EVALUATION OF THE NUTRITIONAL STATUS OF *ASPERGILLUS FLAVUS* INOCULATED KATI, A CEREAL BASED FERMENTED FOOD

Ibitoye, Olukayode Adebola, Akinyele, Bamidele Juliet and Olaniyi, Oladipo Oladiti
Department of Microbiology, Federal University of Technology, P.M.B 704, Akure, Nigeria
*Corresponding author email: microladit@gmail.com

Abstract
The present study aimed at the evaluation of the nutritional status of Aspergillus flavus inoculated and uninoculatedkati made from sorghum grains. The proximate and vitamins compositions of A. flavus inoculated and uninoculatedkati were determined by standard chemical methods and radio-immuno assay respectively. The percentage crude protein increased significantly from 6.42±0.25% in raw sorghum to 8.20±0.83% and 9.05±0.03% in A. flavus inoculated and uninoculated kati (cooked)and 10.70±0.02 and 7.51±0.01% in A. flavus inoculated and uninoculated fermented sorghum gruel (uncooked) respectively. The percentage crude fibre decreased approximately by 97% and 98% in A. flavus inoculated kati (cooked) and fermented sorghum gruel (uncooked) respectively when compared with the raw sample.The gross energy was 17.48±0.08 in the raw sorghum and increased approximately to 6% and 51% in A. flavus inoculated and uninoculated kati (cooked) and 132% in fermented sorghum gruel (uncooked) respectively. The percentage carbohydrate (CHO) in the substrate increased from 2.03±0.08% to 16.40±0.17and 5.06±0.05%A. flavus inoculated and uninoculated kati (cooked) and 18.20±0.01 and 3.72±0.02% in A. flavus inoculated and uninoculated fermented sorghum gruel (uncooked) respectively.The vitamin A, D, E, K, B2 and B6 decreased in all A. flavus inoculated and uninoculated kati when compared with the raw samples. In conclusion, the inoculation of kati with A. flavus enhanced its nutritional composition except for the vitamins.

Keywords: *Aspergillus flavus*, Sorghum, Kati, Proximate composition, Nutritional status

Received: 29.07.2019 Reviewed: 16.10.2019 Accepted: 18.11.2019

1. INTRODUCTION

In the developed and developing countries, cereal grains and the products derived from them are rich with important nutritional parameters (Piotrowska *et al.* 2013). Cereal consumption is moderate in developed countries however in Africa and Asia, it is a daily sustenance (Kumar *et al*., 2011). In Africa,46% of the total energy intake is contributed by cereal; however, this figure could be as high as 78% in some African countries (FAOSTAT, 2010). According to figures made available by the afore mentioned statistics division of FAO, the six most cultivated and hence consumed grains worldwide in order of decreasing production are maize, rice, wheat, barley, sorghum and millet, and of these major grains maize, wheat and rice together account for 87% of all cereal production worldwide and 43% of all food calories (FAOSTAT, 2010). The growing interest in cereal grains and their derivatives is caused by their bioactive components and the potential benefits of regular consumption of cereals and cereal products in their natural form (as in whole grain), which are also rich sources of protein, fiber, carbohydrates, vitamins, fats, oils, minerals and macronutrients, especially magnesium and zinc (Shewry, 2007; Makun *et al*., 2010).

Improvement in the nutritive value and organoleptic properties of grains and agricultural wastes has been reported to be conducted through fungal species (*Rhizopus oligosporus*, *Aspergillus oryzae*, *Penicillium italicum*, *Trichosporonoides oedocephalis*, *A. flavus* etc) via hydrolysis of carbohydrates, secretion of extracellular enzymes and the production of fungal biomass rich in protein (Omid *et al*., 2012; Olaniyi *et al*., 2015). The use of fermentation technology as a food processing method has attained higher significance as a new option compared to other common methods (Khalil, 2006).

The nutritive values of fermented carbohydrate rich substrate depends on various parameters to include the choice of microorganism involved in the fermentation process, inoculum size, age of culture, duration of fermentation, type and size of substrate, suitable culture conditions (temperature, pH, moisture, scale of culture, etc) carbon to nitrogen ratio in the substrate (Krishna 2005; Khalil, 2006).Variety of fermented products is derived from fermentation processwith desired properties to include the following: reduced peptide sizes of protein sources, lowered excretion capacity of digestion enzymes via production of more bioavailable product and presence of growth promoting factors and immune stimulator compounds (Yamamoto *et al*., 2007).

