2011 International Conference on Food Engineering and Biotechnology
IPCBEE vol.9 (2011) © (2011)IACSIT Press, Singapore

Use of Aspergillus terreus for microbial biomass production and its biological evaluation in broiler chicks

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Abstract. The study was aimed to produce microbial biomass using rice as substrate through fermentation with *Aspergillus terreus* and its biological evaluation in broiler chicks to examine its potential as a suitable poultry feed. Chemical and biological potential of the biomass was evaluated through chemical analysis and chick assay, respectively. After optimizing various conditions such as, substrate : water ratio, nitrogen source, carbon : nitrogen ratio , pH and incubation period, *Aspergillus terreus* was grown on broken rice for maximum microbial biomass protein production. Regarding growth kinetics, the specific growth rate (μ) of the *A. terreus* remained 0.451h^{-1}. The Product coefficients (Yx/s) , (Yp/s) , (Yp/x) for the fermentative organism were found 0.553 g cell/g substrate utilized, 0.344 g protein / g substrate utilized and 0.622 g protein / g cell mass formation , respectively. Chemical evaluation of biomass indicated crude protein 43.7 %, true protein 26.60 %, crude fiber 11.35 % with calorific value as 2730 Kcal. Its ash content was 15.20 % with 1.01 % calcium, 3.05 % phosphorus, 0.64 % sodium and 0.98 % chloride. For biological evaluation, biomass was replaced with soybean meal as 30 ad 60% on the basis of protein supply and the birds response in terms of weight gain, feed consumption, feed efficiency, feed conversion ration, protein efficiency ratio and net protein utilization was taken into account. Outcome of the study revealed that microbial biomass produced by *A. terreus* can be replaced upto 30% of the protein supply by soybean meal without any deleterious effects on growing broiler chicks.

Keywords: Microbial protein, Chemical and biological evaluation, Growing broilers

1. Introduction

Population growth, income growth and urbanization are fueling a massive increase in demand for food of animal origin (milk, meat, eggs) in developing countries. The requirement of animal protein for a standard human diet is 30 grams per head per day, but at present the availability of animal protein in Pakistan is 20.2 grams, indicating a shortage of about 33% [1]. Poultry sector has considerable share in providing animal protein to ever growing human population. However, productivity and profitability by this sector can be enhanced by addressing the issues being faced by this enterprise. Poor quality and high cost of feed ingredients particularly that of protein sources, are considered main threats to its proliferation. This necessitates diverting efforts towards exploring new feed resources or improving utilization of available feedstuffs. In this context, biotechnology offers possibilities like production of microbial protein as poultry protein feed. The often quoted comparison that a 0.5t bullock synthesizes less than 0.5Kg of protein in every 24-hours, but 0.5t of soybeans produces the equivalent of 40Kg protein in every 24-hours and 0.5t of yeast generates 50t in this time, illustrates well the main advantage of microbial protein [2]. Keeping in view the current situation the present study was planned to produce microbial biomass from *Aspergillus terreus* with the objectives to determine the potential of broken rice waste as a substrate for the production of biomass protein, nutritional value of biomass by chemical analysis and its suitability as poultry feed.

2. Materials and Methods

2.1 Biomass production

2.1.1 Organism: *Aspergillus terreus*, The microbial culture was maintained on agar slant media [3]. Inoculum was prepared by transferring aseptically the spores on the agar slant to an autoclaved 250ml (Erlenmeyer) flask containing 50 ml inoculum medium

2.1.2 Substrate: Broken rice (rice tips) was used a s substrate. It contained dry matter (92.57%), moisture (7.42%), crude protein (7.65%), crude fiber (1.25%), ash (1.20%), ether extract (1.75%), nitrogen free extract (88.42%), cellulose (0.74%) and caloric value (2788 Kcal/Kg).

