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## Research Article

# Effect of Dietary Supplementation of Probiotic Bacteria Obtained from Fermented *Tchoukoutou* on the Production Performance of Local and Exotic Guinea Fowl

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## Abstract

**Background and Objective:** The use of antibiotic in livestock production has led to the emergence of new strains of pathogenic bacteria resistant to antibiotics. This study aimed to evaluate the effect of feed containing *Lactobacillus casei*, *Lactobacillus fermentum*, *Lactobacillus acidophilus* and *Enterococcus faecium* obtained from fermented *Tchoukoutou* on the performances of local guinea fowl and the exotic guinea fowl (*Numida meleagris*). **Materials and Methods:** The birds were assigned to 3 experimental treatments, namely R<sub>1</sub> group received a diet containing 3% fermented *Tchoukoutou*, R<sub>2</sub> group fed basal diets and R<sub>3</sub> group fed basal diet with an antibiotic (Alfaceryl). A total of 375 guinea-fowl of the local strain and the exotic strain was allotted to each group at the pre-experimental phase were allotted to each group which lasted for one week. **Results:** The results showed that supplementation of probiotic bacteria improved the production performance of local guinea fowl especially at the starter phase whereas the production performance of the exotic birds were not affected. **Conclusion:** It was concluded that the feed containing probiotic bacteria obtained from the fermented *Tchoukoutou* had a beneficial effect on the growth performance of local guinea fowl.

**Key words:** Probiotic, antibiotics, guinea fowl, growth performance, livestock production

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**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Antibiotics is an anti-microbial substance that has inhibitory effect on bacteria growth. As feed additives, they improve growth performance of livestock<sup>1</sup>. However, several studies have highlighted the antibiotic resistance in animals and humans<sup>2</sup>. A study on the use of these antibiotics as growth promoters in chickens revealed the emergence of multi-drug-resistant pathogenic<sup>3</sup>. Antimicrobial resistance has been a public health concern, thus the use of antibiotics has been restricted in the poultry and swine industry<sup>4</sup>.

Several studies have evidenced the presence of antibiotic residues in livestock products due to the use of antibiotics<sup>5</sup>. Thus, whatever the nature of the antibiotic administered, there is a risk of antibiotic residues in animal products (meat, milk and eggs). Therefore, a residual level has been set for each drug and a higher residual percentage endangers the consumer's health. Antibiotics residual percentages in muscles and internal organs varies. In bovine, the meat is contaminated at 38% and the liver at 57%. In meat of avian origin, the gizzards are contaminated at 9% and the livers at 11.7%. The rate of contamination is higher in the liver than the meat and gizzard. The use of antibiotics has been banned in food animals due to the resistance of pathogenic bacteria and consumer concerns<sup>6</sup>. Consequently, there has been a search for possible safe alternative to antimicrobial growth promoters.

Various studies have shown that probiotic microorganisms belonging to the genera *Lactobacillus*, *Streptococcus*, *Bacillus*, *Bifidobacterium*, *Enterococcus*, *Aspergillus*, *Candida* and *Saccharomyces* have beneficial effect on the growth performance of poultry<sup>7-9</sup>. Fermented *Tchoukoutou* containing probiotic bacteria such as *Lactobacillus casei*, *Lactobacillus fermentum*, *Lactobacillus acidophilus* and *Enterococcus faecium*, can serve as alternative to the conventional antibiotics. Previous studies on the use of fermented chukuto as feed additive in the diet of broilers, fish and rabbits have shown the encouraging results<sup>9,10</sup>. The present study therefore aimed to evaluate the effect of fermented *Tchoukoutou* on the production performance of local and exotic guinea fowl.

## MATERIALS AND METHODS

**Experimental location and duration:** This experimental study was carried out at the Poultry Research Laboratory and Zoo-Economy, Faculty of Agricultural Science of University of Abomey-Calavi of Benin. The experiment lasted for 16 weeks for local guinea fowls and 12 weeks for exotic guinea fowls.

