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Assessment of The Performance of Chicks Fed with Wheat Bran Solid Fermented by *Trichoderma longibrachiatum* (SF1)

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THE PRESENT study was carried out to evaluate the effect of feeding fermented wheat bran (WB) on the broiler performance. Nutritional value of wheat bran was improved through fermentation by cellulase secreting fungi isolated from soil. A total number of 90 one-day- old chicks were randomly assigned into 3 experimental groups (30 each, in 3 replicates). Group 1: diet including 0 % wheat bran (control1), Group 2: diet including 10% unfermented wheat bran (control 2), Group 3: diet including 10% wheat bran solid fermented by *Trichoderma longibrachiatum* (SF1). All diets were iso-caloric and iso-nitrogenous. Feed and water were provided *ad libitum*. The statistical evaluation of growth performance at the 5th week of age indicated significant increase in the average live body weight of birds fed with fermented wheat bran which recorded (1640 g) compared with the control-1 (1147.7g) and control-2 (1363.5 g). Feed consumption recorded significant increase to 3915.6 g/ bird, which returned to the best palatability of fermented feed. It was clearly sustained that the best significant feed conversion was recorded in group treated with fermented wheat bran (2.39), while unfermented WB group (2.41) compared to control-1(2.69). The cross sections in duodenum revealed no histopathological features related to such fungi and showing normal histological structure. Concerning the effects of feeding fermented wheat bran on some blood parameters , results showed that most levels are in normal ranges. In conclusion, wheat bran solid fermented by *Trichoderma longibrachiatum* (SF1) could be successfully used in poultry feed without deleterious effect.

Keywords: Broilers, Wheat bran, Solid state fermentation, *Trichoderma longibrachiatum*, Performance

Introduction

Wheat bran is the outer layer ~13% of the wheat seed and is rich in dietary fiber containing ~50.4% neutral detergent fiber (NDF), ~16.7% acid detergent fiber (ADF) and lower amounts, ~4% of acid detergent lignin (ADL) and crude protein of ~18% (Hill et al., 1960). Furthermore, it contains 43.6 % insoluble dietary fibers, 2.5% soluble dietary fibers, 6.7% ash and various essential amino acids for the enzyme production and bacterial growth (Sievert et al., 1990). It may contain up to 12% crude fiber and insertion in the broiler diet usually does not exceed 5% due to its high fiber content (NRC, 1994), which limits its utilization in poultry feed due to ineffective fiber fermentation in gastrointestinal tract.

Fermentation process is treatment method besides as optimal food/feed storage could increase nutrient content. Winarno and Fardiaz (1982) found that fermented feed containing higher nutrient compared to a raw material. Chiang et al. (2010) reported that solid state fermentation improved nutrient digestibility, increased the numbers of *lactobacilli* in the colon and ceca, enhanced the small intestinal structure of the broilers and potentially reducing the cost of broiler production.

Filamentous fungi like *Trichoderma* spp. are well known efficient producers of cellulases (Peij *et al.*, 1998). Most commercial cellulase is of fungal origin and produced by *Trichoderma* species

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(Cherry and Fidantsef, 2003). Filamentous fungi have a greater penetrating power into insoluble substrates and are therefore, more suitable for solid-state fermentation of lignocellulosic materials (Kang *et al.,* 2004). At the present, the various strains of *Trichoderma* spp. are recognized by the researchers as the steadiest and safest fungi for the production of cellulase and hemicellulases. The ability of fungi to degrade lignocellulosic materials is due to their highly efficient enzymatic system (Sánchez, 2009).

In recent years, however, scientists have begun to use fungal inoculum for the fermentation of agricultural by-products in order to raise their nutritional value (Sharma and Arora, 2011). Adeola and Bedford (2004) used either high or low-viscosity wheat and reported a depression in duck growth, especially in high-viscosity wheat. An amelioration of the growth depression, as well as improvement in feed conversion when xylanase from *Trichoderma longibrachiatum* was added to the diet.

However, few studies have reported on the addition of *Trichoderma* fermented wheat bran to the broiler diet, Yi *et al.* (2016) found that 10% fermented wheat bran (FWB) group had lower feed consumption than the other groups but improved feed conversion ratio in the 10% FWB group compared to the control. These results indicated that, fungi excreted exogenous enzymes after solid-state fermentation, it broke down ligno-cellulolytic bonds to increase the amount of soluble carbohydrates. This could provide a positive effect on the growth performance of broilers. The present work was designed to improve the nutritional value of wheat bran by fermentation with *Trichoderma longibrachiatum* (SF1) for poultry feed and assess the growth performance of broiler chicks fed with this new feed source.

Materials and Methods

Isolation and identification of cellulolytic fungi

Trichoderma longibrachiatum (SF1) was isolated from different soil samples by serial dilution method using Mandel's Medium containing 1% Carboxy Methyl Cellulose (CMC) as a sole source of carbon + mineral salt agar (Mandels *et al.,* 1976). The efficient fungal isolate which showed the most efficient result was selected related to clear zone diameter, **(**Belal and El-Mahrouk, 2010) and identified to species level by 18S rRNA gene sequence which carried out at Sigma Scientific Services Co. The rRNA were amplified using Polymer Chain Reaction (PCR) and products were subjected to sequencing and compared with the sequence obtained from the nucleotide database of National Center for Biotechnology Information (NCBI).

