

*Full Length Research Paper*

# **Bio-appraisal of three strains of *Lactobacillus* based probiotics on the growth traits and semen characteristics of local toms**

**Dim C. E.<sup>1</sup>, Ekere S. O.<sup>2\*</sup>, Ugwuoke J. I.<sup>1</sup>, Ndofor-Foleng H. M.<sup>1</sup> and Onyimonyi A. E.<sup>1</sup>**

<sup>1</sup>Department of Animal Science, Faculty of Agriculture, University of Nigeria, Nsukka, Enugu State, Nigeria.

<sup>2</sup>Department of Veterinary Obstetrics and Reproductive Diseases, Faculty of Veterinary Medicine, University of Nigeria, Enugu State, Nigeria.

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The effect of three strains of *Lactobacillus* based probiotics on the growth performance and semen quality of toms was conducted in a thirty-four week study. Eighty day-old (d 1) poults were randomly divided into 4 groups, replicated twice with 10 birds per replicate and assigned to four treatments; T1 (*Lactobacillus delbrueckii* subsp *Bulgaricus*), T2 (*Lactobacillus acidophilus*), T3 (*Lactobacillus sporogens*) and T4 (control), in a completely randomized design (CRD). Feed and water were supplied *ad libitum*. Results showed significant differences ( $P<0.05$ ) in the birds' mean values for average daily weight gain, average daily feed intake, feed conversion ratio and final body weight. Birds on T2 had significantly ( $P<0.05$ ) higher final body weight than other treatment groups. Toms on T4 had significantly ( $P<0.05$ ) higher values for feed conversion ratio than birds on probiotic treatment with T2 recording the least value. However, the effect of treatments on the semen physical characteristics of the birds were also found to be significant ( $P<0.05$ ). The birds on probiotic treatment recorded higher values for the semen quality indices under study than the control group, with T2 having the highest values. It was thus concluded that of the three strains of *Lactobacillus* based probiotics used in the current study, T2 (*L. acidophilus*) improved the growth performance and semen quality of local toms.

**Key words:** Artificial insemination, direct-fed microbial, growth, nutrition, turkey production.

## **INTRODUCTION**

Artificial insemination (AI) is one of the animal production technologies commonly referred as Assisted Reproductive Technology (ART), where off-springs are generated from parents by facilitating the union of gametes (Morrell, 2011). It simply involves the collection of semen from choice male(s) and transferring the semen into the vagina of the females for fertilization to take

place. AI has proved to be an indispensable tool in turkey production owing to the selective breeding for a heavier and broader-breasted commercial turkey, and the consequent incapacitation of toms to natural mating due to their larger body size compared to the hens (Bakst and Dymond, 2013). Moreover, it has also been found to enhance cross breeding programs, improve productive

\*Corresponding author. E-mail: samuel.ekere@unn.edu.ng.

performance and ensure rapid sustainable economic gains from turkey birds (Ngu et al., 2014).

However, to achieve a successful AI program, the need for an outstanding quality of semen cannot be overemphasized (Harstine et al., 2018). Semen of good quality, typified by the quality parameters of semen such as; volume, concentration, color, and motility, guarantees its usefulness in any AI program (Kotłowska et al., 2005). Although the quality of poultry semen can be influenced by a myriad of factors ranging from; age of the bird (Murugesan et al., 2012), breed/strain (Abas et al., 2018), temperature (Karaca et al., 2002), nutrition (Mahmood and Hazim, 2011; Hudson and Wilson, 2003), environmental conditions (Elagib et al., 2012; Santiago-Moreno et al., 2015), etc., it is pertinent to note that poor quality semen represents a great economic loss to production (Khatun et al., 2013). Hence, the need to improve and maintain a superior semen profile in any poultry AI program becomes imperative.

Throughout the last decade, animal nutritionists continued to search and document several dietary managements that promoted poultry semen (Heydari et al., 2015; Sharideh et al., 2015; Deivendran and Yeong, 2015; Murugesan et al., 2016; Amin et al., 2019) and body weight (Dim et al., 2018), as both are positively correlated. These documented researches also included the dietetic use of probiotics to improve poultry semen (Inatomi and Otomaru, 2018; Aalaei et al., 2019). Probiotics have been defined as direct-fed live microbials that confer beneficial effects on the host when administered amply (FAO, 2009). They are principally composed of bacteria and/or fungi organisms, but can also be of protozoan origin (Chabe et al., 2017).

