

In vivo assessment of protein quality and safety of meat derived from broilers fed diet supplemented with probiotics used as substitute to antibiotics

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Abstract

Recent studies demonstrated that antibiotics are misused by farmers leading to public health hazards associated with consumption of meat containing residues. However, there are very few reports concerning the influence that probiotics used as substitute to antibiotics in broiler's diet may have on meat quality and safety. Therefore, we examined this issue by conducting an *in vivo* study during which *Wistar* albino rats were fed diets having as source of protein meat derived from *Cobb*[®] 500 broiler chickens fed diets supplemented with a probiotic preparation containing *Lactobacillus plantarum* MW-18CGZ and *Lactobacillus paracasei* MW-37CGZ. A total of six isonitrogenous diets containing 10% crude protein each were prepared from five meat samples with egg white used as control, while the seventh diet was protein-free. Result of the proximate analysis showed that protein composition of meat from broilers fed diets supplemented with probiotics through various channels wasn't significantly different ($P > 0.05$) but varied significantly as compared to meat derived from broilers fed diet supplemented with antibiotics ($P < 0.001$). All groups recorded positive weight gain except the protein-free group (D_0) that was negative (-15.5 g). The Protein Efficiency Ratio (PER), Food Efficiency Ratio, Net Protein Retention Ratio, True Protein Digestibility and Apparent Protein Digestibility values varied significantly ($P < 0.05$) in all experimental groups (D_0 to D_6). Rats fed protein-free diet recorded a negative PER (-2.75) while those fed diets supplemented with probiotics (D_2 , D_3 , D_4 , D_5 , and D_6) had a value of 2.62, 2.1, 2.57, 2.36 and 2.76 respectively. No difference ($P > 0.05$) was observed in haematology and biochemistry parameters of rats on diets supplemented with meat derived from broilers fed probiotics enriched diets. This implies that probiotics bacteria can be used successfully as biotherapeutic substitute to antibiotics utilized as growth promoters without altering the nutritional quality of meat and prove to be safe for human consumption.

Introduction

In recent years, consumer request for healthy, safe and environmentally friendly produced meat products has put demand on producers [1]. To meet this challenge, attention has been directed toward the use of antibiotics as growth promoters. However, due to growing concern expressed by the public over possible residual effects of antibiotics and the development of drug-resistant bacteria, antibiotics growth promoters have been prohibited and several natural alternative such as probiotics have been evaluated to improve food quality and provide additional health benefits to consumers [2,3].

Probiotics are life microorganisms that provide a beneficial effect on the host by improving the microbial balance of the intestinal tract [4]. The criteria for the selection of probiotics include the lack of pathogenicity, the tolerance to gastrointestinal conditions (acid and bile), the ability to adhere to gastrointestinal mucosa and the competitive exclusion of pathogens. Probiotics have been successfully applied as an alternative to antibiotics in animal farming with Lactic acid bacteria (LAB) being the most commonly used. Some strains of LAB have been successfully used as additives in poultry feed to provide benefits, such as improving growth performance [5] and preventing enteric pathogen infection [6].

The increasing understanding of the relationship between meat and human health has shown that consumption of poor-quality

meat has deleterious public health implications such as coronary heart disease, thrombosis, and cancer. Therefore, the goal in poultry should be to provide meat products that are safe for human, taking in consideration the "One Health" concept which recognizes that the health of humans, animals and ecosystems are interconnected. However, it is unknown whether supplementing broilers feed with probiotic bacteria as a substitute to antibiotics to improve the overall performance may modify meat quality, protein bioavailability and prove to be safe.

Presently, there are several methods utilized to determine protein quality such as the Chemical Score or Acid Amino Score (AAS), Protein Efficiency Ratio (PER), Biological Value (BV) or Protein Digestibility Corrected Amino Acid Score (PDCAAS). However, the rat-based Protein Efficiency Ratio assay had been the most widely used procedure for determining protein quality because it is described as a simple assay that measures the efficiency of utilization of protein for growth as influenced by food intake [7,8].

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We hypothesized that the administration of probiotics to broiler's diet could modify meat quality while at the same time impact their safety. Accordingly, the objective of the study was to evaluate the effects of supplementing *Cobb*[®] 500 broiler diet with a probiotic preparation containing live culture of *Lactobacillus plantarum* MW-18CGZ and *Lactobacillus paracasei* MW-37CGZ on protein quality, bioavailability and safety using rat bioassay.

Materials and methods

Probiotics selection and characterization

Lactobacillus plantarum MW-18CGZ and *Lactobacillus paracasei* MW-37CGZ used in the current study were isolated from fecal samples of farmyard chicken and duck. These two strains exhibited excellent probiotics properties and were selected out of 129 isolates. They displayed high auto-aggregation and coaggregation towards pathogens, survived in low-pH and high bile salt conditions and none exhibited virulent factors. They were also susceptible to chloramphenicol, clindamycin, ampicillin and erythromycin with MIC ranged between the recommended interval established by the European Food Safety Authority [9].

