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## Original Research Article

### Yeast-Mediated Solid-State Fermentation Enhances the Additive Quality of *Dialium guineense* Stem Bark on the Performance of Broilers Aged from 1 to 21 days

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#### ABSTRACT

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The stem bark of Tamarind is a rich source of fibre and phytochemicals, making it a potential feed additive for chickens. The study assessed the effect of yeast-mediated solid-state fermentation (SSF) on the chemical composition of tamarind stem bark and the impact of fermented tamarind bark (FTB) on the growth performance of broilers aged from 1 to 21 days. Two hundred broilers were partitioned into four groups (T0, T0.5, T1.0, and T1.5) of 50 chicks, and each group was replicated five times. Birds on T0 received a diet without FTB supplementation and served as the control, while birds on T0.5, T1.0, and T1.5 received the same control diet but supplemented with FTB at 0.5, 1.0, and 1.5 g/kg feed, respectively, in a completely randomized design. A quadratic optimisation model was used to determine the FTB supplementation levels for optimal growth performance. Results revealed that yeast-mediated SSF improved ( $p < 0.05$ ) dry matter, crude protein, ether extract, ash, flavonoids, phenol content, and DPPH activity in tamarind bark. In contrast, fermentation decreased ( $p < 0.05$ ) the fibre, oxalate, phytate, alkaloids, and saponins content in tamarind bark. Broilers on T0.5, T1.0, and T1.5 had higher ( $p < 0.05$ ) final live weight (FLW), average daily gain (ADG), and average daily feed intake (ADFI) than the T0, and were optimised at 3.05 and 3.03 g FTB/kg feed, respectively. In conclusion, yeast-mediated SSF improved the feed additive quality of tamarind bark, and FTB should be supplemented in the starter broiler diet at 3.05 and 3.03 g feed for the best FLW, ADG, and ADFI.

**Keywords:** Tamarind bark, Fermentation, Broilers, Daily gain, Feed intake, Quadratic function.

#### Introduction

Feed makes up around 60-70% of total costs in intensive broiler farming.<sup>1</sup> As a result, nutrient optimization plays an important part in maximizing broiler productivity. One such strategy to optimize nutrient uptake in broilers is the use of in-feed antibiotics.<sup>2</sup> However, the association of in-feed antibiotics with the development of resistant strains of bacteria has burdened researchers in developing countries to search for alternatives. Among the alternatives usually available in the study region are yeast, organic acids, ginger, turmeric, and tamarind bark.<sup>3-5</sup> Specifically, tamarind (*Dialium guineense*) belongs to the family *Leguminosae* and subfamily *Caesalpinoideae*, and the bark is not used as food by humans and is little known in poultry nutrition. A recent study in our station<sup>5</sup> showed that tamarind bark is high in important phytochemicals such as total phenols (2.02%) and flavonoids (0.34%). The authors also noticed that tamarind bark is moderate in trypsin inhibitors (0.05%), and low in saponins (0.004%), cyanogenic glycosides (0.009%), and alkaloids (0.006%). Trypsin inhibitor reduces protein digestion by hindering the activity of trypsin activity.<sup>6</sup>

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Recent feeding experiments in our station<sup>5,7</sup> revealed that diets supplemented with unfermented tamarind bark at 1.0-1.5 g/kg negatively impacted Ross 308 broiler performance, resulting in decreased growth, blood values, carcass yield, cut-part weights, and intestinal characteristics. These deleterious impacts were partly caused by its high lignocellulosic biomass (>25%), and the existence of trypsin inhibitors<sup>5,8</sup> demonstrated to limit nutrient digestibility and availability in poultry.<sup>7</sup> The ability of enzymes such as cellulases, hemicellulases, and ligninases to degrade cell wall components of fibrous materials into their monomers has been demonstrated.<sup>9</sup> However, most poultry species cannot produce cellulases, hemicellulases, and ligninases in their gastrointestinal tract, and as a result, their potential ability to digest tamarind bark is hindered. To maximize the adoption of tamarind bark as an additive source, strategies are required to degrade its lignocellulosic biomass and antinutrients while improving their nutrient and phytochemical content to increase their supplementation levels in broiler diets. Studies have revealed that Solid-state fermentation (SSF) can be used to improve nutrient and decrease antinutrient content of lignocellulosic biomass.<sup>10-11</sup>

