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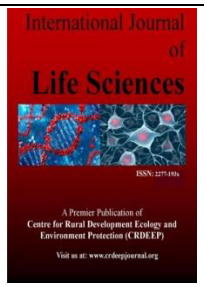

Full Length Research Paper

Isolation of *Bacillus subtilis* from Fermented Locust Beans (*iru*) and its Probiotic effects on Growth Performance, Blood Profile and Carcass yield of Broiler chicken

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ABSTRACT

Probiotics are health promoting bacteria consumed for therapeutic purposes; they promote animal growth and improve meat qualities. The probiotic activity of Bacillus *species has been reported to have pronounced advantages in animal model. This study investigated the probiotic effect of* Bacillus subtilis *from fermented locust beans on growth performance and other growth indices in chicken.* Bacillus subtilis *was isolated, characterized and administered to one hundred and twenty day-old broiler chicks of arbor acre strain randomly segregated into four groups. Different concentrations ranging from 5.0 x 10^5 CFU/mL to 2.0x10^6 CFU/mL were administered only to the treatment group for 42 days. Carcass yield, organ characteristics, haematological and serum biochemical parameters were determined using standard methods. The feed and water intakes of broilers that received 2.0 x 10^6 CFU/mL of* B. subtilis *were significantly higher (p<0.05) compared with broilers in other treatments. Although the weight gain of the broilers among all treatments was not significantly (p>0.05) increased, the weight gain values obtained apparently increased as the concentration of* B. subtilis *increased in the water. There were no significant differences in the final live weight, feed conversion ratio and protein efficiency ratio among all treatments. The albumin content, albumin-globulin ratio and cholesterol levels in the serum were significantly higher (p<0.05) in the treatment groups compared with the control group. The total protein, globulin fractions and haematological parameters were not affected significantly by probiotic treatment. Administration of* B. subtilis *from iru to the broiler chicks had no effect on the relative weights of organs and carcass qualities monitored however, it increases the haematological parameters.*

Introduction

In the last few decades, growth-promoting antibiotics had been used extensively in poultry feeds and water (Muzaffer*et al*., 2003) to improve meat production, energy utilization and to prevent pathogens and diseases. However, the use of dietary growth-promoting antibiotics had resulted in common problems such as development of drug-resistant bacteria (Sorum and Sunde, 2001), drug residues in the body of the birds, and imbalance of normal microflora (Andremont, 2000). Human health can be affected directly through residues of antibiotic in related food (Boerlin and Reid-Smith, 2008; Aderiye and David, 2013a). Thus in order to avoid food borne illness from antibiotic-resistant bacteria and produce antibiotic free chicken, attention is now being focused worldwide on the use of natural prophylactic supplements in place of chemotherapeutics in broiler production. Such preventive products include probiotics which were defined as live microbial feed supplements that beneficially affects the host animal by improving its microbial intestinal balance (Fuller, 1989). Probiotics have been reported to regulate the microbial environment in the gut, reduce digestive upsets and prevent pathogenic gut bacteria, thereby improve live weight gain, feed conversion ratio, reduce mortality and increase egg production in chicken (Samli *et al*., 2007; Bansal *et al*., 2011, Fathi *et al.,* 2018).

Iru is a locust beans seeds fermented condiment consumed in West Africa countries including Nigeria. It could be eating as snack immediately after fermentation. It is also incorporated into

