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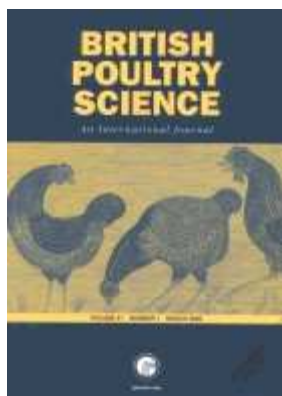
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Effects of fungal (*Aspergillus niger* or *Ceriporiopsis subvermispora*) fermentation on the nutritive value of shea nut (*Vitellaria paradoxa*) meal for broiler chicks

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6 **Effects of fungal (*Aspergillus niger* or *Ceriporiopsis subvermispota*) fermentation on the**
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8 **nutritive value of shea nut (*Vitellaria paradoxa*) meal for broiler chicks**
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24 **RUNNING TITLE: FERMENTATION OF SHEA NUT MEAL**
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3 **Abstract** 1. Shea nut (*Vitellaria paradoxa* Gaertn) meal was fermented for 8 d with either
4 *Aspergillus niger*, *Ceriporiopsis subvermispota*, or a mixture of the two organisms. The
5 fermentation was completed using two methods, an opened container or a closed container.
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10 2. Each of the 6 samples was dried and incorporated into basal broiler diets at 90g/kg.
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12
13 1 3. In addition, the unfermented shea nut meal was incorporated in the diet at 90 g/kg and the
14 basal diet (maize and soybean meal-based) was also provided as an 8th dietary treatment to
15 2
16 3 individually caged broiler chickens.
17

18
19 4 4. All fermented fungi-treated shea nut meals had similar proximate nutrient compositions to
20 5 the unfermented shea nut meal, but there were substantial decreases in their hydrolysable
21 6 tannins and saponin contents. Both fermentation methods gave similar reductions in the
22 7 concentrations of tannins and saponins.
23
24

25 8 5. Shea nut meal fermented with individual or both fungal organisms gave greater ($P<0.001$)
26 9 growth performance than that of unfermented shea nut meal. However, all shea nut meals
27 10 including the unfermented meal gave lower ($P<0.001$) growth variables than those for the
28 11 maize-soybean meal control.
29
30

31 12 6. The nutritional improvement of shea nut meal achieved in this study still falls far short of
32 13 what is expected for it to become valuable for the poultry feed industry. These fermentation
33 14 methods using *A. niger* or *C. subvermispota* require further improvements to provide
34 15 satisfactory feed products.
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39 16 INTRODUCTION

40 17 Shea nut (*Vitellaria paradoxa*, Gaertn.) meal is a by-product obtained from the shea fat
41 18 processing industry in West Africa. It is increasingly available in large quantities for feeding
42 19 animals and it has the potential to supply significant amounts of dietary protein and energy to
43 20 poultry feeds in Western Africa. However, its utilisation in the diet is limited by the presence
44 21 of high concentrations of anti-nutritive factors such as tannins and saponins (Annongu *et al.*,