2.1.3 Batch culture cultivation: The Erlenmeyer flask (250ml) containing 50 ml growth medium plugged with cotton and covered with aluminum foil was autoclaved for 15 minutes at 15 lb/in^2 .The pH of medium was adjusted at 6.0 with sterilized 1 N HCl. A loop full of fungal spores of *Aspergillus terreus* was added to each flask. The flask was then incubated for three days (72-hours) on orbital shaker working at the rate of 100 -120 rpm at 35°C temperature. Dulpicate flasks were harvested after 3 days of incubation. Flasks were centrifuged. Intra-cellular protein and enzymes (amylase & glucoamylase) were determined in the broth (supernatant) of cell mass after centrifugation. Duplicate flasks were harvested after three days incubation .The liquid protein was separated by centrifugation .The protein content and cell mass was measured

2.1.4 Cell mass determination: The cell mass was determined gravimetrically. The sample was harvested after three days of incubation from seprate flasks .The whole sample was centrigugated at 10,000 rpm for 20 minutes The supernatant was separated and the pellet was washed with saline and resuspended and dried in a pre weighed dry crucible at 100°C to a constant mass in duplicate .For insoluble substrate like broken rice the whole 50 ml sample was squeezed from a fine cloth, to get a pellet from which biomass was carefully separated, suspended in saline and dried in an oven using crucible.

2.1.5 Protein determination: The biomass protein was determined by Bradford method.(1976) using bovine serum albumen as standard.

2.2 Optimum Conditions: The conditions like substrate: water ratio, nitrogen source, carbon: nitrogen ratio, pH and incubation period were optimized.

2.3 Large Scale Production of Biomass: Large scale fermentation was carried out in twenty liter capacity glass fermentor.

2.4 Assay for alpha-amylase activity: One unit of alpha-amylase activity in each case was defined as amount of enzyme required to liberate one µmol of reducing sugars /min/ml. Alpha-amylase assay was preformed by some modifications of DNS method.

2.5 Growth kinetics: Growth kinetics of Aspergillus terreus, yield of products and specific substrate uptake rate during the fermentation were also calculated.

2.6 Biological evaluation: For biological evaluation the biomass was fed to ten days old broiler chicks for a period of ten days. To determine protein quality of biomass, it was replaced with soybean meal with the proportion of 30% and 60% respectively.

2.6.1 Composition of experimental rations: Four experimental rations viz; A, B, C and D were prepared. A control ration containing 23.0 percent crude protein and 3200 Kcal metabolizable energy Kcal /Kg was prepared and designated as ration "A". Rations B and C were formulated containing 23 percent crude protein and 3200 Kcal metabolizable energy /Kg.In ration B , 30 percent of the soybean meal was replaced with biomass protein while in ration C, 60 percent of the soybean protein was replaced with biomass. A nitrogen free ration (D) was formulated having 3200Kcal metabolizable energy /Kg.

2.6.2 Experimental birds and data collection

Day-old-broiler chicks (Hubbard) were procured from local market and raised on maize for ten days. Then t wenty birds were selected on weight uniformity basis and were randomly allotted to rations , A, B, C and D in such a way that each ration was fed to five birds. Each chick was kept in a separate pen considering

it as a replicate. Initial body weight, feed offered & refused, bird weight were taken into account to study feed conversation ratio, net protein utilization and protein efficiency ratio.

2.7 Statistical analysis: The experiment data were subjected to statistical analysis using Completely Randomized Design .For this purpose the mean values of each treatment replicate were calculated. These replicate means were then used for statistical analysis on the computer and analysis of variance table were prepared for each experiment. Finally the treatment means were computed by Duncan's Multiple Range Test [4].

3. Results and Discussion

3.1 Optimum conditions for micriobial biomass

3.1.1 Substrate: water ratio: Protein contents obtained on different substrate levels revealed that biomass protein and cell mass was maximum at 2% substrate to water ratio and biomass true protein and cell mass production was decreased with the increase in substrate: water ratio beyond 2%. Similar findings have been reported by Saima [5] who reported decrease in biomass protein concentration with fermented wheat bran using *Candida utilis* as a fermentative organism, when used beyond 6 %.

