

Effect of Different Fermentation Methods on Growth Indices and Serum Profile Of Broiler Chickens

Ari, M. M^{1*}, Ayanwale, B.A.², Adama, T. Z² and Olatunji, E.A³

* ¹Department of Animal Science, Faculty of Agriculture, Nasarawa State University Keffi,

²Department of Animal Production Technology, School of Agriculture and Agricultural Technology, Federal university of Technology Minna, Niger State, Nigeria

³Department of Animal Science, Faculty of Agriculture, University of Abuja, FCT. Nigeria

*Corresponding author address: E- mail : arimaikano@yahoo.com; Tel: +234 803 625 3270

ABSTRACT

This experiment was conducted to compare the effects of different fermentation methods of soyabeans on growth and serum indices using 240 days-old Anak broilers that were randomly divided into four (4) experimental groups of three replicates each. Dietary treatments were as follows: T1, T2, T3 and T4 representing *lactobacillus* (control), *Cooking and fermenting*, *Daddawa fermentation* and *Cooking with potash before fermentation* based groups at both starter and finisher phases and fed starter (1- 35 d) and finisher (36- 63 d) diets. T2, T3 and T4 significantly ($P < 0.05$) increased SGR and GE when compared to T1 at the starter phase. FCR, PER and EER were significantly affected by dietary treatments ($P > 0.05$). Variations in serum profile were significant ($P > 0.5$) except cholesterol. This suggest that fermentation processes provides effective mechanism for the improvement in growth indices, PER, EER and reduction in serum cholesterol in broilers

Key Words: Broilers, Fermentation, Growth Indices and Serum Profile

1.0 INTRODUCTION

The efficiency of poultry to convert feed into meat plays a key role in the economics of broiler industry (Ashayerizadeh et al. 2011). This therefore requires significant improvement in feed utilisation efficiency of the key ingredients of poultry feeds such as soyabeans for growth and tissue development. Raw soybean however contains several anti-nutritional factors (ANF) in variable amounts that affect utilization of soybean products (Refstie et al.1999; and Caprita, et al. 2001, Ari *et al.*, 2012) by monogastrics. Therefore, inactivation or minimization of the ANFs through different processing methods is required (Caine et al. 1998; Fabgemi et al., 2005; and Feng et al., 2007).

Different types of fermentation procedures have been adopted in the processing of oil seeds being feed materials of importance to both human and livestock. Waters-Bayer (1998), Campbell-Platt (1980) and Ayanwale and Ari (2002) reported the fermentation of soyabean and African Locust Beans seeds into Daddawa, fermentation of soyabeans by inoculation with microbial substrates was reported by Caine et al. (1998), while Ayanwale and Kolo (2001) reported the use of simple process of cooking soyabeans with or without potash before ensiling with basket and leaves. This processes converts food compounds into structurally related but financially more viable food through the activities of microbial cells (Stanbury and Whitaker, 1984; Ayanwale and Ari, 2002; Barde and Ari, 2004).

Various findings on the effect of different fermentation methods as well as probiotics and prebiotics inclusion on the health and growth responses of broiler chickens were also reported (Kabir et al., 2004; Piray et al., 2007), the health promoting and food enrichment effects of fermented soyabean products stands out as the overall benefit as protein efficiency is increased by the activities of intestinal microflora that impaired the activities of pathogenic bacteria through a competitive exclusion process (Miller,2002) leading to decrease in the breakdown of proteins to nitrogen and increase in efficiency of dietary protein utilization (Mikulec et al., 1999). The application of growth studies and serum profile in measuring the quality of nutrients and its utilisation had been widely used for both ruminant and monogastric animals (Tona et al., 2002; Abiola and Tewe, 2003, Ashayerizadeh et al. 2011). The aim of this study was to compare the effects of three different natural fermentation methods (uncontrolled fermentation) and a controlled fermentation on the growth indices, efficiency of dietary energy and protein utilization and blood biochemical parameters of broiler chickens