Yeast (*Saccharomyces cerevisiae*) was chosen in this experiment trial for its production of hydrolytic enzymes, including invertase, lactase, lipase, raffinase, pectinases, amylases, cellulases, ligninase, and xylanase<sup>12</sup> and its established use in feed applications.<sup>3</sup> These enzymes break down lignocellulosic biomass, thereby improving nutrient and additive values. Presently, no study has examined the impact of yeast-mediated SSF on the additive value of tamarind bark and its supplementation effect on broiler growth. Therefore, the impacts of FTB supplementation on the growth characteristics of broilers aged from 1 to 21 days were also studied.

#### Materials and Methods

This experiment was done at the Poultry Unit of FUTO, Nigeria, and was approved by the University Animal Ethics committee with ethics number: FUTO/2024/24. FUTO lies between latitudes 5°20'N - 5°25'N and longitudes 7°00'E - 7°05'E with 2400 mm mean rainfall. The mean

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daily temperature ranges from 19-24°C (minimum) to 28-35°C (maximum).<sup>13</sup> Proximate and phytochemical composition of unfermented VB and fermented BV (FVB) were conducted at the Precision Food and Feed Analysis Laboratory, Ibadan, Nigeria.

#### Production of unfermented and fermented tamarind bark

The tamarind bark was harvested in FUTO, Nigeria, cut into bits, sundried, and milled as described by Ogbuewu and Mbajorgu.<sup>5</sup> A portion was mixed with dried baker's yeast (STK Royal®) at a 10 kg:1 g ratio.<sup>5</sup> Thereafter, the sample was fermented for 8 days<sup>14</sup> and sun-dried for 3 days to 14% moisture content.<sup>15</sup> The other portion, unfermented and without yeast, served as a control. Fermented and unfermented tamarind bark were analysed for proximate, fibre, and phytochemical composition.

#### Proximate composition and fibre fractions

Dry matter (DM) (method no 930.15), crude protein (CP) (method 990.03), crude fibre (CF) (method 962.09), ether extract (EE) (method 960.39), and crude ash (method no 924.05) of fermented and unfermented tamarind bark were determined in triplicates and recorded in percentages according to the methods of AOAC.<sup>16</sup> The fibre fractions assessed were neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), cellulose, and hemicellulose. Samples were oven-dried at 105°C to determine moisture content. EE content was extracted using a Soxhlet Extractor (DW-MSZF-M06C; Houston, Texas, USA) with petroleum ether. Ash content was determined by incinerating samples at 550°C in a muffle furnace. The samples were heated in a furnace at 550°C to quantify the ash value. Total organic nitrogen (TON) was assessed via the macro-Kjeldahl technique, and CP was derived as TON × 6.25. NDF and ADF values were assessed by serial reflux with neutral and acid detergent solutions.<sup>17</sup> The fibre fractions in the samples were measured via an ANKOM200 Fibre Analyzer (Model: ANKOM200 Fibre Analyzer, New York, USA). Fibre fractions were determined as follows: ADL content = ADF - cellulose; hemicellulose = NDF - ADF; Cellulose = ADF - ADL.

#### Determination of phytochemical contents

The phytochemical components analysed included DPPH, tannins, phenols, flavonoids, alkaloids, phytate, saponins, and oxalate. The tannins and saponins were measured using the standard methods.<sup>18-19</sup> Phytate concentration was evaluated using a modified colorimetric method<sup>20</sup>, while total phenol, flavonoid, and antioxidant activity content were determined as described by others.<sup>21-22</sup> Alkaloid content was measured using Wagner's reagent test as described by Amaza<sup>23</sup>, while the total oxalate was quantified using the method of Ruan et al.<sup>24</sup>