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2
3 1 1996; Atuahene *et al.*, 1998). The adverse effects of these anti-nutritive factors in shea nut
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5 2 meal can be minimised by hot water treatment (Okai *et al.*, 1995), but this also gives a
6
7 3 considerable loss of dry matter. *Aspergillus niger* (Pinto *et al.*, 2001) or *Ceriporiopsis*
8
9 4 *subvermispota* (Gamble *et al.*, 1996) fungi can degrade tannins. Microbial fermentation can
10
11 5 also reduce the concentration of saponins in a feedstuff (Reddy and Pierson, 1994). Solid state
12
13 6 fermentation using fungal cultures such as *Aspergilli* can improve the nutritive value of
14
15 7 feedstuffs for broiler chickens (Mathivanan *et al.*, 2006; Yamamoto *et al.*, 2007). The
16
17 8 potential uses of these two organisms to improve the nutritional value of shea nut meal by
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19 9 fermentation have not been studied.
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25 10 If fermentation of shea nut meal is valuable, a practical system of fermentation would
26
27 11 need to be applied at the oil extraction factory or on-farm. It is unlikely that controlled
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29 12 fermentation conditions would be available at these sites. There is a need to examine whether
30
31 13 the method of fermentation is an important factor in the nutritional improvement of shea nut
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33 14 meal. The simplest practical fermentation method would be to apply the fungal organisms to
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35 15 the shea nut meal and then leave windrowed. Alternatively, after the application of the fungal
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37 16 organisms, the material could be compacted and sheeted to exclude some, but not all, air and
38
39 17 reduce other fungal or bacterial contamination. Two laboratory-based fermentation methods
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41 18 were devised to examine these two fermentation options.
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45 19 Therefore, this study was undertaken to determine if the fermentation of shea nut meal
46
47 20 using *A. niger* or *C. subvermispota*, or a combination of the two organisms could change
48
49 21 tannin and saponin contents or change the growth performance of broiler chickens relative to
50
51 22 the unfermented shea nut meal or a basal diet (containing no shea nut meal). A second
52
53 23 objective was to compare two methods of fermentation for the same variables of chemical
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55 24 composition, nutrient availability and broiler chicken growth performance for each of the
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1 three fungal organism treatments (*A. niger*, *C. Subvermispora* or a combination of both
2 organisms). Eight dietary treatments were compared.

3 MATERIALS AND METHODS

4 **Culturing of fungal species**

5 *Ceriporiopsis subvermispora* (ATTC number 90466) was obtained from the American Type
6 Culture Collection (ATTC, 10801 University Blvd., Manassas, VA 20110-2209), cultured in
7 ATTC medium (#200 YM or Difco 0712) and incubated at 24°C for 7 d. The *Aspergillus*
8 *niger* was a laboratory strain isolate obtained from the University of Wolverhampton, UK,
9 cultured by agar plating technique using Sabouraud dextrose agar (Oxoid Ltd, Basingstoke,
10 UK) and incubated at 24°C for 7 d. *A. niger* spores were harvested by tapping, whereas those
11 of *C. subvermispora* were scraped off. Spore counts were determined using an
12 haematocytometer according to the Fuchs-Rosenthal technique to be approximately 1.6×10^6
13 spores, which were equivalent to 0.25 g (*A. niger*) and 0.49 g (*C. subvermispora*). The spores
14 were preserved at 4°C until the organisms were used for solid medium inoculation.

15 **Preparation of fermented fungi-treated shea nut meal samples**

16 An expeller shea nut meal (Shebu-Loders Croklaan Ltd, Savelugu-Ghana) was used for this
17 study (Dei *et al.*, 2008) and this was divided into 7 lots. Six of these shea nut meal samples
18 were mixed with water in the ratio 1:2 (1 part shea nut meal: 2 parts water) and the spores of
19 *A. niger* (0.25g per kg of shea nut meal) were added to two samples. The spores of *C.*
20 *subvermispora* (0.49g per kg of shea nut meal) were added to two further shea nut meal
21 samples and a mixture of spores of *A. niger* (0.25g) and *C. subvermispora* (0.49g) were added
22 to the two remaining shea nut meal samples. Each of the two fungal treatment samples was
23 allocated to one of two fermentation treatment methods. Method 1 involved fermentation of
24 the wet material in an opened plastic container, whereas in Method 2, the material was gently
25 compacted and then the plastic container was closed and sealed with adhesive film. All the

1 samples were kept in a room at ambient temperature (24°C) for 8 d. Although *A. niger* is an
2 aerobic organism, there would be a production phase under the micro-aerobic conditions that
3 existed in the closed container (David *et al.*, 2003).

4 The pH of the fermented samples was determined using a pH Meter (Model 3510,
5 Jensway, UK). The mats of fungal growth on the surface of samples in the opened containers
6 were scraped-off before drying. Each sample was dried at 56°C and ground.

