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ORIGINAL RESEARCH

Biochemical characterization of solid state fermented maize cob (*Zea mays*) using *Rhizopus oligosporus* and its application in poultry feed production

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Abstract

Purpose This study aimed to evaluate the biochemical characterization of solid state fermented maize (*Zea mays*) cob and its use in poultry feed production.

Method Solid state fermentation was carried out at room temperature for 72 hour using *Rhizopus oligosporus* inoculum with a well prepared phosphate buffer (50Mm, pH 6).

Results Results showed a significant ($p<0.05$) increase in glucose and decrease in reducing sugars and soluble proteins concentration in the *R. oligosporus* fermented maize cob. At 10% inoculum, the highest concentrations of glucose, reduced sugars and soluble proteins were: 1.15±0.21, 45.7±0.6 and 12.9±0.3 mgg^{-1}, respectively. Similarly, the total phenol, flavonoid content and antioxidant activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) and amylase activity of maize cob increased significantly ($p<0.05$) with fermentation. Broiler chickens fed with fermented maize cob of 10% inoculum had the same weight gain as the control (1.4±0.1kg). Fermentation did not induce a significant difference ($p<0.05$) in the activities of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP).

Conclusion The observed increase in proteins and sugars in the fermented products suggests their suitability as excellent components for animal feed production. Thus, the livestock feed formulation industries might benefit from the usage of fermented maize cob as a raw material in animal feeds as well as a protein fortifier.

Keywords Maize cob, Poultry feed, Raw material, Solid state fermentation, *Rhizopus oligosporus*

Introduction

In Nigeria, poultry production has been limited by the lack of grains. Feed cost is an important component in influencing the level of chicken survival and profitability and accounts for up to 80% of total production costs in poultry (Yafetto 2018; Olugbemi et al. 2010). Maize is one of the major basic foodstuffs used as a raw material in several industries such as poultry food production,

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its demand usually exceeds its supply, resulting in a price increase of over 2000% over the last 20 years (Okhonlaye and Foluke 2016). It has consistently led to the high cost of poultry feeds as well as an increase in the costs of poultry products (Avwioroko et al. 2016). As a result, poultry feed producers are confronted with the challenge of locating alternative feedstock that will not compromise quality. Therefore, attention is given to the ability of poultry to utilize alternative cheaper feedstuffs including fibrous crops by-products such as maize cob. Maize cobs are a byproduct of the maize crop that comprise the female inflorescence's core fibrous rachis (the maize "ear"), and are traditionally used in the formulation of animal feeds in Nigeria (Adelekan and Nnamah 2019). This is due to the tight interweaving of cellulose (45 - 55%), hemicellulose (25 - 35%), and lignin (20 - 30%) in maize cobs, they are classed as lignocellulose biomass (Kanengoni et al. 2015). Another byproduct from wheat, wheat offal, is composed of wheat germ, bran (the majority of the wheat offal), coarse middling, and fine middling (Alawa and Umunna 1993). It is made up of 14.80-17.60% crude protein (CP), approximately 10% crude fiber and 3.4-6.40% crude ash (Olomu 1995; Yin et al. 1993). The relatively low crude fiber content of maize cob in comparison to other byproducts might be a rationale for its use as animal feed. However, its insignificant protein content seems to be a barrier to its use by broiler feed production enterprises (Adelekan and Nnamah 2019; Tonukari et al. 2016). This has resulted in some environmental damage caused by current feed formulation firms' inefficient use of maize cob. As a result, urgent scientific action is necessary to boost the application and protein level of maize bran and cob. This will enables animal feed production companies to utilize maize cob and bran for animal feeds, decreasing pollution produced by household and industrial trash (Obi et al. 2016; Negewo et al. 2018; Elipopoulos et al. 2021; San Martin et al. 2021). Recent scientific studies have concentrated on the modification of agro industrial waste obtained from cereals and grains into more valuable outcome and better animal feed (Oyarekua 2016) The altered waste products have also been found to represent significant biofuel sources (Obi et al. 2016). Previous research has also demonstrated that biotransformation of grains improves nutritious value (Oyarekua 2016; Abegaz 2007). Solid state fermentation is a technical approach developed for the transformation of waste products from domestic and agricultural sources, in which waste materials undergo biotransformation via an oxidative process. Agro-industrial wastes are turned into biologically-active byproducts in the lack or presence of water in this fermentation process. Solid state fermentation enhances cereal protein content and improves digestibility (Anigboroet al. 2020; Egbune et al. 2022). Solid state fermentation is potential contemporary biotechnology for converting household and food industry wastes into valuable byproducts and enhanced meals. Little is known about this procedure in Africa, particularly in Nigeria. Therefore, the goals of this research are first to evaluate the biochemical characteristics of solid state fermented maize (*Zea mays*) cob produced using *Rhizopus oligosporus* and second, to evaluate the fermented cobs in poultry feed production.