Preparation of the fermented wheat bran

Fungal inoculums were prepared from 7 days old slants on PDA at 30 °C, by adding 10 ml of sterilized distilled water containing 0.01% (v/v) Tween 80 (Shaibani et al., 2011).The spores were scratched with the help of a sterilized wire loop to make a homogeneous suspension. The obtained suspensions were used to inoculate 250 ml flask containing 200 ml sterilized Potato dextrose medium (PD). Flasks were incubated under shaking condition at 30^{o}C for 7 days. The spore suspension concentration was adjusted at 1x 10^{7} spores/ml.

Techniques of solid state fermentation were applied using 2kg of wheat bran packed in polyethylene bags. Bags were moistened using calculated amount of Mandel's liquid medium to obtain 75% final relative humidity (FRH). Bags were plugged with cotton and autoclaved at 121 for 20 min. Wheat bran was inoculated with 200 ml (included in FRH calculation) of inoculums containing 1x 10^{7} spores/ml for fungi. Bags were incubated under the optimum temperature (30^{o}C) for 14 days. At the end of incubation period the fermented substrate was sun dried for 48 hours until weight stability and analysis of crude protein and crude fiber content were carried out by the methods of A.O.A.C (1990). The dried fermented wheat bran was inserted as feed ingredient with 10% level in the experimental diets.

The experimental diets

The composition and calculated analysis of the used basal diets are presented in Table 1. The starter grower diet was introduced from 1 day old to 25 day old, and the finisher diet was introduced from 26 to 35 day of age. The wheat bran (10 % fermented or un fermented) were well mixed with other ingredients of feed before introduced.

Birds and management

The experiment was carried out at the Poultry Breeding Farm, Faculty of Agriculture, Kafrelsheikh University in Completely Randomized Design (CRD).A total number

of ninety unsexed broiler chicks one day old (Cobb strain) were obtained from local hatchery Alwatania Company, Egypt. Average chick weight was 40 ± 0.7 g /bird. The experimental chicks were randomly divided into 3 experimental groups. Each group was represented by 30 chicks (divided into 3 replicates). Treatment (1): diet including 0 % wheat bran (control-1). Treatment (2): diet including 10% unfermented wheat bran (control-2). Treatment (3): diet including 10% wheat bran solid fermented by fungal isolate *Trichoderma longibrachiatum* (SF1) isolated from soil. Vit. B_6 3000 MG, Vit. B_{12} 15 MG, Panotothenic acid 10.000 MG, Nicotenic acid 40.000 MG, Biotin 75 MG, Folic acid 1500 MG, , Selenium 0.3 GM, Manganese 100 GM, Zinc 80 GM, Iodine 1GM, Iron 40GM, Copper 10 GM, Cobalt 0.15 GM and carrier $CaCO_3$ to 3000 GM. The anticoccidosis (Diclazoril 0.5%) was added as 200 g /ton feed.

TABLE 1. Composition and calculated analysis* of the Starter grower and Finisher diets

Ingredients	Starter grower diet %			Finisher Diet %		
	0% WB Control 1	Unfermented 10 %WB Control 2	Fermented 10% WB	0% WB Control 1	Unfermented 10% WB Control 2	Fermented 10% WB
Yellow corn	62.49	50	51.37	71.08	57.97	59.66
Soybean meal (CP 44%)	22.59	21.36	20.37	13.17	12.48	11.19
Corn gluten	10	10	10	10	10	10
Wheat bran	**0**	**10**	0	**0**	**10**	0
Fermented wheat bran	0	0	**10**	0	0	**10**
Soya oil	0.4	4.15	3.73	1.27	5.15	4.67
Di-calcium phosphate	1.76	1.66	1.67	1.8	1.7	1.71
Limestone	1.3	1.34	1.34	1.22	1.25	1.26
Salt	0.33	0.32	0.32	0.36	0.35	0.35
Choline chloride 60%	0.1	0.1	0.1	0.1	0.1	0.1
Sodium bicarbonate	0.1	0.1	0.1	0.1	0.1	0.1
D-L Methionine	0.12	0.15	0.15	0.09	0.1	0.11
L- Lysine	0.51	0.52	0.55	0.51	0.5	0.55
Premix**	0.3	0.3	0.3	0.3	0.3	0.3
Total	**100**	**100**	**100**	**100**	**100**	**100**
Calculated analysis (%):						
Crude protein %	21.5	21.5	21.5	18	18	18
Metabolizable Energy (kcal/kg)	3025	3025	3025	3175	3175	3175
Crude fat %	3.33	6.98	6.60	4.39	7.95	7.30
Crude fiber %	3.28	3.80	3.76	2.8	3.55	3.28
Calcium %	0.95	0.95	0.95	0.95	0.95	0.95
Chloride %	0.18	0.18	0.18	0.22	0.22	0.22
Sodium %	0.17	0.17	0.17	0.19	0.19	0.19
Available phosphorus %	0.45	0.45	0.45	0.45	0.45	0.45
Lysine %	1.3	1.3	1.3	1.05	1.05	1.05
Methionine %	0.55	0.55	0.55	0.43	0.43	0.43
Methionine + cystine %	0.95	0.95	0.95	0.82	0.82	0.82

*The calculated analysis was performed according to the methods of Central lab. for food and feed.