7 **Broiler chick assay**

8 The 6 fermented and one unfermented shea nut meal samples were each substituted for the
9 basal diet (Table 1) at 90 g/kg. Ross 308 male broiler chicks were reared in a litter-floored
10 pen and fed a proprietary broiler starter feed for 10 d. At 10 d of age, 64 broilers of similar
11 body weight were individually caged (0.3m x 0.3m x 0.36m) and fed one of the 8 meal-form
12 experimental diets (8 replicates) to 18 d of age. During the last 4 d of the 8-d experiment, the
13 droppings were collected daily and stored at 4°C until the combined 4-d sample was dried in a
14 force-draught oven at 60°C.

Table 1 near here

15 **Chemical analyses**

16 The ground shea nut meal samples were analysed using standard methods (AOAC, 2000) for
17 dry matter (Method 934.01), nitrogen (Method 968.06), ash (Method 942.05) and gross
18 energy (adiabatic bomb calorimeter Model 1261; Parr Instrument Co., Moline, IL). Ether
19 extract content was determined after an hydrochloric acid (4M) digestion (Method 920.39).
20 The neutral detergent fibre content was determined by the method of van Soest (1963) using
21 neutral detergent solution. Each sample of bird droppings was also analysed for dry matter,
22 nitrogen and gross energy.

23 Tannin concentrations of the shea nut meal samples were determined at the Institute of
24 Animal Reproduction and Food Research of the Polish Academy of Sciences, Olsztyn,
25 Poland. The content of total soluble phenolics was determined according to the method

1 described by Naczek and Shahidi (1989) using catechin as a standard. The bound plus soluble
2 proanthocyanidins were assayed colorimetrically by the method of Price *et al.* (1978) using
3 catechin as a standard. The soluble proanthocyanidins were determined by the acid-butanol
4 reagent method of Porter *et al.* (1986) as cyanidins using an HPLC (Beckman DU 7500,
5 Fullerton, CA) and detected at a 550 nm wavelength. Hydrolysable tannins were determined
6 as gallic acid after enzymatic hydrolysis by tannase according to the procedure of Karamać *et*
7 *al.* (2006) using a Shimadzu HPLC system (Shimadzu Corp., Kyoto, Japan) and detected at a
8 280 nm wavelength. The saponin in the samples was determined at the Institute of
9 Agricultural Research and Training, Ibadan, Nigeria) by the method of Wall *et al.* (1952). The
10 sample was extracted with ethanol in a soxhlet apparatus, and the extract was defatted with
11 benzene in a continuous liquid-liquid extractor. The addition of butanol formed a butanol-
12 saponin extract that was quantified using rat red blood cells.

13 **Statistical analysis**

14 A randomised block analysis of variance was used to compare the treatment means using
15 GENSTAT (10th version). The tier level of cages was considered as a blocking factor. The
16 three fungal treatments and two methods of fermentation were considered as treatment factors
17 with their interactions. Two further orthogonal contrasts were used to compare the means of
18 all the fermentation treatments with each of the two additional controls (maize-soybean meal
19 diet and unfermented shea nut meal diet).

20 **RESULTS**

21 The pH of the fermented shea nut meals were 5.3 (opened container) and 5.6 (closed
22 container) indicating that a significant amount of fermentation had taken place in each of the 6
23 samples. The proximate nutrient compositions of all fermented fungi-treated and unfermented
24 shea nut meal samples were similar (Table 2). However, there were appreciable reductions in
25 hydrolysable tannins (44-79%) as well as saponins (10-87%) in the fermented samples (Table

1
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3 1 3). The reduction in hydrolysable tannins was greatest when both organisms were present in
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5
6 2 the meal fermented by method two (closed container). The fungal degradation of
7
8 3 proanthocyanidins was less than that of hydrolysable tannins in both fermentation methods
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10 4 (Table 3).