3.1.2 Nitrogen source: In order to know the suitable carbon source for the maximum production of biomass protein , various nitrogen sources viz., ammonium nitrate, ammonium sulphate, ammonium di-hydrogen phosphate, fish meal, urea and corn steep liquor were used in the growth medium @ 1.2 g N/L. The results revealed that maximum biomass protein (5.09 g/L) was produced when corn steep liquor was used as a nitrogen source. The possible reason may be that CSL might have some additional growth factors (some amino acids, NFE , minerals, trace elements , certain vitamins etc.) due to which it gave better results as compared to other nitrogen sources.

3.1.3 Carbon: nitrogen ratio: The results revealed that maximum biomass protein was formed when carbon : nitrogen was 2.4 g N/L as compare to other ratios. Similar observations have been observed by Mehmood et al.[6] who reported higher production of microbial biomass protein by fermentation of rice straw with *Arachniotus sp*.

3.1.4 pH.The results revealed that maximum biomass yield and biomass protein (4.75 g/L) was obtained with pH- 4.0. The results of the present study are in line with those of Sana [7] who produced biomass protein by corn stover with *Arachniotus* sp.

3.1.5 Incubation period: The results revealed that the maximum biomass true protein (6.09) and cell mass was obtained with 72-hour of incubation. The results are in line with that of Esabi [8] who produced single cell protein from Ram Horn Hydrolysate.

3.2 Large scale production of biomass:

After optimizing various conditions (substrate: water ratio, carbon : nitrogen ratio , nitrogen source, optimum pH and incubation period) for the production of microbial biomass protein, large scale microbial biomass was produced using fermentor of 20-liters capacity with 100percent oxygen provision with rpm (120). Dried biomass, obtained after fermentation, was subjected to analysis for crude protein , true protein , crude fiber , ether extract , ash , NFE , calorific value , RNA contents and minerals (calcium , sodium , phosphorus and chloride) using standard methods.

3.3 Biological evaluation: Average gain in body weight ,feed consumption , feed efficiency (FE), feed conversion ratio (FCR), protein efficiency ratio (PER) and net protein utilization (NPU) for standard and test rations have been summarized in Table 1.

Table 1 Average weight gain, feed consumption, feed efficiency, protein efficiency ratio, Feed efficiency, feed conversion ratio, protein efficiency ratio and net protein utilization for standard and test diet Rations

Description	A	B	C
Weight gain per chick.(g)	207.6	225.80	230.80
Feed consumption (g)	475.20	501.20	521.20
Feed efficiency (gain/feed)	0.438	0.450	0.443
Feed conversion ratio (feed/gain)	2.282	2.218	2.316
Protein efficiency ratio (gain/protein)	1.899	1.952	1.925
Net protein utilization	38.718	37.270	34.144

3.3.1 Weight gain: The highest weight gain was recorded in the chicks fed on ration containing 60 percent replacement of biomass protein with soybean meal (C) followed in descending order by those fed on ration 30 percent replacement of biomass protein with soybean meal (B) and control ration (A). It indicated that microbial biomass protein can be partially replaced by soybean meal in the rations of broiler chicks without having any detrimental effects on weight gain of birds. The results of the present study are in line with that of Joshi et al [9] who produced animal feed from apple pomace on large scale by solid state fermentation with five yeasts(*Saccharomyces cerevisiae. Candida utilis. Torula utilis. Schizosaccharomyces pombe* and *Kloeckera spp)*. Fermented apple pomace mixed with a standard broiler feed (1:1 ratio) was comparable to the standard feed. The broilers gained weight regularly upto 8 weeks and no mortality/ abnormality was observed.

3.3.2 Feed consumption: The lowest feed consumption was recorded on standard ration (A). Maximum fed was consumed by chicks fed on ration (C) in which 60 percent of the soybean protein was replaced with microbial biomass. The results of the present study is also in line with that of Ikram-ul-Haq [10] who produced single cell protein from *Brevibacterium flavum* and *Arachniotus sp.* while the substrate used for the microbial protein production was rice polishing. He replaced this microbial protein with that of Fish meal.