Meat samples origin

Meat samples used in this study came from previous study which aimed to evaluate the effect of dietary supplementation of two probiotics strains - *Lactobacillus plantarum* MW-18CGZ and *Lactobacillus paracasei* MW-37CGZ on growth performance, carcass characteristics, antioxidant enzymes, hematologic and biochemical parameters of *Cobb*[®] 500 broiler chickens. Two hundred and fifty five day-old chicks were randomly selected and distributed into five groups of 51 day-old chicks each: T⁻ (Standard Diet Alone); T⁺ (Standard Diet+Reference antibiotic, Oxytetracycline); T_{Food} (Standard Diet+Probiotics given in Food); T_{H₂O} (Standard Diet+Probiotics given in Drinking Water); T_{Formulation} (Standard Diet+Formulation (Kernel oil+Probiotics) given in Food. Birds were housed in an environmentally controlled poultry house with floor covered with wood shavings. The shavings were kept dry throughout the experimental period by routine replacement of the spoiled litter. Ambient temperature and relative humidity were monitored, and chicks were administered with respective vaccines. The *Lactobacillus* probiotics were combined and administered to each experimental group through drinking water, food or formulation made up of probiotics mixture and kernel oil used as vehicle. Chicks were fed a commercial broiler starter diet for the first 28 days of age and finisher diet from 29 to 42 days of age. Birds had 15 h of light and 9 h of darkness throughout the experimental period. After 42 days of feeding, birds were humanely slaughtered by mechanical stunning (contusion at the region of the occipital and atlas bones), followed by cutting of the blood vessels (carotid artery and jugular vein). After severing the neck, the birds were scalded using hot water. The skeletal muscle of individual chicken from each treatment group was removed using a knife, including the skin. The carcass was analysed for chemical composition. This included moisture (%), crude fat (%), crude protein (%), crude Fiber (%), ash (%) and some minerals (%). The chemical determination was done on combination of the muscles and the skin of the chickens.

Chemical analyses of meat samples

Moisture determination

The standard reference method for measurements of moisture in meat is oven drying [10]. In this experiment, ground sample (about 6

g) was dried in an oven at 105°C for 16 h. Then after drying, the samples were re-weighed. The difference between the weight of the undried meat and the dried was the moisture content of the meat. Moisture percentage was calculated using the formula bellow.

$$\text{Moisture (\%)} = \frac{(X - Y)}{X} \times 100$$

Where: X=weight of undried meat sample and Y=weight of oven dried meat sample.

Crude protein determination

This experiment used the Kjeldahl method [11] which involved digestion of 2 g of ground meat samples (using a food blender), distillation, titration and calculating the crude protein by:

$$\text{Crude Protein (\%)} = \text{protein content} = \frac{[N \times 14.007 \times (V_s - V_b) \times 6.25 \times 250] \times F}{W \times 1000} \times 100$$

Where N: Normality (0.01) of Standard HCL acid; V_s: Volume of standard HCL acid to titrate a sample; V_b: Volume of standard HCL acid used to titrate a blank; W: Weight (g) of dry sample used; F= Dilution factor.

Crude fat determination

Crude fat was determined by gravimetric measurement [12] whereby 20 g of ground and homogenized chicken meat, passed through 1 mm sieve were weighed and put into extraction thimbles and ether extraction followed using Soxhlet apparatus. Then the ether was evaporated from the extract (at 105°C) and the remains were weighed. The crude fat (%) was calculated using the formula bellow;

$$\text{Crude Fat (\%)} = \frac{(W - Z)}{W} \times 100$$

Where W (g): Weight of extraction thimble and sample before extraction; Z: Weight of extraction thimble and sample after fat extraction.

Ash determination

In ash determination, 2 g of oven dried and ground muscle (passed through 1 mm sieve), dried at 100 °C, were weighed using a digital scale balance and then put in muffle furnace. The samples were incinerated at 550 °C for 5 hours [13]. The incinerated samples were weighed using digital scale balance. The ash was calculated using the formula below:

$$\text{Ash (\%)} = \frac{(WCSB - WCSA)}{WCSB} \times 100$$

Where WCSB: Weight (g) of crucible+sample before incineration

WCSA: Weight (g) of crucible+sample after incineration

Crude fiber determination

Crude fiber was determined by the method of James (1995) [14]. Sample (5.0 g) processed sample was boiled in 150 mL of 1.25% H₂SO₄ solution for 30 min under reflux. The boiled sample was washed in several portions of hot water using a two-fold cloth to trap the particles. It was returned to the flask and boiled again in 150 mL of 1.25% NaOH for another 30 min under same condition. After washing in several portion of hot water, the sample was drained before being transferred quantitatively to a weighed crucible where it was dried in the oven at 105°C to a constant weight. It was thereafter taken to a muffle furnace where it was burnt, only ash was left of it. The weight of the fiber was

determined by difference and calculated as a percentage of the weight of sample analysed thus:

$$\text{Crude Fiber (\%)} = \frac{(W_2 - W_3)}{\text{Weight of sample}} \cdot 100$$

Where:

W_2 = Weight of crucible+sample after washing, boiling and drying

W_3 = Weight of crucible+sample of ash

Available carbohydrate

Available carbohydrate represents that fraction of carbohydrate that can be digested by enzymes, absorbed and enters intermediary metabolism. Available carbohydrate can be arrived at in two different ways: it can be estimated by difference or analysed directly. Here, we calculate available carbohydrate by difference using the following formula:

$$\text{Available carbohydrate (\%)} = 100 - (\text{Moisture} + \text{Protein} + \text{Lipids} + \text{Ash} + \text{Dietary Fiber})$$

Mineral analysis

For the analysis of minerals (Ca, Mg, P and Fe), the samples were initially homogenized in a food processor and dried in a drying oven at 100°C. The samples were then burned and oven-dried at 450°C to constant weight. Atomic absorption spectrophotometry was used to determine calcium, magnesium, iron and phosphorus contents [15].

Rats diet preparation

A total of seven diets (7) was formulated using the procedures for PER as outlined by the Association of Official Analytical Chemists with cooked egg white used as reference protein [7]. Of these, six isonitrogenous diets, containing 10% crude protein each, were prepared from five meat sample and egg white to cover the rat's needs in growth while the seventh diet was protein free [16]. Table 1 below shows the composition of the seven experimental rations. The D_0 diet was protein-free; ration D_1 represented the ration having as protein source egg white; ration D_2 had as a source of protein chicken meat from group T; D_3 ration had as its source of protein the meat chicken meat from T⁺; D_4 had as a source of protein chicken meat from group

T^{Food}; D_5 ration had as its source of protein the meat chicken meat from group T^{H₂O}; and finally the D_6 ration had as a source of protein chicken meat from group T^{Formulation}. Calculation of protein proportion in the diet formulation of each ration was based on the proximate analysis of the test protein. After diet preparation, the protein proximate analysis was repeated to ensure the diet formulation was done correctly.