** HY-MIX feed additives produced by MISR Company, Egypt was used. Each 3 kg contain: Vit. A 12000.000 IU, Vit. D_3 3000.000 IU, Vit. E 15.000 MG, Vit. K3 3000 MG, Vit. B_1 2000 MG, Vit. B_2 6000 MG, Vit. B_6 3000 MG, Vit. B_{12} 15 MG, Panotothenic acid 10.000 MG, Nicotenic acid 40.000 MG, Biotin 75 MG, Folic acid 1500 MG, , Selenium 0.3 GM, Manganese 100 GM, Zinc 80 GM, Iodine 1GM, Iron 40GM, Copper 10 GM, Cobalt 0.15 GM and carrier $CaCO_3$ to 3000 GM. The anticoccidosis (Diclazoril 0.5%) was added as 200 g /ton feed.

Parameters

Growth performance parameters (*e.g.*, average of weekly live body weight, feed consumption and feed conversion ratio), digestibility coefficient, production efficiency factor and economical efficiency were recorded and calculated during the experimental period. Growth performance index was calculated according to North (1978) by using the following equation:

$$\text{Performance Index (PI)} = \frac{\text{Live body weight (kg)}}{\text{Feed conversion}} \times 100$$

Health and physiological parameters including mortality, health disorders, counting of cecum microflora and blood parameters (SGPT, SGOT, Cholesterol, Albumin, Uric acid, Total antioxidant capacity and Haemagglutination Inhibition (HI) test were detected at the end of experiment according to Grimes (2002), in addition to histopathological features of intestine (duodenum) were examined.

Results and Discussion

Filamentous fungi particularly *Aspergillus* and *Trichoderma* spp. are well known efficient producers of cellulases (Peij *et al.*, 1998) and have a greater penetrating power into insoluble substrates, therefore more suitable for solid-state fermentation of lignocellulosic materials (Kang *et al.,* 2004). In the current study**,** the efficient fungal isolate showed the most efficient cellulolytic activity was selected and identified to specie level by 18S rRNA gene sequence which compared with the sequence obtained from the nucleotide database of (NCBI). The fungal isolate was identified as *Trichoderma longibrachiatum* isolate BG-21which showed in the following Phylogenetic tree (Fig. 1).

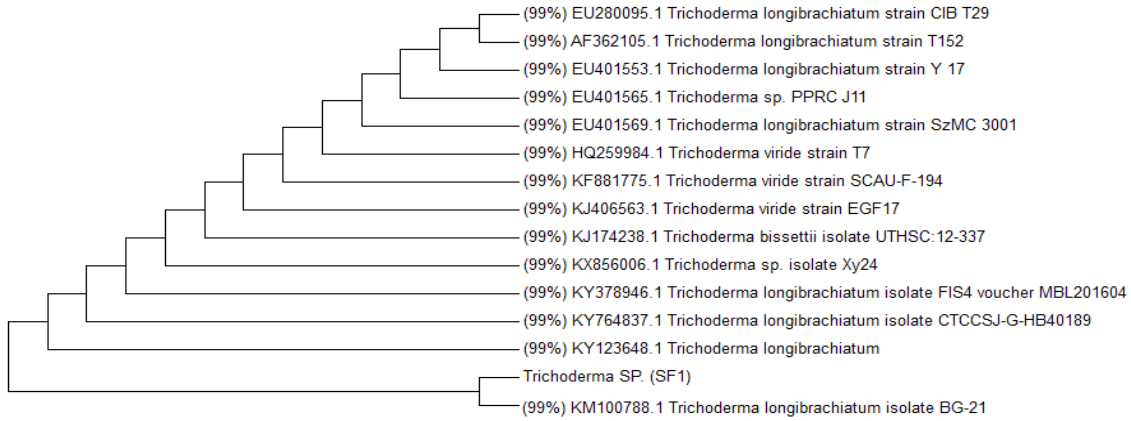

Fig.1. Phylogenetic tree of 18S rRNA gene sequence of strain *Trichoderma longibrachiatum* **(SF1).**

Microbial growth on substrate through fermentation process causes separation of enzymes from microbial cells and complex changes to the nutritive value of fermented products by changing the composition of proteins, fats and carbohydrates (Fellows, 1990). In the present study, fermentation of wheat bran by *Trichoderma longibrachiatum* (SF1) increased crude protein from 12.49% to 15.0% and reduced crude fiber from 15.28% to 12.37% due to degradation of cellulose through fungal growth which also increased protein by single cell protein. Dry matter and ash were increased from 85.34% and 4.42 to 88.20% and 5.01% , respectively. The increase of dry matter caused by water evaporation during fermentation process. Increasing substrate temperature due to carbohydrate breakdown releasing water from substrate. The increase of substrate dry matter was also affected by mycelium growth of microbe Hardini (2010). The improvement seen in ash (minerals) bioavailability may be explained by the ability of fungi to elicit enzyme like phytase which increases the bioavailability of phytate phosphorus and this may invariably lead to improvement in the bioavailability of other minerals (Ferket, 1993).