In Nigeria, 'kati' is a traditionally cereal based fermented food made from sorghum, millet and yellow maize. 'Kati', a staple delicacy of the Southwestern Nigerian, originated from Ondo State (7^o 35' 0'' North 5^0 48' 0'' East) (Adetimehin and Akinyele, 2014). 'Kati' production is a new research area with no or scanty information. The aim of this study was to evaluate the impact of *A. flavus* inoculation on the nutritional composition of 'kati' made from sorghum.

2. MATERIALS AND METHODS

2.1 *Isolation of Aspergillus flavus*

The *A. flavus* was isolated from different spoilt food samples that were collected from different locations in Akure, Ondo State, Nigeria. The total fungal counts from the samples were determined using the pour plate method on potato dextrose agar PDA (BIOMARK, India). The samples were serially diluted and 1ml of an appropriate dilution was inoculated on agar plates in duplicate according to Lateef *et al.* (2004).

2.2 *Identification of Aspergillus flavus*

At the end of incubation, the colonies were sub-cultured from the mixed cultures and identified on the basis of cultural characters (colour, shape of colony, surface and reverse pigmentation and texture of the colony) as well as microscopic structure (septate or nonseptate hyphae, structure of hyphae and conidia) (Olaniyi *et al*., 2015).

2.3 *Preparation of spore suspension*

An actively growing *A. flavus* on agar plate was washed with gentle agitation with 10 ml sterile distilled water. The washed spore suspension was added to 90 ml of sterile distilled water considered to be 10^{-1} dilution. Serial dilutions ranged from 10^{-2} to 10^{-6} were made to determine the number of sporesrequired for the inoculation of fermented sorghum, 'kati'. Ten ml of the original spore suspension containing 10^6 spores inoculum concentration was used (Olaniyi *et al*., 2015).

2.4 *Grain collection*

Sorghum (*Sorghum bicolor*) used for the production of 'kati' were collected from Oja Oba market in Akure, Ondo State, Nigeria.

2.5 *Preparation of 'kati'*

350 grams of sorghum (*Sorghum bicolor*) was used for the production of 'kati'. The production of 'kati' involves:

Washing: The grains were thoroughly picked to remove the stones and other dirty and also washed to ensure that clean grains were used.

Cold water steeping: The grains were cold water steeped for 72 h.

Wet milling: The cold water steeped grains were grounded into paste before it was fermented

Fermentation: The pastes were distributed into different treatments.

Treatment 1: Uninoculated 'kati' prepared from fermented sorghum

Treatment 2: Inoculate 'kati' prepared from fermented sorghum

Mixing: This was to ensure even distribution of the additive introduced.

Precooking: This was done by stirring the fermented gruel on the fire for 15 minutes until it formed a cooked solid-like substance.

Wrapping: The pre-cooked substance was wrapped in leaves using "ewe eran" (*Thaumatococcus daniellii).*

Cooking: The precooked 'kati' was cooked for two hours under smoldering fire wood.

2.6 *Proximate analysis of the treatments*

The proximate composition of the treatments: crude fat, crude protein, crude fiber, free fatty acid, energy contents, pH, moisture content, ash contents and carbohydrate content were carried out according to the method of AOAC (2012).

2.7 *Determination of vitamins*

The vitamin contents of the samples were determined using the radioimmunoassay method as described by El-Sheekh *et al*. (2013).

2.8 *Analysis of data*

The statistical analysis was performed using the general linear model function of statistical package for social science (SPSS), Version 16.0.All data generated were subjected to One Way Analysis of Variance (ANOVA) while Statistical differences of treatment were determined using Duncan's Multiple Range.

3. RESULTS AND DISCUSSION

3.1 *Fungi counts from different samples*

Table 1 shows the total fungal counts from the samples collected. Grape extract with code B (1.00×10^7sfu/ml) had the highest number of fungal population, while soya bean (1.00×10^4sfu/g) recorded the least.