The lactic acid bacteria are the most dominant class of colonizing bacteria species used as probiotics to improve production traits in farm animals (Ezema, 2013; Panda et al., 2007; Ehrmann et al., 2002). Reports of Duncan et al. (2004) suggests that they exhibit the cross-feeding mechanism, where the lactic acids they produce are utilized by the strictly anaerobic butyrate producing bacteria for the assemblage of large concentrations of butyric acid. These assembled butyrate function to change the intracellular pH, thus leading to the exclusion of the pathogenic bacteria cells in the gut (Panda et al., 2009). Also, the development of epithelial cells with improved gastrointestinal health has been linked to butyric acid metabolism in the gut (Bron et al., 2002). These protocols justify why lactic acid bacteria administration can significantly enhance performance indices in food animals. Conversely, Triplett et al. (2016), Kiess et al. (2016) and Haines et al. (2015) made some interesting observations on the decreased quality of poultry semen in-relation with lactic acid bacteria. They suggested that *Lactobacillus* organisms negatively affected the quality of poultry semen under the conditions of their respective studies.

However, there is a dearth of credible literatures on the

improvement of the indigenous local turkey (*Meleagris gallopavo*) despite its hardy and resilient adaptation features to the tropical humid environment. These birds adapt under unfavorable environmental conditions and meager nutritional status better than most poultry species (Perez-Lara et al., 2013; Yakubu et al., 2013). The meat percentage of the toms is better expressed through their massive, stocky and long-legged indices (Damaziak et al., 2014). Therefore, a study designed to bio-assess the growth rate and semen quality of local toms raised in the tropics, fed three selected strains of *Lactobacillus* based probiotics is prompt and of paramount significance.

## MATERIALS AND METHODS

### Location and duration of study

The study was conducted at the turkey unit of Veterinary Teaching and Research Farm, University of Nigeria, Nsukka. Geographically, Nsukka is located within longitude 07°54 'E and latitude 05°22 'N, with annual rainfall range of 966- 2098mm (Momoh et al., 2010). Agbagha et al. (2000) reported the study area to be typically tropical, having a mean daily temperature of 26.8°C, and relative humidity percentage values that ranges from 65-80%. Duration of study lasted a period of 28 weeks.

### Test strains

The three selected strains of the *Lactobacillus* – based probiotics used in the study included *Lactobacillus sporogenes* NRRL – 4496 ( $1 \times 10^8$  CFU/ml), *Lactobacillus delbrueckii* subspecies *bulgaricus* NRRL B – 4527 ( $1 \times 10^8$  CFU/ml) and *Lactobacillus acidophilus* NRRL – 4495 ( $1 \times 10^8$  CFU/ml). They were obtained and constituted in an MRS broth from the Microbial Genomics and Bio-processing Research Unit, National Centre for Agricultural Utilization Research, Agricultural Research Service, United States Department of Agriculture, University St., Peoria, Illinois. Upon receipt, the probiotics were maintained in the broth medium and kept in a cold room at a temperature of 18°C.

### Experimental diets

The experimental diets comprised starter, grower and finisher diets, with their respective percentage compositions are presented in Table 1. The poults were fed the starter diets from day-old (d-1) to eight weeks of age before carefully replacing their feed gradually to the grower diets. The birds were placed on the finisher diets from sixteen weeks of age till the completion of the study.

