



# Effects of 4BAC-Extra Probiotic Supplementation Levels on Nutrient Digestibility and Blood Parameters of Sasso Laying Hens

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## Abstract

This study was conducted to evaluate the effects of 4Bac-Extra Probiotic supplementation levels on nutrient digestibility and blood parameters of sasso laying hens using One Hundred and Sixty (160) Sasso layers that were randomly assigned into four treatments with four replicates each representing T1- 0, T2- 5, T3- 10 and T4- 15% supplementation of basal feed with 4Bac-Extra probiotics. During the sixty two days (62 d) experimental period, the birds were managed on deep litter and provided with the experimental feeds and water *ad libitum*. The parameters measured were nutrient digestibility, biochemical and hematological indices. Nutrient digestibility indicates a significant ( $P < 0.05$ ) difference between treatments with better and higher values in diet  $T_4$  for most nutrients measured while Hematological and serum biochemical showed no significant ( $P > 0.05$ ) difference between treatments. The results obtained from this experiment indicate that the effects of 4-Bac Extra probiotics were only slight. However, inclusion of 5 – 10% was found to have satisfactory effects on birds.

## INTRODUCTION

The use of In-feed antibiotic supplements to achieve a balance between birds' productive performance and health improvement is gradually in the decline in most countries of the world due to its effects on the animal and humans who are the consumers of animal products. Therefore, researchers are looking in the direction of natural means of replacing the use of in- feed antibiotics in poultry nutrition (Genedy Salwa and Zeweil, 2003; Ibrahim *et al.*, 2005). Thus, the use of exogenous probiotics in poultry feeds to improve bird performance is fast gaining acceptability as a good replacement (Ari *et al.*, 2016).

The utilization of feeds and additives are measured by their conversion into useful nutrients readily available for uptake and conversion for maintenance and production needs of the animals. Nutrient digestion and serum assay provides biological platforms for assessment. Dierick (1989) reported that probiotics increase activity of intestinal enzymes and digestibility of nutrients. Similarly O'Sullivan *et al.* (1992) reported that probiotics change complex foods into simpler forms in body and they are able to complement many deficiencies in our digestive system such as production of vitamin K and absorption of certain ions. It is important to note that probiotics maintain normal intestinal microbiota by competitive exclusion and antagonism against disease causing microbes, and therefore this role is exploited in maintaining health of man and animals or birds. Studies have shown that probiotics influences serum profile and composition of haematological parameters (Corcoran *et al.*, 2005; Jain, 2010; Aro and Akinmoegun, 2012). This study was aimed at evaluating the effects of 4bac-extra probiotic supplementation levels on the nutrient digestibility and serum profile of sasso laying hens.

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### MATERIALS AND METHODS

#### Experimental Site

The study was conducted at the Livestock Teaching and Research Farm of Faculty of Agriculture, Shabu-Lafia Campus, Nasarawa State University, Keffi. It is located on latitude 08.35°N and longitude 08.33°E in the Guinea Savannah Zone of North Central Nigeria.

#### Source of Feed Ingredients

Maize grain was purchased from Doma market, groundnut cake, premix, methionine, lysine, limestone and bone meal were purchased in Jos Plateau State while 4-Bac Extra (probiotics) was bought from Mid-Century Agro-Allied Venture Limited Lagos, Lagos State.

#### Experimental Diets and Feed Preparation

The experimental diets were formulated using *Feed win Software* to provide 2735kcal of metabolizable energy and 16.25% of crude protein as basal diets. The experimental treatment diets were divided into four treatment groups representing 0.00, 0.125, 0.250, and 0.357 mg/100g supplementation of diet with 4Bac-Extra probiotic through addition to basal diet; this represents experimental treatments T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>. The formulated diets were prepared using hammer mill to grind, the probiotic was add accordingly prior to mixing and pelleting. The feeds were pelleted using a 2mm single screw extruder.