Table 1: Fungal counts from different agricultural wastes

Sources	Fungal count (sfu/g) / (sfu/ml)
Orange A	8.00×10^4
Orange B	2.00×10^5
Soya bean	1.00×10^4
Maize A	1.80×10^6
Maize B	1.05×10^6
Almond A	1.70×10^6
Almond B	8.50×10^6
Grape A	1.00×10^7
Grape B	2.00×10^6
Sorghum A	1.00×10^6
Sorghum B	5.00×10^6

3.2 *Cultural characterization and microscopic observation of Aspergillus flavus*

The cultural and microscopic features of *A. flavus* associated with the sample collected are shown in Table 2. The microscopic features of the fungal strains revealed upright conidiophores that terminate in a davate swelling bearing phialides at the apex or radiating from the entire surface; conidia are 1-celled and globose. The cultural observation revealed yellow mycelia growth.

Table 2: Cultural characterization and microscopic observation of *Aspergillus flavus*

Cultural characteristics	Microscopic observation	Suspected organisms
Yellow mycelia Growth	An upright conidiophores that terminates in a davate swelling bearing phialides at the apex or radiating from the entire surface; conidia are 1-celled and globose.	*A. flavus*

3.3 *Proximate composition of Aspergillus flavus fermented sorghum (cooked) (% dry weight)*

The proximate composition of *A. flavus* inoculated and uninoculated 'kati' is shown in Table 3. The percentage crude protein increased significantly from 6.42±0.25% in raw sorghum to 8.20±0.83% and 9.05±0.03% in *A. flavus* inoculated and uninoculated 'kati' respectively. The percentage ash content in raw sorghum decreased in *A. flavus* inoculated 'kati' by approximately 90%, and increased by 4% in uninoculated 'kati' when compared with the raw sorghum. The percentage crude fibre decreased approximately by 97% in *A. flavus* inoculated 'kati' when compared with the raw sample. The gross energy was 17.48±0.08 in the raw sorghum while there was increase in the values by approximately 6% and 51% in *A. flavus* inoculated and uninoculated 'kati' respectively made from the same sample.

Table 3: Proximate composition of *Aspergillus flavus* fermented sorghum (cooked) (% dry weight)

Sample	Ash	Protein	Fat	Fibre	Matter	CHO	Moisture	G. energy
DSC	16.31±0.12^{b}	6.42±0.25^{a}	2.72±0.18^{c}	31.08±0.14^{b}	4.80±0.14^{c}	2.03±0.08^{a}	14.87±0.17^{b}	17.48±0.08^{a}
IK	1.66±0.08^{a}	8.20±0.83^{b}	1.05±0.04^{a}	0.83±0.07^{a}	0.54±0.06^{a}	16.40±0.17^{c}	12.30±0.10^{a}	18.49±0.39^{b}
UK	17.03±0.07^{c}	9.05±0.03^{c}	2.02±0.05^{b}	33.64±0.08^{c}	4.46±0.06^{b}	5.06±0.05^{b}	12.02±0.03^{a}	26.38±0.03^{c}

DSC=Dried sorghum (Control), UK=Uninoculatedkati, IK=Inoculatedkati

3.4 *Proximate composition of Aspergillus flavus fermented sorghum gruel (uncooked) (% dry weight)*

The proximate composition of fermented sorghum gruel inoculated with *A. flavus* during the course of fermentation is shown in Table 4. The percentage crude protein increased significantly in all the treatments. It increased from 6.42±0.25% in raw sorghum to 10.70±0.02 and 7.51±0.01% in *A. flavus* inoculated and uninoculated fermented sorghum gruel respectively.The percentage crude fiber and dry matter in raw sorghum were 31.08±0.14 and 4.80±0.14% respectively. These values reduced significantly in *A. flavus* inoculated and uninoculated fermented sorghum gruel.

3.5 *Vitamin composition of Aspergillus flavus fermented sorghum (cooked) (µg/g)*

The vitamin composition of *A. flavus* inoculated and uninoculated 'kati' is shown in Table 5. There were significant reduction in vitamin A, D and K in *A. flavus* inoculated and uninoculated 'kati' made from sorghum in comparison with the raw samples. The vitamin E content decreased significantly by 92% in *A. flavus* inoculated 'kati' in comparison with the raw sample. The uninoculated 'kati' made from sorghum had an appreciable increase in Vitamin B1 when compared with raw samples. The Vitamin B2 content of both *A. flavus* inoculated and uninoculated 'kati' had a significant decrease. Significant reduction in vitamin B3 was observed in the *A. flavus* inoculated 'kati' while the uninoculated sample had its vitamin B3 content increased while vitamin B6 content in *A. flavus* inoculated 'kati' and uninoculated 'kati' decreased compared with raw samples.