**Experimental design and diets:** In this experimental study, 375 day-old keets (local guinea fowl) and 375 day-old keets (exotic guinea fowls) were used. The birds were weighed and those of similar weights were randomly assigned to 3 experimental treatments. Each treatment was further divided into 5 replicates with 25 birds per replicate using a completely randomized design. The birds in the treatments R<sub>1</sub> were fed with diet containing 3% fermented *Tchoukoutou*. R<sub>2</sub> (Control group) were fed with a standard diet and R<sub>3</sub> were also fed with standard diet but with an antibiotic (Alfaceryl). The microbiological and physico-chemical analysis of samples of fermented "kpètè-kpètè" was carried out by N'tcha *et al.*<sup>11</sup>. This analysis showed that there was a great variability between the parameters considered, with the exception of the pH which was relatively the same for all these samples. The fermented "kpètè-kpètè" had the following characteristics: pH = 4.03; Tribal Acidity = 0.14 mg g<sup>-1</sup>; Dry Matter = 7.31%; the load in Log CFU of lactic acid bacteria = 6.42, Yeasts and Moulds = 6.2; and Streptococci = 3.84. The feed containing probiotic bacteria obtained from the fermented *Tchoukoutou* and the traditional "kpètè-kpètè" (fermented) contains four species of bacteria (*Lactobacillus casei*, *Lactobacillus fermentum*, *Lactobacillus acidophilus* and *Enterococcus faecium*) was used in this study<sup>9,10</sup>. The feed and water were provided ad libitum and a lighting program of 23L: 1D was adopted. Feed intake, mortalities, weekly body weight (BW), weight gain (WG), feed cost per kg of BW and economic feed efficiency were recorded during the experiment. Composition of the experimental diet is shown in Table 1.

Table 1: Composition of Experimental diet

Ingredients	Starter diet	Grower diet
Maze	58.30	59.40
Wheat bran	3.00	5.00
Soybean meal	30.00	25.00
Cotton cake	4.00	6.00
Palm oil	1.00	1.00
Oyster shell	1.80	1.80
Lysine	0.20	0.10
Methionine	0.20	0.20
Dicalcium phosphate	1.00	1.00
NaCl	0.30	0.30
Prémix (CMV)	0.20	0.20
Total	100.00	100.00
<b>Nutritional composition</b>		
Dry matter (%)	87.00	87.00
Crude protein (%)	20.22	19.17
Lysine (%)	1.19	1.02
Methionine (%)	0.50	0.50
Amino acid sulfur (%)	0.87	0.85
Calcium (%)	1.00	1.00
Phosphorus (%)	0.60	0.70
Metabolizable energy (kcal kg <sup>-1</sup> )	2879.00	2858.00

**Growth performance:** During rearing period, daily feed intake, weekly body weight and weight gain and mortality were recorded. Feed conversion ratio, feed cost and feed efficiency ratio were calculated using the following formulas<sup>12-14</sup>.

$$\text{Feed conversion ratio} = \frac{\text{Feed consumption}}{\text{Weight gain}}$$

$$\text{Feed cost} = \frac{\text{Daily feed intake} \times \text{cost of kg of feed}}{\text{Daily weight gain (kg)}}$$

**Statistical analysis:** Effect of fermented *Tchoukoutou* on the production performance of local and exotic guinea fowl was analyzed using one way ANOVA with the help of GLM procedure of R software 4.0.2 version. Where significant treatment effects were observed ( $p < 0.05$ ), differences between treatment means were compared using Tukey's test. All parameters were expressed as Mean  $\pm$  standard deviation.

## RESULTS

**Feed intake (g day<sup>-1</sup>) measurement:** At the starter phase, feed intake of the two strains of guinea fowl was not significantly influenced by all experimental treatments (Table 2). However, at the growing phase, feed intake of local guinea fowl fed R<sub>1</sub> diet was significantly ( $p < 0.05$ ) lower than

those of the birds fed R<sub>3</sub> diet. Feed intake of exotic guinea fowls fed R<sub>3</sub> diet was significantly ( $p < 0.05$ ) higher than those of the other treatment groups (Table 2).

**Body weight (g):** In the local guinea fowl, the body weight at the starter phase was significantly ( $p < 0.05$ ) higher in birds fed with R<sub>1</sub> diet than those of the birds fed with R<sub>3</sub> and R<sub>2</sub> diets. However, the body weight at the growing phase, was significantly ( $p < 0.05$ ) higher in birds fed with R<sub>3</sub> diet than those of R<sub>1</sub> and R<sub>2</sub> groups. In the exotic guinea fowl, body weight of birds in R<sub>3</sub> group both at starter and growing phase were significantly ( $p < 0.05$ ) higher than those of the R<sub>1</sub> and R<sub>2</sub> groups. Also, the body weight of guinea fowl in R<sub>2</sub> group was lower in all rearing phases irrespective of strain (Table 3).