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13 5 The nutrient metabolisabilities of the experimental diets were greater ($P<0.05$) for the
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15 6 control (maize-soybean meal diet) than any of the shea nut meal containing diets (Table 4).
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17 7 However, dietary AME of the unfermented meal was similar to that of the other control.
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19 8 Although there were no significant differences between the fermented and unfermented
20
21 9 meals, dietary AME of the fermented meals tended ($P=0.052$) to be lower. This may have
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23
24 10 been due to the utilisation of some of more available nutrients by the fungal organisms (Egli
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26
27 11 and Zinn, 2003). Tables 2 & 3 near here

28
29 12 A correlation matrix of the chick growth and nutrient metabolisability variables of the
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31 13 fermented samples and their contents of anti-nutritive factors is shown in Table 4. There were
32
33 14 no significant correlations between the variables. However, the correlation ($r = -0.794$)
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35 15 between crude protein metabolisability and saponin approached a significant level ($P =$
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37 16 0.059).

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41 17 The birds fed the fermented shea nut meal treatments had higher ($P<0.001$) feed
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43 18 intakes, weight gains and feed conversion efficiencies compared to the birds fed the
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45 19 unfermented shea nut meal (Table 5). Also, all growth variables of birds fed the unfermented
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47 20 meal were far lower ($P<0.001$) than those of birds fed the basal diet (maize and soybean
48
49 21 meal-based). Although the birds fed the shea nut meal fermented with both fungal organisms
50
51 22 had lower ($P<0.05$) feed intakes, there were no significant differences in their weight gains or
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53 23 feed conversion efficiencies compared to birds fed the fermented meals using the individual
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55 24 fungal organisms (Table 5). Tables 4 & 5 near here

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DISCUSSION

The amounts of tannins and saponins in some feeds can be reduced by fermentation (*i.e.* wet incubation of a feedstuff), although the mechanism by which these components are eliminated is not fully understood (Reddy and Pierson, 1994). The fermentation process can create conditions for the growth of microbes (*Bacillus*, *Corynebacterium*, *Klebsiella*, *Aspergillus*, *Penicillium*, *Fusarium* and *Candida*) that break down tannins (Bhat *et al.*, 1998). However, the microbial degradation of condensed tannins (proanthocyanidins) is less than that of hydrolysable tannins in both aerobic and anaerobic environments (Bhat *et al.*, 1998). In this study, the fungal fermentation treatments resulted in higher reductions in hydrolysable tannins than proanthocyanidins (Table 3). Annongu *et al.* (1996) reported a poor reduction (34%) in proanthocyanidins in naturally fermented shea nut meal, probably because of the action of endogenous micro-organisms. *A. niger* strains are noted for tannase synthesis ability (Pinto *et al.*, 2001; Ramirez-Coronel *et al.*, 2003) that can degrade hydrolysable tannins into glucose and gallic acid. As is evident from the results, gallic acid concentrations increased (21-93%) in the fermented samples. Also, *A. niger* is capable of ligno-cellulolytic enzyme production such as cellulases and xylanases (Villena and Gutierrez-Cornea, 2007), which can degrade tannins associated with cell wall polysaccharides. *C. subvermispora* can degrade condensed tannins (Gamble *et al.*, 1996) as well as hydrolysable tannins (Aikin *et al.*, 1995) through the production of ligno-cellulolytic enzymes that degrade fibre. *C. subvermispora* is among the most selective lignin-degraders in the wood industry.

The fermentation process can produce organic acids that break down saponins (Delaude, 1974). The reduction in saponin concentrations in the fermented shea nut meals may have been due to the production of organic acids. Many authors have reported the importance of *A. niger* in the industrial production of citric acid using agro-industrial by-products as substrates (Leangon *et al.*, 1999; Vandenberghe *et al.*, 2000). Heng *et al.* (2006)