### Management of experimental birds

The conduct of the current study adhered strictly to the provisions of the ethical committee on the use of animals and humans for biomedical research of the University of Nigeria. 80 day old (d-1) male poults were randomly divided into 4 groups of 20 birds each. Each group was randomly assigned to four treatment groups, tagged; T1, T2, T3 and T4, in a completely randomized design (CRD). Each treatment group was replicated twice with 10 birds per replicate, placed in a deep litter system. Birds on T1, T2 and T3 received probiotics of *L. delbrueckii* subspecies *bulgaricus*, *L. acidophilus* and *L. sporogenes*, respectively at inclusion levels of

**Table 1.** Percentage composition of experimental diets.

Ingredients	Starter (%)	Grower (%)	Finisher (%)
Maize	45.00	48.00	50.00
Soybean meal	40.00	37.00	24.10
Fishmeal	7.30	4.50	1.50
Wheat offal	0.00	4.00	17.55
Bone meal	4.30	3.20	3.65
Oyster shell	2.50	2.50	2.50
Vitamin Premix*	0.25	0.25	0.25
Salt	0.25	0.25	0.25
DL-methionine	0.30	0.20	0.10
Lysine	0.10	0.10	0.10
Total	100	100	100
<b>Calculated composition</b>			
Crude protein (%)	26.00	22.40	19.50
Crude fibre (%)	3.90	4.26	4.65
Ether extract (%)	5.00	4.52	4.31
ME (K cal/Kg)	2845.00	3100.00	3150.00

\*Each 2.25 kg of vitamin premix contains; 10,000,000 I.U Vitamin A, 2,200,000 I.U Vitamin D3, 10,000 mg Vitamin E, 2000 mg. Vitamin K3, 1500 mg Vitamin B1, 5000 mg Vitamin B2, 1500 mg, Vitamin B6, 10 mg Vitamin B12, 15,000 mg Niacin, 20 mg biotin, 125,000 mg Anti-Oxidant, 500 mg, Folic acid, 5000 mg Calpan.

0.5ml/L in their drinking water for 3 consecutive days from 1 - 3, 10 - 12 and 21 - 23 days of age to achieve the recommended microbial concentration ( $10^6$  CFU/ml) as stipulated by the supplier. 0.5 kg of milk powder/1000 L of the birds' drinking water served as substrates to protect the lactic acid bacteria from oxidative damage during the duration of administration. T4 served as control with no strain of *Lactobacillus* inclusion. Feed and water were provided *ad libitum*. Birds were weighed at the start of the experiment and also at weekly intervals to obtain their weekly bodyweight gain. Daily feed intake was also recorded for the birds all through the period of study.

#### Semen analysis

The Burrows and Quinn method of abdominal massage as outlined by Yahaya et al. (2013) was used for the semen collection. Semen volume was graduated using a conical test tube. The progressive motility and spermatozoa count was determined using a haemocytometer (450 × magnifications) and a light microscope with warm stage. The Ernst and Ogasawara technique as cited by Ngu et al. (2014) was used to determine the percentage values of live/dead and normal/abnormal sperm cells.

#### Statistical analysis

One way analysis of variance (ANOVA) was used to analyze the data generated in the current study, using a statistical package (SPSS) windows version 20.0. The differences in the mean values were separated using the Duncan New Multiple Range Test as put forward by Obi (2002). The experimental model of the CRD used in the current study is:

$$X_{ij} = \mu + T_i + \sum_{ij}$$

Where  $X_{ij}$  = any observation or measurement taken,  $\mu$  = population mean,  $T_i$  = Treatment effect,  $\sum_{ij}$  = Experimental error,  $i$  = number of treatments,  $j$  = number of replicates.

## RESULTS AND DISCUSSION

### Effect of three strains of *Lactobacillus* based probiotics on the growth traits of local toms

Table 2 shows the growth performance of local toms fed three strains of *Lactobacillus* based probiotics (LBP). Results showed significant differences ( $P < 0.05$ ) in the birds' values for final body weight, average daily weight gain, average feed intake, and feed conversion ratio (FCR) across the treatment groups. Birds on LBP treatments were observed to be heavier ( $P < 0.05$ ) than those on the control group, with toms on T2 (*L. acidophilus*) recording the most superior values. Despite the differing numerical values recorded for birds on T1 (*L. delbrueckii* subspecies *bulgaricus*) and T3 (*L. sporogenes*), their mean values were found to be statistically similar ( $P < 0.05$ ). However, toms on LBP treatments consumed less feed ( $P < 0.05$ ) than birds on the control group (T4), with birds on T2 recording the least feed intake values across the treatment groups under study. Toms on T1 and T3 also had comparable ( $P < 0.05$ ) average daily feed intake values while varying numerically. Hence, it follows that birds on LBP treatments ate less feed to weigh more than the birds on