**Daily weight gain:** In the local guinea fowl, at the growing phase, the daily weight gain was not significantly affected by treatment. However, weight gain was significantly higher in R<sub>1</sub> group at the starter phase compared to those of R<sub>3</sub> and R<sub>2</sub> groups at the growing phase. However, in the exotic guinea fowl, the daily weight gain was significantly ( $p < 0.05$ ) higher in the R<sub>3</sub> group compared to R<sub>1</sub> and R<sub>2</sub> group. Independent of the strain, the daily weight gain of birds in R<sub>2</sub> group was lower during all the rearing phases (Table 4).

**Feed conversion ratio (FCR):** Feed conversion ratio of both strains in all rearing phases was significantly ( $p < 0.05$ ) lower in

Table 2: Average feed intake of local guinea fowl and exotic guinea fowl

Phases	Treatments			p-value
	Local guinea fowl			
	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	
Starter	4.38±1.00	4.71±1.00	4.67±1.00	0.900
Grower	11.78±0,06 <sup>b</sup>	15.08±2.01 <sup>ab</sup>	18.17±1.99 <sup>a</sup>	0.008
<b>Exotic guinea fowl</b>				
Starter	13.68±0.01	17.36±2.10	16.59±2.01	0.070
Grower	24.78±0.10 <sup>a</sup>	29.89±0.08 <sup>b</sup>	30.47±0.06 <sup>c</sup>	0.001

Means with different superscripts a, b, c across rows are significantly ( $p < 0.05$ ) different, R means group or treatment

Table 3: Average body weight (g) of local and exotic guinea fowl

Phases	Treatments			p-value
	Local guinea fowl			
	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	
Starter	90.77±5.01 <sup>b</sup>	66.27±6.01 <sup>a</sup>	69.01±4.02 <sup>a</sup>	0.001
Grower	261.31±6.04 <sup>b</sup>	231.43±3.02 <sup>a</sup>	294.01±3.01 <sup>c</sup>	0.001
<b>Exotic guinea fowl</b>				
Starter	187.97±0.01 <sup>b</sup>	174.19±2.10 <sup>a</sup>	190.91±2.01 <sup>c</sup>	0.001
Grower	459.20±0.10 <sup>b</sup>	436.40±0.08 <sup>a</sup>	476.03±0.06 <sup>c</sup>	0.005

Means with different superscripts a, b, c across rows are significantly ( $p < 0.05$ ) different, R means group or treatment

Table 4: Average daily weight gain (g) of local and exotic guinea fowl

Table 1: Average daily weight gain (g) of local and exotic guinea fowl				
	Treatments			
	Local guinea fowl			
Phases	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	p-value
Starter	1.38±1.01 <sup>c</sup>	0.96±0.11 <sup>a</sup>	1.02±0.20 <sup>b</sup>	0.001
Grower	2.69±1.08	2.55±1.06	3.51±1.10	0.500
<b>Exotic guinea fowl</b>				
Starter	3.94±0.30 <sup>b</sup>	3.63±1.10 <sup>a</sup>	4.07±0.10 <sup>c</sup>	0.001
Grower	5.72±2.01 <sup>b</sup>	5.53±0.20 <sup>a</sup>	5.87±1.01 <sup>c</sup>	0.001
Means with different superscripts a, b, c across rows are significantly (p<0.05) different, R means group or treatment				

Means with different superscripts a, b, c across rows are significantly (p<0.05) different, R means group or treatment

Table 5: Feed conversion ratio (FCR) of local and exotic guinea fowl

Table 3: Feed conversion ratio (FCR) of local and exotic guinea fowl				
	Treatments			
	Local guinea fowl			
Phases	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	p-value
Starter	1.05±0.20 <sup>a</sup>	1.63±1.30 <sup>c</sup>	1.52±0.02 <sup>b</sup>	0.001
Grower	1.45±1.11 <sup>a</sup>	1.97±0.02 <sup>c</sup>	1.72±2.02 <sup>b</sup>	0.001
<b>Exotic guinea fowl</b>				
Starter	1.15±1.06 <sup>a</sup>	1.59±2.10 <sup>c</sup>	1.35±1.40 <sup>b</sup>	0.001
Grower	1.44±0.70 <sup>a</sup>	1.80±1.02 <sup>c</sup>	1.72±2.10 <sup>b</sup>	0.001