Results of feeding 10% fermented wheat bran by *Trichoderma longibrachiatum* (SF1) showed a significant increase in average of weekly live

body weight at 5th week recording 1640 g/bird compared to diet with no wheat bran (control 1) and diet with 10% unfermented wheat bran (control 2) which recorded 1147.7 and 1363.5 g/ bird, respectively (Table 2). These results were in agreement with Kayode *et al*. (2012) who reported that there was noticeable weight gain improvement in Mango Kernel Cake (MKC) group fermented by *Aspergillus niger* and *Penicillium chrysogenum* and disagreement with Adesua and Onibi (2014) who reported that fermentation of wheat bran with rumen liquor marginally increased the crude protein content of the wheat bran, but birds fed wheat bran based diets had lower total weight gain than the birds in control diet.

TABLE 2. Average of weekly live body weight of broiler chicks as affected by feed containing 10% fermented wheat bran by *Trichoderma longibrachiatum* (SF1).

Experimental diets	Average of weekly live body weight (g)/bird				
	1 wk	2 wk	3 wk	4 wk	5 wk
Diet with no wheat bran (Control 1)	113.6 b	248.5 b	476.3 c	743.3 c	1147.7 c
Diet with 10% unfermented WB (control 2)	123.6 a	270.5 ab	535.9 b	854.4 b	1363.5 b
Diet with 10% WB fermented by *Trichoderma longibrachiatum* **(SF1)**	116.0 ab	279.3 a	620.3 a	1033.7 a	1640.0 a
Significance	0.065	0.053	0.002	0.000	0.002

Note: a,b,c… Means with similar litters in each column are not significantly different.

Average of weekly feed consumption (g feed/bird) as showed in Table (3) revealed that fermented wheat bran by *Trichoderma*

TABLE 3. Average of weekly feed consumption of broiler chicks as affected by feed containing 10% fermented wheat bran by *Trichoderma longibrachiatum* (SF1).

Experimental diets	Average of weekly feed consumption (g feed/bird)					
	1 wk	2 wk	3 wk	4 wk	5 wk	Total
Diet with no wheat bran (Control 1)	110.3	247.0 c	542.6 b	966.8 b	1228.7 b	3095.4 b
Diet with 10% unfermented WB (control 2)	123.7	289.3 b	589.4 b	977.0 b	1295.0 b	3274.5 b
Diet with 10% WB fermented by *Trichoderma longibrachiatum* **(SF1)**	117.7	308.7 a	682.2 a	1232.6 a	1574.4 a	3915.6 a
Significance	NS	0.001	0.002	0.009	0.045	0.003

Note: a,b,c… Means with similar litters in each column are not significantly different.
NS: Not significant.

Feed conversion ratio (g feed / g gain) was improved in chicken group fed with fermented wheat bran by *Trichoderma longibrachiatum* (SF1) through all the experimental period which recorded at 5th week 2.39 compared to diet with no wheat bran (control 1) which recorded 2.69 (g feed / g gain) as showed in Table 4.

Wheat bran contains 43.6 % insoluble dietary fibers (Sievert *et al*., 1990), which decreased digestibility coefficient in broilers feed. Oldale, (1996) reported that the inclusion level of raw materials in animal's diet is restricted by many factors including the quality and digestibility of the materials, the species concerned and the age of the animals. Barley, wheat and farm byproducts can be fermented to improve nutrient digestibility and enrich the quality of protein (Canibe and Jensen, 2007). As shown in Table 5 the improvement in feed digestibility was cleared through nutrient digestibility coefficient of crude protein which significantly increased in broiler chicken group fed with wheat bran fermented by *Trichoderma longibrachiatum* (SF1) at the end of experimental period which recorded 79.63% compared to diet with no wheat bran (control 1) and diet with 10% unfermented WB (control 2) which recorded 70.40 and 71.46%, respectively. Nutrient digestibility

coefficient of crude fiber in fermented wheat bran group was also improved which recorded 47.60 % compared to diet with no wheat bran (control 1) and diet with 10% unfermented WB (control 2) which recorded 28.96 and 34.73%, respectively (Table 5). These results were in accordance with Hidayat (2007), who reported that fermentation is the activity of microbes in food or feed to produce high quality products through increasing the nutrient content and nutritional value of the products. Accordingly, inclusion of the fermented products were improved the nutritional value of feeds and increased different substrates for their utilization in poultry feed (Kompiang *et al.,* 1995). Hong *et al*. (2004) also reported that fermentation of feed using *Aspergilus oryzae* increased digestibility of its dry matter and crude protein.