**Table 2.** Growth performance of toms fed three strains of *Lactobacillus* based probiotics.

Parameter	Treatments				SEM
	T1	T2	T3	T4	
Initial Body Weight (g)	60.01	60.03	60.02	60.00	1.63
Final Body weight (g)	9450.00 <sup>b</sup>	9900.00 <sup>a</sup>	9500.00 <sup>b</sup>	8000.00 <sup>c</sup>	200.51
Average Daily Weight Gain(g)	39.70 <sup>b</sup>	41.59 <sup>a</sup>	39.91 <sup>b</sup>	33.61 <sup>c</sup>	1.02
Average Daily Feed Intake (g)	125.45 <sup>b</sup>	120.61 <sup>c</sup>	124.91 <sup>b</sup>	134.45 <sup>a</sup>	2.80
Feed Conversion Ratio	3.16 <sup>b</sup>	2.90 <sup>c</sup>	3.13 <sup>b</sup>	4.00 <sup>a</sup>	0.96

<sup>abc</sup>Means on the same row with different superscripts are significantly ( $P<0.05$ ) different. SEM= Standard error of Mean. T1= *L. delbrueckii* subspecies *bulgaricus*; T2= *L. acidophilus*; T3= *L. sporogenes*; T4= control.

the control group. This translated to the improved FCR values ( $P<0.05$ ) observed in birds on LBP treatments, with toms on T2 recording the best-quality values. The increased growth performance indices observed in birds on LBP treatments can be linked to the role of these bacteria in enhancing bio-availability of nutrients, nutrient assimilation and metabolism. Literature evidence suggests that lactic acid bacteria utilize carbohydrates as substrates for growth (Watson et al., 2013), while most pathogenic bacteria primarily employ secreted proteins as their substrate-media (Figaj et al., 2019; Cezairliyan and Ausubel, 2017). By producing bacteriocins and a gut pH that limits the favorable proliferation of the pathogenic bacteria in the gastrointestinal tract, the lactic acid bacteria competitively excludes the harmful bacteria from the tract, thus allowing for total absorption of their secreted substrate proteins by the body of the birds. This consequently transformed to the amplified growth response observed in birds on the *Lactobacillus* based probiotics (LBP) treatments. These results confirm the observations of Li et al. (2014) and Vantsawa et al. (2017), who documented a major increase in bodyweight and decrease in FCR for birds fed/treated with *L. acidophilus*. The outcome of the present study substantiates the reports of Ahmad (2006) and Eckert et al. (2010) who demonstrated the positive effect of probiotics on poultry birds.

#### Effect of three strains of *Lactobacillus* based probiotics on semen quality of local toms

Table 3 shows the effect of the three strains of *Lactobacillus* based probiotics (LBP) on semen characteristics of local toms. Results from the study illustrated significant differences ( $P<0.05$ ) in the toms values for all the semen quality indices studied, ranging from semen volume, semen concentration, progressive motility, percentages of live, dead, normal and abnormal spermatozoa. Toms on LBP treatments (T1, T2 and T3) produced more concentrated semen ( $P<0.05$ ) with greater percentages of live and normal sperm cells that are highly motile than the control group. Birds on T2 (*L.*