3.6 *Vitamin composition of Aspergillus flavus fermented sorghum gruel (uncooked) (µg/g)*

The vitamin composition of sorghum gruel inoculated with *A. flavus* during the course of fermentation is shown in Table 6. The vitamin A vitamins; D, E, K, B1, B2, B3 and B6 reduced significantly in all the fermented *A. flavus* inoculated samples when compared with the raw sample. However, there was an increase in vitamin B1, B2 and B3 in fermented uninoculated sorghum.

Table 4: Proximate composition of *Aspergillus flavus* fermented sorghum gruel (uncooked) (% dry weight)

Sample	Ash	Protein	Fat	Fibre	matter	CHO	moisture	G. energy
DSC	16.30±0.12^{c}	6.42±0.25^{a}	2.72±0.18^{c}	31.08±0.14^{c}	4.80±0.14^{c}	2.03±0.08^{a}	14.87±0.17^{a}	17.48±0.08^{b}
ISG	0.44±0.00^{a}	10.70±0.02^{c}	1.55±0.08^{a}	0.60±0.02^{a}	0.21±0.02^{a}	18.20±0.01^{c}	58.10±0.03^{c}	12.51±0.02^{a}
USG	12.25±0.06^{b}	7.51±0.01^{b}	1.87±0.08^{b}	28.02±0.02^{b}	2.27±0.06^{b}	3.72±0.02^{b}	19.61±0.01^{b}	23.05±0.04^{c}

DSC=Dried sorghum (Control), ISG=Inoculated sorghum gruel, USG=Uninoculatedsorghum gruel

Table 5: Vitamin composition of *Aspergillus flavus* fermented sorghum (cooked) (µg/g)

Sample	Vit.A	Vit.D	Vit.E	Vit.K	Vit.B1	Vit.B2	Vit.B3	Vit.B6	Vit.C
DSC	5.31±0.01^{c}	2.60±0.04^{c}	6.10±0.00^{c}	6.81±0.04^{c}	0.21±0.01^{b}	0.61±0.01^{c}	0.41±0.01^{b}	1.31±0.01^{c}	0.02±0.01^{b}
IK	2.01±0.01^{a}	0.77±0.02^{a}	0.51±0.01^{a}	1.61±0.01^{a}	0.03±0.00^{a}	0.26±0.01^{a}	0.00±0.00^{a}	0.22±0.02^{a}	0.00±0.00^{a}
UK	3.50±0.01^{b}	1.80±0.01^{b}	2.04±0.02^{b}	2.37±0.01^{b}	0.30±0.01^{c}	0.41±0.01^{b}	0.56±0.01^{c}	0.78±0.01^{b}	0.06±0.01^{c}

DSC=Dried sorghum (Control), IK=Inoculated kati, UK=Uninoculatedkati

Table 6: Vitamin composition of *Aspergillus flavus* fermented sorghum gruel (uncooked) (µg/g)

Sample	Vit.A	Vit.D	Vit. E	Vit.K	Vit.B1	Vit.B2	Vit.B3	Vit.B6	Vit.C
DSC	5.31±0.01^{c}	2.60±0.04^{c}	6.10±0.00^{c}	6.81±0.04^{c}	0.21±0.01^{b}	0.61±0.01^{b}	0.41±0.01^{b}	1.31±0.01^{c}	0.02±0.01^{b}
IK	3.31±0.02^{a}	1.73±0.05^{a}	3.12±0.03^{a}	4.01±0.05^{a}	0.00±0.00^{a}	0.36±0.02^{a}	0.15±0.02^{a}	0.94±0.04^{a}	0.00±0.00^{a}
USG	4.71±0.01^{b}	2.21±0.02^{b}	5.81±0.02^{b}	4.92±0.03^{b}	0.23±0.01^{b}	0.64±0.03^{c}	0.55±0.01^{c}	1.17±0.01^{b}	0.03±0.01^{c}

DSC=Dried sorghum (Control), IK=Inoculated kati, USG=Uninoculatedsorghum gruel

Fungal bioconversion of carbohydrate rich substrates had been connected to the production of consortium of extracellular enzymes secreted by the various organisms involved in the process. Predigesting fiber compounds via secreting carbohydrases increases bioavailablity of these compounds for target microorganism and consequently causes to produce nutritive protein biomass. Fungal based fermentation produces different excellence food products with the functional and nutraceutical properties to solve challenges of malnutrition in the developing countries (Olaniyi, 2014; Olaniyi *et al*., 2015).