TABLE 4. Average of weekly feed conversion of broiler chicks as affected by feed containing 10% fermented wheat bran by *Trichoderma longibrachiatum* (SF1)

Experimental diets	Average of weekly feed conversion (g feed / g gain)					
	1 wk	2 wk	3 wk	4 wk	5 wk	Total
Diet with no wheat bran (Control 1)	1.50	1.83	2.38	3.65	3.03	2.69
Diet with 10% unfermented WB (control 2)	1.47	1.97	2.24	3.07	2.56	2.41
Diet with 10% WB fermented by *Trichoderma longibrachiatum* (SF1)	1.57	1.89	2.00	2.97	2.60	2.39
Significance	0.477	0.209	0.182	0.088	0.105	0.100

Note: a,b,c… Means with similar litters in each column are not significantly different.

Average of weekly growth performance index (%) showed the best performance index through all the experimental period in broiler chicken group fed with wheat bran fermented by *Trichoderma longibrachiatum* (SF1) which recorded through the experimental period 68.98% compared to diet with no wheat bran (control 1) and diet with 10% unfermented WB (control 2) which recorded 42.71% and 56.95% , respectively, (Table 6). These results are in agreement with Kayode *et al*. (2012) who reported that there was noticeable improvement by solid state fermentation in feed consumption, weight gain and feed conversion ratio compared to the results obtained in previous works using raw mango seed kernels in broilers rations.

Mortality was recorded only in group fed diet with no wheat bran (control 1) which recorded 10% as 3/30 bird. On the other hand, rickety and wryneck cases were recorded as 6.7% and 3.35% respectively, in group fed diet with 10% unfermented wheat bran (WB) (control 2) as showed in Table (7). Limping cases were recorded only in birds fed diet with no wheat bran (control 1) and diet with 10% unfermented WB (control 2) which recorded 20% and 30% respectively.

Economical efficiency of feeding broilers wheat bran solid fermented by *Trichoderma longibrachiatum* (SF1) recorded high score (0.328) at the end of experiment compared to diet with no wheat bran (Control 1) and diet with 10% unfermented WB (control 2) which recorded 0.148 and 0.253, respectively (Table 8). Accordingly, production efficiency factor was highly increased by fermentation of wheat bran and using fermented substrate in broiler feed resulted 196 production efficiency factor compared to 106.5 in control 1 and 161.6 in control 2 as presented in Table 8. Yi *et al.* (2016) suggested that replacing 10% of a basal diet with fermented wheat bran (FWB) by *Trichoderma pseudokoningii* could not only improve growth performance and nutrient contents but also had economical benefits. This was in agreement with the present study which showed high economical efficiency and production efficiency factor of fermented wheat bran compared to control groups.

Regarding to cecum microflora count through different periods at 15, 25 and 35 days of age, Coliform count significantly decreased at 25 day of age in cecum of birds fed with wheat bran solid fermented by *Trichoderma longibrachiatum* (SF1), which recorded 8.55 log CFU/g cecum content, compared to diet with no wheat bran (control 1) and diet with 10% unfermented WB (control 2) which recorded 9.51 and 9.55 log CFU/g cecum content respectively, as shown in Table 9. These results were in accordance with Yi *et al.* (2016) who revealed that, the number of coliform bacteria was decreased in the ilea and caeca of

broilers supplemented with 10% fermented wheat bran (FWB) with *Trichoderma pseudokonngii* compared to the control group. Lactobacilli count at the 35th day of age was significantly increased to 8.71 log CFU/g cecum content in birds fed with wheat bran solid fermented by *Trichoderma longibrachiatum* (SF1), compared to diet with no wheat bran (control 1) and diet with 10% unfermented WB (control 2) which recorded 8.17 and 8.43 log CFU/g cecum content respectively, (Table 9). Cellulolytic bacterial count was not significantly increased at 25 and 35 day of age between groups, while at the15th day of age, cellulolytic bacterial count significantly increased in cecum content of birds fed with wheat bran solid fermented by *Trichoderma longibrachiatum* (SF1), which recorded 6.64 log CFU/g cecum content, compared to diet with no wheat bran (control 1) and diet with 10% unfermented WB (control 2) which recorded 5.74 and 5.85 log CFU/g cecum content respectively, (Table 9). These results are in agreement with Van Winsen *et al.* (2001) and Canibe & Jensen (2003) who indicated that supplementation with fermented feed increased the gastrointestinal Lactobacilli population. Accordingly, Chiang *et al.* (2010) reported that inclusion of fermented rapeseed meal successfully enhanced the growth of lactobacilli in the colon and ceca compared with either the control diet or the unfermented rapeseed meal diet that induced a balanced microbial population which support a healthy intestinal tract resulting in better control of intestinal pathogens.

TABLE 5 Nutrient digestibility coefficient of broiler chicks as affected by feed containing 10% fermented wheat bran by *Trichoderma longibrachiatum* (SF1)

Experimental diets	Nutrient digestibility coefficient				
	Crude Protein	Crude Fiber	Ether Extract	ASH	Dry Matter
Diet with no wheat bran (Control 1)	70.40[b]	28.96[c]	39.60[b]	45.36[c]	73.43
Diet with 10% unfermented WB (control 2)	71.46[b]	34.73[b]	45.10[a]	56.06[b]	72.83
Diet with 10% WB fermented by *Trichoderma longibrachiatum* **(SF1)**	79.63[a]	47.60[a]	47.16[a]	60.73[a]	75.50
Significance	0.006	0.00	0.020	0.00	0.472

Note: a,b,c… Means with similar litters in each column are not significantly different.