3.3.3 Feed efficiency(gain/feed): The data on feed efficiency of chicks ration A showed better efficiency as compared to other two experimental rations. Analysis of variance of feed efficiency shows non-significant differences. It shows that soybean meal may be replaced successfully upto 60percent with microbial protein of *Aspergillus terreus.* The results of the present study are in line with Khan et.al [11] who had observed that feed efficiency was not affected by the dietary yeast biomass. Similar results were also found by Safdar [12] who found non-significant differences in feed efficiency of two experimental rations when up to 50 percent of the fish meal of the control diet was replaced by biomass protein in the diet of broiler chicks. The results of the present study are inagreement with that of Saima [5] who produced single cell protein from *Candida utilis* and *Brevibacterium flavum* using wheat bran as a substrate. Fish meal of the diet was replaced upto 50 percent with microbial biomass and non-significance results were recorded.

3.3.4 Feed conversion ration. (feed/gain): Analysis of variance shows that non-significant difference among experimental rations.The results of the present study are in line with that of Chavez et.al [13] who reported non-significance differences among the standard and test diet when *Chaetominum cellulolyticum* biomass was fed to chicks.

3.3.5 Protein efficiency ratio: Analysis of variance revealed that PER differs non-significantly difference among various experimental diets. The results of the present study are in line with that of Khan et al. [11] who replaced 25 and 50 percent of the vegetable protein with microbial biomass produced from rice polishing with *Candida utilis.*

3.3.6 Net protein utilization: The findings of the present study are in line with that of Ikram-ul-haq [10] who produced microbial biomass protein from *Brevibacterium flavum* and *Arachniotus sp*. using rice polishing as a substrate. He replaced this biomass, 0, 25, 50, 75 and 100 percent with fish meal. The ANOVA of the variance shows significant difference among treatments.

Growth kinetics: In order to explain the trend of growth and efficiency of substrate utilization for the synthesis of protein by *Aspergillus terreus*, the growth kinetics was worked out. Various parameters were calculated for cell mass and protein production and also for amylases production (amylase and glucoamylase) (Table-2). The grams of product formed per gram of substrate utilized for fungi is usually reported as 0.5 $\pm$ 0.05 [14,15]. Yogo et al. [16] however showed different values because of the reason that the substrate they used contained less quality of carbon required for protein synthesis. The rate of product formation (Yx/s) was found to be 0.553 which is closely related with that of Ather et.al [15] who reported 0.614.The product coefficient (Yp/s) observed during the present study was 0.344 which is closely related to that of Varavinit et.al. [17] who reported Yp/s value as $0.4 gg^{-1}$

Table 2 Various growth kinetics parameters (cell mass and protein formation , amylases production) of Aspergillus terreus.

Parameters	*Aspergillus terreus*
μ	$0.451\ h^{-1}$
Yx/s	0.553 gram cell mass /gram substrate utilized
Yp/s	0.344 gram protein /gram substrate utilized
Yp/x	0.622 gram protein /gram cell mass formation
YGAM/s	1487.5 IU/gram of substrate utilized
YGAM/x	2689.80 IU/ gram cell mass formation
YAMY/s	937.50 IU/ gram substrate utilized
YAMY/x	1695.30 IU/ gram cell mass formation
QGAM	2060 volumetric glucoamylase formation rate
QAMY	3038 volumetric amylase formation rate
Qp	1.212 volumetric protein formation rate
Qx	1.445 volumetric cell mass formation rate
q p	0.2805 gram protein /gram cell mass/h
q GAM	1213.09 IU/gram cell mass /h
q AMY	164.58 IU/ gram cell mass / h

4. References

[1] B. B. Khan, A. Iqbal, and M. I. Mustafa. Sheep and goat production. TM Graphics, Al-Rehman plaza, St. # 6, Munshi Mohallah, Aminpur Bazar, Faisalabad. 232-233, 2003.