#### Animal management and experimental design

One day, 200 Ross 308 were distributed equally into four groups, with five birds per replication. Birds in the T0 group received a diet (Table 1) without FTB supplementation, which served as the control, while birds on T0.5, T1.0, and T1.5 groups received a control diet with FTB at 0.5, 1.0, and 1.5 g/kg feed, respectively. Broilers in each group were allotted to one of the diets in a completely randomised design. Each replicate was housed in a pen with dimensions of 1×2 m, in a dwarfed walled poultry house with an open side covered with wire mesh. The pens were cleaned and disinfected with Polidine® (Animal Care Nigeria) and thereafter covered with wood shavings to a depth of 2 cm. Drinkers and feeders were thoroughly cleaned and assembled a day before the commencement of the feeding study. A broiler diet (Table 1) was formulated to meet Ross 308 nutrition specifications<sup>25</sup>. The experimental birds were vaccinated following the standard method.<sup>26</sup> Birds were offered feed and clean water without restriction for three weeks. Metabolizable energy (ME) value of the ration was computed through the formula of Pauzenga<sup>27</sup>: ME (Kcal/kg) = 37 × %CP + 81.8 × %EE + 35.5 × %NFE, where NFE (nitrogen free extract) is calculated as 100 - %moisture - %CP - %EE - %ash - %CF.

**Table 1:** Ingredient and nutrient content of experimental diet

Ingredients	percent
Maize (9% CP)	55.00
Soybean meal (44% CP)	31.00
Wheat offal	2.00
Palm kernel cake	2.00
Fish meal	4.00
Bone meal	3.00
Oyster shell	2.00
Common salt	0.25
Vitamin/mineral premix*	0.25
Lysine	0.25
Methionine	0.25
Total	100
Calculated nutrient composition (%)	
Crude protein	23.00
Crude fibre	3.98
Ether extract	3.39
Lysine	0.97
Methionine	0.45
Calcium	1.63
Phosphorus	1.14
Metabolizable energy (Kcal/kg)	2965.10

\*Having the following/ kg feed: Vitamin A (1,200,000 iu), cholecalciferol (350,000 iu), tocopherol (4000 mg), thiamine (250 mg), riboflavin (800 mg), pyridoxine (600 mg), cobalamin (3.2 mg), menadione (450 mg), nicotinic acid (4.5 g), calcium pantothenate (1.5 g), folic acid (120 mg), biotin (5 mg), choline chloride (55 g), iron (3 g), copper (2 g), manganese (10 g), zinc (8 g), iodine (120 mg), selenium (0.3 mg), and cobalt (40 mg).

#### Data collection

The birds' live weights were recorded at the start and weekly using an AG500 electronic scale ( $\pm 0.5$  g precision). Average daily feed intake (ADFI) was determined as the amount of feed offered on a particular day less the amount in the feeder the next morning. Average daily gain (ADG) was determined weekly, while feed conversion ratio (FCR) was calculated as ADFI/ADG per replicate.

#### Statistical analysis

Results obtained on proximate composition, fibre fraction, phytochemical contents, and growth metrics were analysed with IBM SPSS<sup>28</sup> Version 27. For chemical composition, means were separated with Student's t-test via the equation:  $Y_{ij} = \mu + T_i + E_{ij}$ . Where  $Y_{ij}$  is proximate, fibre fraction, phytochemical characteristics;  $\mu$  = population mean,  $T_i$  = impact of yeast-mediated SSF, and  $E_{ij}$  = random error. Growth performance was assessed using the general linear models (GLM) procedure of the same software. Means were separated using Tukey's test using the model:  $Y_{ijk} = \mu + T_i + E_{ijk}$ , where  $Y_{ijk}$  is the growth performance,  $\mu$  is the population mean,  $T_i$  is the supplementation levels of FTB in the diets ( $i = 4$ ; T0, T0.5, T1.0, and T1.5), and  $E_{ijk}$  is the random error linked with observation. The dose-related responses to increasing supplementation levels of FTB for significant variables were modeled using the quadratic formula:  $y = a + c_1x + c_2x^2$ , where  $y$  is the FLW, ADG, and ADFI;  $a$  is the intercept;  $x$  = FTB supplementation levels;  $c_1$  and  $c_2$  = coefficients of the optimisation equation; and  $-c_1/2c_2$  = FVB level for optimum starter broiler performance. These models were chosen as they gave the best fit, with significance at 5% level.