1.1 MATERIALS AND METHODS

Soyabean Collection, Processing and Diet Preparation:

Soyabeans seeds (*Glycine max*) were procured from a local market in Lafia metropolis of Nasarawa State, Nigeria. The collected seeds were cleaned by winnowing and hand picking of stones and debris. Equal quantities of the raw soyabeans were subjected to three uncontrolled (natural) fermentation processing methods viz: Cooking and fermenting (T2); Daddawa fermentation (T3) and Cooking with potash before fermentation (T4). Each of these processing methods serving as experimental treatment groups were compared with controlled fermentation (T1) process (Fermentation by culture organisms) which served as experimental control. The different fermentation processes are described as thus::

Fermentation by culture organisms (*Lactobacillus bulgaricus*, *Saccharomyces cerevisiae* and *Streptococcus lactis*) – Treatment (1):

The fermentation procedure was undertaken at the Animal Science Laboratory of the College of Agriculture, Lafia. The cleaned soyabean samples were tempered using the procedure described by Ari *et al.* (2012) before inoculation.

Inoculation of sample: The organisms used as starter culture were *Lactobacillus bulgaricus*, *Saccharomyces cerevisiae* and *Streptococcus lactis*. The tempered soyabean sample was inoculated using the procedure described by Pelczar *et al.* (1999) and adopted by Ari *et al.* (2012). The samples were sun dried and milled.

Cooking and fermenting – Treatment 2:

The raw soyabeans were sorted to ensure homogeneous cleaned grains. Cleaned soyabeans were cooked according to the methods described by Kaankuka *et al.* (1996) and fermented according to the method described by Ayanwale and Kolo (2002). The samples were sun dried and milled.

Daddawa fermentation – Treatment (3):

The raw soyabeans seeds were briefly fried in a hot dry pan (common driers) for about 3 minutes. The fried beans were grinded to remove the skins and then boiled for 6 hours before fermenting as documented by Campbell – Platt (1980) and Water-Bayer (1988) as an indigenous innovation by Nigerian housewives in the fermentation of soyabeans into *daddawa* (local magi).

Cooking with potash before fermentation – Treatment (4):

This method is a modification of the method described by Ayanwale and Kolo (2002). The raw soyabeans were cooked with 10 grams of potash / 100kg of soyabeans at 100°C for 15 minutes. The boiled grains were drained, cooled to room temperature and placed in a leaf-lined basket covered with further leaves and kept for 72 hours. The products were sun dried and milled.

Diet Preparation

The experimental fixed and variable ingredients were grinded using single grinding with Vent mill and a screen size of 3mm before mixing. The compounded experimental feeds were packed in polythene bags, sealed, labelled and stored until required.

Experimental Treatment:

240 days-old Anak broilers that were randomly divided into four (4) experimental groups of three replicates each. Dietary treatments were as follows: T1, T2, T3 and T4 representing *lactobacillus* (control), *Cooking and fermenting*, *Daddawa fermentation* and *Cooking with potash before fermentation*

A total of 240 days-old Anak broilers that were randomly divided into four (4) experimental groups of three replicates each. Dietary treatments were as follows: T1, T2, T3 and T4 were representing *lactobacillus* (control), *Cooking and fermenting*, *Daddawa fermentation* and *Cooking with potash before fermentation* based groups at both starter and finisher phases using Randomized Complete Block design having the test ingredients incorporation as the main source of variation.

The starter diets were fed for five (5) weeks (1- 35 days) and the finisher diets were fed for four (4) weeks (36- 63 days). Experimental diets were formulated to be *isocaloric* and *isonitrogenous* diets having 3,000 Kcal, 24% CP at starter stage and 3,100 Kcal, 22% at finisher level. The experimental feeds were formulated using at least cost feed formulation software *Feedwin*.