All the experimental diets were prepared by incorporating the meat and egg white powder into the protein free diet to obtain the required 100 g by volume. The diets also provided cellulose, mineral and vitamin fortification mixes. In the protein-free diet, corn-starch-sucrose mixture replaced the test protein.

Rat bioassay

Experimental design and animal management

Twenty-eight *Wistar* albino rats (3-4 weeks old), weighing 50-67 g were divided into seven groups of four animals (two males and two females) with average weight per group calibrated to be identical. They were purchased from the Department of Animal Biology of the University of Dschang and were bred in the animal house of the Department of Biochemistry with 12 hours of light and 12 hours of darkness in a day. Temperature was maintained at 21-25°C while a humidifier was used to maintain the humidity between 40-70%. Rats were placed in individual cages and randomly assigned by treatment to individual cages as described by Sarwar and Estakra [17] which enabled collection of faeces as well as food residues not consumed. The cage consisted of a 30 cm diameter basin whose bottom was lined with absorbent paper towels. The basin was 20 cm deep and had a wire mesh serving as floor on which was fixed a food feeder. The Basin was covered with a wire mesh for roof on which was suspended a drinking trough for water (Figure 1).

The growth and digestibility studies were carried out using the Completely Randomized Design (CRD). Each experimental group was randomly assigned a ration, then food and water were distributed *Ad libitum*. Prior to feeding the experimental diets, the rats were placed on an adaptation diet for a period of three days. The studies were conducted according to the ethical guidelines of the Committee for control and supervision of experiments on animals of the Faculty of Agronomy and Agricultural Sciences (FASA) of the University of Dschang, Cameroon.

Table 1. Formulation of experimental diets (g/100g)

Experimental Diets							
Ingredients (g)	D_0 (protein-free)	D_1 (reference)	D_2	D_3	D_4	D_5	D_6
Maize Flour	15	15	15	15	15	15	15
Maize Oil	5	5	5	5	5	5	5
Vitamin and Mineral Mix	5	5	5	5	5	5	5
Cellulose	7.9	7.9	7.9	7.9	7.9	7.9	7.9
Saccharose	67.1	53.73	54.34	53.15	55.02	54.74	54.79
Egg white	0	13.37	0	0	0	0	0
MT	0	0	12.76	0	0	0	0
MT ⁺	0	0	0	13.95	0	0	0
MT ^{H₂O}	0	0	0	0	12.08	0	0
MT ^{Food}	0	0	0	0	0	12.36	0
MT ^{Formulation}	0	0	0	0	0	0	12.31
TOTAL (g)	100	100	100	100	100	100	100

D_0 : Protein-free; D_1 : Egg white as protein source; D_2 : Meat from group T as protein source; D_3 : Meat from group T⁺ as protein source; D_4 : Meat from group T^{H₂O} as protein source; D_5 : Meat from group T^{Food}; D_6 : Meat from group T^{Formulation} as protein source. MT: Meat from broiler feed with standard Diet Alone; MT⁺: Meat from broiler feed with standard Diet+Reference antibiotic, Oxytetracycline; MT^{Food}: Meat from broiler feed with standard Diet+Probiotics given in Food; MT^{H₂O}: Meat from broiler feed with standard Diet+Probiotics given in Drinking Water; MT^{Formulation}: Meat from broiler feed with standard Diet+Formulation (Kernel oil+Probiotics) given in Food.

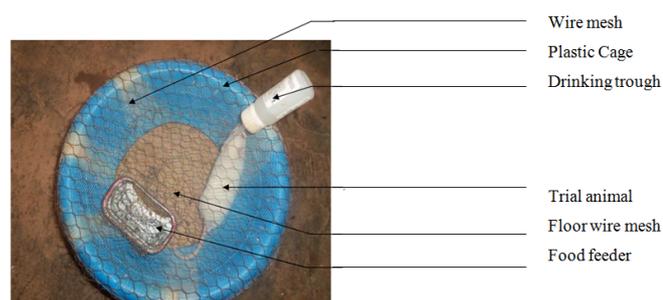


Figure 1. Experimental cage

Growth study

Growth study lasted 28 days. Before the start of the experiment, the weight of the rats was taken using an electronic balance (Sartorius, Germany) and repeated on alternate days throughout the study. The data collected from this study were used to determine the weight gained during the study which helped in the determination of the Food Efficiency Ratio (FER), Protein Efficiency Ratio (PER) and Net Protein Retention Ratio (NPRR) as follows:

$$\text{Protein Efficiency Ratio (PER)} = \frac{\text{g of weight gain}}{\text{g of protein consumed}}$$

$$\text{Net Protein Retention Ratio (NPRR)} = \frac{\text{g of weight gain} + \text{g of weight loss in protein free diet}}{\text{g of protein consumed}}$$

Digestibility study

The protein digestibility study lasted seven days from day 7 – day 13 of the growth study. During this period, food given to each rat per day were recorded. The food remnants were also collected at the end of the day. The difference between food allowance and food remnants was used to calculate the food consumed per rat daily. The faecal material for each rat was collected daily, dried in an open air and stored for faecal nitrogen determination. Faecal nitrogen was determined by Kjeldahl method as described above. Digestibility parameters were analysed using the model below:

$$\text{Apparent Protein Digestibility (\%)} = \frac{I - F}{I} * 100$$

$$\text{True Protein Digestibility (\%)} = \frac{I - (F - F_y)}{I} * 100$$

$$\text{Faecal Protein (\%)} = \frac{F - F_y}{I} * 100$$

Where:

I=Nitrogen Intake (calculated from the diet composition)

F=Faecal Nitrogen Output on the experimental diets

F_y=Faecal Nitrogen Output on a protein-free diet

Haematological and biochemical parameters analysis

At the end of the feeding period, rats were deprived for 18 h and then sacrificed. Blood was withdrawn from the heart by cardiac puncture from each animal under anaesthesia with chloroform, thereafter, was transferred into labelled heparinized and non-heparinized vacutainer tubes.