*acidophilus*) recorded the top values ( $P<0.05$ ) for volume of semen, concentration of semen, and percentage live spermatozoa among the treatment groups, with birds on T1 (*L. delbrueckii* subspecies *bulgaricus*) and T3 (*L. sporogenes*) recording similar ( $P<0.05$ ) comparable statistical values irrespective of their contrasting numerical values. However, toms on T1 and T2 were observed to produce the most progressive motile spermatozoa ( $P<0.05$ ) across the groups under study. Also, percentage values for dead and abnormal spermatozoa were observed to be higher ( $P<0.05$ ) in birds on the control group, with birds on T2 having the least values. Moreover, toms on *L. acidophilus* administration had the best-quality values ( $P<0.05$ ) for normal sperm cells across the groups under study. These quality values were also found to be statistically the same ( $P<0.05$ ) with the values recorded for birds on *L. delbrueckii* subspecies *bulgaricus* and *L. sporogenes*. Nevertheless, birds on the control group also had the lowest values ( $P<0.05$ ) for normal sperm cells, which were however observed to be statistically similar ( $P<0.05$ ) with the values recorded for birds on T1 and T3. The improved semen quality indices observed in birds on the LBP treatments can however be linked to the role of these bacteria in the synthesis of trace minerals and vitamins in the birds' gut that supported quality of semen. Reports have it that *Lactobacillus* bacteria synthesize trace minerals (Nagy et al., 2016), antioxidant vitamins (E and C) and B-complex vitamins (LeBlanc et al., 2011). These vitamins ( $B_{12}$ , E and C) have been found to guarantee improved spermatozoa motility and count in food animals (Surai et al., 2001; Eid et al., 2006; Deivendran and Yeong, 2015; Banihani, 2017). Trace minerals like selenium, zinc and manganese produced by these bacteria also work to boost spermatogenesis in poultry (Barber et al., 2005). Furthermore, the secreted substrate proteins absorbed by the birds' gastrointestinal tract as a result of the pathogenic exclusion exhibited by the lactic acid bacteria, ultimately supported the improved quality of semen observed in birds on LBP treatments. It is evident from the results observed in the current study, that probiotic organisms support superior quality of semen as opined by Khan et al. (2012) and Emmanuel

**Table 3.** Effect of three strains of *Lactobacillus* based probiotics on semen quality of local toms.

Parameter	Treatments				SEM
	T1	T2	T3	T4	
SV (ml)	0.39 <sup>b</sup>	0.48 <sup>a</sup>	0.38 <sup>b</sup>	0.27 <sup>c</sup>	0.03
SC (x10 <sup>9</sup> /ml)	5.11 <sup>b</sup>	5.82 <sup>a</sup>	5.13 <sup>b</sup>	4.78 <sup>c</sup>	0.09
PM (%)	82.20 <sup>a</sup>	82.93 <sup>a</sup>	81.17 <sup>b</sup>	70.13 <sup>c</sup>	1.57
LS (%)	93.20 <sup>b</sup>	94.13 <sup>a</sup>	93.07 <sup>b</sup>	87.73 <sup>c</sup>	1.08
DS (%)	6.80 <sup>b</sup>	5.87 <sup>c</sup>	6.93 <sup>b</sup>	12.27 <sup>a</sup>	0.63
NS (%)	90.00 <sup>ab</sup>	91.38 <sup>a</sup>	90.32 <sup>ab</sup>	82.33 <sup>b</sup>	0.18
ABS (%)	10.00 <sup>b</sup>	8.62 <sup>d</sup>	9.68 <sup>c</sup>	17.67 <sup>a</sup>	0.12

<sup>abc</sup> Means on the same row having different superscripts are significantly different (P<0.05); SEM = Standard Error of Mean. SV = Semen volume; SC = Sperm concentration; PM = Progressive motility; LS = Live sperm; DS = Dead sperm; NS = Normal sperm; ABS = Abnormal sperm. T1= *L. delbrueckii* subspecies *bulgaricus*; T2= *L. acidophilus*; T3= *L. sporogenes*; T4= control.

et al. (2018). These results debunk the findings of Triplett et al. (2016), Haines et al. (2015) and Kiess et al. (2016) who documented decreased quality of poultry semen when associated with lactic acid bacteria. The differences might be attributed to a collage factors like breed/strain of the experimental birds, strain of the lactic acid bacteria used, climatic condition of the study area, among others. However, the superiority of semen observed in the present study validates the report of El-Deep et al. (2011), who observed improved semen quality indices in birds fed probiotic organisms.

## CONCLUSION AND RECOMMENDATION

It is glaring from the results obtained in the present study that birds on T2 (*L. acidophilus*) recorded the most superior values for the birds' growth traits and semen quality indices studied among the treatment groups. *L. acidophilus* based probiotics should therefore be used in local toms' nutrition to improve their growth performance and of course, the quality of semen. Nevertheless, further research should be carried out to understand how these bacteria affect their host beneficially even at the genomic level. A better understanding of how they affect the genes responsible for production traits in birds will bring poultry research closer to the field of nutrigenomics.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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