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3 1 reported degradation of saponins in peas at acidic pH. Annongu *et al.* (1996) attributed the
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5 2 reduction in saponin (50%) in naturally fermented shea nut meal to organic acids.
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8 3 The fermentation of shea nut meal using both fungal organisms in the same culture
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10 4 failed to elicit far greater reductions of tannins or saponins than individual organisms. This
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12 5 was probably due to antagonistic activity between the two fungal organisms. Although the
13
14 6 mode of the antagonism was not known, the modes of action proposed for microbial
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16 7 antagonism in other studies (Shrivastava *et al.*, 1996; Sipiczki, 2006) include competition for
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18 8 space and nutrients, and production of enzymes that break down cell wall. Thus microbial
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20 9 fermentation of shea nut meal should preferably involve single micro-organisms.
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24 10 Generally, the effects of the fungal treatments on the tannins in particular were not as
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26 11 great as expected. The residual tannins in the fermented meals could, therefore, still have
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28 12 adverse effects on the growth of broiler chickens. Fungal fermentation processes can be
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30 13 affected by several factors such as type of substrates (Lu *et al.*, 1995; David *et al.*, 2003),
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32 14 aeration conditions (Meijer *et al.*, 2007) and water activity (Romero *et al.*, 2007) on fungal
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34 15 growth and productivity. Also, as the fungal cells divide and the culture develops, the amount
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36 16 of biomass makes the culture congested. This is a recognised practical problem with fungal
37
38 17 fermentation (Ward *et al.*, 2006). This may limit mass-transfer processes in the culture system
39
40 18 of enzymes and metabolites produced or retard growth of the fungal organisms. Thus there is
41
42 19 a need to subject fermentation methods of the shea nut meal to further studies. These may
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44 20 include the use of other natural or genetically-engineered fungal organisms and manipulation
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46 21 of the growth conditions of the microbe in order to provide acceptable feed products.
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53 22 The poor growth performance of birds fed on unfermented shea nut meal was to be
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55 23 expected. Many studies involving shea nut meal have shown reduced feed intakes, weight
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57 24 gains and feed conversion efficiencies in broiler chickens (Annongu *et al.* 1996; Atuahene *et*
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59 25 *al.*, 1998; Olorede *et al.*, 1999), rats (Okai *et al.*, 1995) and pigs (Okai and Bonsi, 1989).
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3 1 These authors have attributed the poor utilisation of shea nut meal in monogastric diets
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5 2 variously to the presence of anti-nutritive factors such as tannins and saponins. The biological
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7 3 significance of tannins and saponins in poultry nutrition is related to their characteristic
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9 4 adverse effects on feed intake (Cheeke, 1971; Armstrong *et al.*, 1974) and nutrient utilisation
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11 5 (Shimoyamada *et al.*, 1998; Smulikowska *et al.*, 2001). In this study, the adverse effects of
12
13 6 tannins and saponins probably resulted in low feed intakes as well as low nutrient
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15 7 metabolisability in the unfermented meal.
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20 8 The chicks fed diets containing shea nut meal fermented with the fungal organisms
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22 9 had an improved growth performance compared to the unfermented shea nut meal group.
23
24 10 Annongu *et al.* (1996) also reported significant improvements in feed intakes and growth rates
25
26 11 of adult broilers when fed shea nut meal that had undergone natural fermentation. Other
27
28 12 reports have shown that chicks fed on diets containing guar meal (Nagra *et al.*, 1998),
29
30 13 soybeans (Mathivanan *et al.*, 2006) or koji feed (Yamamoto *et al.*, 2007) cultured with
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32 14 *Aspergilli* significantly improved growth rates of broiler chickens. In this study, there were
33
34 15 substantial improvements in the feed conversion efficiencies of the fermented meals. This
35
36 16 may have been due to protein-sparing effects, as a result of reductions in the concentrations of
37
38 17 anti-nutrients (Nelson *et al.*, 1975). All anti-nutrients in the fermented meals tended to be
39
40 18 negatively correlated with crude protein metabolisability, particularly saponins ($P=0.059$).
41
42 19 However, the residual concentrations of these anti-nutrients were still high. Thus growth rates
43
44 20 of birds fed the fermented meals were relatively poor compared to that of birds fed on the
45
46 21 control diet. Within the 6 fermented shea nut meal treatments, there were no strong
47
48 22 correlations between bird growth performance and determined tannin and saponin levels. This
49
50 23 may have been just a reflection of the high variability in animal-based experiments; but the
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52 24 tannin break-down products may also have had significant effects on bird growth
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54 25 performance. Despite the lack of correlations between the available tannins and growth
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3 1 variables, dietary tannins have been shown to inhibit *in vivo* activities of trypsin and α -
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5 2 amylase (Longstaff and McNab, 1991), which may affect digestion of proteins and starch.
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8 3 Also, total phenolics may include non-tannin polyphenolic compounds or tannin precursors.
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10 4 These low molecular weight polyphenol components associated with tannins are more readily
11
12 5 absorbed from chick diets than tannins, and may account for the major anti-nutritive effects
13
14 6 (Butler and Rogler, 1992).