#### Experimental Treatment and Data Collection

One hundred and sixty (160) pre-peak sasso laying birds were randomly allocated to four treatment groups of Four (4) replicate, each treatment group was fed *ad libitum* treatments diets T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> respectively during experimental period. The birds were raised under the deep litter while routine management practices and water was provided throughout. The following data were taken:

#### Nutrient Digestibility

The nutrient utilization of diets containing different levels of probiotic evaluation was conducted using sixteen (16) birds from each treatment and four (4) per replicates birds that were transferred to the metabolic cages. A 24 hr adjustment period in cages was allowed, dried sacks were spread below the cages to collect faecal droppings daily for seven (7) days. Faecal collections were dried and weighed after which it was put in dessicator. All the samples were bulked by replicates weight and thoroughly mixed and sub samples were taken for analysis of their proximate constituents. Apparent nutrient digestibility was calculated using the formula:

$$\text{Apparent Nutrient Digestibility} = \frac{\text{Nutrient Consumed} - \text{Nutrient Voided}}{\text{Nutrient Consumed}} \times 100$$

#### Haematological and Serum Biochemistry Parameters

Blood samples were collected from the wing vein using 2ml syringe and needle at the end of the experiment from each replication of the treatment and stored in plastic sample bottles containing EDTA (ethylene diamine tetraacetic acid) for haematological studies. While another 3ml each was deposited into anticoagulant free plastic tubes and allowed to clot at room temperature, within 3 hours of collection. The serum samples were stored at a temperature of -20°C prior to biochemical analysis. The haematological analysis was carried out according to the procedure by Jain (1986) to determine: packed cell volume (PVC), Haemoglobin concentration (HC), Erythrocytes (RBC), Leucocytes Count (WBC). The biochemical parameters were analyzed according to

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the method of Ajagbonna *et al.* (1999), Uko *et al.* (2000) and Ahamefule *et al.* (2008) to determine: total protein, albumen, globulin, urea, creatinine, Alanin Amino Transferase (ALT), Aspartate Amino Transferase (AST) and total cholesterol.

### Chemical Analysis

The proximate composition for each of the experimental treatment diets were determined according to Association of Official Analytical Chemists (AOAC) (2006) methods, while Metabolizable Energy of the experimental diets was calculated using Ponzenga (1985) equation.

### Statistical Analysis

Results obtained from the study were subjected to Analysis of Variance (ANOVA) using SPSS 20 while Duncan's multiple range test (DMRT) was applied to separate the means were applicable (Duncan, 1955).

## RESULTS AND DISCUSSION

The result obtained indicate significant ( $P < 0.05$ ) differences in the mean digestibility values of crude protein in diet T<sub>1</sub> (control, 90.35), and the highest value, followed by diet T<sub>4</sub> (15%, 85.84 probiotics, diet T<sub>2</sub> (5% probiotics, 82.89), Diet T<sub>3</sub> (10% probiotics, 81.98) are similar. Ether extract in diet (T<sub>4</sub>) with 15% probiotics 90.36, diet (T<sub>2</sub>) with 5% probiotic has 87.29 and to diet T<sub>1</sub> (control with 88.02) are similar and higher compared to diet T<sub>3</sub> with 10% probiotics and 80.66.

Dry matter digestibility in diet T<sub>4</sub> with 15% probiotic has the highest value of (80.44) while diet T<sub>3</sub> (10%) with 70.92 value, diet T<sub>2</sub> (72.37) with (5%) probiotics and diet T<sub>1</sub> (0%) which is the control with 72.25 are similar. Crude fibre in diet (T<sub>1</sub>) is similar to T<sub>4</sub> (15% probiotics) with 91.05 but higher compared to diet T<sub>3</sub> (10% probiotic) 88.52 and diet T<sub>2</sub> (5% probiotics) which are similar. Nitrogen free extract in diet T<sub>4</sub> (15% probiotics, 97.72) has the highest value. Diet T<sub>1</sub> (control) with 91.02 has the lowest value, while diet T<sub>3</sub> (10% probiotic, 94.00) and diet T<sub>2</sub> (5% probiotics 93.20) have similar value.

Calcium in diet T<sub>2</sub> (5% probiotics 94.46), diet T<sub>1</sub> (control, 93.44) and diet T<sub>4</sub> (15% probiotic) 94.29 are similar while T<sub>3</sub> has the lowest value. Diet T<sub>3</sub> (10%, 92.18<sup>b</sup>) has the lowest value.

Phosphorus in diet T<sub>4</sub> (15% probiotics) is higher compared to T<sub>3</sub>. T<sub>1</sub>, T<sub>2</sub>, and T<sub>3</sub> are similar diet T<sub>2</sub> (5%, 93.94).