High microbial loads observed from all the samples evaluatedwere envisaged hence contaminations from different sources might be involved, majorly during harvesting; packaging coupled with others human activities. The high microbial counts in the samples might be connected with their compositions with respect to the growth promoting factors embedded in them (Arotupin, 2007; Arotupin and Olaniyi, 2013).

Percentage increase in crude protein of *A. flavus* inoculated and uninoculated 'kati' might be attributed to the secretion of certain extracellular enzymes which are proteineous in nature into the substrates during their breakdown (Akinfemi *et al*., 2010; Akinyele *et al*., 2011). The differences in crude protein content between the treatments may be due to physical and environmental factors which are known to induce differences in the physiology of the organisms involved (Akharaiyi and Omoya, 2008).The reduction in crude fibre of fermented cereal-based foods through natural fermentation has been documented. The reduction in crude fibre of *A. flavus* inoculated and uninoculated fermented sorghum could be due to the ability of the fermenting microorganisms to secrete hydrolyzing and oxidizing enzymes, which could convert the complex compounds in the substrates into utilizable compounds (Olaniyi *et al*., 2015). Akharaiyi and Omoya (2008) reported appreciable reduction in crude fibre of 'Ogi', a fermented product from maize, Oyarekua (2011) reported a reduction in crude fibre of co-fermented cereals/cowpea 'Ogi' while Babalola and Giwa (2012) reported a reduction in crude fibre of fermented soy beans. Decrease in crude fiber of fungal treated sorghum stover was reported by Akinfemi *et al*. (2010), and this was attributed to the effect of cellulase enzymes secreted by cellulolytic fungi.

In this study, more than ninety percent of the vitamins evaluated from fermented grains were negatively affected by spontaneous fermentation. The reduction in the vitamin contents of few fermented products has been documented and this could be associated with lactic acid bacteria fermentation (Abdulaziz *et al*., 2014). The findings from this study areconsistent with those reported by Magdi and Osman (2010), who found that lactic acid bacterial fermentation resulted in a marked decrease in vitamin B6, B12 and vitamin C level, while only small changes in vitamin A, B1, B2 and niacin took place. According to Makun (2010), cereals are rich sources of minerals, vitamins, carbohydrates, oils and proteins but when processed through wet-milling and fermentation majority of the nutrients especially water soluble vitamins are lost leaving mostly carbohydrates and are therefore grown mainly for energy.

4. CONCLUSION

In conclusion, the inoculation of kati with *A. flavus* enhanced its nutritional status, although

there was reduction in some vitamins. *A. flavus*used as an inoculum in this study might be utilized as a starter culture once it has been established that it is not an aflatoxin-producing strain.Periodic monitoring of aflatoxin concentration is therefore recommended for fermented 'kati' and other related products made from cereals. Similarly, bioassay using experimental animals might be considered for future research to evaluate the effect of fermented sorghum 'kati' on their organs and blood.

5. REFERENCES

[1]. Abdulaziz, S. B., Abdelrahim, A. A. and Abdallah, A. A. (2014). Effect of fermentation process on the improvement of nutrition value of camel milk. International Journal of Multidisciplinary and Current Research. 2: 78-82.

[2]. Adetimehin, A. M., & Akinyele, B. J. (2014). Evaluation of Microbial harzard associated with Processing of kati; A cereal based fermented food. M. Tech Food Microbiology thesis, Department of Microbiology, Federal University of Technology Akure. 60pp.

[3]. Akharaiyi, F. C., & Omoya, F. O. (2008). Effect of processing methods on the microbiological quality of liquid pap "ogi" prepared from maize. Trends in Applied Sciences Research, 3(4), 330-334.

[4]. Akinfemi, A., Adu, O. A., & Doherty, F. (2010). Conversion of sorghum stover into annual feed with white-rot fungi: Pleurotusostreatusand Pleurotuspulmonarius. African Journal of Biotechnology, 9(1), 1706-1712.

[5]. Akinyele, B. J., Olaniyi, O. O., & Arotupin, D. J. (2011). Bioconversion of selected agricultural wastes and associated enzymes by Volvariellavolvacea: An edible mushroom. Research Journal of Microbiology, 6(1), 63-70.