TABLE 6. Growth performance index (%) of broiler chicks as affected by feed containing 10%fermented wheat bran by *Trichoderma longibrachiatum* (SF1)

Experimental diets	Average of weekly growth performance index (%)					
	0-1 wk	1-2 wk	2-3 wk	3-4 wk	4-5 wk	0 -5 wk
Diet with no wheat bran (Control 1)	7.60	13.58	19.99[c]	20.64[c]	38.00[c]	42.71[c]
Diet with 10% unfermented WB (control 2)	8.36	13.72	24.46[ab]	27.91[b]	53.67[ab]	56.95[ab]
Diet with 10% WB fermented by *Trichoderma longibrachiatum* **(SF1)**	7.42	14.80	31.11[a]	34.76[a]	63.65[a]	68.98[a]
Significance	0.29	0.421	0.036	0.003	0.021	0.013

Note: a,b,c… Means with similar litters in each column are not significantly different.

TABLE 7. Mortality and health disorders of broiler chicks as affected by feed containing 10% fermented wheat bran by *Trichoderma longibrachiatum* (SF1)

Experimental diets	Mortality %	Rickety%	Limping %	Wryneck%
Diet with no wheat bran (Control 1)	10%	0	20.0%	0
Diet with 10% unfermented WB (control 2)	0	6.7%	30.0%	3.35%
Diet with 10% WB fermented by *Trichoderma longibrachiatum* **(SF1)**	0	0	0	0

TABLE 8. Economical efficiency and production efficiency factor of broiler chicks as affected by feed containing 10% fermented wheat bran by *Trichoderma longibrachiatum* (SF1)

Treatment items	Control (1)	Control (2)	T.longibrachiat-um (SF1)
fixed price/chick L.E	7.0	7.0	7.0
Aver. Feed (g) consumed. Starter	1452	1560	1813
Aver. Feed (g) consumed. Finisher	1643	1714	2103
feed cost/ton starter L.E	3463	3741	3800
feed cost/ton finisher L.E	3325	3613	3667
Total feed cost/chick L.E	10.49	12.02	14.60
Total cost/ chick L.E	17.49	19.02	21.60
average live BW g /bird	1147.7	1363.5	1640
price/kg live BW L.E	17.5	17.5	17.5
total revenue/chick L.E	20.08	23.86	28.70
Net revenue/chick L.E	2.59	4.83	7.09
Economical Efficiency	**0.148**	**0.253**	**0.328**
Relative E.E to control 1	100	171.28	221.64
Relative E. E to control 2	----	100	129.40
Production Efficiency Factor	**106.5**	**161.6**	**196**

TABLE 9. Cecum microflora of broiler chicks as affected by feed containing 10% fermented wheat bran by *Trichoderma longibrachiatum* (SF1)

Experimental diets	Age in day								
	15	25	35	15	25	35	15	25	35
	*Coli form**			*Lactobacilli*			*Cellulolytic bacteria*		
Diet with no wheat bran (Control 1)	8.09 [a]	9.51 [a]	9.21	5.95	7.49 [b]	8.17 [b]	5.74 [b]	7.35	7.06
Diet with 10% unfermented WB (control 2)	7.72 [b]	9.55 [a]	9.05	6.20	6.67 [b]	8.43 [b]	5.85 [b]	7.29	7.35
Diet with 10% WB fermented by *Trichoderma longibrachiatum* **(SF1)**	7.57 [b]	8.55 [b]	9.22	6.23	8.18 [a]	8.71 [a]	6.64 [a]	7.57	7.54
Significance	0.001	0.025	0.635	0.74	0.048	0.009	0.005	0.30	0.230

Notes: a,b,c… Means with similar litters in each column are not significantly different.

* The cecum bacterial counts were expressed as log CFU/g cecum content.

Cross sections in intestine (duodenum) of chicken treated diet with no wheat bran (control-1) are presented in Fig. 2 and showed that intestinal glands (IG) and villi (V) are normal. However, normal histological architecture with elongated intestinal glands (EG) and villi (V) were observed in intestine cross section (duodenum) of chicken treated diet with 10% unfermented wheat bran (control-2) (Fig. 3). Regarding to histopathological examination of chickens treated with 10% wheat bran diets fermented by cellulolytic fungi *Trichoderma longibrachiatum* (SF1), the cross section in intestines (duodenum) revealed no histopathological features related to such fungi and showing normal histological structure, muscularis mucosa (MM), serosa (Se) and activated in intestinal glands (crypts of Lieberkulm) (Fig 4). Yi *et al.* (2016) was determined that *Trichoderma pseudokoningii* might adhere to the epithelial cells of the chicken crop and this mechanism will be investigated in the near future. The improvement in intestinal morphology also may be mostly due to the decrease of anti-nutritional factors and the degradation of structural polysaccharides (Yi *et al.,* 2016). A larger villus height increases the surface area, allowing for greater absorption of available nutrients and further improving intestinal health (Baurhoo *et al.*, 2007).