The total protein in the blood showed no significant ( $P > 0.05$ ) diet T<sub>1</sub> which is the control shown the highest value 56.75. Diet T<sub>2</sub> (5% probiotic 51.25 and diet T<sub>3</sub> (10% probiotics) with 51.60 are similar, with diet having higher value. Diet T<sub>4</sub> (15% probiotics) have the lowest value of 48.50 when compared to other treatments. The mean of Albumin (g/l) shows no significant ( $P < 0.05$ ) diet T<sub>3</sub> (10% probiotics) shows the highest value of Albumin (g/l). Diet T<sub>2</sub> (5% probiotic) have 20.85 while diet T<sub>1</sub> (0% control) 20.08 and diet T<sub>4</sub> (5% probiotic) 20.03 are similar with diet T<sub>4</sub> with the lowest value. The mean of Globulin (g/l) shows no significant diet T<sub>4</sub> (15%) 20.03 have the lowest value, followed by diet T<sub>3</sub> (10% probiotic), 30.15 diet T<sub>2</sub> (5% probiotic) 30.63 and diet T<sub>1</sub> (control 0% probiotic) 36.68 with highest value respectively. The mean values obtained for urea (Mmol/L shows no significance). The diet T<sub>1</sub> (control 0% probiotics 2.80 have the highest value. Diet T<sub>4</sub> (15% probiotics 2.63, diet T<sub>2</sub> 5% probiotics 2.60, diet T<sub>3</sub> 10% probiotic 1.78 have lower value with diet T<sub>3</sub> with lowest respectively.

The mean of creatinine (Mmol/L) shows no significant treatment T<sub>2</sub> (5% probiotic 60.25) have the highest value. Diet T<sub>1</sub> (control 54.50), T<sub>4</sub> (15%) diet 10% probiotic 53.50 with the lowest value.

The mean of ALT (g/l) value in diet T<sub>4</sub> (15% probiotics, 37.75%) have the highest value. Diet T<sub>1</sub> (control 25.75

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and diet T<sub>2</sub> (5% probiotics, 25.60) are similar with slight increase in diet T<sub>1</sub> 25.75 which is the control while diet T<sub>3</sub> (10% probiotic 13.00) with lowest value respectively. The mean of AST (g/l) value shows no significant diet T<sub>2</sub> (5% probiotic 32.50 and diet T<sub>4</sub> (10% probiotic) have same value. Diet T<sub>4</sub> (15% probiotic 46.13) have increase in the volume of the AST (g/l) while their decrease in diet T<sub>1</sub> (0%, 25.75). The mean total cholesterol (Mmol/L) shows no significant diet T<sub>2</sub> (5% probiotic 3.20) have the highest total cholesterol (Mmol/L) Diet T<sub>1</sub> (control, 2.85 and diet 10% probiotic 2.63 increase. Diet T<sub>4</sub> (15% probiotic 2.00) show decrease in Total cholesterol level.

**Table1.** Experimental diets for layer birds

Ingredient	T1	T2	T3	T4
<i>Based diet</i>	Kg/100Kg			
Maize	55.52	55.52	55.52	55.52
Groundnut cake	19.8	19.8	19.8	19.8
Maize bran	8.00	8.00	8.00	8.00
Rice bran	4.00	4.00	4.00	4.00
Limestone	7.00	7.00	7.00	7.00
Bone meal	5.00	5.00	5.00	5.00
L-lysine	0.10	0.10	0.10	0.10
DL-methionine	0.08	0.08	0.08	0.08
Salt	0.25	0.25	0.25	0.25
*Premix	0.25	0.25	0.25	0.25
<b>Total</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>
<i>Probiotic supplements</i>				
**4Bac-Extra	0.00	0.125	0.250	0.357
<b>Calculated nutrient and energy composition</b>				
Energy (kcal/kg, ME)	2735.43	2735.43	2735.43	2735.43
Crude protein (%)	16.24	16.24	16.24	16.24
Crude fibre (%)	4.10	4.10	4.10	4.10
Crude fat (%)	4.63	4.63	4.63	4.63
Methionine (%)	0.33	0.33	0.33	0.33
Lysine (%)	0.61	0.61	0.61	0.61
Ca (%)	3.63	3.63	3.63	3.63
(P) (%)	0.75	0.75	0.75	0.75