[2] K.S. Bilgrami, and A. K. Pandey. Introduction to Biotechnology. First Edition, CBS Publishers and Distributors, Delhi-110032(India).pp-159-160, 1992.

[3] K. S. Siddiqui, M. J. Azar, M. H. Rashid, and M.I. Rajoka. Purification and the effect of Mn++ on the activity of carboxymethyle cellulose from *Aspergillus niger* and *Cellulomonas biazotea*. *Folia Microbiol.*1996, **42** (4) : 303-311.

[4] R. G. D. Steel, and J. H. Torrie. Principles and Procedures of Statistics. A Biometrical Approach (2nd Ed.) McGraw Hill Book Co. Inc., New York, 1984.

[5] Saima. Bioconversion of wheat bran to biomass protein, its biological evaluation and detoxification of aflatoxin in broiler chicks. M.Sc. Thesis , Deptt. of Animal Nutrition , University of Agriculture , Faisalabad, 1996.

[6] Z. Mahmood, A. S. Hashmi, and M. Akthar. Isolation of mycotoxins produced by an antagonistic fungal organism , *Arachniotus species*. *Pak. J. Agri. Sci.*1990, **27** (4) : 314- 316.

[7] A. Sana, M. Rashid, M. Asghar and M.Yaqub. A study of optimum condition for the production of biomass protein from maize Stover by *Arachniotus sp*. *Pak. J. Biol.Sci.*1998, **1** (2) : 78 – 80 , 1998.

[8] B. Esabi. Production of single cell protein from ram horn hydrolysate. *Turk.J.Biol.* pp:371-377, 2001.

[9] V. K. Joshi, K. Gupta, A. Devrajan, B.B. Lal, and S.P. Arya. 2000. Production and evaluation of fermented apple

pomace in the feed of broilers. *J food Sci. Technol*. 2000, **37** (6): 609-612.

[10] Ikram-ul-Haq. Protein enrichment of rice polishing through metabiosis and its biological evaluation.Ph.D.Thesis. Deptt. of Animal Nutrition , University of Agriculture , Faisalabad, Pakistan, 2000.

[11] S. H. Khan, A. S. Hashmi, Z.A.Khan and A.H.Gilani. Bioconversion of defatted tice polishing to protein concentrate and its chemical and biological evaluation. Paper presented at *All Pakistan Science Conference*, Aitchison college , Lahore, 1993.

[12] M. Safdar. Production of biomass protein from beet pulp on pilot scale through metabiosis and its biological evaluation on broiler chicks. M.Sc. Thesis, Deptt. of Animal Nutrition .University of Agriculture , Faisalabad, 1995.

[13] E. R. Chavez, S.P. Touchburn, and M. Moo-Young. Microbial biomass protein from the fungus Chaetomimum cellulolyticum. Nutritive value for chicks and piglets. *Anim. Feed Sci. and Technol.*1988, **22**:23-32.

[14] A. Rieche, W. Nordheim, A.Martini, and G. Muller. Total processing of potatoes in production of high quality feed with *Candida utilis.Ztschr.Tierphysiol.Tierernahrung Futtermittelk.*1966, **21**: 144 – 153.

[15] M. Ather , A. S. Hashmi, A. H. Galani, N .Ahmed and K. H. Malik. Bioconversion of beet pulp into microbial biomass in feed batch culture and its biological evaluation in broiler chicks .Proceedings ,Second National Seminar on Nature Farming, University of Agriculture, Faisalabad ,Pakistan, 1995

[16] M. Yogo, Y. Miyable. and M.Tanaka.Yeast cells .*Japan.Kokai*.1976, 3pp.

[17] A. Varavinit, P. Srithongkum, C. De-Eknamkul, A.Assavanig. and K.Charoensiri. Production of single cell protein from cassava starch in air lift fermentor by *Cephalosparium eichhorniae*. Starch/Starrke.1996, **48**(10): 379 – 382.