Materials and methods

Maize cobs collection and characteristics

Maize cobs (*Zea mays*) were harvested from a local farm in the Abraka, Delta State, Nigeria. Harvesting was done by the separation of the two fractions, grain and cobs, directly in the field by physical means (Bergonzoli et al. 2020). Authentication of cobs was carried out at Department of Botany, Delta State University, Abraka, Delta State Nigeria. The cobs were dehydrated, crushed and then kept at room temperature (37°C).

Chemical analysis of maize cob

The dry matter of the samples was evaluated after 8 hours at 105ºC. The nitrogen (N) content of milled dry samples was evaluated using the standard Kjeldhal technique (AOAC 1995), and crude protein (CP) was computed (N x 6.25). The ash content was calculated using a muffle furnace. The technique published by Van Soest et al. 1991 was used to determine neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL). The difference between NDF and ADF was used to calculate hemicellulose, while the difference between ADF and ADL was used to calculate cellulose.

Fermentation of maize cob

Fermentation of maize cob was performed using *R. oligosporus* according to the method described by Ofuya and Nwajiuba (1990). Harmony Path Laboratory (Sapele, Delta State) provided the *Rhizopus oligosporus* (made by PT Aneka FermentasiIndustri, Bandung, Indonesia). One gram (1 g) of *R. oligosporus* (1.4×10^2 CFU) at different inoculum sizes (5, 10 and 15%) was mixed in 15 ml phosphate buffers (50 Mm, pH 6) and added to a petri dishes containing 10 g of the ground maize cob. The inoculated petri dishes were covered and allowed to ferment for 72 hour at room temperature (37°C). Control treatment (containing grounded maize cob and buffer only, without inoculation) was prepared alongside the test samples. After fermentation, each petri dish with a varied inoculum size received 6 g of the mixture; 40 ml of distilled water was added and homogenized using a mortar and pestle. After which, a supernatant was obtained by centrifuging 10 ml of the mixture at 3500 rpm for 10 minutes. The supernatant was used as the crude extract or sample for the various tests that were performed in triplicate.

Total soluble proteins of the fermented maize cob were determined according to the method described by Gornall et al. (1949). Glucose was determined using the Randox glucose kit (Randox® Laboratories Ltd. Ardmore, United Kingdom) following the manufacturer's recommendations. Reducing sugar was estimated using the method by Miller (1959). Antioxidant property was determined by DPPH assay using the method described by Hatano et al. (1988). The total phenol and flavonoid contents were determined using the procedures given by Singleton and Rossi (1965) and Jia et al. (1999). Reducing sugar was estimated by dinitrosalicylic method as described by Miller (1959).

Experimental design and set up for feed formulations

Five broiler starter feed diets were formulated for 0 - 4 weeks old Cornish broiler chicks by replacing maize with fermented and unfermented maize cob. All other feed ingredients were kept constant (Table 1). The feeds were administered for 4 weeks, to a total of 25 chicks divided into five groups (n = 5). The broilers chicks were purchased from Songhai Delta's hatchery Amukpe Sapele, Delta State. The diet was administered throughout the study and the weights of the broiler chicks were obtained on day 1 and at the end of week 4 using an electronic sensitive balance (Havard Trip Model). The birds were housed in a cage of 100 feet x 45 feet x 14 feet dimension for proper ventilation. Upon arrival, the chicks were kept in a deep litter house. Flat tray feeders and tube drinkers were used to administer feed and water *ad libitum*.

Blood sampling and biochemical analysis

At the end of the feeding session, venous blood was taken with a sterile syringe and needle from pronounced veins in the chicks' wings and/or legs and transferred to a test tube. After allowing the blood to coagulate for a

while, it was dislodged and centrifuged at 2000 g for 10 minutes to get the serum as supernatant. The supernatants (sera) were kept at ±4°C and utilized for biochemical investigations. The Randox® kits were used to measure serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) activity. The assay methods were meticulously carried out following manufacturer's manual.