human diet as soup condiment serving as an alternate source to animal protein (Ajayi, 2014; Ihekeet al., 2017). *Bacillus* spp have been reported to be the major group of organism responsible for the fermentation of locust bean (Aderiye and David, 2013b; David *et al.,* 2016). *Bacillus subtilis* is among the wide variety of microbial species that have been characterized and used extensively as probiotics (Korosi *et al*., 2005; Xu *et al*., 2006; Husam, 2010; Zhou *et al*., 2010; David *et al.,* 2016). However, there is a dearth of information regarding the effect of suspension of *Bacillus subtilis* fed through drinking water on poultry. Most studies involving the use of *Bacillus subtilis* were conducted using the probiotic as feed supplement (additive in feed) and have emphasized a reduction in mortality, increase body resistance to infectious diseases, improve feed conversion and reduce abdominal fat. It has been reported that the efficacy of a probiotic application depended on factors such as species composition and viability, administration level, application method, frequency of application among others (Patterson and Burkholder, 2003). In poultry industry, the efficiency of feed conversion to meat, particularly, by broilers has been the focus of some research over time. Broiler chicken meat is one of the most common animal protein sources because of its nutritional value and relative reasonable prices in Nigeria. Therefore, this study was attempted to investigate the effect of *B. subtilis,* isolated from iru using infusion method, at different concentrations on growth performance, carcass yield, haematological and serum biochemical parameters of broiler chickens.

Materials and Methods

Study area

"Iru" a fermented product of locust beans (*Parkia biglobosa*) was purchased from Oja Oba, Ado-Ekiti, Nigeria (7.6124°N, 5.2371° E) and put in a sterile polythene bag. The sample was immediately transported to the Microbiology Laboratory of Ekiti State University, Ado-Ekiti, Nigeria for analyses.

Isolation and Identification of Bacillus *Species from Iru*

The modified method of Barbosa *et al.* (2005) was used for the isolation of *Bacillus* spp. from *iru* samples. Under a sterile condition, one gram (1 g) of freshly prepared *iru* was measured and ground to form a smooth paste and serially diluted to the factor of 10. Tubes with different dilutions were heated to 65 °C for 45 min and later diluted in absolute ethanol (1:1, v/v) and allowed to stand for 1 h at 25 °C. One millilitre of the solutions was aseptically inoculated on Hi-Chrome *Bacillus* Agar (HCBA) (HiMedia M1651, India) and incubated aerobically at 37 °C for 24 h. Isolates on the plates were sub-cultured on HCBA and discrete colonies were transferred into slants. To determine the identity of the isolates, the different biochemical procedures were carried out which include Gram reactions, catalase, indole, Voges-Proskauer and Methyl-Red test, utilization of citrate, fermentation of carbohydrate (arabinose, fructose, galactose, inositol, mannitol, mannose, rhamnose, ribose, sorbose and xylose). The results were interpreted according to Holt *et al.* (1994).

Qualitative and Semi-Quantitative Detection of Adherence of isolated Bacillus *Species*

Biofilm formation among the isolates was detected by the method of Chaleb *et al.* (2007). Isolates identified as *Bacillus subtilis* were radially streaked on nutrient agar supplemented with 4 % Congo red dye. The plates were incubated for 24 h at 37 °C. Isolates with black colonies on Congo red agar were taken for biofilm production. The quantity of biofilm formed by isolates was further determined by inoculating them into Mueller Hilton broth (MHB) (HiMedia, India) and incubated at 37 °C for 72 h; a sterile MHB was used as control. The broth was discarded and adherent bacterial cells were stained with 1 % crystal-violet (Merck, France) for 10 m. Excess stain was rinsed off and air dried. The dry tube was bleached with absolute ethanol and the optical density was measured at 520 nm (OD_{520}) using spectrophotometer (Jenway 6505, England). The cut-off OD (ODc) was defined as three standard deviations above the mean OD of the negative control. The results were interpreted according to Siegrifreld *et al.* (1994) as follows: strong biofilm former ($4 \times ODc<OD$), moderate biofilm former ($2 \times ODc<OD \leq 4 \times ODc$), weak biofilm former ($ODc<OD \leq 2 \times ODc$) andnon-biofilm former ($OD \leq ODc$).

Determination of Acid and Bile Tolerance of the Isolates

The ability of moderate biofilm former and strong biofilm former *B. subtilis* isolates to tolerate bile was determined according to Liong and Shah (2005) with little modification. The pH of Mueller-Hinton agar was adjusted by 1 M HCl (BDH) to achieve pH values of 3.0, 4.0 and 5.0 and the standardized (0.5 MacFarland standard) *B. subtilis* inocula were inoculated. The culture was incubated for 24h at 37 °C. Growth on the agar plates was taken to be a positive result. The method of Meei-Yn and Tseng-Wei (2000) was used to determine the effect of bile on the growth of isolates. Each of the standardized isolates was inoculated onto Mueller-Hinton agar and supplemented with 0.5 ml (% v/w) sterile fresh bovine bile. The culture was incubated at 37 °C for 24 h. the results were read as stated above. All the experiments were conducted in triplicates and repeated twice.