The heparinized blood was used for haematological analysis (haematocrit, total red cell (RBCs), total white blood cell (WBCs), lymphocytes, neutrophils, monocytes, eosinophils and basophils) [18].

The non-heparinized blood was allowed for complete clotting and then centrifuged at 3000 × g for 5 min. The supernatants (serum samples) were collected and frozen at -20°C. The serum was assayed for creatinine, aspartate amino transferase (AST), alanine amino transferase (ALT), total cholesterol, high density lipoprotein (HDL) and triglycerides using commercial kits (IMNESCO GmbH, Germany).

Tissues proteins analysis

Immediately after blood collection, the liver, lungs, heart, kidneys and spleen were isolated, freed of blood, and weighed using an electronic balance (Mettler PE 160, France). A section of each organ was used for estimation of protein concentration. For this purpose, the homogenate of each organ was prepared in 0.9% NaCl solution at 10% (i.e. 10 g organ in 100 mL of solution). The protein concentrations were determined by the Biuret method [19].

Animal used and ethics

All experiments described, respected the rules formulated under the Animal Welfare Act by the United States Department of Agriculture (USDA) and by adopting ARRIVE guidelines [20].

Statistical analyses

Results were expressed as mean value ± standard deviation. Within group, comparisons were performed by the analysis of variance (ANOVA) test using GraphPad InStat version 7.0. Significant difference between control and experimental groups were assessed by Tukey's multiple comparison test with statistical significance set at 0.05.

Results and discussion

Proximate analyses

In the present study, we evaluated the proximate analyses of meat from various broilers feed diets supplemented with probiotics and antibiotics as shown in Table 2.

Table 2 indicates that among the meat samples used in this study, the protein content of meat from broilers feed diet supplemented with probiotics given in water (82.78%) was the highest, followed by probiotics given through the formulation (81.25%) and food (80.93%). On the other hand, the protein content for egg white used as the reference protein was significantly higher (P<0.001) than various samples. In addition, meat from broilers feed diet supplemented with probiotics had a protein content significantly higher (P<0.001) compare to meat from broiler supplemented with antibiotics. However, the protein content of meat from broilers feed diet supplemented with probiotics through various channels wasn't significantly different (P>0.05). Similarly, there was no significant difference (P>0.05) in lipid and ash content between all meat from broilers feed diet supplemented with antibiotics and probiotics. Finally, data for the mineral analysis of various meat samples show that there wasn't a significant difference (P>0.05) between the minerals content of all meat samples with P value greater than 0.05 (Table 3). Our findings correlate with results reported by Hidayat *et al.* [21] indicating that the addition of liquid turmeric extract (LTE) at 2% to 10% did not significantly change the meat protein content with range of 21.49% to 23.94% and fat content with the range of 1.41% to 2.21%.

Rat bioassay

Growth study

In growth study, 92.86% of rats survived until the end of the study (26/28) and 85.1% gained positive body weight (24/28). However, two

rats died in the protein free group during the study. Based on the data shown in Figure 2, rats fed with protein free diet (D_0) showed the highest negative body weight gain (-15.5 g) at the end of the growth study which was significantly different ($P < 0.001$) from the weight gain by rats fed egg white as reference protein as well as those having as source of protein meat from various broilers meat. Moreover, rats in experimental groups having as source of protein meat from broilers fed diet supplemented with probiotics didn't shows any significant difference ($P > 0.05$) in total weight gained at the end of the assay. Our observation agrees with findings described by Ingbian and Adegoke [22], who fed albino rats with protein-enriched traditional cereal food in order to evaluation their nutritional quality. In addition, Nwabueze [23] observed similar trends with rats fed with extruded African bread fruits-based diets.

Table 4 shows the PER values for rats fed with 7 respective experimental diets. The results from this study showed PER values ranging between 2.08 to 2.76. The PER of rats fed meat from broilers whose diet was supplemented with probiotics (D_4 and D_5) was not significantly different ($P > 0.05$) to the PER value of rats fed diet supplemented with reference protein (D_1) with the exception of group D_6 where the PER value was significantly different ($P = 0.02$) to that of the reference protein group (D_1). In addition, no significant difference ($P > 0.05$) was found on the NPRR values for rats fed with meat from various broilers cluster (D_4 , D_5 , and D_6) and the value from the reference protein group (D_1). Furthermore, the FER values for rats fed reference protein (D_1) was not significantly difference to the values for rats fed various meat samples with P value greater than 0.05. The protein efficiency ratio (PER) measures the relationship between

Table 2. Proximate Composition of meat samples and reference protein (dry matter basis)

	Moisture	Protein	Carbohydrate	Lipids	Ash	Dietary Fiber
Egg white	9.02± 0.01 ^a	88.00±0.03 ^a	0.51±0.02 ^a	0.96±0.03 ^a	1.02±0.03 ^a	0.49±0.01 ^a
MT	5.05± 0.40 ^{bc}	78.38±1.59 ^{bc}	1.13±0.34 ^{bc}	11.58±1.11 ^b	2.3±0.13 ^{ab}	1.56±0.01 ^b
MT+	6.11± 2.20 ^b	71.68±1.73 ^c	3.77±0.05 ^c	13.66±0.16 ^b	2.29±1.1 ^{ab}	2.49±0.15 ^c
MT _{H₂O}	2.50± 0.27 ^c	82.78±0.68 ^{dc}	1.67±0.03 ^{df}	11.01±0.32 ^b	1.36±0.8 ^{ab}	0.68±0.05 ^d
MT _{Food}	3.12±1.24 ^c	80.93±1.46 ^c	1.13±0.02 ^c	12.39±0.36 ^b	1.53±0.05 ^{ab}	0.90±0.04 ^c
MT _{formulation}	2.30±0.27 ^c	81.25±0.99 ^c	1.54±0.04 ^f	11.39±2.82 ^b	2.64±0.46 ^b	0.88±0.03 ^c

MT: Meat from broiler feed with standard Diet Alone; MT+: Meat from broiler feed with standard Diet+Reference antibiotic, Oxytetracycline; MT_{Food}: Meat from broiler feed with standard Diet+Probiotics given in Food; MT_{H₂O}: Meat from broiler feed with standard Diet+Probiotics given in Drinking Water; MT_{Formulation}: Meat from broiler feed with standard Diet+Formulation (Kernel oil+Probiotics) given in Food. Data are presented as mean±standard deviation (n=3). Values in a column followed by different letter as superscript are significantly different according to Tukey's multiple comparison test ($P < 0.05$).