Means with different superscripts a, b, c across rows are significantly (p<0.05) different, R means group or treatment

Table 6: Feed cost per kg of body weight of local and exotic guinea fowl

Table 8. Feed cost per kg of body weight of local and exotic guinea fowl				
	Treatments			
	Local guinea fowl			
Phases	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	p-value
Starter	332.42±4.30 <sup>a</sup>	416.13±2.60 <sup>c</sup>	387.72±2.20 <sup>b</sup>	0.001
Grower	459.61±0.51 <sup>b</sup>	502.49±1.20 <sup>c</sup>	440.07±0.14 <sup>a</sup>	0.001
<b>Exotic guinea fowl</b>				
Starter	364.08±3.10 <sup>b</sup>	406.66±2.03 <sup>c</sup>	346.09±4.01 <sup>a</sup>	0.001
Grower	454.54±1.01 <sup>b</sup>	459.40±2.06 <sup>c</sup>	440.69±2.30 <sup>a</sup>	0.001

Means with different superscripts a, b, c across rows are significantly (p<0.05) different, R means group or treatment

R<sub>1</sub> treatment group compared to R<sub>2</sub> and R<sub>3</sub>. Irrespective of the strain and rearing phase, feed conversion ratio of exotic guinea fowl in R<sub>1</sub> group was lower compared to R<sub>2</sub> and R<sub>3</sub> groups (Table 5).

**Feed cost evaluation:** In indigenous guinea fowl, feed cost was significantly (p<0.05) lower in R<sub>1</sub> group compared to other groups at the starter phase. At the grower phase, feed cost was significantly lower in R<sub>3</sub> group in contrast to other treatments. In the exotic guinea fowl, feed cost of R<sub>3</sub> group was significantly (p<0.05) lower than R<sub>1</sub> and R<sub>2</sub> during the two production phases (Table 6).

**Feed efficiency ratio:** In the local guinea fowl, at the starter phase, the feed efficiency ratio was significantly (p<0.05) higher in the R<sub>1</sub> group compared to the R<sub>2</sub> and R<sub>3</sub> groups. But in the exotic guinea fowl, the feed efficiency ratio was significantly (p<0.05) higher in the R<sub>3</sub> group at the onset and growth phase compared to the R<sub>1</sub> and R<sub>2</sub> groups (Table 7).

## DISCUSSION

The objective of this research was to evaluate the effect of feed supplemented with fermented *Tchoukoutou* on the production performance of local and exotic guinea fowl (*Numida meleagris*) without antibiotics. In the local guinea fowl, at the starter phase, there was an improvement in the body weight of R<sub>1</sub> group, which received a diet containing 3% fermented *Tchoukoutou* compared to R<sub>2</sub> and R<sub>3</sub> groups. These results are similar to those of Patterson *et al.*<sup>15</sup> and Houndonougbo *et al.*<sup>9</sup> who noticed an improvement in the weight of chicken fed diet containing probiotic residues (*Bacillus subtilis* C-3102) or *Tchoukoutou* compared to the control group at starter phase. In addition, similar results were obtained by Marion and Jean-Paul<sup>16</sup>; Al-Dobaib and Mousa<sup>17</sup> and Krehbiel *et al.*<sup>18</sup> who reported that probiotic bacteria have growth promoting effects on the pig, bovine and poultry. This finding could also be explained by the fact that probiotics improved the feed efficiency. Indeed, independent of the

Table 7: Feed efficiency ratio of local and exotic guinea fowl

Table 7: Feed efficiency ratio of local and exotic guinea fowl				
	Treatments			
	Local guinea fowl			
Phases	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	p-value
Starter	1.00±1.08 <sup>c</sup>	0.80±2.01 <sup>a</sup>	0.85±1.10 <sup>b</sup>	0.001
Grower	0.72±2.55 <sup>b</sup>	0.66±2.03 <sup>a</sup>	0.75±1.04 <sup>c</sup>	0.200
<b>Exotic guinea fowl</b>				
Starter	1.06±2.03 <sup>b</sup>	0.95±2.01 <sup>a</sup>	1.12±2.05 <sup>c</sup>	0.001
Grower	0.85±1.50	0.84±1.06	0.88±1.70	0.100