Table 1 Gross composition of diets used for the broiler chicks

Components	**Formulated Diets**				
	Control	**Unfermented**	**5% Inoculum**	**10% Inoculum**	**15% Inoculum**
Wheat offal	40	-	-	-	-
Empty maize cob	-	40	-	-	-
5% Fermented empty maize cob	-	-	40	-	-
10% Fermented empty maize cob	-	-	-	40	-
15% Fermented empty maize cob	-	-	-	-	40
Maize (kg)	30	30	30	30	30
Soybean cake (kg)	25	25	25	25	25
Bone meal	1.4	1.4	1.4	1.4	1.4
Limestone	2	2	2	2	2
Dicalcium phosphate	0.7	0.7	0.7	0.7	0.7
Salt	0.3	0.3	0.3	0.3	0.3
Premix	0.2	0.2	0.2	0.2	0.2
Lysine	0.2	0.2	0.2	0.2	0.2
Methionine	0.2	0.2	0.2	0.2	0.2
Total (kg)	100	100	100	100	100

(-)Indicated the component was not added to the formulated diets. Values presented are in kg.

Statistical analysis

Data analyses were done using the SPSS 19.0 program (SPSS Inc., Chicago, IL, USA). Values were reported as Mean ± Standard deviation and the outcomes of the experiments were evaluated using analysis of variance (ANOVA). Fischer Last Significant Difference (LSD) was carried out to compare the group means. Results were considered significant at $p< 0.05$.

Results and discussion

The results showed that glucose concentration was significantly ($p<0.05$) increased after 72 hours of *R. oligosporus* fermentation in all the inoculum size treatments (Fig. 1). This is in line with previous report on *R. oligosporus* fermentation of maize (*Zea mays*) offal by Anigboro et al. (2020). The results showed that solid state fermentation (SSF) increases glucose concentration at the different inoculum sizes. The fungus, following the consumption of simple sugars, initiates the hydrolysis of starch chains by the enzymatic action of extracellular enzymes such as α-amylase, linamarase and cellulase (Soccol et al. 2017). Hydrolysis causes the

breakdown of large starch chains into several units, which is reflected by an increase in sugars.

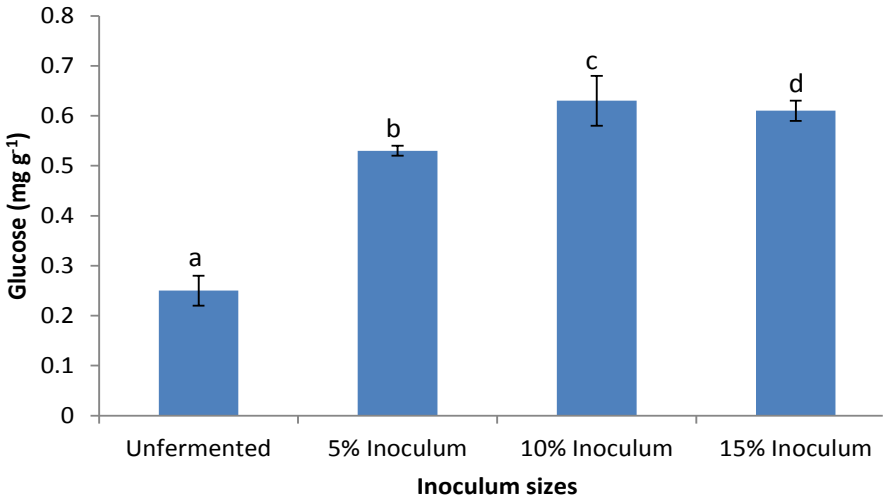

Fig. 1 Glucose levels in fermented maize cob with *R. oligosporus*

Bars having different letters mean statistically significant differences at $p < 0.05$.

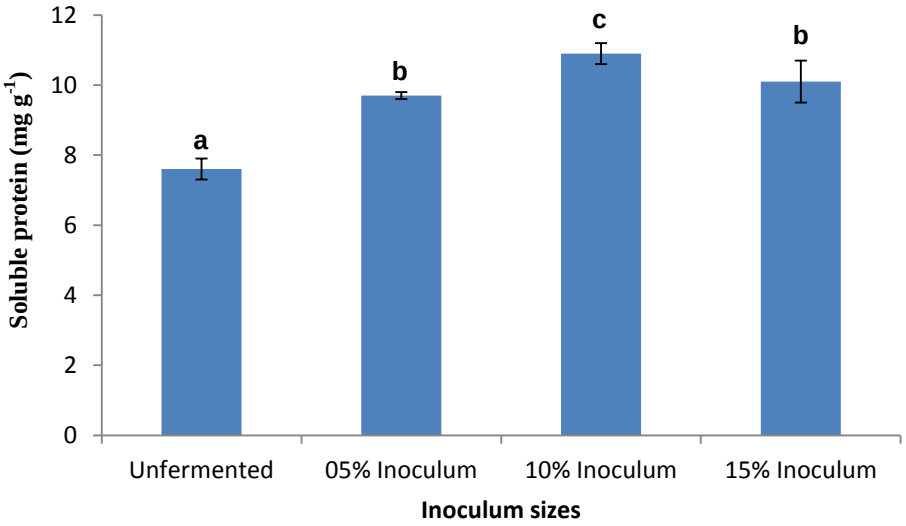

Fig. 2 Levels of soluble proteins in the *R oligosporus* fermented maize cob

Bars having different letters mean statistically significant differences at $p < 0.05$.