Table 3. Mineral composition (mg/100 g dry weight) of various meat samples

Microelement	Minerals (mg/100g)			
	Fe	P	Ca	Mg
MT	2.77±0.25 ^a	865±18.02 ^a	20.57±3.26 ^a	122.67±1.53 ^a
MT+	2.3±0.46 ^a	889.67±10.5 ^a	21.33±2.08 ^a	126.67±7.02 ^a
MT _{H₂O}	2.2±0.6 ^a	872±2.0 ^a	18.47±0.83 ^a	123.67±9.07 ^a
MT _{Food}	2.4±0.56 ^a	886±14.53 ^a	22.67±1.53 ^a	122.67±8.62 ^a
MT _{formulation}	2.6±0.68 ^a	803.33±6.68 ^b	25.33±4.75 ^a	118.67±8.02 ^a

MT: Meat from broiler feed with standard Diet Alone; MT+: Meat from broiler feed with standard Diet+Reference antibiotic, Oxytetracycline; MT_{Food}: Meat from broiler feed with standard Diet+Probiotics given in Food; MT_{H₂O}: Meat from broiler feed with standard Diet+Probiotics given in Drinking Water; MT_{Formulation}: Meat from broiler feed with standard Diet+Formulation (Kernel oil+Probiotics) given in Food. Data are presented as mean±standard deviation (n=3). Values in a column followed by different letter as superscript are significantly different according to Tukey's multiple comparison test ($P < 0.05$).

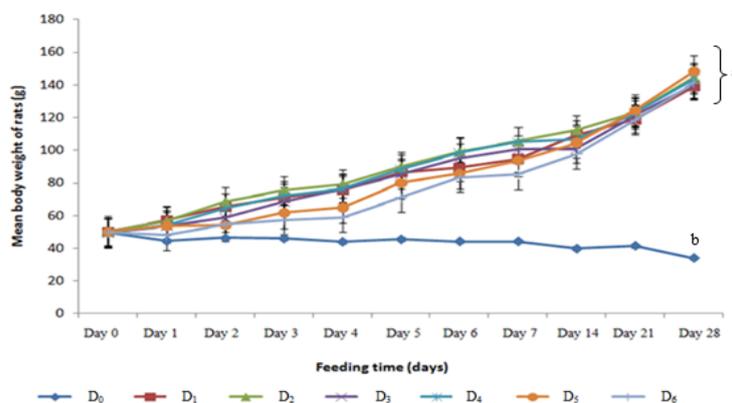


Figure 2. Growth of animals fed on various experimental diets (D_0 : Protein free diet; D_1 : Egg white as protein source; D_2 : Meat from group T as protein source; D_3 : Meat from group T+ as protein source; D_4 : Meat from group T_{H₂O} as protein source; D_5 : Meat from group T_{Food}; D_6 : Meat from group T_{Formulation} as protein source. Data are presented as mean±standard deviation (n=4). Values at day 28 followed by different letter as superscript are significantly different according to Tukey's multiple comparison test ($P < 0.05$))

weight gain by test animals and the corresponding protein as N-intake. Values of less than 2.0 or more than 2.0 are attributed to low and normal quality protein foods respectively [24,25]. However, PER has been heavily criticized, and NPRR (Net protein retention ratio) has been the recommended method by the AOAC and the Protein Advisory Group of the United Nations [26,27]. The NPRR credits the protein used for both growth and maintenance as it considers the weight loss of the rats on the protein-free diet [24].

Digestibility study

The true protein digestibility (TPD) and the apparent protein digestibility (APD) obtained with this *in vivo* rat bioassay are shown in Table 4. The reference protein group (D₁) showed no significant difference for value of TPD as compared to other diet treatment (p>0.05). Meanwhile, the values for APD for rats fed meat from various broilers clusters (D₂, D₃, D₄, D₅ and D₆) showed no significant difference as compared to the reference protein with P value greater than 0.05. Our findings affirm earlier digestibility study reports which showed that TPD and APD values for rats fed protein fortified foods were higher than those in the basal diet group [28].

Relative organ weight, haematology and biochemical parameters of rats

The results of the relative organ indices of rats are summarized in Table 5. There was no significant difference (p>0.05) in the liver, lung, kidney, and heart to body weight ratios in all experimental groups. However, the spleen weight of rats in the protein-free group was significantly decreased as compared to other groups D₁, D₂ and D₅ with P values of 0.018, 0.01 and 0.015 respectively. This decrease

may indicate an impaired functioning of the immune system evidence by the death of two rats of this group as the spleen is paramount in sustaining protective immunity [29].

The haematological analysis indicated that total RBC, total white blood cells count (WBCs), haematocrit, red blood cells count (RBCs), lymphocytes, eosinophils, basophils and monocytes values for rats fed diet supplemented with meat from various broilers cluster were not significantly different (p>0.05) as compared to the reference protein group. However, these values were different (p<0.03) as compared with rats from the protein-free diet (Table 6). Similar findings were reported [30].