## Results and Discussion

FTB had higher ( $p < 0.05$ ) CP, EE, and ash, but lower DM than unfermented tamarind bark (Table 2), which supports of previous results by Ahire et al.<sup>15</sup> The decreased DM value of FTB is likely because yeast enzymes broke down complex compounds in tamarind bark into simpler molecules (volatile fatty acids, gases, alcohols) during fermentation.<sup>29-30</sup> The results showed that FTB had lower CF, NDF, ADF, ADL, and hemicellulose compared to unfermented tamarind bark. Fibre limits feed digestion by preventing enzyme access to nutrients<sup>30</sup>, making high-fibre feed resources undesirable for poultry.<sup>31</sup> The activities of yeast during SSF may have reduced the concentrations of CF, NDF, ADF, ADL, and hemicellulose in tamarind bark, which yeast may use as a carbon source, leading to increased CP level, enhanced by microbial mass. Results revealed fermentation didn't change cellulose levels in tamarind bark. This finding is at variance with the findings of Gbenle et al.<sup>14</sup>, who found reduced cellulose levels in marama beans because of the actions of *Aspergillus oryzae* during fermentation. The EE results support the findings of Gbenle et al.<sup>14</sup> that fermentation with fungi enhances the EE value of fibre-rich feed ingredients. This variation in results may be linked to the type of substrate used.<sup>20</sup> These results imply that yeast fermentation of tamarind bark yields a low-fibre biomass suitable as a poultry feed additive. Phytochemical values of unfermented and FTB are displayed in Table 3. FTB had significantly ( $p < 0.05$ ) higher levels of phenol, flavonoids, and DPPH, but lower levels of tannins, oxalate, phytate, alkaloids, and saponins compared to unfermented tamarind bark. These findings support the earlier results that fungal-mediated SSF improved the feed/food value of marama beans.<sup>14</sup> The significantly low levels of tannins, alkaloids, and saponins in tamarind bark indicate the ability of yeast-mediated SSF to biodegrade tannins, alkaloids, and saponins into simpler, less harmful compounds. Yeast decreases the concentrations of tannin and saponin in feed during fermentation via enzymatic activity and changes in pH.<sup>32</sup> In similar research, Arjmand et al.<sup>33</sup> revealed that yeast strains with  $\beta$ -glucosidase activity can biodegrade saponins, whilst fermentation activity creates an acidic medium that aids in saponin breakdown. Oxalates, a naturally occurring compound in plants, can bind with calcium in feed or feedstuffs to form calcium oxalate, an insoluble compound, making minerals less available for absorption.<sup>34</sup> The reduction in oxalate content of tamarind bark during fermentation might be due to the yeast's ability to synthesize enzymes that break down oxalic acid or oxalate-mineral complexes, hence reducing the overall oxalate level.<sup>35</sup> The decreased phytate level upon fermentation is likely due to the yeast producing phytase, an enzyme that degrades phytate.<sup>36</sup> Phytate binds to proteins, carbohydrates, and minerals, making them less available to digestive enzymes.<sup>37</sup> Thus, a decline in the phytate level can improve the availability of important minerals in fibrous biomass such as tamarind bark. This is quite important given that poultry does not synthesize phytase in their digestive system.

The significant increase in phenolics upon fermentation of tamarind bark could be related to the ability of yeast to produce lignocellulolytic enzymes such as cellulases, hemicellulases, and ligninases<sup>38</sup> that release bound forms of phenols and flavonoids during cell wall breakdown.<sup>34</sup> Phenolic compounds act as antioxidants and can enhance the antioxidant activity of functional feed ingredients, as confirmed by the DPPH results in this study. This observation confirms that fermentation increased the DPPH level in lignocellulosic biomass.<sup>5</sup> The higher contents of phenolics in FVB could provide a plausible explanation for the increased DPPH activity in FTB.