Determination of Virulence Factors

Gelatinase production among biofilm forming isolates was detected by streaking the isolates radially on Mueller-Hinton agar supplemented with 0.4 % gelatin (BDH) as described by Su *et al.* (1991). The plates were incubated for 48 h at 37 °C. The cultures were observed for growth and subsequently flooded with 10 mL of Frazier solution (Mercuric chloride, 15.0 g in 20 mL of 37 % v/v hydrochloric acid, made up to 100 mL by adding distilled water). Clear zone of clearance was observed around the gelatinase positive strains and recorded positive. Detection of haemolysin production was detected in the isolates by streaking the isolates on Brain Heart Infusion agar (Oxoid, UK) supplemented with 5 % human blood. The plates were incubated at 37 °C for 24 h and observed for red blood cell lytic activity around the bacterial colonies. Detection of caseinase among the test bacterial strains were done by inoculated them onto Trypticase Soy agar (TSA) (Oxoid, UK) supplemented with 1 % skim milk (w/v) using streaking method. The plates were incubated at 37 °C for 24 h and caseinase production was observed with zone of clearance around isolates as described by Abderrahmen *et al.* (2014). Production of catalase among the isolates was assayed by simply smear a loopful of the test organism onto a slide and add a drop of 3.0 % hydrogen peroxide. The production of visible bubbles indicates the presence of catalase (Olutiola *et al.,* 2002).

Determination of Antibiotic Susceptibility Pattern of Biofilm forming Bacillus subtilis

The isolates grown at 37 °C in Mueller-Hilton broth (Oxoid) for 18 h was diluted to an OD_{600} of 0.1 (0.5 McFarland Standard) and stored at 4 °C. The disc diffusion method was used for susceptibility testing as described by Clinical and Laboratory Standard Institute (2012). The isolates were tested against eight commercial antibiotic disks (Abtek Biologicals Limited) with different concentrations which included: Ampicillin [(AMP) 25 µg], amoxycilin clavulanic acid [(AMC) 30 µg], ceftazidine [(CEF) 30 µg], cefuroxime [(CEX) 30 µg], ciprofloxacin [(CIP) 5 µg], gentamycin [(GEN) 10 µg], nitrofurantoin [(NIT) 300 µg] and ofloxacin [(OFL) 5 µg]. The diameters of the zone of inhibition were measured to the nearest whole millimeter and interpreted according to CLSI guideline (CLSI, 2012).

Preparation of Bacillus subtilis *Cells*

Bacillus subtilis strain with the best probiotic properties was selected, cultured on Mueller-Hinton broth and incubated for 24 h at 37 ^{o}C. The cells were harvested by centrifuging at 4000 rpm for 30 min at 15 ^{o}C and washed three times in physiological saline (0.85 % NaCl) at 4000 rpm for 10 min. The cells were re-suspended in sterile physiological saline solution. The absorbance of the bacterial suspensions at 600 nm was adjusted to optical density of 0.500 with an average cell count of 1.5×10^9 CFU/mL. Three different concentrations of *B. subtilis* were prepared from the stock, quantified and infused into a litre of sterile drinking water to make a final concentrations of 0.5×10^6, 1.0×10^6 and 2.0×10^6 CFU/mL (representing the three treatment groups) and stored at 4 ^{o}C until used. During the experiment the birds were allowed unrestricted access to water and water intake was monitored for a period of six weeks. The control group was not exposed to the test probiotic bacterium.