[6]. AOAC (2012). Official methods of analysis of the Association of Official Analytical chemists, Washington, USA. 2: 1234.

[7]. Arotupin, D. J., & Olaniyi, O. O. (2013). Screening and identification of mannanase-producing fungi isolated from selected agricultural wastes. British Microbiology Research Journal. 3(4), 635-644.

[8]. Arotupin, D. J. (2007). Evaluation of microorganisms from cassava water for production of amylase and cellulase. Research Journal of Microbiology, 2(5), 475-480.

[9]. Babalola, R. O., & Giwa, O. E. (2012). Effect of fermentation on nutritional and anti-nutritional properties of fermenting soy beans and the antagonistic effect of the fermenting organism on selected pathogens. International Research Journal of Microbiology, 3(10),333-338

[10]. El-Sheekh, M. M., Abd-Elsalam, I. S., Shabana, S., & Zaki, A. (2013). Production of vitamin B12 and folic acid from agricultural wates using new bacterial isolates. African Journal of Microbiology Research, 7(11), 966-973.

[11]. FAOSTAT (2010). Crops primary equivalent. Retrieved on 16th May, 2011 from www.faostat.fao.org

[12]. Khalil, A. (2006). Nutritional improvementof an Egyptian breed of mung beanby probiotic lactobacilli. AfricanJournal of Biotechnology. 5 (2): 206-212.

[13]. Krishna, C. H. (2005). Solid-statefermentation systems-an overview. Critical Review on Biotechnology. 25: 1-30.

[14]. Kumar, P., Yadava, R. K., Gollen, B., Kumar, S., Verma, R. K.., & Yadav, S. (2011). Nutritional contents and medicinal properties of wheat: A review of life science. Medical Resources, 22,1-10.

[15]. Lateef, A., Oloke, J. K., & Gueguim-Kana, E. B. (2004). Antimicrobial resistance of bacterial strains isolated from orange juice products. African Journal of Biotechnology, 3(6), 334-338.

[16]. Magdi, A. and Osman. (2010). Biochemical changes occurring during fermentation of camel milk by selected bacterial starter cultures. African Journal of Biotechnology. 9(43): 7331-7336.

[17]. Makun, H. A., Anjorin, S. T., Moronfoye, B., Adejo, F. O., Afolabi, O. A., Fagbayibo, G., Balogun, B. O. and Surajudeen, A. A. (2010). Fungal and aflatoxin contamination of some human food commodities in Nigeria. African Journal of Food Science. 4: 127-135.

[18]. Olaniyi, O. O., Bankefa, O. E., &Ibitoye, O. A. (2015). Bioconversion of orange peels into a nutritionally enriched substrate by Trichosporonoidesoedocephalis, Penicilliumitalicum and Aspergillusflavus. Nigerian Journal of Microbiology, 29, 3075-3088.

[19]. Olaniyi, O.O. (2014). Effect of Beta-Mannanase Treatment on Nutritive Quality of Palm kernel Meal. African Journal of Microbiology Research. 8(25):2405-2410.

[20]. Omid, S., Mehrdad, F., Chris, C., Bagher, Y. and Masoomeh, M. S. (2012). Studyon the effect of solid statefermentation with Aspergillusnigeron antinutritional factors of canolaprotein concentrate with aim ofusing in the diet of rainbow trout(Oncorhynchusmykiss). The 11th International and the 4th National Congress on Recycling of OrganicWaste in Agriculture 26–27 April2012 in Isfahan, Iran. Pg 1-12.

[21]. Oyarekua, M. A. (2011). Evaluation of the nutritional and microbiological status of co-fermented cereals/cowpea "ogi". Agricultural and Biological journal of North America, 2(1), 61-73.
[22]. Piotrowska, M., Śliżewska, K. and Biernasiak, J. (2013). Mycotoxins in cereal and soybean-based food and feed. In tech open science/ open mind.
[23]. Shewry, P. R. (2007). Improving the protein content and composition of cereal grain. Journal of Cereal Science, 46, 239–250.
[24]. Yamamoto, M., Saleh, F., Tahir, M., Ohtsuka, A. and Hayashi, K. (2007).The effect of koji-feed (fermented distillery by-product) on the growth performance and nutrient metabolizability in broiler. Journal of Poultry Science. 44: 291-296.