Fermentation also resulted in a significant ($p<0.05$) increase in soluble protein (Fig. 2). The solid state fermentation of maize cob increases the content of essential amino acids and soluble proteins, reduces mycotoxins and prevents oxidative rancidity, among others (Savón and Scull 2006).

Due to the enzymatic activity of microorganisms during SSF, texture, flavor and nutritional composition were modified as indicated by changes in the content of proteins, lipids and carbohydrates (Egbune et al. 2021; Thomas et al. 2013). The increase in soluble proteins after fermentation supports the findings of Anigboro et al.

(2020), who found an increase in soluble proteins following SSF of maize offal with *R. oligosporus*. The improved protein content in the fermented maize cob could be attributed to possible secretions of extracellular enzymes (proteins) such as amylases, linamarase and cellulase into the maize cob substrate by the fermenting organisms in an attempt to utilize the cassava starch as a source of carbon (Hawashi et al. 2019).

Therefore, the significantly enriched maize cob could be integrated into animal feed formulations provided it is acceptable and highly digestible by farm animals. Solid state fermentation is a type of microbial fermentation that occurs in the lack or near absence of free water, mimicking the natural environment to which the chosen microorganisms (particularly fungus) are suited. The solid substrate in solid state fermentation provides nutrients to the microbial culture growing in it while also acting as anchoring for the cells (Sadh et al. 2018).

Similarly, a substantial rise in reducing sugar level was observed following the fermentation of maize cob with *R. oligosporus* (Fig. 3). The study revealed that fermented maize cob yielded more reducing sugars in 72 hours compared to the control probably because the substrate was degraded and used as source of carbon to liberate various enzymes during the process. This yield is in line with the 98% increase in reducing sugar reported by Olanbiwoninu and Odunfa (2012).

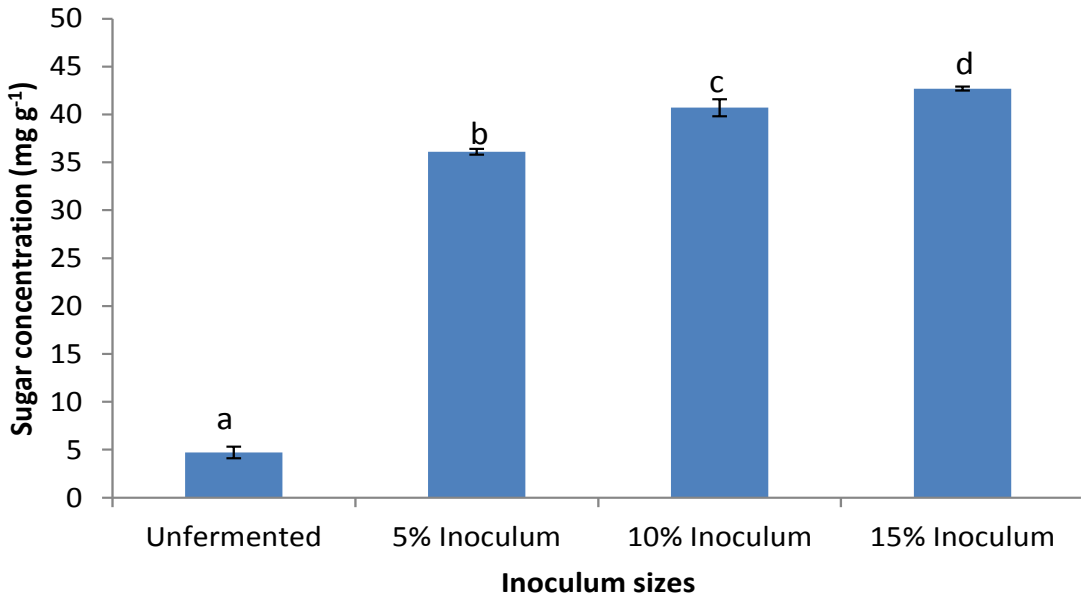

Fig. 3 The Reducing sugar concentration in the *R oligosporus* fermented maize cob

Bars having different letters mean statistically significant differences at $p < 0.05$.