Broilers and feed intake were weighed weekly. Energy and protein efficiency ratios and specific growth rate were calculated as follows:

$$\text{Specific growth rate (SGR)} = 100 \times (\text{FBW} - \text{IBW}) / t$$

Where, FBW is final body weight (g), IBW is initial body weight (g) and t is time in days.

$$GE = WG / LW$$

Where, GE is growth efficiency for time period, WG is weight gain for specific time period and LW is initial weight as a covariate.

Feed conversion efficiency (FCR) = (Average feed intake per week (g) / (Average weight gain per week (g)

Protein intake (PI) = total feed intake \times (CP% of diet / 100)

Protein efficiency ratio (PER) = weight gain/total protein intake

Energy intake (EI) = (kcal ME of per kg diet \times feed intake) /1000

Energy efficiency ratio (EER) = weight gain \times 100 / total ME intake

Analytical Procedures

Chemical Analysis: Chemical composition of each of the fermented soyabeans samples and experimental diets were determined following standard methods (AOAC, 1995). Crude protein (N \times 6.25) was determined by the Kjeldahl method after acid digestion (Gerhardt, Königswinter, Germany). Crude lipid analysis without acid hydrolysis was determined by the ether-extraction method using a Soxtec System (Gerhardt, Königswinter, Germany). Moisture was determined by oven drying at 105 °C until a constant weight was achieved. Ash content was estimated by incinerating the samples in a muffle furnace at 600 °C for 6 h. Total Carbohydrates (NFE) was determined by difference and Calculated as thus : 100% - %(CP+ Ash + Crude Fat + Moisture).

Serum Analysis: Blood samples were obtained from the experimental chickens by neck decapitation. The samples from each group were collected into a labeled Ethylene Diamine tetra acetic acid (EDTA) bottles for analysis at Dalhatu Araf Specialist Hospital hematological laboratory, Lafia. Packed Cell Volume (PCV), the hemoglobin content and cholesterol were determined by the methods described by Aletor and Ogunyemi (1988).

Statistics: Data collected were subjected to one-way analysis of variance (ANOVA), means were separated where there were significant differences using Duncan's Multiple Range Test (Duncan, 1955) using SPSS 16.0.

1.2 RESULTS

Table 1 presents the chemical composition of fermented soya beans. Dry matter (DM) values ranged from 89.57 to 93.77% while crude protein (CP) ranged from 32.91% to 40.35%. Crude fibre (CF) on the other hand had values ranging from .94% to 17.22%. The highest value of ether extract (EE) was obtained in cooked and fermented soyabeans while the least (9.32%) was obtained in lactobacillus fermented soyabeans. Total ash ranged from 2.64 to 6.33% while total carbohydrates expressed as nitrogen free extract (NFE) ranged from 20.13 to 39.04%. The highest calcium (Ca) and phosphorous (P) values were 0.51 and 0.29% in daddawa and cook with potash and ferment groups respectively.

The chemical composition of fermented soyabean based starter and finisher diets are presented in Table 2. The DM values ranged from 90.47% to 92.78% and 92.43% to 94.19% in the starter and finisher diets respectively, while the CP values ranged from 20.13 to 22.91% in the starter diets, the finisher diets had the CP range of 21.06 to 23.24%. The lowest CF value in the starter diets was 6.06% (cooked and ferment) and 5.67% (lactobacillus) in the finisher diets. Values for EE were highest in daddawa based diets (13.50%) in the starter diets and finisher diets (13.30%). Total ash values in the starter diets were from 12.79% to 17.76% and 8.79% and 15.03% in finisher diets; the range for NFE was between 35.08 and 46.36% in the starter diets; and 41.62 and 44.22% in the finisher diets. Ca and P had highest values of 5.39 and 2.68% respectively in the starter diets and 2.69 and 1.35% in the finisher diets. The energy values of the starter diets ranged from 3320.35 and 3598.78Kcal/Kg ME while the energy value range of the finisher diets was from 34086.30 to 3752.35Kcal/Kg ME.

