of protease.

### 2.5. Effect of fermented culture and its crude enzymes in chicken growth

#### 2.5.1. Preparation of chicken house

The chicken house was constructed with wire guaze partitioned with plywood around its body. Its roof was covered with aluminium zinc and fumigated with Hypo detergent and Lambda cyhalothrin 2.5 % (Nanjing Red Sun Company Limited China).Thereafter, it was left for 1 wk prior to use.

### 2.6. Application of whole probiotic culture and crude enzymes in chicken feeding

The broiler chickens were first labeled on their legs and wing feathers with permanent markers designating the type of supplemented feed to be exposed to. A set of 3 chickens was respectively fed with feed supplemented with crude enzyme, supplemented whole culture and control at 2 % concentration. The control sample represented feed without supplement. All the chickens were exposed to the same conditions and treatment. The growth of each set of chicken was determined weekly with a weighing balance (Medifield Equipment and Scientific Ltd, England). A mean mass of the chicken was obtained per week from the duplicated samples.

## 3. Results and discussion

### 3.1. Primary screening of *Lactobacillus plantarum* CS for amylase and protease production

The result revealed that the organism yielded amylase by hydrolyzing, the starch present in starch nutrient agar plate. (figure 1). This result is similar to other reports that revealed *L. plantarum* as a starch hydrolyzing organism on a starch-agar plates [22, 23].

**Figure 1** Starch hydrolysis by *Lactobacillus plantarum* CS (mean result of 29 mm)

The organism also hydrolyzed the protein present in skim milk agar plate (figure 2). The diameter of hydrolyzed zone around the organism was 19 mm.The proteolytic ability of *L. plantarum* was in line with the studies conducted by [24,25] that showed that *L. plantarum* had protease potentials. However, the hydrolytic potential of the test organism (19 mm) was less than that (26 mm) reported by [26].

**Figure 2** Protein hydrolysis of *L. plantarum* CS (19mm)

The recorded zones of hydrolysis indicated a higher amylolytic than proteolytic potential of *L. plantarum*. This could be a reason for its common utilization in starch conversion processes.

### 3.2. Secondary screening of *Lactobacillus plantarum* CS by submerged fermentation

The secondary screening was based on the quantification the enzymes in the fermentation media. The results revealed that the organism produced more amylase (22.50 U/ml) than protease (20.92 U/ml) although there was no significant different between them ($p<0.05$). The production of these enzymes by *Lactobacillus plantarum* CS is an evidence of its potential for many industrial applications. Therefore, it is necessary to optimize this organism for higher enzyme yield.