The antioxidant capacities of fermented maize cob as measured by DPPH scavenging assay were generally increased compared to the control. From the results, fermentation resulted in a significant ($P<0.05$) increase in free radical scavenging activities from 17.38±0.4 (%) in the unfermented control to 24.32±0.5 (%) in the fermented maize cob at 15% inoculum size (Fig. 4). This can be attributed to an increase in the total phenol and flavonoid contents (Figs. 5 and 6, respectively) of the fermented maize cob compared to the control. This is in agreement with earlier reports on the ability of these compounds to neutralize oxygen radicals (Adebo and Medina-Meza 2020). Consequently, phenol, flavonoids

and other polyphenolics in the fermented medium function as good electron and hydrogen atom donors and as such possess the ability to end radical chain reaction by converting free radicals to more stable products. The findings of this study are comparable to those of prior investigations on fermented seeds, in which fermentation enhanced the seeds' total phenolic content (Moktan et al. 2008; Plaitho et al. 2013). In their native condition, phenolic compounds are joined with sugar, restricting their accessibility to organisms. The proteolytic enzymes hydrolyze phenolic compounds during fermentation, converting them into soluble-free phenols and other simpler and more physiologically active substances that are easily metabolized (Shrestha et al. 2010; Lasekan and Shabnam 2013). This might be as a result of phenolics migrating in cell fluid and being oxidized by the action of a polyphenol oxidase enzyme.

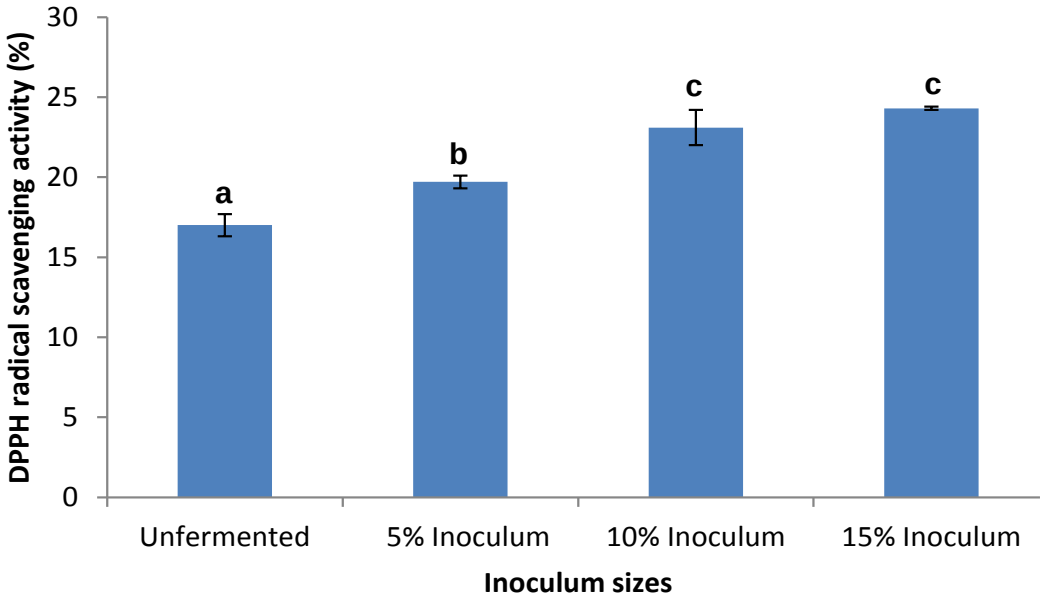

Fig. 4 Antioxidant activities inhibition of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical of maize cob

Bars having different letters mean statistically significant differences at $p < 0.05$.

According to the results of the amylase assay for this study, amylase activity increased considerably ($P<0.05$) after 72 hours of fermentation at all inoculum sizes. At 15%, the sample showed the highest amylase activity (Fig. 7). This increase is due to the fact that amylase (an enzyme that catalyses the hydrolysis of starch into sugars) is secreted by *R. oligosporus* to degrade the starch present in the fermented maize cob into glucose which the microorganism uses as a growth substrate (Egbune et al. 2022). A similar pattern was obtained by Saxena and Singh (2011) where fermentation of agro-industrial wastes with *Bacillus sp.* led to increasing amylase yields secretion. These findings support the notion that agro-industrial wastes can be used for the production of amylase through solid state fermentation.