The results of the effects of different fermentation methods on growth indices of broilers are presented in the Table 3. Uncontrolled fermentation significantly increased the SGR and GE as compared to the control fermentation during the starter period ($p < 0.05$). Moreover, these indices were highest in broilers fed different uncontrolled fermented soyabeans based diets than those of controlled fermented soyabeans based diet group from 1-35 d of experiment ($p < 0.05$). At the finisher (36 to 63 d) SGR and GE were also a significant ($p < 0.05$) variation with T4 (cook with potash and ferment) given the lowest values of 29.14 and 0.59 respectively. FCR were significantly affected by dietary treatments ($p > 0.05$) at both the starter and finisher phases with daddawa having the best FCR values of 2.04 and 2.20 at the starter and finisher phases respectively which were closely followed by values of cook and ferment group at both phases (2.31 and 2.90). PER values significantly ($P < 0.05$) differ at both starter and finisher phases with

values ranged from 1.54 to 2.27 and 1.37 to 2.17 respectively. Similarly, EER followed the same trend ($P < 0.05$) at both the starter and finisher phases with values ranging from 10.29 to 15.93 and 9.40 to 13.24 respectively.

The results of the serum and blood profile analysis for broilers fed differently fermented soyabean based diets are presented in table 4. There were significant ($P > 0.5$) variations in most of the serum and blood parameters measured. Cook with potash and ferment (T4) had highest mean for urea and creatine while packed cell volume and haemoglobin was highest in the cook and ferment (T2) group. Treatment effects did not differ significantly ($p > 0.05$) in cholesterol levels.

1.3 DISCUSSIONS

The results of the chemical composition of differently fermented soyabeans were consistent with the reports of HNIS (1989) and Ensminger et al. (1990) who observed variations in the proximate composition of soyabeans subjected to different processing methods. Similar variations were also reported by Ayanwale and Kolo (2002) and Feng et al. (2007) for different fermentation processes of soyabeans.

The variation in the nutrient contents can be attributable to the differences in fermentation microbes associated with the different methods. The different microbes might have used different nutrient to different extents as sources of energy and protein for their growth and survival. Matsui (1996) similarly attributed differences in products that are fermented to differences in fermentation microbes. Ayanwale and Kolo (2002) reported similar range of values of observed in this experiment for DM, CP, CF and total ash for differently fermented soyabeans seeds while differences in the chemical composition of different fermentation products were also reported by Murwan and Ali (2011). Fermentation was also reported Sahlin (1999) and Abou –Arab and Abou- salem (2010) to result in a lower proportion of DM in the food and the concentrations of minerals and protein appear to increase when measured on a dry weight basis, this was similarly observed in this study.

In spite of the variations in the nutrient composition of the differently fermented soyabean based diets at both starter and finisher phases were within recommended range for the tropics as reported by Oluyemi and Roberts (2000). Similar variations in the nutrient composition of differently fermented soya beans based diets were reported (Ayanwale and Kolo, 2002). The high digestibility recorded for nutrients especially crude fibre in all the fermentation groups can be linked to microbial action on the fibrous nutrients before enzymatic action in the gastro – intestinal tract.