The biochemical values of rats fed different experimental diet are presented in Table 7. The lipid profile of rats fed diet supplemented with broilers meat was not significantly difference (p>0.05) as compared to values for rats from the reference protein groups. However, the AST levels of all rats were not significantly different (p>0.05) in all groups even though the values for ALT for rats of the protein-free diet was different (p<0.008) as compared with values from all groups. In addition, the concentration of hepatic, spleen and kidney proteins was not significantly different (p>0.05) in all groups fed diet supplemented with meat from broilers as compared to the reference protein group. These results demonstrate the safety properties of probiotics as rats fed with meats from various broilers treatments didn't exhibited toxicity. In addition, our findings indicate that the supplementation of probiotics to broilers diet wouldn't modify the cholesterol balance of meat as high concentration of total cholesterol and triglycerides increase risk of coronary diseases in consumers [31].

Table 4. Effect of consumption of various experimental diets on growth and protein quality Indices

Treatment	Weight gain rats (g/rat)	Total feed intake (g/rat/28 days)	Protein consumed (g/rat/28 days)	PER	NPRR	FER	TPD (%)	APD (%)
D ₀ (protein-free)	-15.5±1.91 ^a	214.12±12.41 ^a	-	-	-	-0.07±0.01 ^a	-	-
D ₁ (reference)	89.25±8.62 ^b	285.60±25.35 ^{bd}	42.84±3.80 ^a	2.08±0.27 ^a	1.72±0.34 ^a	0.31±0.04 ^b	98.17±0.38 ^a	97.56±0.39 ^a
D ₂	94.50±6.35 ^b	282.80±22.04 ^{bd}	36.06±2.81 ^{bcd}	2.62±0.26 ^{ab}	2.19±0.57 ^a	0.33±0.04 ^b	98.08±0.15 ^a	97.34±0.28 ^a
D ₃	90.50±9.03 ^b	308.70±7.36 ^{bcd}	43.03±1.02 ^a	2.10±0.21 ^a	1.74±0.2 ^a	0.29±0.03 ^b	98.51±0.22 ^a	97.80±0.23 ^a
D ₄	94±4.24 ^b	302.40±7.92 ^{bcd}	36.47±0.96 ^{bcd}	2.57±0.13 ^{ab}	2.15±0.14 ^a	0.31±0.02 ^b	97.84±0.47 ^a	97.01±0.47 ^a
D ₅	98.25±5.85 ^b	336.70±28.95 ^{cd}	41.68±3.58 ^{bc}	2.36±0.25 ^{ab}	1.96±0.22 ^a	0.29±0.03 ^b	97.88±0.68 ^a	97.09±0.72 ^a
D ₆	103±13.73 ^b	303.10±19.31 ^d	37.31±2.38 ^{cd}	2.76±0.41 ^b	2.35±0.40 ^a	0.34±0.05 ^b	97.87±0.26 ^a	97.14±0.21 ^a

D₀: Protein free diet; D₁: Egg white as protein source; D₂: Meat from group T as protein source; D₃: Meat from group T⁺ as protein source; D₄: Meat from group T_{H2O} as protein source; D₅: Meat from group T_{Food}; D₆: Meat from group T_{Formulation} as protein source. Data are presented as mean±standard deviation (n=4). Values in a column followed by different letter as superscript are significantly different according to Tukey's multiple comparison test (P<0.05). PER: Protein Efficiency Ratio; NPRR: Net Protein Retention Ratio; FER: Food Efficiency Ratio; TPD: True Protein Digestibility; APD: Apparent Protein Digestibility.

Table 5. Effect of consumption of various experimental diets on Relative organ weights indices (g/100g) of rats

Experimental Diets	Liver	Spleen	Kidneys	Lungs	Heart
D ₀ (protein-free)	2.65± 0.79 ^a	0.15±0.03 ^a	0.26±0.01 ^a	0.49±0.09 ^a	0.30±0.02 ^a
D ₁ (reference)	4.12± 0.55 ^a	0.37±0.09 ^{bcd}	0.50±0.08 ^{bc}	0.61±0.10 ^a	0.49±0.10 ^{ab}
D ₂	3.65± 0.74 ^a	0.39±0.10 ^{bcd}	0.49±0.04 ^{bc}	0.65±0.04 ^a	0.57±0.07 ^b
D ₃	3.09± 0.85 ^a	0.31±0.05 ^{ccc}	0.43±0.04 ^{cc}	0.63±0.06 ^a	0.53±0.10 ^b
D ₄	3.00±0.45 ^a	0.31±0.07 ^{adc}	0.45±0.09 ^a	0.60±0.04 ^a	0.53±0.13 ^b
D ₅	3.32±1.15 ^a	0.36±0.12 ^c	0.45±0.03 ^c	0.63±0.05 ^a	0.51±0.09 ^b
D ₆	3.49±0.51 ^a	0.33±0.09 ^{ac}	0.42±0.16 ^{cc}	0.62±0.14 ^a	0.54±0.09 ^b

D₀: Protein free diet; D₁: Egg white as protein source; D₂: Meat from group T as protein source; D₃: Meat from group T⁺ as protein source; D₄: Meat from group T_{H2O} as protein source; D₅: Meat from group T_{Food}; D₆: Meat from group T_{Formulation} as protein source. Data are presented as mean±standard deviation (n=4). Values in a column followed by different letter as superscript are significantly different according to Tukey's multiple comparison test (P<0.05).