\*Premix to provide the following per Kg of diet Calcium 27.3 %, Crude fibre 0.02 %, Vitamin A (E672) 4000000 IU, Vitamin D3 (E671) 1000000 IU. Vitamin E (all-rac- $\alpha$ -tocopheryl acetate) (3a700) 6000 IU. Vitamin B1 (thiamine mononitrate) 600 mg. Vitamin B2 (riboflavin) 1600 mg. Vitamin B6 (pyridoxine hydrochloride) (3a831) 1200 mg. Vitamin B12 (cyanocobalamin) 6000  $\mu$ g. Vitamin K3 (menadione nicotinamide bisulfite) 800 mg. Pantothenic acid (calcium-D-pantothenate) (3a841) 3200 mg. Niacinamide (3a314) 8000 mg. Biotin (3a316) 40000 mg. Folic acid (3a316) 200  $\mu$ g. Choline chloride (3a890) 80000 mg. Iron (E1; as ferrous carbonate) 16000 mg. Iodine (E2; as calcium iodate anhydrous) 600 mg. Copper (E4; as cupric sulphate pentahydrate) 4000 mg. Manganese (E5; as manganous oxide) 32000 mg. Zinc (E6; as zinc oxide) 20000 mg. Selenium (E8; as sodium selenite) 60 mg.

\* Composition of Probiotic 4Bac-Extra

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Vitamin  $\alpha$  tocopherol 700 100mg, lactobacillus acidophilus 45,000 Million, live yeast cultures of Saccharomyces cerevisiae sc 47, 125 million c.f.u, protein 11.6%, crude fibre 1.3%, moisture 10.9%, crude ash 14.0%, crude oil and fat 2.6%, sodium 0%, Lysine 0%, Methionine 0% total sugar 70%.

**Table2.** Chemical composition of experimental diets

Treatment	DM	NFE	CP	EE	CF	Ash	Ca	P
T1	95.52	62.85	16.52	1.80	9.4	4.95	3.55	2.11
T2	95.00	60.35	16.49	2.46	11.1	4.6	2.95	2.00
T3	95.20	59.98	16.49	3.00	10.7	5.03	3.75	2.06
T4	71.20	54.96	16.69	2.80	11.7	5.05	3.7	2.156

DM- Dry Matter; CP – Crude Protein; EE – Ether Extract; CF – Crude Fibre; NFE – Nitrogen Free Extract; Ca – Calcium; P – Phosphorous.

**Table3.** Nutrients digestibility by Sasso hens fed diets containing different levels of probiotics

Digestibility	T <sub>1</sub> (0%)	T <sub>2</sub> (5%)	T <sub>3</sub> (10%)	T <sub>4</sub> (15%)	SEM
Crude protein (%)	90.35 <sup>a</sup>	82.89 <sup>c</sup>	81.98 <sup>c</sup>	85.84 <sup>b</sup>	0.89*
Ether extract (%)	88.02 <sup>a</sup>	87.29 <sup>a</sup>	80.66 <sup>b</sup>	90.36 <sup>a</sup>	1.14*
Dry matter (%)	72.25 <sup>b</sup>	72.37 <sup>b</sup>	70.92 <sup>b</sup>	80.44 <sup>a</sup>	1.11*
Crude fibre (%)	94.00 <sup>a</sup>	87.37 <sup>c</sup>	88.52 <sup>bc</sup>	91.05 <sup>ab</sup>	0.79*
Nitrogen-free extract (%)	91.02 <sup>c</sup>	93.20 <sup>b</sup>	94.00 <sup>b</sup>	97.72 <sup>a</sup>	0.64*
<b>Absorption</b>					
Calcium (%)	93.44 <sup>a</sup>	94.46 <sup>a</sup>	92.18 <sup>b</sup>	94.29 <sup>a</sup>	0.29*
Phosphorus (%)	93.70 <sup>ab</sup>	93.94 <sup>ab</sup>	93.17 <sup>b</sup>	94.92 <sup>a</sup>	0.26*

NS- Not Significant (P>0.05),

\*- Significant Difference (P<0.05),

\*\* - Highly Significant (P<0.01), SEM- Standard Error of Means,

abcd- Means denoted by Different alphabets in the same row are significant.

**Table4.** Effect of 4Bac-extra probiotic on serum biochemical parameters of Sasso hen fed different levels

	T <sub>1</sub> (0%)	T <sub>2</sub> (5%)	T <sub>3</sub> (10%)	T <sub>4</sub> (15%)	SEM
Total Protein (g/l)	56.75	51.25	51.00	48.50	2.24 <sup>NS</sup>
Albumin (g/l)	20.08	20.63	20.85	20.03	0.37 <sup>NS</sup>
Globulin (g/l)	36.68	30.63	30.15	28.48	2.20 <sup>NS</sup>
Urea (Mmol/L)	2.80	2.60	1.78	2.63	0.18 <sup>NS</sup>
Creatinine (Mmol/L)	54.50	60.25	53.50	54.50	2.68 <sup>NS</sup>
Alanine Aminotransferase ALT (U/L)	25.75	25.00	13.00	37.75	4.81 <sup>NS</sup>
Aspartate Aminotransferase AST (U/L)	25.75	32.50	32.50	46.13	4.48 <sup>NS</sup>
Total Cholesterol (Mmol/L)	2.85	3.20	2.63	2.00	0.19 <sup>NS</sup>

NS- Not Significant (P>0.05); SEM- Standard error of means.