Experimental Birds and Design

One hundred and twenty day-old broiler chicks of arbor acre strain procured from a reputable hatchery were used in this study. The chicks were brooded and allowed a physiological adjustment period of two weeks. All chickens with similar initial weights were randomly divided into four treatments, with each treatment having 30 broiler chicks in 3 replicates of 10 broilers each. The chicks were fed with the broiler starter diet from days 1 to 28 and broiler finisher diet from 29 to 56 days. Feed consumption and weight gain of the birds were recorded on weekly basis. Fresh water infused with *B. subtilis* according to the respective treatments was provided on daily basis throughout the experimental period. Remaining water from the previous day was measured and discarded before adding fresh water and the water intake was measured on daily basis.

Data Collection

Daily feed intake (DFI), daily weight gain (DWG) and feed conversion ratio (FCR) of the birds were estimated accordingly from the weekly data collected. Protein efficiency ratio was estimated as the ratio of weight gain to total protein intake or consumed. At the end of the feeding trial, 12 birds (4 birds per replicate) were randomly selected from each treatment group for the purpose of blood collection and carcass evaluation.The birds were starved overnight, in order to attain a stable serological evaluation and for easy handling. Blood samples were collected from the birds using 5 mL syringe into vacutainer tubes containing Ethylene Diamine Tetra acetic Acid (EDTA) for haematology and plain tubes (without EDTA) for serological analyses. Serum samples were assayed for estimation of total protein, albumin and cholesterol by using Standard Kit (Randox, England). The haematological variables; Red Blood Cells (RBC), Packed Cells Volume (PCV), White Blood Cells (WBC) Haemoglobin (Hb), and blood constants (MCV, MCH and MCHC) were determined according to the methods described by Ogunlade and Egbunike (2013). After blood collection, the birds were slaughtered by severing the jugular vein and the visceral were removed. After standard cutting, the breast, back, thigh, drumstick, neck, shank, head, wings, gizzard, liver, lung, heart, intestine, spleen, crop and abdominal fat were obtained and weighed accordingly from the four treatments.

Statistical Analyses

The data obtained were subjected to one-way analysis of variance (ANOVA) procedure of SAS (1999). The treatment means were compared using Duncan multiple range test of the same software.

Results

The total spore former bacteria in the samples ranged from 3.28 Log_{10} CFU/g to 3.54 Log_{10} CFU/g. Nineteen isolates belonging to the genus *Bacillus* consisting of four species bases on the appearance on *Bacillus* differential agar and biochemical tests were isolated from the sample. The *Bacillus* spp. recovered included: *Bacillus cereus* (large blue colonies), *B. coagulans* (pink colonies with mucoid surface), *B. megaterium* (yellow colonies with mucoid surface) and *B. subtilis* (green colonies) with 10.52 %, 26.32 %, 5.26 % and 52.62 % respectively. None of the six selected *B. subtilis* strains was susceptible to AMP, AMC, CAZ, CPR, GEN, NIT and OFL. Strain DMLB12 and DMLB31 were susceptible to all the antibiotic tested. Only two of the isolates were resistant to CAZ. None of the isolates produced gelatinase while three produced haemolysin. Two of the isolates produce caseinase and catalase. Strains DMLB12 and DMLB32 were unable to tolerate acid and bile respectively as shown in Table 1.

Table 1: *In vitro* probiotic properties of biofilm producing *B. subtilis*strain isolated from fermented locust beans

Properties	**Antibiotics**	***B. subtilis* strains**					
		DMLB12	**DMLB32**	**DMLB19**	**DMLB31**	**DMLB23**	**DMLB27**
Antibiotic	AMP	--	--	--	--	--	--
Resistance	AMC	--	--	--	--	--	--
	CEF	--	--	--	--	--	--
	CEX	--	--	++	--	++	--
	CPR	--	--	--	--	--	--
	GEN	--	--	--	--	--	--

	NIT	--	--	--	--	--	--
	OFL	--	--	--	--	--	--
Extracellular protein	Caseinase	(--)	(++)	(--)	(++)	(--)	(--)
	Catalase	(--)	(--)	(--)	(++)	(--)	(++)
	Gelatinase	(--)	(--)	(--)	(--)	(--)	(--)
	Haemolysin	(++)	(++)	(--)	(--)	(++)	(--)
Tolerance	Acid	G	NG	G	G	G	G
	Bile	NG	G	G	G	G	G