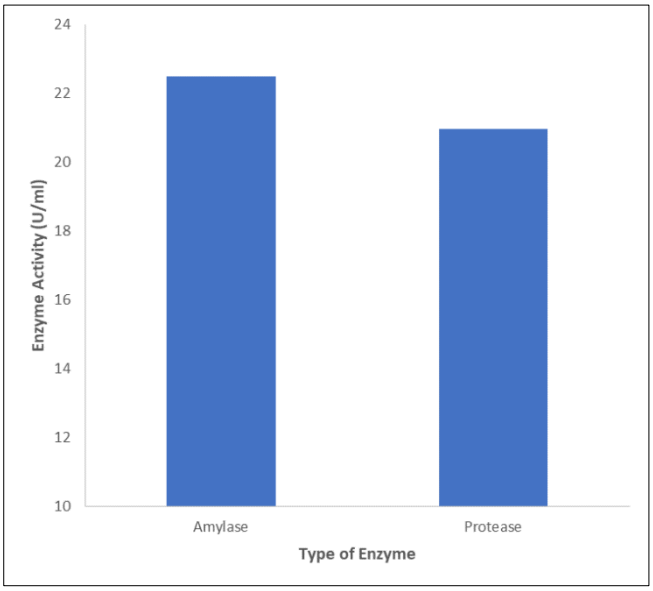


**Figure 3** Secondary screening of amylase and protease production by *Lacto bacillus plantarum* CS

### 3.3. Effect of fermented culture and crude enzymes on chicken growth

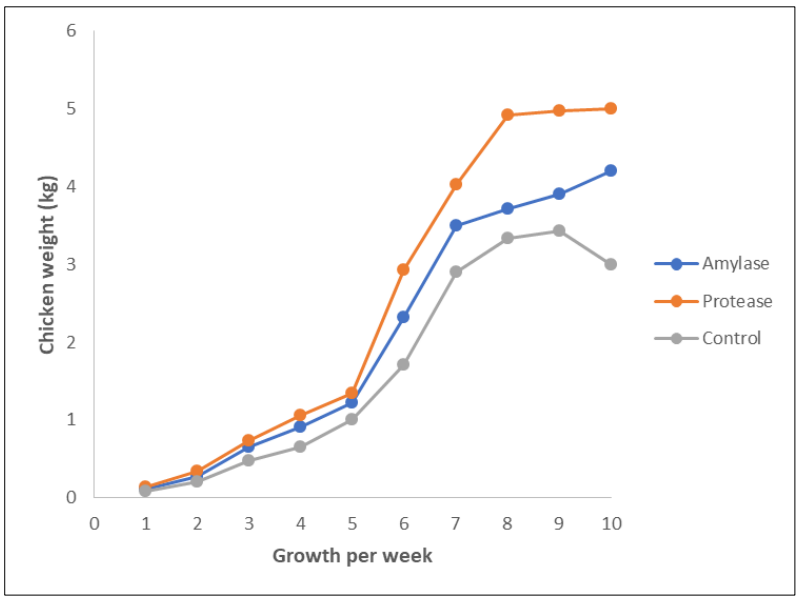


**Figure 4** Effect of Whole *L. plantarum* CS fermented culture on chicken growth

The result revealed that both the fermented culture (fig. 4) and the crude enzymes (fig. 5) enhanced the chicken growth but the fermented culture enhanced the chicken growth more than the crude enzymes. A higher growth rate associated with the fermented culture could be attributed to the findings of [27] which stated that probiotics can be used in poultry feed as a growth promoter. It can also be explained to the fact that the fermented culture contained both the probiotic cell and its crude enzymes. On the part of the enzyme-supplemented feeds, the protease (3.86 kg/10 wk), enhanced the growth of the chicken more than that of amylase (3.52 kg/10 wk)., although not significant.

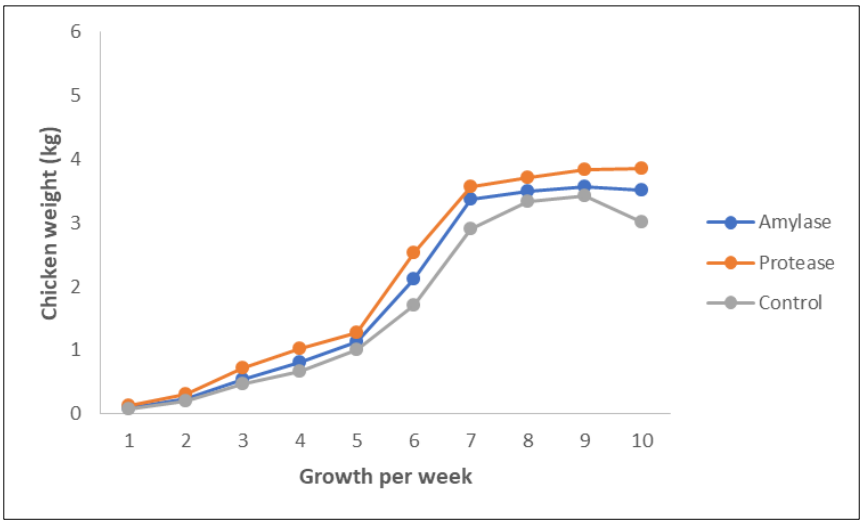


**Figure 5** Effect of crude enzymes on chicken growth

## 4. Conclusion

The present study indicated the potential of *L. plantarum* CS fermented culture and its crude enzymes as good chicken growth promoters and thus, suggests their scale-up production especially for the fermented culture for its application in poultry feed industries in Nigeria.

## Compliance with ethical standards

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### *Disclosure of conflict of interest*

The authors have no conflict of interest to disclose.

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