Table 4 shows the initial live weight (ILW), FLW, ADG, ADFI, FCR, and percentage mortality of birds fed FTB supplemented diets are shown in Table 4. Birds on T0.5, T1.0, and T1.5 performed better in terms of FLW, ADG, and ADFI than birds on T0.5. The enhanced ADG may be due to yeast breaking down complex fibre in tamarind bark into simpler, more readily absorbable nutrients, lignocellulosic complex reduced antinutritional factors in tamarind bark, as confirmed by our fibre fraction and phytochemical results. However, this finding was at variance with Ogbuewu and Mbajorogu<sup>5</sup>, who observed that inclusion of unfermented tamarind bark to broiler rations at the rate of 1.0 - 1.5 g/kg decreased ADG. This discrepancy suggests that fermentation improves physicochemical values of fibrous biomass, leading to

improved nutrient utilization and uptake in poultry.<sup>15</sup> The FCR of broilers on T0.5, T1.0, and T1.5 was numerically lower ( $p > 0.05$ ) than that on T0, indicating a high ability of broilers to utilize FTB-supplemented diets, leading to high FLW and ADG. There were no differences ( $P > 0.05$ ) in ILW and percentage mortality between broilers offered FTB and control diets.

**Table 2:** Proximate composition and fibre fractions of unfermented and fermented tamarind bark

Parameters (%)	Unfermented	Fermented	Mean	SD	SEM	p-value
Dry matter	93.47 <sup>a</sup>	91.51 <sup>b</sup>	92.4 9	1.1 4	0.5 7	<0.001
Crude protein	6.16 <sup>b</sup>	8.16 <sup>a</sup>	7.16 6	1.1 8	0.5 1	0.00
Ether extract	0.61 <sup>b</sup>	0.68 <sup>a</sup>	0.65 4	0.0 2	0.0 8	0.03
Crude ash	9.91 <sup>b</sup>	10.60 <sup>a</sup>	10.2 5	0.4 0	0.2 0	<0.001
Crude fibre	30.80 <sup>a</sup>	20.20 <sup>b</sup>	25.5 0	6.1 2	3.0 6	0.01
Neutral detergent fibre	61.36 <sup>a</sup>	48.39 <sup>b</sup>	54.8 7	7.5 3	3.7 7	0.006
Acid detergent fibre	50.29 <sup>a</sup>	46.34 <sup>b</sup>	48.2 7	2.4 1	1.2 0	0.02
Acid detergent lignin	24.04 <sup>a</sup>	20.47 <sup>b</sup>	22.2 5	2.0 6	1.0 3	0.001
Cellulose	26.26	25.77	26.0 1	0.5 7	0.2 9	0.512
Hemicellulose	11.07 <sup>a</sup>	2.16 <sup>b</sup>	6.61 0	5.3 5	2.6 9	0.029

**Table 3:** Phytochemical composition of unfermented and fermented tamarind bark

Parameters	Unfermented	Fermented	Mean	SD	SEM	p-value
Tannins (mg/g)			5.23	4.04	2.0	<0.001
	8.73 <sup>a</sup>	1.73 <sup>b</sup>			2	0.001
Phenols (mg/g)			12.1	4.51	2.2	0.004
	8.22 <sup>b</sup>	15.99 <sup>a</sup>	1	5	4	
Flavonoids (mg/g)			2.75	1.34	0.6	<0.001
	1.60 <sup>b</sup>	3.91 <sup>a</sup>			7	0.001
Oxalate (mg/g)			0.38	0.12	0.0	<0.001
	0.48 <sup>a</sup>	0.28 <sup>b</sup>	6	0.0	0.0	
Phytate (mg/g)			0.10	0.01	0.0	<0.001
	0.11 <sup>a</sup>	0.09 <sup>b</sup>	1	0.0	0.0	
Alkaloids (%)			14.2	11.7	5.8	<0.001
	9	6	8	0.0	0.0	
Saponins (%)			10.8	3.19	1.6	0.001
	24.48 <sup>a</sup>	4.10 <sup>b</sup>	0	0	0	
	13.56 <sup>a</sup>	8.04 <sup>b</sup>			1	

DPPH ( $\mu\text{g/ml}$ )	41.1 35.71 <sup>b</sup>	6.26 46.52 <sup>a</sup>	3.1 3	0.00 3
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<sup>a,b</sup> Means with common superscripts do not statistically differ ( $P > 0.05$ ). DPPH -2,2-Diphenyl-1-picrylhydrazyl; SD- standard deviation; SEM – standard error of the mean; p - probability.