17 7 The nutritional improvement of shea nut meal achieved in this study still falls far short
18
19 8 of what is expected for it to become valuable for the feed industry. The fermentation methods
20
21 9 presently used require further research to enable a nutritionally valuable feedstuff to be
22
23 10 produced.
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30
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32
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36
37 16 University of Agriculture, Abeokuta, Nigeria) is gratefully acknowledged.
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Table 1. *Composition of the experimental diets*

Ingredients (g/kg)	Basal diet	Shea nut meal diets
Maize	515.5	469.1
Dehulled soybean meal	250.0	227.5
White fishmeal	60.0	54.6
Wheatfeed	150.0	136.5
Lysine hydrochloride	1.0	0.9
D,L-methionine	2.0	1.8
Dicalcium phosphate	10.0	9.1
Limestone	5.5	5.0
Vitamin and trace element premix ¹	3.0	2.75
Sodium chloride	3.0	2.75
Experimental shea nut meal sample ²	-	90.0
Calculated composition (g/kg)		
Crude protein	230.8	223.0
Lysine	13.5	12.8
Methionine	5.9	5.6
Methionine + Cystine	9.8	9.4
Calcium	7.5	7.4
Phosphorus	7.6	7.2
Sodium	1.8	1.7
Metabolisable Energy (MJ/kg)	12.2	12.4

¹Vitamin-trace mineral premix for broilers (Ian Hollows Feed Supplement, UK) added per kg: 800 mg retinol, 150 mg cholecalciferol, 1.25 g tocopherol, 150 mg thiamin, 500 mg riboflavin, 150 mg pyridoxine, 750 mg cyanocobalamin, 3 g nicotinamide, 0.5 g pantothenic acid, 75 mg folic acid, 6.25 g biotin, 12.5 g choline chloride, Iron 1 g, 50 mg cobalt, 5 g manganese, 0.5 g copper, 4 g zinc, 50 mg iodine, 10 mg selenium 25 mg molybdenum.

²Nutrient compositions of the experimental shea nut meal samples were assumed to be similar. Dietary formulation was based on an expeller shea nut meal (Dei *et al.*, 2008).

Table 2. Chemical composition of unfermented and fermented fungi-treated samples of shea nut meal (g/kg dry matter basis)

Treatments		Crude protein	Ether extract	Ash	Neutral detergent fibre	Gross energy (MJ/kg)
Unfermented shea nut meal		143.6	101.4	52.3	455.7	22.8
<u>Fermented shea nut meals</u>						
<i>A. niger</i>	Method 1	143.1	117.0	49.6	498.1	23.9
	Method 2	141.9	113.5	50.0	470.3	23.6
<i>C. subvermispora</i>	Method 1	143.0	116.3	55.4	448.6	23.8
	Method 2	141.2	115.3	53.6	467.0	23.6
<i>A. niger</i> + <i>C. subvermispora</i>	Method 1	142.0	111.2	53.6	451.9	23.6
	Method 2	139.3	120.0	49.4	467.4	24.2

Table 3. Total soluble phenolics, proanthocyanidins, hydrolysable tannins, free gallic acid and saponins in unfermented (control) and fermented fungi-treated samples of shea nut meal (dry matter basis)