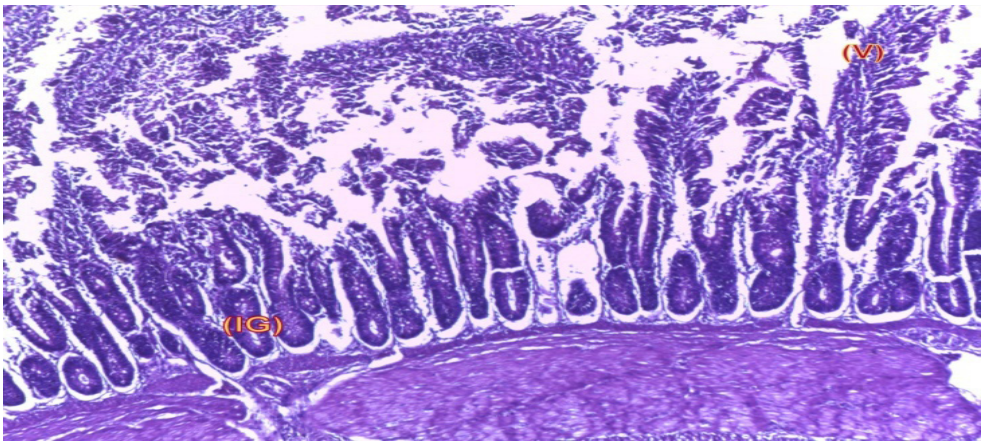

Fig. 2. Cross section in intestine (duodenum) of chicken treated diet with no wheat bran (WB) (Control -1) showing intestinal glands (IG) and normal villi (V). H&E (X100)

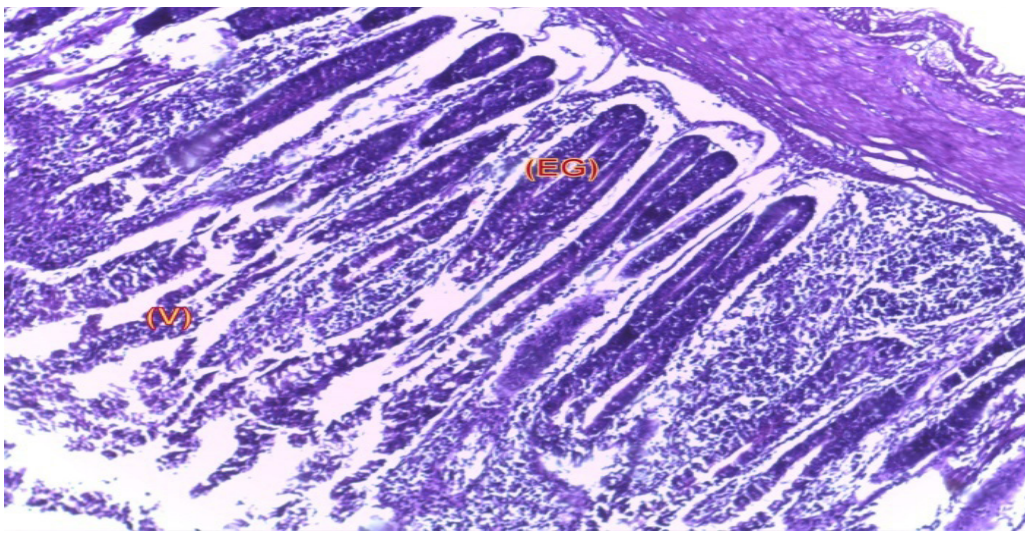

Fig. 3. Cross section in intestine (duodenum) of chicken treated diet with 10% unfermented wheat bran (WB) (control -2) showing normal histological architecture with elongated intestinal glands (EG) anv villi (V). H&E (X100)