**Table 4:** Performance of starter broilers fed diets supplemented with fermented tamarind bark

Parameters	Dietary fermented tamarind bark Levels				SE M	p val
	T0	T0.5	T1.0	T1.5		
ILW (g/bird)	44.15 0.06	44.12 0.01	44.15 0.06	44.15 0.05	0.01	0.84
FLW (g/bird)	511.15 38.72 <sup>b</sup>	616.08 35.49 <sup>a</sup>	614.51 3.25 <sup>a</sup>	610.98 11.61 <sup>a</sup>	14.9 7	0.00
ADG (g/bird)	24.34 1.84 <sup>b</sup>	29.34 1.69 <sup>a</sup>	29.26 0.15 <sup>a</sup>	29.09 0.55 <sup>a</sup>	0.71	0.00
ADFI (g/bird)	38.04 2.17 <sup>b</sup>	44.86 1.65 <sup>a</sup>	44.77 0.46 <sup>a</sup>	44.41 0.73 <sup>a</sup>	0.94	0.00
FCR	1.56 0.04	1.53 0.05	1.53 0.01	1.53 0.02	0.01	0.50
Mortality (%)	1.00	0.00	0.00	1.00	-	-

<sup>a,b</sup> Means  $\pm$  SD in the same row with the same superscript are significant at  $p < 0.05$ . FTB fermented tamarind bark; T0 = 0 g FTB/kg feed; T0.5 = 0.5 g FTB/kg feed; T1.0 = 1.0 g FTB/kg feed; T1.5 = 1.5 g FTB/kg feed; ILW initial live weight; FLW final live weight; ADG average daily gain; ADFI average daily feed intake; FCR feed conversion ratio; SD standard deviation; SEM standard error of the mean

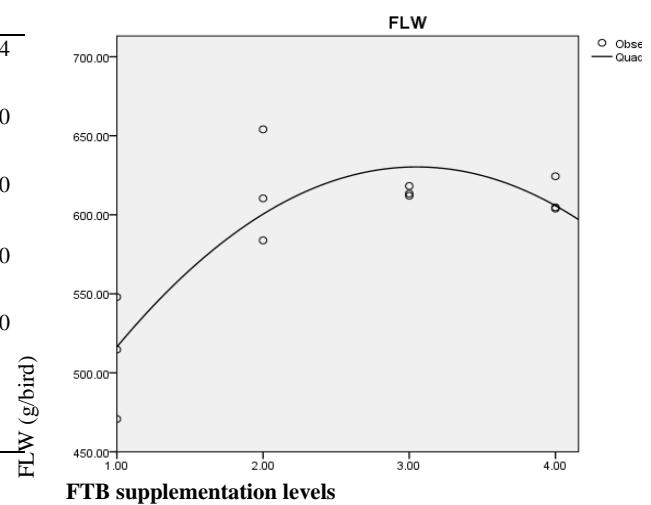
Results of the impact of FTB supplementation level on optimal FLW, ADG, and ADFI of starter broilers are presented in Figures 1 to 3 and Table 5. The results indicated that growth performance had high (74.8–80.2%) coefficients of determination ( $r^2$ ). Not many studies have used quadratic regression to find the best tamarind bark levels that support broiler performance and health<sup>5,7</sup>. Presently, there's a gap in research on using regression analysis to determine the optimal FTB levels for starter broiler growth. FLW and ADG were optimised at 3.05 g FTB/kg feed (Figures 1 and 2). This is expected since live weight and ADG are indirectly related. In addition, result indicates that ADFI was optimized at a level of 3.03 g/kg feed (Figure 3), indicating that the response of broilers to dietary FTB supplementation may depend on the production variable in question. The high  $r^2$  levels for growth parameters suggest a strong relationship between FTB and improved growth variables. These results have implications for formulating broiler feed with FTB to improve growth parameters, thereby reducing additive wastage. The high  $r^2$  values of FLW, ADFI, and ADG on FTB diets indicate that these results could be predicted at a given amount of FTB supplemented to the diets of starter broilers.