Key: *-- = Susceptible, ++ = Resistance, (--) = Absent, (++) = Present, G= Growth, NG = No growth*

Table 2 shows the initial live weight (ILW), Final live weight (FLW), Feed intake (FI), Weight gain (WG), Water intake (WI), Feed conversion ratio (FCR) and Protein efficiency ratio (PER) of broilers infused with varied concentrations of viable *B. subtilis*. There were no significant differences in ILW, FLW, FCR and PER of birds across the treatments groups. Although birds infused with $2.0x10^6$ CFU/mL (T4) of *B. subtilis* had an apparently higher WG value (55.75g/b/d/), the birds were not significantly higher in weight as compared with other groups. With respect to feed intake, the birds that received the probiotic with microbial concentration of $2.0x10^6$ CFU/mL (T4) showed a significantly higher ($p<0.05$) feed intake (116.76g/bird) compared with those that received $1.0x10^6$ CFU/mL, $0.5x10^6$ CFU/mL and the control group respectively. The water intake values of the experimental birds followed similar trend with those of feed intake except for those of treatments 2 and 3 which were statistically similar to that of treatment 1.

Table 2: Growth performance of broilers infused with different concentration of *B. subtilis* DMLB31

Parameter	**Control**	**Treatment groups (10^6cfu/mL)**			**SEM**
		0.5	**1.0**	**2.0**	
Initial live weight (g)	348.12±12.88	354.02±13.10	350.06 ±12.95	346.10±95.45	20.21
Final live weight (g)	2683.30±99.28	2816.70±74.22	2850.00±75.45	2500.00±4.13	207.44
Feed intake (g/bird/day)	$111.14±8.11^b$	$111.05±4.1^b$	$111.72±12.10^b$	$116.76±1.8^a$	1.49
Weight gain (g/bird/day)	46.76±2.73	49.47±1.83	50.48±2.10	55.75±7.72	4.19
Water intake (ml/bird/day)	$200.07^b±7.40^b$	$202.67±7.50^{a,b}$	$208.67±30.40^{ab}$	$209.33± 0.08^a$	2.72
Feed conversion ratio	1.99±0.07	1.94±0.07	2.09±0.30	2.17±0.10	0.08
Protein efficiency ratio	2.38±0.09	2.54±0.09	2.66±0.50	2.55±0.30	0.23

a,b = Means on the same row with different superscripts are significantly different (p<0.05).SEM: Standard Error of Mean.

Weight gain, Protein efficiency ratio (PER) and Feed conversion ratio (FCR) were not significantly affected by the inclusion levels of *B. subtilis*. The serum biochemical parameters of broiler infused with different concentration of *B. subtilis* is shown in Table 3. Albumin value obtained for birds on treatment 4 was significantly superior ($p<0.05$) to those on treatments 3, 2 and 1 respectively. Albumin/Globulin ratio and cholesterol levels increased significantly ($p<0.05$) as the inclusion level of *B. subtilis* increased. However, total protein and globulin components of the serum of the experimental birds were unaffected by the inclusion levels of *B. subtilis* Table 4 shows the haematological parameters of broilers infused with different concentration of *B. subtilis*. The results showed that the various inclusion levels of *B. subtilis* in the water of the broilers did not significantly ($p>0.05$) influence their haematological parameters. Table 5 shows the relative carcass weight of broilers infused with different concentrations of *B. subtilis*. The result revealed that the relative weights of the back, neck and fat of the experimental birds were significantly influenced by the inclusion levels of *B. subtilis* while other parameters investigated were not significantly affected. Back weight and fat weight increased significantly as the inclusion levels of *Bacillus subtilis* increased in the water of the experimental birds.The relative organ weights of the experimental birds are presented in Table 6. The various inclusion levels of *B. subtilis* in this study had no significant influence on the relative wright of all the organs investigated.