Treatments	Total soluble phenolics (mg catechin/g)	Bound+soluble proanthocyanidins (mg catechin/g)	Soluble proanthocyanidins (mg cyanidin/g)	Hydrolysable tannins (mg gallic acid/g)	Free gallic acid (mg gallic acid/g)	Saponins (mg/g)
Unfermented shea nut meal	116.7	253.5	18.2	3.4	1.4	6.0
Fermented shea nut meals						
<i>A. niger</i> Method 1	103.2	186.7	16.6	1.5	2.5	3.7
Method 2	91.1	166.0	13.7	1.6	1.8	0.8
<i>C.</i> Method 1	102.6	200.2	15.1	1.5	2.7	1.1
<i>Subvermispora</i> Method 2	83.3	166.8	11.5	1.6	1.7	1.7
<i>A. niger</i> + <i>C.</i> Method 1	93.9	191.3	14.1	1.9	2.7	0.8
<i>Subvermispora</i> Method 2	96.7	166.4	12.7	0.7	2.5	5.4

Table 4. Correlation matrix¹ of some chick growth and nutrient metabolisability variables, and anti-nutrients in the fermented shea nut meal samples

	Feed intake	Weight gain	CPM	AME	HT	Pas	TPas	TSP
Weight gain	0.968							
CPM	-0.016	-0.122						
AME	0.290	0.044	0.484					
HT	0.689	0.578	-0.314	0.430				
PAs	0.284	0.315	-0.633	-0.045	0.219			
TPAs	0.029	-0.045	-0.480	0.240	0.415	0.709		
TSP	-0.155	-0.120	-0.413	-0.092	-0.221	0.862	0.677	
Saponins	0.010	-0.028	-0.794	0.030	0.583	0.386	0.487	0.131

¹Coefficients between -0.81 and +0.81 were not statistically significant ($P>0.05$).

CPM-crude protein metabolisability, AME-apparent metabolisable energy, HT-hydrolysable energy, Pas-soluble proanthocyanidins, TPas-bound + soluble proanthocyanidins, TSP-total soluble phenolics.

Table 5. Growth performance of broiler chicks (10-18 d), apparent metabolisable energy, dry matter and crude protein metabolisability coefficients of basal diet and diets containing 90 g/kg of unfermented and fermented fungi-treated samples of shea nut meal

Treatments		Feed intake (g/b/d)	Weight gain (g/b/d)	Gain: Feed (g/g)	Dry matter metabolisability coefficient	Crude protein metabolisability coefficient	AME (MJ/kg)
Basal diet (no shea nut meal)		59.0	41.3	0.71	0.80	0.72	13.4
Unfermented shea nut meal		23.0	5.0	0.21	0.75	0.57	12.7
Fermented shea nut meals							
<i>A. niger</i>	Method 1	34.5	14.3	0.39	0.68	0.44	11.1
	Method 2	37.9	15.4	0.37	0.73	0.57	12.3
	Mean	36.2	14.9	0.38	0.71	0.51	11.7
<i>C. subvermispora</i>	Method 1	33.2	12.2	0.36	0.72	0.54	12.1
	Method 2	33.5	13.1	0.39	0.68	0.53	11.4
	Mean	33.4	12.7	0.38	0.70	0.54	11.8
<i>A. niger</i> + <i>C. subvermispora</i>	Method 1	32.2	11.1	0.33	0.71	0.49	12.3
	Method 2	27.3	8.8	0.31	0.69	0.55	11.5
	Mean	29.8	10.0	0.32	0.70	0.52	11.9
Probability of treatment differences							
<u>Orthogonal treatment comparisons</u>							
Basal vs. Fermented meals		***	***	***	*	**	*
Unfermented vs. Fermented meals		***	***	***	ns	ns	ns
<u>Fermented meals only</u>							
Methods of fermentation (M)		ns	ns	ns	ns	ns	ns
Fungal organisms (F)		*	ns	ns	ns	ns	ns
M x F interaction		ns	ns	ns	ns	ns	ns
SED for comparing:							
Individual treatment means (n=8)		2.865	2.665	0.056	0.034	0.063	0.678
Fermentation method means (n=24)		1.654	1.663	0.032	0.020	0.036	0.391
Fungal organisms means (n=16)		2.026	2.037	0.039	0.024	0.044	0.479

SED-significant error difference, n-number of replicates, ns-not significant ($P>0.05$), * $P<0.05$, ** $P<0.01$, *** $P<0.001$.