The SGR, GE and FCR values recorded were consistent with fermentation based diets fed to broilers and feed ingredients or probiotic additives (Ashayerizadeh et al. 2011). The high PER and EER values presented in this study by broilers confirms the beneficial effects of fermentation, probiotic and prebiotic products on broiler performance. These are in agreement with previous studies (Ayanwale and Kolo 2002, Zulkifli et al., 2000; Thitaram et al., 2005; Nayebpor et al., 2007; Falaki et al., 2010). It must be noted that part of the beneficial effect of using fermented products in the broiler ration had significant effects on growth performance of broiler chickens because of the synergistic effect of beneficial fermentation microbes and host microorganisms leading to reduction the count of pathogenic bacteria and increase in the population of useful microflora in gut through a process of competitive exclusion (Miller, 2002). The health promoting effects of fermented food products to monogastrics were also reported (Ashayerizadeh et al. 2011; Williams et al. 2001; Fairchild et al., 2001). Therefore, it can be inferred that by removing pathogenic bacteria that can adhere to the gastrointestinal track wall, Immune system may be less stimulated and a favourable medium is provided for the use of energy and nutrients by birds (Savage and Zakrzewska, 1996; Fairchild et al., 2001) and also reduce the breakdown of proteins to nitrogen. In this way, the utilization of proteins (amino acids) is improved, particularly from food that does not contain them in optimum quantities (Mikulec et al., 1999). The effects of feeding fermentation based diets on intestinal morphology indicates an increase in length of the intestinal mucosa, thereby increases the absorption areas and improves the birds energy and protein efficiency ratio (Santin et al., 2001). The observed variations among treatments could be attributed to differences in the growth and activities of fermentation microbes associated with each of the fermentation processes including the controlled fermentation.

The variations in the serum profile of experimental birds were attributed to dietary nutrient variations as reported by Aletor and Ogunyemi (1988) and Jiang et al (1990). The Observed low cholesterol levels among both the controlled and uncontrolled fermentation based diets confirms the efficiency of fermentation and related products in reducing cholesterol as earlier observed by Panda et al. (2001), Kalavathy et al.(2003); and Jin et al.(1998).

1.4 CONCLUSION

Fermentation processes provides effective mechanism for the improvement in growth indices; PER, EER and reduction in serum cholesterol in broilers. The synergistic effect of beneficial fermentation microbes and host microorganisms led to reduction in the count of pathogenic bacteria and increase in the population of useful micro flora in gut, resulting to improvement in the gastrointestinal health and performance of boilers. Therefore, fermentation could be introduced as a safe and natural alternative to antibiotic growth promoters in broiler diets.

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Table 1: Effect of fermentation on the Chemical Composition of Soyabean

Fermentation methods	Chemical Composition (%)							
	Dry Matter	Crude Protein	Crude fibre	Ether Extract	Total Ash	NFE	Ca	P
Lactobacillus	91.12	40.35	4.96	9.32	6.33	39.04	0.38	0.25
Cook & ferment	89.57	32.91	14.34	19.41	4.21	29.13	0.50	0.23
Daddawa	93.77	37.9	10.32	16.82	2.64	32.32	0.51	0.22
Cook + potash & ferment	90.11	37.86	17.22	20.22	4.57	20.13	0.48	0.29

Table 1	Composition of experimental diets							
	Starter phase				Finisher phase			
Ingredients	T1	T2	T3	T4	T1	T2	T3	T4
Maize	32.75	38.3	37.5	38.25	36	41.5	41.5	41.5
Maize Bran	13	8	10.25	10.25	13	9	11.25	11.5
Rice Bran	5	2.5	2.85	2.55	5	1.25	2.25	2.5
Soya lactobacillus	29.35	-	-	-	29.35	-	-	-
Soya Cook & ferment	-	32	-	-	-	31.5	-	-
Soya daddawa	-	-	31	-	-	-	30.25	-
Soya ferment + K	-	-	-	29.5	-	-	-	30
Blood Meal	3.75	5	4.25	4.75	2	3.5	2.25	2.25
Fish Meal	4	4.75	4	4.25	3.25	3.8	3.3	3.3
Bone Meal	4	4	3.5	3.5	4	3.5	3.5	3.25
Limestone	4.7	0.5	1.7	1	3.2	-	-	-
Palm Oil	0.5	2	2	3	1.25	3	2.75	2.75
L-Lysine	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
DL-Methionine	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Salt	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Premix*	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100	100	100	100
Calculated analysis	3000.93	3000.22	3014.23	3008.08	3093.93	3083.07	3115.02	3028.85
ME/Kcal/kg	24.01	24	24.01	24.08	22.05	21.98	21.92	21.82
CP%								
Determined analysis	90.47	92.78	91.4	92.05	92.93	92.43	94.19	92.57
Dry Matter (%)	22.91	22.3	22.47	20.13	21.23	21.66	21.06	23.24
Crude Protein (%)	6.78	6.06	6.74	6.82	5.67	6.9	6.93	5.91
Crude Fibre (%)	9.88	12.01	13.5	9.48	11.5	11.23	13.3	11.53
Ether extract (%)	12.79	17.25	17.76	17.21	15.03	12.11	8.76	11.19