Table 6. Effect of consumption of various experimental diets on haematological parameters of rats

Parameters studied	Experimental Diets						
	D ₀ (protein-free)	D ₁ (reference)	D ₂	D ₃	D ₄	D ₅	D ₆
Total RBC (x 10 ⁶ /mm ³)	1.95±0.12 ^a	3.16±0.84 ^{ab}	3.38±0.35 ^{ab}	3.18±0.31 ^{ab}	3.2±1.23 ^{ab}	3.33±0.93 ^{ab}	3.9±0.74 ^b
Total WBC (x 10 ³ /mm ³)	152.25±18.26 ^a	203±11.60 ^{ab}	216.5±22.78 ^b	206.5±18.41 ^{ab}	223±33.75 ^b	219.75±32 ^b	247.25±42.62 ^b
Haematocrit (%)	30.25±4.11 ^a	45.5±4.04 ^b	46.5±5.06 ^b	44±3.37 ^b	46.75±6.65 ^b	45.5±2.64 ^b	50±3.56 ^b
Eosinophils (%)	0.6±0.14 ^a	1.0±0.0 ^b	1.1±0.27 ^b	1.0±0.29 ^b	1.0±0.0 ^b	1.0±0.0 ^b	1.0±0.0 ^b
Neutrophils (%)	22.75±4.85 ^a	26.75±2.75 ^a	27±6.27 ^a	28.75±6.8 ^a	27.25±6.6 ^a	28.75±6.13 ^a	27.5±7.0 ^a
Basophils (%)	0.33±0.13 ^a	0.43±0.17 ^a	0.45±0.13 ^a	0.45±0.24 ^a	0.4±0.10 ^a	0.48±0.30 ^a	0.4±0.10 ^a
Monocytes (%)	5.88±0.85 ^a	6.45±0.53 ^a	6.75±0.96 ^a	6.25±0.95 ^a	6.45±0.41 ^a	6.28±0.86 ^a	6.5±1.0 ^a
Lymphocytes (%)	65.38±1.55 ^a	70.4±6.28 ^a	64.5±1.29 ^a	63.5±1.91 ^a	65±1.63 ^a	64±0.82 ^a	64.5±0.58 ^a

D₀: Protein-free; D₁: Egg white as protein source; D₂: Meat from group T as protein source; D₃: Meat from group T⁺ as protein source; D₄: Meat from group T_{H₂O} as protein source; D₅: Meat from group T_{Food}; D₆: Meat from group T_{Formulation} as protein source. Data are presented as mean±standard deviation (n=4). Values in a row followed by different letter as superscript are significantly different according to Tukey's multiple comparison test (P<0.05). WBC= White blood cells count; RBC=Red blood cells counts.

Table 7. Effect of consumption of various experimental diets on biochemical parameters of rats

Parameters studied	Experimental Diets						
	D ₀ (protein-free)	D ₁ (reference)	D ₂	D ₃	D ₄	D ₅	D ₆
Total cholesterol (mg/dl)	97±5.41 ^a	117.75±5.32 ^b	118.25±1.25 ^b	115.25±10.17 ^b	116.5±5.45 ^b	118.5±12.92 ^b	116.75±4.99 ^b
HDL cholesterol (mg/dl)	89±8.28 ^a	90.25±3.30 ^a	90.5±4.04 ^a	90.75±2.21 ^a	90±4.54 ^a	90±3.16 ^a	90.5±1.29 ^a
LDL cholesterol (mg/dl)	3.5±1.3 ^a	4.5±1.29 ^a	4.75±0.96 ^a	4.5±1.28 ^a	5.25±0.96 ^a	4.5±1.73 ^a	5±0.81 ^a
Triglycerides (mg/dl)	133.75±4.34 ^a	141.5±1.29 ^{ab}	141.25±2.98 ^{ab}	141.5±2.64 ^{ab}	140.5±4.5 ^{ab}	141.75±4.92 ^b	139.5±1.29 ^{ab}
ALT(U/L)	50.0±3.76 ^a	59.89±0.30 ^b	59.95±1.22 ^b	59.17±6.10 ^b	59.96±3.22 ^b	59.38±2.26 ^b	59.20±1.35 ^b
AST (U/L)	35.75±2.62 ^a	43.25±6.65 ^a	47±5.48 ^a	46.5±4.36 ^a	46.75±3.95 ^a	46.25±4.03 ^a	46±6.21 ^a
Hepatic protein (mg/g)	257.5±38.41 ^a	539.75±3.09 ^b	533.5±5.80 ^b	535.5±7.23 ^b	535.5±10.63 ^b	532±11.74 ^b	533±23.94 ^b
Spleen protein (mg/g)	279.25±8.99 ^a	343.25±7.27 ^b	333±4.08 ^b	332±6.05 ^b	333.25±4.85 ^b	332.3±13.67 ^b	334±2.87 ^b
Kidney protein (mg/g)	39.5±1.29 ^a	88.5±1.30 ^b	89.25±2.63 ^b	89±2.16 ^b	89.25±8.14 ^b	88.5±1.29 ^b	88.75±5.25 ^b

D₀: Protein-free; D₁: Egg white as protein source; D₂: Meat from group T as protein source; D₃: Meat from group T⁺ as protein source; D₄: Meat from group T_{H₂O} as protein source; D₅: Meat from group T_{Food}; D₆: Meat from group T_{Formulation} as protein source. Data are presented as mean±standard deviation (n=4). Values in a row followed by different letter as superscript are significantly different according to Tukey's multiple comparison test (P<0.05).

Conclusion

It may be concluded that *Lactobacillus plantarum* MW-18CGZ and *Lactobacillus paracasei* MW-37CGZ could be utilized as biological feed additives in replacement of antibiotics used as growth promoters in poultry as they did not modify protein quality, protein bioavailability and prove to be safe. Their use will further reduce antibiotic residues in food derived from poultry as well as the generation and spread of antibiotic resistance in zoonotic pathogens.

Authorship and contributorship

Zambou Ngoufack Francois, Fonteh Anyangwe Florence, Kaktcham Pierre Marie, Vittorio Colizzi and Raoul Emeric Guetiya Wadoum conceived and designed the experiments. Raoul Emeric Guetiya Wadoum, Mube Herve and Takam V. Fotsouba carried out most of the experiments, including *in vitro* and *in vivo* experiments. The manuscript was written by Raoul Emeric Guetiya Wadoum in consultation with all authors. All authors approved the final version of the manuscript.

Conflict of interest

The authors declare that they have no competing interests.