Table 3: Serum biochemical parameters of broilers infused with different concentration of *B. subtilis* DMLB31

Parameter	**Control**	**Treatment groups (10^6cfu/mL)**			**SEM**
		0.5	**1.0**	**2.0**	
Total Protein (g/100ml)	26.08±0.96	26.21±1.01	26.42±2.98	27.09±2.04	0.61
Albumin (g/100ml)	$13.01±0.65^c$	$16.42±0.61^b$	$16.74±0.62^a$	$17.37±2.64^a$	0.37
Globulin (g/100ml)	9.72±0.36	9.79±0.39	9.68±0.36	9.72±1.36	0.39
Alb/Glob ratio	$1.34±0.21^c$	$1.67±0.19^b$	$1.72±0.06^{a,b}$	$1.79^a±0.09^a$	0.10
Cholesterol (mg/dL)	$0.26±0.16^b$	$0.36±0.01^a$	$0.39±0.01^a$	$0.39^a±0.03^a$	0.02

a,b: Means with different superscripts across the row are significantly different (p<0.05).SEM: Standard Error of Means.

Table 4: Haematological parameters of broilers infused with different concentration of *B. subtilis* DMLB31

Parameter		**Treatment groups (10^6cfu/mL)**			
	Control	**0.5**	**1.0**	**2.0**	**SEM**
ESR (x10 ^{6}mm^3)	2.33±0.11	30.20±2.39	30.33±12.56	30.33±5.99	0.33

Packed cell volume (%)	30.00±2.37	281.67±22.25	283.35±26.18	283.33±22.38	2.99
Erythrocyte ($x10^6$ mm^3)	280.67±22.17	10.00±0.79	10.10±0.80	10.20±0.51	2.16
Haemoglobin (g/dL)	10.00±0.79	61.67±4.17	61.67±4.87	61.67±4.37	0.99
Lymphocyte ($x10^3$ mm^3)	61.67±4.87	21.33±1.19	21.00±2.00	21.67±1.41	0.28
Heterophils ($x10^3$ mm^3)	21.67±1.71	13.30±1.05	13.00±1.03	13.00±1.23	0.08
Monocytes ($x10^3$ mm^3)	13.67±1.08	4.20±1.33	4.53±0.36	4.67±0.37	0.32
Eosinophil ($x10^3$ mm^3)	4.30±0.34	106.52±9.42	106.39±8.40	106.7±8.13	0.27
MCV (μ^3)	106.94±8.34	34.81±2.75	36.70±2.90	35.88±2.03	1.99
MCH (µg)	35.86±2.83	33.31±3.63	33.27±2.63	33.62±2.56	0.71
MCHC (%)	33.34±2.63	30.20±2.39	30.33±2.40	30.33±3.40	0.19

ESR: Erythrocyte Sedimentation Rate, MCV: Mean Cell Volume, MCH: Mean Corpuscular Haemoglobin, MCHC: Mean Corpuscular Haemoglobin Concentration

Table 5: Relative (%) Carcass weight of broiler infused with different concentrations of *B. subtilis* DMLB31

Parameter	**Treatment groups (10^6cfu/mL)**				
	Control	**0.5**	**1.0**	**2.0**	**SEM**
Plucked weight	92.55±2.47	92.90±7.34	94.74±4.48	91.33±7.92	1.05
Eviscerated weight	81.99±2.19	86.39±6.82	82.46±6.51	81.33±6.47	1.02
Dressed carcass weight	74.54±1.99	76.92±6.38	76.02±6.51	76.67±10.06	7.84
Drum stick	10.55±0.28	11.41±0.48	10.96±1.87	10.94±0.86	0.55
Breast weight	23.77±0.63	24.81±1.96	24.16±3.91	24.84±2.46	0.93
Wing weight	7.90±0.21	8.51±0.67	7.95±0.43	8.39±0.96	0.17
Back weight	$11.10±0.30^{b}$	$13.23±1.05^{a}$	$13.92±2.30^{a}$	$13.82±2.09^{a}$	0.47
Thigh weight	11.53±0.31	11.22±0.89	11.13±1.08	11.67±1.92	0.44
Neck weight	$5.27±0.14^{a}$	$4.72^{ab}±0.37^{a,b}$	$4.63±0.37^{b}$	$4.60±0.56^{b}$	0.17
Abdominal fat weight	$0.59±0.02^{b}$	$0.97^{ab}±0.08$	$1.07±0.08^{ab}$	$1.35±0.11^{a}$	0.24
Shank weight	3.97±0.11	4.37±0.35	3.73±0.29	4.03±0.52	0.45