Means with different superscripts a, b, c across rows are significantly ( $p < 0.05$ ) different, R means group or treatment

strain of guinea fowls and feed treatments, the feed intake was statistically similar in local and exotic guinea fowl at the starter phase. But the feed intake of birds in R<sub>1</sub> group which received the feed containing 3% fermented *Tchoukoutou* was lower than those of R<sub>2</sub> and R<sub>3</sub> groups. Finally, probiotic treatment reduced feed intake in local and exotic guinea fowl. This might be due to the taste of feed and its characteristics<sup>19</sup>. Similar results were obtained by Benali *et al.*<sup>20</sup> and Houndonougbo *et al.*<sup>9</sup> who noticed that diet supplemented with probiotic bacteria was more digestible and easily excreted and the daily feed consumption decreased significantly with the increase in the energy level of feed in rabbits of Hyplus strain. These bacteria therefore help the guinea fowl of the R<sub>1</sub> group to digest the energy contained in the feed which was supplemented with fermented *Tchoukoutou*<sup>20</sup>. Probiotic bacteria therefore made it possible to establish a balance in the intestinal flora in guinea fowl<sup>21</sup>.

The efficiency of the diet containing *Tchoukoutou* residues could be explained by decreased feed conversion ratio and increased daily weight gain, especially during the starter phase of local guinea fowl in R<sub>1</sub> group. It could be alleged that diet containing fermented probiotics "*Tchoukoutou*" improved their daily weight gain. These results are consistent with those of Chavez *et al.*<sup>22</sup>; Atela *et al.*<sup>23</sup> and Manafi *et al.*<sup>24</sup> who reported better feed conversion ratio ( $p < 0.05$ ) in chicken. Moreover, the increase in daily weight gain resulted in lowering the efficiency of diet (fermented *chukuto*) containing the probiotic bacteria. These results are consistent with those of Kalavathy *et al.*<sup>7</sup>; Awad *et al.*<sup>25</sup>; Tayeb *et al.*<sup>26</sup>; Ledezma-Torres *et al.*<sup>27</sup>; Gninkpo *et al.*<sup>10</sup> and Balamuralikrishnan *et al.*<sup>28</sup> who observed an increase in the daily weight gain in chickens and rabbits which received probiotic bacteria in drinking water or in feed compared to the control groups (without probiotic). When feed intake and feed conversion ratio are lower, then the feed efficiency ratio is higher<sup>13</sup>. This rise explained that breeders spend less and earn

more. Thus, the diet containing the bacteria (fermented *Tchoukoutou*) was more beneficial for the local guinea fowl at the starter phase while this benefit decreased at the growing phase. However, the use of feed containing probiotic bacteria was not beneficial for exotic guinea fowl during the starter and growing phases. Furthermore, the increase in the feed efficiency ratio explained that the feed cost recorded at the starter phase was lower in the local guinea fowl. These results are in agreement with those of Gninkpo *et al.*<sup>10</sup> who carried out an experiment in red-eyed rabbits with the incorporation of fermented *Tchoukoutou* in the diet and Dehou<sup>29</sup> who incorporated palm nut fibers in the diet of fattening rabbits. All the experimental treatments led to the reduction of feed cost. A contradictory result was reported by Chrysostome *et al.*<sup>30</sup> who conducted an experiment using the R<sub>c</sub> and R<sub>o</sub> cockerels which received a full ration compared to the cafeteria system. Results of the present study showed that the feed containing the probiotic bacteria obtained from fermented *Tchoukoutou* was much more beneficial to the local guinea fowl especially at the starter phase compared to the exotic guinea fowl.

## CONCLUSION

The incorporation of 3% fermented *Tchoukoutou* in the ration of local and exotic guinea fowl improved the production performances with an increase in body weight, daily weight gain and feed efficiency ratio. Also, this resulted in a reduction in feed intake, feed conversion ratio and feed cost especially at the starter phase in local guinea fowl.

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