**Table 5:** Fermented tamarind bark levels for optimal performance characteristics of starter broilers

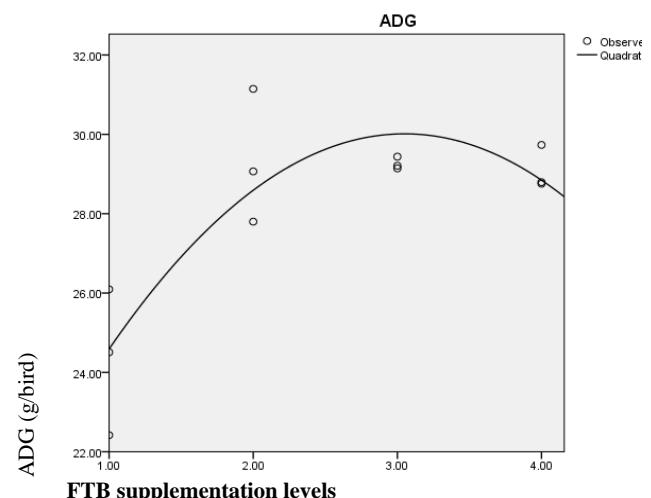
Parameter	Equation	X	Y	$r^2$	pval
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FLW (g/bird)	Y=378.118 +165.370x 27.115x <sup>2</sup>	3.0 - 5	630.2 6	0.74 8	0.00 3
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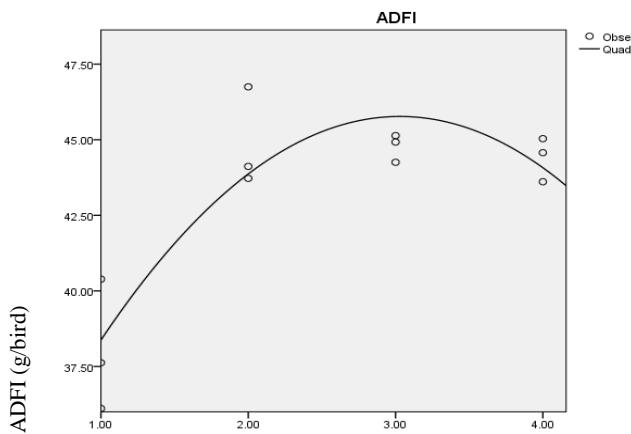
X level optimal FTB level; Y value optimal growth metric;  $r^2$  coefficient of determination; pval probability.



**Figure 1:** Relationship between dietary fermented tamarind bark supplementation level and FLW in starter broilers



**Figure 2:** Relationship between dietary fermented tamarind bark supplementation level and ADG in starter broilers



#### FTB supplementation levels

**Figure 3:** Relationship between dietary fermented tamarind bark supplementation level and ADFI in starter broilers

#### Conclusion

In conclusion, yeast-mediated SSF improved proximate composition, fibre fractions, beneficial phytochemicals (flavonoids and phenols), DPPH activity, and decreased antinutrient content in tamarind. Results also indicated that FTB supplementation at 0.5, 1.0, and 1.5 g/kg feed enhanced FLW, ADG, and ADFI in broilers without negatively impacting FCR and percentage mortality. The quadratic optimization results suggested that FTB should be supplemented in the starter broiler diet at 3.05 and 3.03 g/kg feed for the best FLW, ADG, and ADFI. Blood characteristics, plasma antioxidative status, gut histology, and microbiota composition of starter broilers fed FTB-supplemented diets are recommended, as such published data is lacking in the literature.

#### Conflict of Interest

The authors declare no conflict of interest.

#### Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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