a,b: Means with different superscripts across the row are significantly different ($p<0.05$).CFU: Colony Forming Unit.SEM: Standard Error of Means.

Table 6: Relative (%) organ weights of broiler infused with different concentrations of *B. subtilis* DMLB31

Parameter	**Treatment groups (10^6cfu/mL)**				
	Control	**0.5**	**1.0**	**2.0**	**SEM**
Gizzard	2.05	2.01	2.03	2.05	0.20
Proventriculus	0.32	0.30	0.32	0.33	0.11
Liver	1.86	1.80	1.80	1.85	0.06
Lung	0.68	0.59	0.60	0.60	0.05
Heart	2.19	2.43	2.22	2.53	0.15
Intestine	4.81	4.61	4.84	4.89	0.38
Spleen	0.14	0.13	0.14	0.14	0.01
Crop	0.54	0.46	0.48	0.55	0.06

SEM: Standard Error of Mean.

Discussion

The ability of the organism (probiotic) to produce exopolysaccharide (biofilm) assists it in preventing the colonization and establishment of intestinal pathogens (Hong *et al.,* 2008). Biofilm-producing *B. subtilis* strains have been reported to remain longer in the digestive system than none producers (Tam *et al.*, 2006) hence has a better probiotic properties. *Bacillus* has been reported to be an efficient producer of catalase (Abderrahmen *et al.,* 2014). Catalase mops free and reactive oxygen molecules. This scavenging ability impact on host a health benefit (Patel *et al.,* 2009). The probiotic properties of the *B. subtilis* used in this study is similar to those isolated from Tunisian hypersaline environments as reported by Abderrahmen *et al.* (2014).

Strain DMLB31 of the *B. subtilis* isolated from the fermented locus beans (iru) was susceptible to all tested antibiotics in their various standard concentrations. This is considered to be an attributes of a potential probiote (Patel *et al.,* 2009). In this case there will not be transfer of genetic element between related and unrelated bacteria in the gut.The test organism isolates under study showed excellent resistance to bile and acid at the gastric conditions. Deshpande *et al.* (2014) reported that a good probiotic organism should be able to withstand 0.5 % of bile salt and other conditions that are similar to what is obtainable in the gut.

Improvements in growth performance and feed conversion ratio of broiler chicken fed probiotics have been well documented (Samli *et al.*, 2007; Awad *et al.*, 2009; Bansal *et al.*, 2011; Hayashi *et al.,* 2018; Fathi *et al.,* 2018). In the present study the lack of significant improvements in the final live weight, weight gain, feed conversion ratio and protein efficiency ratio of broilers infused with different concentrations of *B. subtilis* over the

control birds are contrary to the results obtained in previous studies. Bansal *et al.* (2011) and Awad *et al.* (2009) reported that dietary probiotic stimulates body weight gain and improves feed conversion ratio in broiler chickens. This could be associated with variation in the composition of microbial species, administration method, frequency of application, bird age, concentration of bacteria in probiotic dosage and environmental stress. However, the absence of beneficial effect of *B. subtilis* on body weight gain, final live weight, feed conversion ratio and protein efficiency ratio obtained in this study are in agreement with the findings of Priyanka *et al.* (2003) and Kumprechtova *et al.* (2000) who reported that administration of *B. subtilis* and *Saccharomyces cerevisiae* (Sc 47) respectively on broiler chicken could not improve the growth performance and live weight at 21 and 42 days of age.