*Premix to provide the following per KG of diet: Vitamin A, 9,000 IU; Vitamin D3, 2,000,IU; vitamin E, 18 IU; vitamin B1, 1.8 mg; vitamin B2, 6.6 mg B2,; vitamin B3, 10 mg; vitamin B5, 30 mg; vitaminB6, 3.0 mg; vitamin B9, 1 mg; vitamin

Growth indices	Treatments					SEM
	T1	T2	T3	T4		
SGR(%d ⁻¹)						
	Starter phase	14.07 ^c	20.26 ^a	19.11 ^b	19.16 ^b	±0.72*
	Finisher phase	53.36 ^b	52.55 ^c	61.27 ^a	29.14 ^d	±3.62*
GE						
	Starter phase	5.70 ^d	6.57 ^b	9.18 ^a	6.34 ^c	±0.40*
	Finisher phase	0.72 ^b	0.59 ^c	0.93 ^a	0.59 ^c	±0.04*
FCR						
	Starter phase	2.84 ^a	2.31 ^c	2.04 ^d	2.48 ^d	±0.09*
	Finisher phase	3.44 ^a	2.60 ^b	2.20 ^b	3.24 ^a	±0.16*
Protein Intake(g)						
	Starter phase	158.69 ^b	135.51 ^c	164.03 ^a	126.62 ^d	±4.70*
	Finisher phase	279.22 ^b	272.31 ^c	305.68 ^a	305.42 ^a	±4.55*
PER						
	Starter phase	1.54 ^d	1.95 ^c	2.26 ^a	2.02 ^b	±0.08*
	Finisher phase	1.37 ^d	1.61 ^b	2.16 ^a	1.38 ^c	±0.10*
Energy Intake						
	Starter phase	2474.40 ^b	2017.60 ^d	2627.00 ^a	2177.90 ^c	±72.25*
	Finisher phase	4483.10 ^d	4677.00 ^c	5446.30 ^b	46488.00 ^a	±5434.75*
EER						
	Starter phase	9.86 ^d	13.10 ^b	14.08 ^a	11.72 ^c	±0.48*
	Finisher phase	8.55 ^c	9.38 ^b	12.13 ^a	0.91 ^d	±1.25*

abcd means on the same row with the same superscript are not significantly (P>0.05) different

SEMPooled standard Error of Means

* Significantly (P>0.05) different

NS Not Significantly (P>0.05) different

Table 4: Effect of fermentation methods of Soyabeans on Serum and Blood Profile of Broilers

Parameters	Fermentation Methods				SEM
	T1	T2	T3	T4	
Urea (mmn/l)	2.83 ^b	2.83 ^b	2.07 ^c	3.00 ^a	±0.11*
Cholesterol (mmn/l)	2.70	2.80	2.97	2.87	±0.11 ^{NS}
Creatine (mn/l)	85.33 ^a	98.67 ^b	85.33 ^a	115.33 ^a	±3.73*
Packed Cell Volume (PCV)	29.33 ^b	32.33 ^a	25.33 ^a	27.33 ^c	±0.81*
Haemoglobin (Hb) (g/dl)	9.77 ^a	9.80 ^a	8.67 ^c	9.03 ^b	±0.15*

abc means on the same row with the same superscript are not significantly (P>0.05) different

SEMPooled standard Error of Means

* Significantly (P>0.05) different

NS Not Significantly (P>0.05) different

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