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**Table 5.** Haematological characteristics of Sasso hens fed diets containing different levels 4Bac-Extra of probiotics

	T <sub>1</sub> (0%)	T <sub>2</sub> (5%)	T <sub>3</sub> (10%)	T <sub>4</sub> (15%)	SEM
Packed Cell Volume (%)	26.50	32.00	28.50	24.50	3.02 <sup>NS</sup>
Haemoglobin (g/dL)	8.45	7.60	6.83	8.03	1.18 <sup>NS</sup>
Red Blood Cells (x10 <sup>12</sup> /L)	2.47	2.81	1.92	2.34	0.33 <sup>NS</sup>
White Blood Cells (x 10 <sup>9</sup> /L)	94.25	97.50	104.50	72.00	11.68 <sup>NS</sup>

SEM- Standard Error of Means, NS- Not Significant (P>0.05)

### Discussion

The result obtained indicate significant (P>0.05) differences with inclusion of probiotic in the experimental diet at the rate ranging from 0% to 15%. This result agreed with the study reported by Nawaz *et al.* (2016) that observed significant (P>0.05) effect on overall performance and nutrient digestibility in birds fed diet containing probiotic. It also agreed with observation made by Zhang *et al.* (2015) that an increased in apparent digestibility of crude protein and total phosphorus was reported following probiotic administration.

The observation made in this study are in line with the reports of Mountzouris *et al.* (2010) who reported that probiotic inclusion level had a significant effect on broilers growth response, apparent nutrient digestibility.

The layers fed with zero probiotic (Treatment 1) value in total protein, Globulin G/L compared to those on treatment T<sub>2</sub>, T<sub>3</sub>, and T<sub>4</sub> diet. However Albumin, total protein, globulin G/L did not differ significantly. Also Urea (Mmol/L), Creatinine (Mmol/L), ALT (U/L) ASL (U/L) and total cholesterol showed no significant difference (P<0.05) and the value obtained. This finding is not in agreement with (Mansuls, 2010 and Kurtoghu, 2004) who observed disease in some serum especially cholesterol.

Albumin values recorded for all diets were not statistically significant (P<0.05) from each other, this is similar to the findings of Al-Saiady (2010) who reported that probiotics supplementation did not have any effect on Albumin and hematological parameters. Globulin values recorded for the diet were not statistically significant (P<0.05) from each other. Similarly Al-Saiady (2010) reported that probiotics supplementation did not have any effect on globulin and haematological parameters.

This is in agreement with result by Al-Saiady (2010) who reported that probiotic supplementation did not have any effect on globulin and haematological parameters. The mean values for urea were not significantly (P<0.05) different. Creatinine, ALT (U/L), AST (U/L) were also similar between treatments.

The result obtained showed that probiotic (4-Bac Extra) supplementation had no significant effect on any of the haematological traits measured (P<0.05). This is similar to reports of Chen *et al.* (2005) that haematology and serum chemistry parameters, RBC, WBC and lymphocyte were not affected by the dietary treatments (P>0.05). According to La Ragione *et al.*, 2001, Dimcho *et al.* (2005) and Knowles *et al.* (2000) the addition of probiotic did not affect RBC, WBC, haemoglobin and platelet, total protein and total cholesterol concentrations significantly.

### CONCLUSION

The results obtained from the experiment conforms to the suggestion by some authors that some effects of 4-bac Extra probiotics are only slight. However, because of the abuse of antibiotic, it has provided safe alternative and reduced spread of diseases. It suffice to state that there is a relationship between 4-bac Extra supplementation levels and nutrients digestibility, biochemical, and hematological characteristics as the 15% inclusion level of 4Bac-Extra level in this trial had positive effect on digestibility of nutrients by the layers. The use of 4-bac Extra probiotics at all levels in diets of layers had higher economic advantage compared with the control. Therefore,



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the use of probiotics at 10% inclusion rate is recommended to enhance the health status of birds against infection, diseases, preserve the birds and ensured stability on serum indices of the birds

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