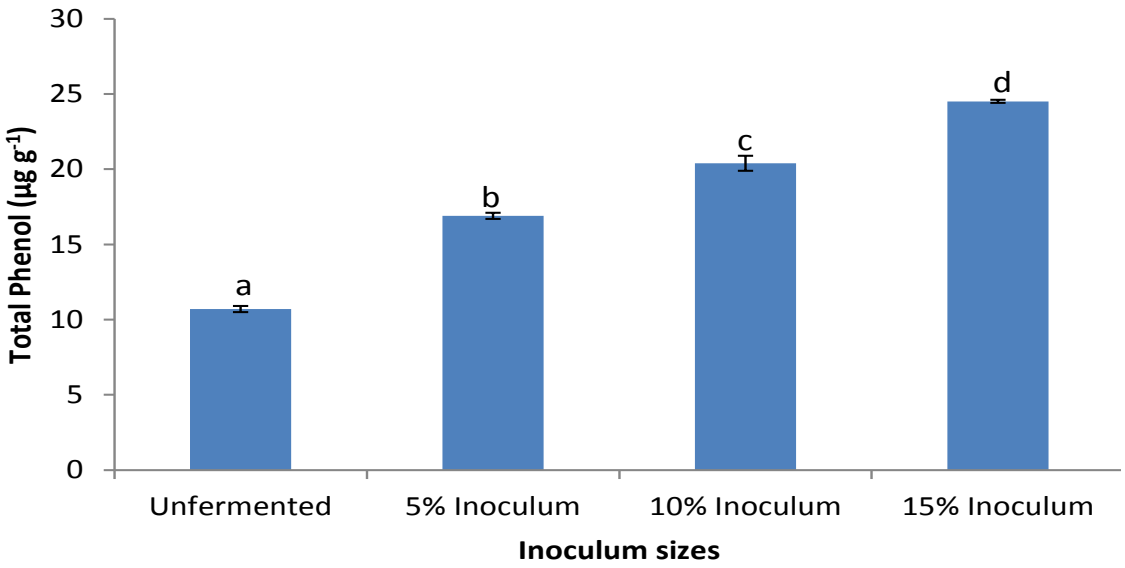

Fig. 5 Total phenol content in the *R oligosporus* fermented maize cob

Bars having different letters mean statistically significant differences at $p < 0.05$.

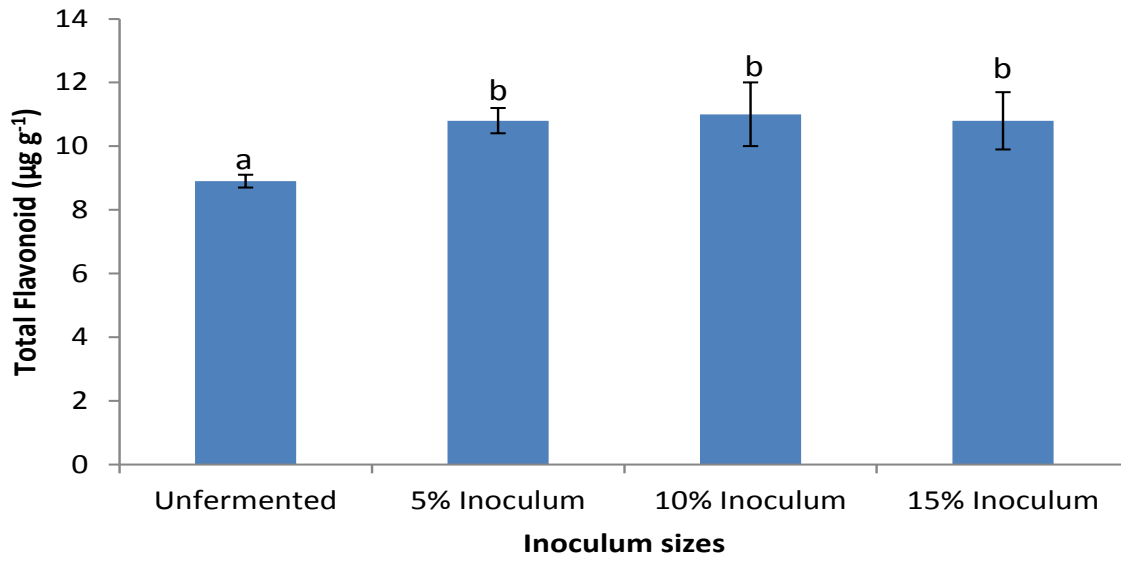

Fig. 6 Total flavonoid content in the *R oligosporus* fermented maize cob

Bars having different letters mean statistically significant differences at $p < 0.05$.