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References

- Pelin E, Gulden B, Birol K (2016) Utilization of lactic acid bacteria and probiotics on meat products. *Journal of Hygienic Engineering and Design* 5: 78-82.
- Guetiya WRE, Zambou NF, Anyangwe FF, Njimou JR, Coman MM, et al. (2016) Abusive use of antibiotics in poultry farming in Cameroon and the public health implications. *Bri Poultry Sci* 57: 483-493.
- Xiaozhuo Z, Rong X, Huali L, Ting L, Yong Liao, et al. (2017) Effect of a novel strain of *Lactobacillus brevis* M8 and tea polyphenol diets on performance, meat quality and intestinal microbiota in broilers. *Italian Journal of Animal Science* 17: 396-407.
- FAO/WHO (2002) Guidelines for the evaluation of probiotics in food, Report of a Joint FAO/WHO working group on drafting guidelines for the evaluation of probiotics in food. Food and Agriculture Organization/World Health Organization. <http://ftp.fao.org/es/esn/food/wgreport2.pdf>. Accessed 26 January 2019.
- Nafees M, Pagthinathan M (2018) Effect of dietary supplementation of *Lactobacilli* and *Streptococci* cultures on the performance of broiler chickens. *Journal of Agricultural Sciences* 11: 34-40.
- Wang L, Long L, Yan L, Qiaoling C, Junchang F, et al. (2018) *Lactobacillus plantarum* restores intestinal permeability disrupted by salmonella infection in newly-hatched chicks. *Scientific Reports* 8: 2229.
- Association of official analytical chemists international (1984) Official methods of analysis. 14th Ed. Association of official analytical chemists, pp: 871- 881.
- Hackler LR (1984) Rat bioassay methods for assessing protein quality of meat and poultry products. *Am J Clin Nutr* 40: 711-717.
- Guetiya Wadoum R, Fonteh F, Kaktcham P, Ulrich Landry BM, Momo Kenfack CH, et al. (2019) *In Vitro* antimicrobial characterization of *Lactobacillus* isolates towards their use as probiotic alternatives to antibiotic growth promoters. *International Journal of Microbiology and Biotechnology* 4: 72-86.
- Association of official analytical chemists international (2003) Method 960-48. 417 official methods of analysis of the association of analytical chemists 418 international, 17th edn. AOAC International: Gaithersburg, ML.
- Official methods of analysis (1995) 16th Ed., AOAC international, Gaithersburg, MD, sec. 33.2.11, Method 991.20.
- AOAC official method of analysis, method 920.39, Fat (crude) or ether extract in animal feed. 18th ed., AOAC International, Gaithersburg, MD (2005).

13. Official methods of analysis (2000) 17th Ed., AOAC international, Gaithersburg, MD, sec. 33.2.11, Method 991.20.
14. James C (1995) The analytical chemistry of foods. Chapman and Hall Press, New York, pp: 86.
15. Association of official analytical chemists (AOAC) (2000) Official methods of analysis of AOAC international, 17th ed.; AOAC International: Gaithersburg, MD, USA.
16. Fasuyi AO (2005) Nutritional evaluation of Cassava (*Manihot esculenta*, Crantz) leaf protein concentrates (CLPC) as alternative protein sources in rat assay. *Pakistan Journal of Nutrition* 4: 50-56.
17. Sarwar G, Estakra S (2002) Protein digestibility and quality in product containing antinutritional factors are adversely affected by old age in rats. *Journal of Nutrition* 133: 220-225.
18. Manjarekar PN, Jolly CI, Narayan S (2000) Comparative studies of immunomodulatory activity of *Tinospora cordifolia* and *Tinospora sinensis*. *Fitoterapia* 71: 254-257.
19. Gornall A, Barwill G, David M (1949) Determination of serum protein by means of the biuret reaction. *J Biol Chem* 177: 751-766.
20. Kilkenny C, Browne W, Cuthill I, Emerson M, Altman Dg (2011) Animal research: reporting *in vivo* experiments-the ARRIVE guidelines. *Journal of Cerebral Blood Flow & Metabolism* 31: 991-993.
21. Hidayat M, Kurniawati A, Wati AK, Kusmayadi A (2017) The effect of liquid tumeric extract supplementation on carcass production and chemical quality of broiler meat. *Journal of the Indonesian Tropical Animal Agriculture* 42: 6-13.
22. Ingbian E, Adegoke G (2007) Nutritional quality of protein-enriched *mumu* – a traditional cereal food product. *International Journal of Food Science and Technology* 42: 476-481.
23. Nwabueze TU (2008) Weight analysis and nitrogen balance assay in rats fed extruded African Bread fruit (*Treculia Africana Decue*) based diets. *Nigeria Food Journal* 26: 27-41.
24. Sarwar G, McDonough F (1990) Review of protein quality evaluation method: evaluation of protein digestibility – corrected amino acid score method for assessing protein quality of foods. *Journal of the Association of Official Analytical Chemists* 73: 347-356.
25. Sakanata S, mitsuya T, Gutierrez M, Juneja R (2000) Protein quality determination of dilapidated egg York. *Journal of food Composition and Analysis* 13: 773-781.
26. PAG (1971) Guidelines on protein rich mixtures for use in weaning foods. Protein Advisory Group, United Nations, pp: 45-76.
27. Pallet P, Yang V. Evaluation of protein quality. NAS-NRC publication 1100, Tokyo United Nations University Press.
28. Elijah H, Charlotte S, Florence W (2017) Rat bioassay for evaluation of protein quality of soy-fortified complementary foods. *Journal of Food Research* 6: 35-44.
29. Sellers R, Morton D, Bindhu M, Roome N, Johnson J, et al. (2007) Society of toxicologic pathology position paper: Organ weight recommendations for toxicology studies. *Toxicologic Pathology* 35: 751-755.
30. Alada A, Akande O, Ajayt F (2004) effect of soya bean diet preparations on some haematological and biochemical indices in the rat. *African Journal of Biomedical Research* 7: 71-74.
31. Micha R, Michas G, Mozaffarian D (2012) Unprocessed red and processed meats and risk of coronary artery disease and type 2 diabetes - an updated review of the evidence. *Curr Atheroscler Rep* 14: 515-524.