Findings of this study showed that the use of *B. subtilis* at a concentration of $2x10^9$ CFU/mL significantly increased the feed and water intakes of the broiler chickens than those broilers in the other treatments. The pattern of increase in the feed intake, water intake and body weight gain of the experimental birds followed similar trend. This suggests an increased digestive efficiency (Banday and Risam, 2001) in the gut of the experimental birds. Mountzouris *et al.* (2010) documented that probiotic can improve intestinal microbiota and digestive function of intestine in broilers. Similar improvements in feed intake had been reported for broiler chicken fed probiotics (Nahanshon *et al*., 1993; Sharma *et al*., 2003; Knarreborg *et al.,* 2008). Different concentrations of *B. subtilis* had significantly different effects on albumin, albumin/globulin ratio and cholesterol. Statistically superior values were recorded for albumin, albumi-globulin ratio and cholesterol of broilers orally infused with varying levels of *B. subtilis* in comparison with those of the control group. This suggests that oral infusion of *B. subtilis* to broilers will significantly improve albumin and albumin-globulin ratio. This result agrees with those of Zeweil *et al.* (1993), Abdel *et al.* (2001) and Tolba *et al.* (2004) who reported a significant increase in the value of albumin as a result of feeding microbial probiotic to Japanese quail and broilers respectively. Djouvinov *et al.* (2005) and Aluwong *et al.* (2012) reported that the total protein of birds fed diets supplemented with *Lactobacillus rhamnosus* was not significantly affected.

However, the increase in cholesterol value with increased concentration of *Bacillus subtilis* in this study suggests that fatty acid synthesis in the liver of the broiler was not influenced. This result is at variance with the reports of Husam (2010) and Kannan *et al.* (2005) who reported decrease cholesterol values for broilers fed diets supplemented with *Saccharomyces cerevisiae* and *B. subtilis*. This result further indicates that *B. subtilis* may lack anticholesterolaremic property and may have impeded assimilation of cholesterol.Infusion of varying concentrations of *B. subtilis* to broilers as used in this study had no significant effect on the haematological parameters investigated. Values obtained for the haematological parameters were within the normal range reported by Jain (1993) for chicken. Chen *et al.* (2006) reported similar results for haematological parameters of finisher pigs fed diets supplemented with Bacillus-based probiotics. In this study, broilers infused with graded concentrations of *B. subtilis* had significantly higher back weight than those of control. Dressed carcass weight of the experimental birds (although not significantly influenced) followed similar trend. These results may be attributed to statistically superior values recorded for the feed and water intakes of the birds infused with *B. subtilis* as compared with those on control diet. These findings were in agreement with the previous reports of Knap *et al.* (2011), Hardy *et al.* (2013) and Sadeghi *et al*. (2015).

The significantly higher values obtained for the fat component of birds on dietary *B. subtilis* in this study could be linked with increase in cholesterol fraction in the serum of birds fed dietary *B. subtilis*. Cholesterol has been reported to be a type of fat (Husam, 2010). This result is however contrary to the report of Santos *et al.* (1995) who noted that inclusion of dried *Bacillus subtilis* culture to broilers diet resulted in reduced abdominal fat. Korosi *et al.* (2005) observed no significant difference in abdominal fat between broilers fed diets supplemented with *B. subtilis* and the control diet.The relative weight of organs investigated in this study were unaffected by the inclusion levels of *B. subtilis*. This result is consistent with that of Awad *et al.* (2009) for broilers fed diets supplemented with probiotic.

Conclusion

It can be concluded that suspension of *Bacillus subtilis* administered orally to broiler in the range ($5.0x10^5$ - $2.0x10^6$ CFU/mL) used in this study significantly increased feed intake, water intake, serum albumin, albumin-globulin ratio and cholesterol of the birds. No significant effect of the probiotics suspension was observed on the haematology and carcass yield of the broilers except the back, neck and abdominal fat weights. Furthermore, the relative weights of organs of broilers monitored in this study were not significantly affected. It would appear that suspensions of *B. subtilis* at the inclusion levels used in this study did not have significant effect on most performance and carcass qualities parameters monitored, further investigation should be carried out using *B. subtilis* at higher concentration and longer period of application.

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