In this study, the solid state fermentation of maize cob was carried out at different inoculum sizes, after fermentation for 72 hours using *R. oligosporus.* Results show no significant ($p>0.05$) difference between birds maintained at 10% inoculum (1.4±0.1 kg) and the control (1.4±0.1kg) (Fig. 8). These data suggested that the broiler chicks were able to ingest and successfully metabolize the feed ration with wheat offal substituted with fermented maize cob throughout the length of the trial as compared to the control feed. These results are similar to those of Avwioroko et al. (2016), Tonukari et al. (2016), Orororo et al. (2014), Svihus et al. (2004).

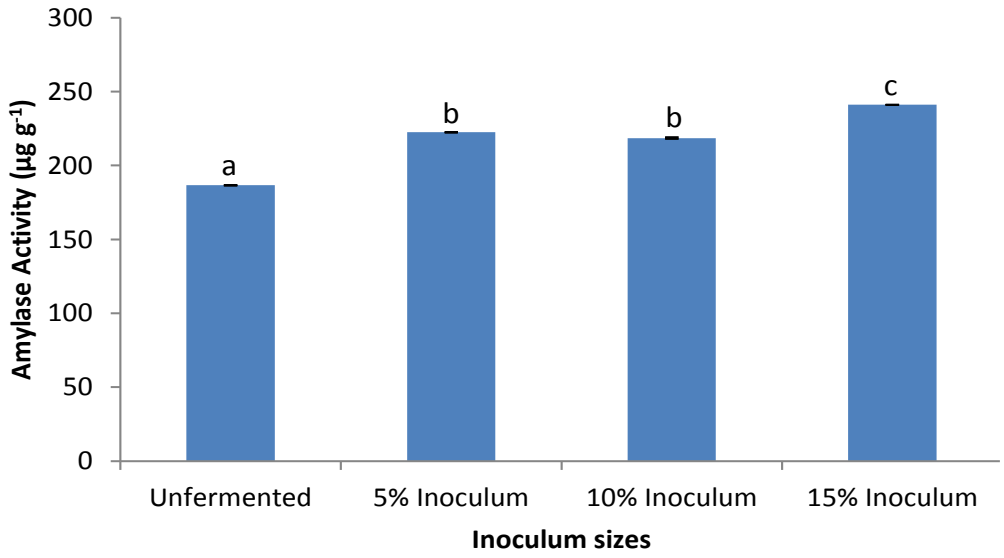

Fig. 7 Amylase activities at different inoculum sizes during solid state fermentation of maize cob with *R oligosporus*

Bars having different letters mean statistically significant differences at $p < 0.05$.

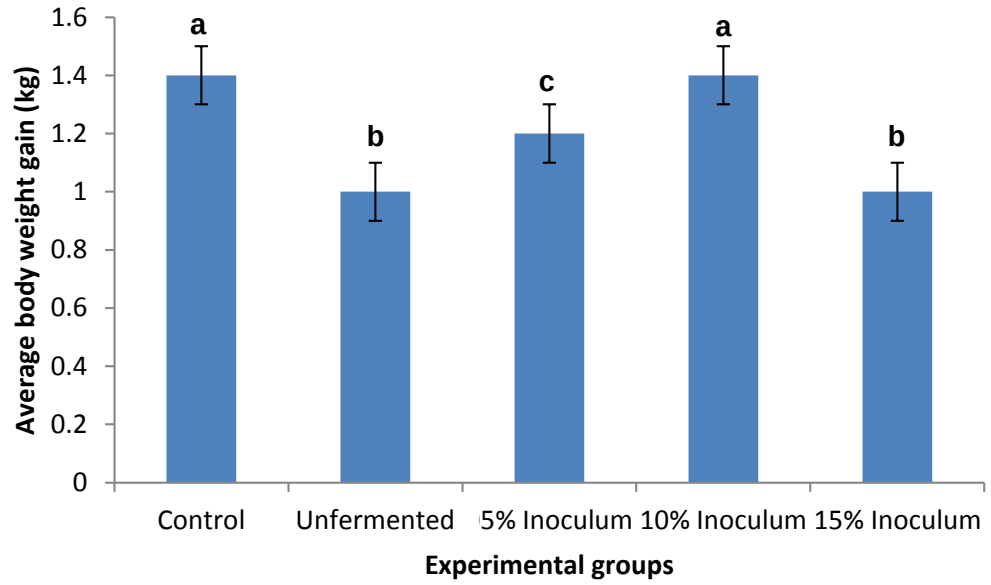

Fig. 8 Weight gains (kg) by the chicks (n = 5)

Bars having different letters mean statistically significant differences at $p < 0.05$.