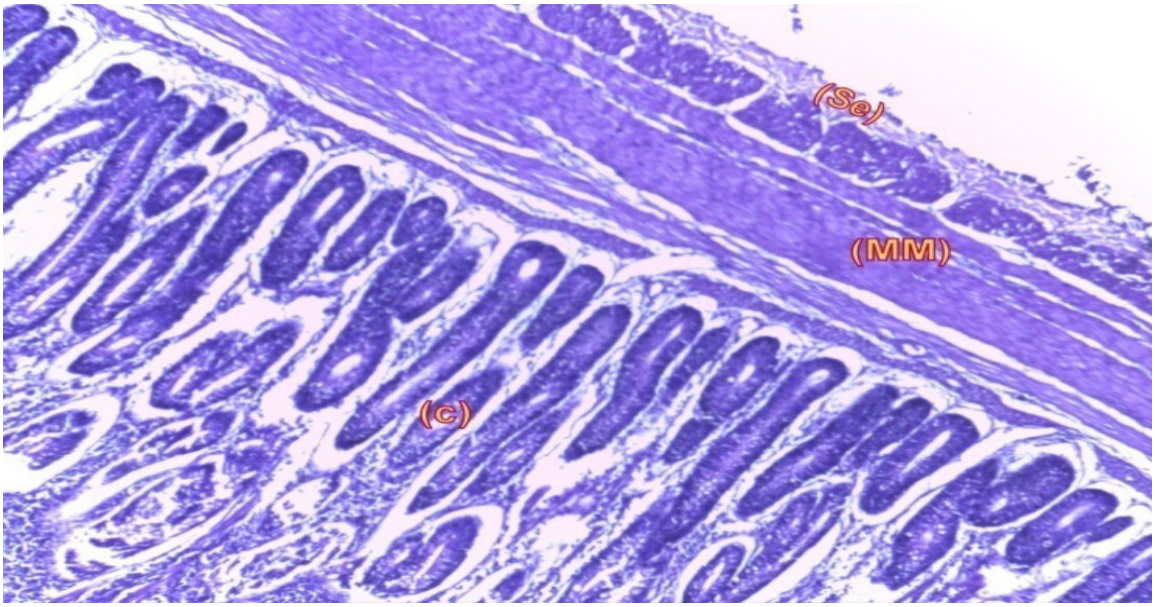

Fig. 4. Cross section in intestine (duodenum) of chicken treated diet with 10% wheat bran (WB) fermented by *Trichoderma longibrachiatum* (SF1) showing normal histological structure, muscularis mucosa (MM), serosa (Se) and activated in intestinal glands (crypts of Lieberkulm) (C). H&E (X100)

Blood biochemical parameters are important indicators of the physiology and health of livestock (Etim *et al.*, 2013). Serum chemical parameters, such as SGOT, SGPT, Total Protein (TP) and Albumin (ALB) are mainly evaluated for liver and kidney function. Results concerning to effects of feeding wheat bran solid fermented by *Trichoderma longibrachiatum* (SF1) on some blood biochemical constituents of Cobb broiler chicks were estimated at the end of the experimental period and presented in Table 10. There were increased in plasma levels of SGPT and SGOT (U/L), but all levels could be located among the referred normal ranges of the healthy chickens. Plasma levels of cholesterol and albumin in broiler chicks treated with 10% wheat bran solid fermented by *Trichoderma longibrachiatum* (SF1) were comparable to control (1) and control (2) which mean that significant differences were not observed in levels of cholesterol and albumin related to the treatment. Uric acid levels in plasma were significantly increased in 10% unfermented wheat bran (control-2) which recorded 7.31compared to diet with no wheat bran (control-1) which recorded 4.94 and in group fed with 10% wheat bran solid fermented by *Trichoderma longibrachiatum* (SF1) uric acid recorded 5.82. Uric acid levels (mg/dl) in plasma could be in normal range according to Kubena *et al*. (1990) which reported that uric acid levels (mg/dl) in chicken plasma could be reached to 6.2.

Total antioxidant capacity was significantly increased in birds treated with 10% unfermented wheat bran (control 2) and in control 1 (diet with no wheat bran) being 0.557 and 0.317 mM/L, respectively. However, no difference was observed between birds treated with 10% wheat bran solid fermented by *Trichoderma longibrachiatum* (S1) and unfermented wheat bran group (control 2). Although wheat bran fermented by white rot fungi *Pleurotus eryngii* could hold the promise of antioxidant activity and its antioxidant capacity would be evaluated as free radical scavenging capacity (Wang *et al.,* 2016). Concerning to (HI) test (immune response to New castle disease virus), results indicated that birds treated with 10% wheat bran fermented by *Trichoderma longibrachiatum* (S1) not significantly increased HI antibody response (256.0), when compared to control-1 treatment and unfermented wheat bran which recorded the same titre (256.0).

Data presented in the present study were in agreement with Yi *et al.* (2016) who suggested that replacing 10% of a basal diet with fermented wheat bran (FWB) by *Trichoderma pseudokoningii* could provide optimal intestinal morphology and normality blood chemical in broilers.

TABLE 10. Blood parameters of broiler chicks as affected by feed containing 10% fermented wheat bran by *Trichoderma longibrachiatum* (SF1)

Experimental diets	GPT U/L	GOT U/L	Album. μ.mol/L	Cholest mg/dl	Uric acid mg/dl	Total antiox. mM/L	HI titers
Diet with no wheat bran (Control 1)	10.66 [b]	36.33	1.16	134.3	4.94 [b]	0.317 [b]	256.0
Diet with 10% unfermented WB (control 2)	20.03 [a]	59.56	1.10	133.3	7.31 [a]	0.557 [a]	256.0
Diet with 10% WB fermented by *Trichoderma longibrachiatum* **(SF1)**	16.70 [ab]	53.40	1.30	127.0	5.82 [ab]	0.507 [ab]	256.0
Significance	0.198	0.281	0.483	0.814	0.130	0.152	0.422

Note: a,b,c… Means with similar litters in each column are not significantly different.

Conclusion

It could be concluded that wheat bran solid fermented by *Trichoderma longibrachiatum* (SF1) could be successfully used as poultry feed without deleterious effect. Thus, it could be indicated that microbial fermentation process could be used as a biological treatment for conversion of the agro-waste materials to useful and healthy broiler feed.

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