The activities of ALT, AST, and ALP in the serum of broiler chicks fed a diet prepared with fermented maize cob are shown in Fig. 9. The blood activities of ALT, AST, and ALP in the control and experimental meals were not significantly ($p>0.05$) different. Khempaka et al. (2014) examined AST and ALT activities as indices of liver health and discovered no difference in the activities among birds fed with fermented maize cob and the control. In this experiment, ALT/AST activities are within the range reported by Nicoll (2007). Elevated serum AST and ALT activity indicate hepatic injury, some inflammatory illness, or hepatic cellular damage (Lala et

al. 2021; Ezedom and Asagba 2016). Both aminotransferases (ALT and AST) serve as connections between carbohydrate and protein metabolisms by the process of transamination using key molecules such as -ketoglutarate and alanine to pyruvic acid and glutamic acid, respectively (Hagopian et al. 2003).

Since there was no significant difference ($p>0.05$) in serum ALT and AST activities in birds fed the compounded feed against the control feed, it was concluded that the experimental feed diet would be as safe to the liver as the control feed.

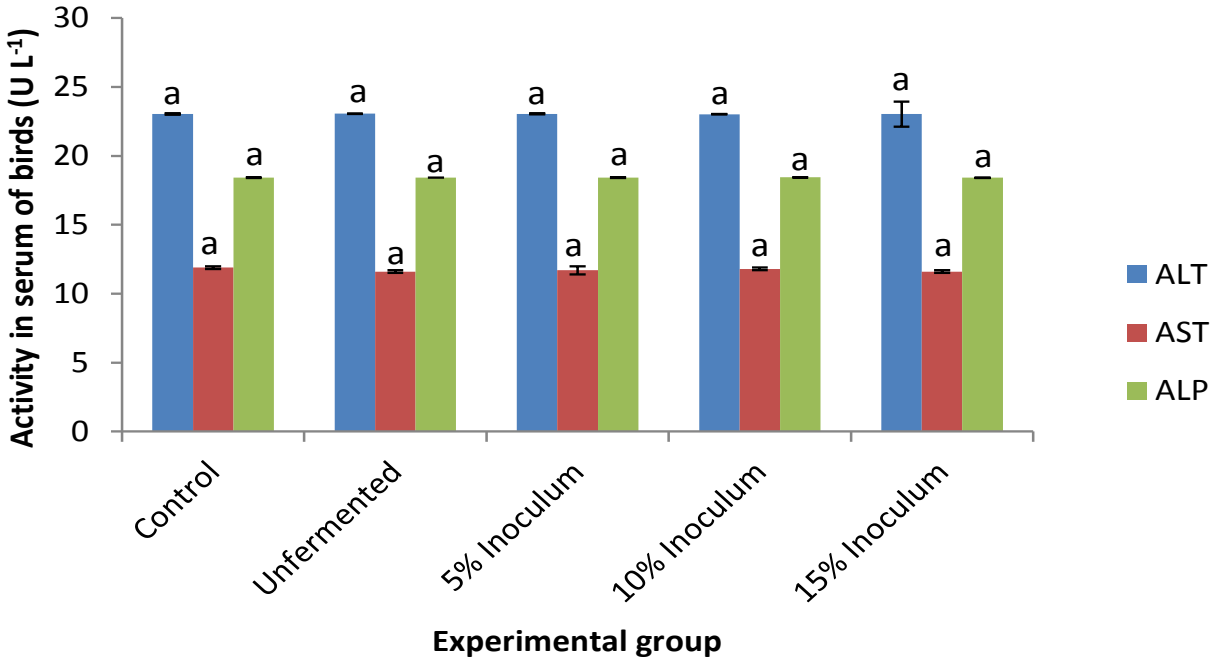

Fig. 9 Serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT) and serum alkaline phosphatase (ALP) activity (UL^{-1}) of birds (n = 5)

Bars having different letters mean statistically significant differences at $p < 0.05$.

Conclusion

Fermentation is a low-cost method of increasing the nutritional value of innovative and unusual feed components for broiler chickens. According to the findings of this research, fermentation of maize cob using *R. ligosporus* boosts its proteins, glucose, amylase activities, and antioxidant characteristics. This approach has the potential to increase the utilization of maize cob in livestock feed components as well as the synthesis of protein from a single cell. The modification of agro industrial waste from maize and other industries into value-added byproducts has the potential to dramatically reduce environmental pollution. The livestock feed formulation industry might benefit from the use of fermented maize cob with a 10% inoculum as a feed ingredient in animal feed formulations, as well as feed fortifier for improved protein.

Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest associated with this study.

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