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BIOCHEMICAL, HAEMATOLOGICAL AND HISTOLOGICAL STUDIES ON BROILER CHICKS FED SINGLE CELL PROTEIN BIOMASS BY *PENICILLIUM EXPANSUM*

M. Yakoub Khan, S.H. Ahmed Naqvi* and M. Umar Dahot*

Institute of Biochemistry and *Institute of Biotechnology and Genetic Engineering, University of Sindh, Jamshoro, Pakistan.

ABSTRACT

The objective of this study was to determine the effect of feeding diet containing single cell protein of *Penicillium expansum* on broiler chicks. Single cell protein was used at a level of 10 % dry mass mixed with basal diet, showed that weight gain improved for chicks. After four weeks feeding trial, the chickens were slaughtered to study the carcass characteristics. The serum samples, cellular portion of blood and the tissues of major organs were used for the biochemical, hematological and histological analysis respectively. The hematological parameters particularly Hb and RBC no longer influenced significantly ($p>0.05$) by diet effect except for WBC ($p<0.05$), lower count in the chicks fed diet without SCP biomass. The serum enzymes (amylase, ALP, CPK $p>0.05$) and LDH ($p<0.05$) were significantly higher in chicks diet supplemented with 10 % SCP except GPT ($p<0.05$) and (GGT, GOT $p>0.05$) values did not significantly differ between these groups. The waste metabolites (Urea, creatinine, Uric acid $p>0.05$), electrolytes (sodium, potassium, bicarbonates calcium $p>0.05$ and chloride $p<0.05$) and main energy metabolism parameters (cholesterol, triglycerides $p>0.05$) were not significantly affected by supplemented diet. The A/G ratio ($p<0.05$) was significantly lower due to higher concentration of globulin ($p<0.05$) in chicks fed supplemented with 10 % SCP biomass, while total protein ($p>0.05$) in both the groups having no major change during growth. No pathological changes were observed in the organs (Liver, heart, kidney and pancreas) of broiler chickens fed on single cell protein biomass. The chicks on single cell protein based diet had blood profile; hematology and histology were comparable with the chicks fed basal diet except the few parameters like enzymes (amylase, ALP, CPK, and LDH) and the globulin. Consequently, we can suggest that the SCP of *Penicillium expansum* has a promoting effect on the growth, weight gained and the death ratio of the chicks was rare.

Key word: Broiler Chickens, Single cell protein,Hematology, Biochemistry and histology.

ALP = Alkaline phosphatase , GPT = Glutamic pyruvic transaminase, GGT = γ- Glutamyl transferase. GOT = Glutamic oxaloacetic transaminase, LDH = Lactate dehydrogenase. SCP = Single cell protein, Hb = Haemoglobin, RBC=Red blood cells, WBC = White blood cell count. A/G ratio = Albumin/globulin ratio.

INTRODUCTION

Poultry industry facing many challenges in the developing world regarding the improvement the efficiency of production. To meet this challenge and maintain the efficiency of feed utilization, series of attempts have been made by researchers. The feed prepared for poultry production is rich in essential nutrients required by chicks to grow and the constituents of feed can easily be digested. (Card, & Nesheim., 1972) It has been reported by researchers (Mary, & James., 1969; Nobou, 1969; Reade *et al*., 1972;

Ravinder, *et al.* 2003; Soloman, 1973) that the mycelial biomass is the suitable source of protein, when fungal species were grown for the production of single cell protein by incorporating the waste celluloses as carbon source. Single cell protein is a source of protein and it contains all micro and macro nutrients, which are useful for the growth of chicks. But it was also reported that single cell protein contains secondary metabolites such as patulin, citrinin, ocratoxin A, roquefortine, rubratoxin B, and penicilic acid which may cause acute and chronic toxicity, especially to the kidney and liver tissues of rat, guinea pigs, mice and cockerels (Cole, *et al*., 1972; Saito, *et al.*, 1971; Scott, *et al*., 1976).

Therefore, the physiological statuses of the animals were checked after feeding trial of alternate feed sources, by the analysis of blood chemistry and hematology. The blood analyses in avian is less common in comparison to veterinary animals .The blood parameters may provide the valuable information for differential diagnosis of nutritional diseases (Quintavalla, *et al*., 2001), anti-toxic effects (Aqawane, et al., 2004) and the health status of birds (Kral & Suchy, 2000). The imbalances in blood biochemistry of chicken's may be due the several factors including poultry diseases (Burnham, *et al.*, 2003; Koinarski, *et al*., 2001; Kumar, *et al.*, 2003; Panigrahy, *et al*. 1986), feed additives (Cetin, *et al.*,2002; Oguz, *et al.*, 2000), some feed nutrients (Al-Homidan, *et al*., 2002; Eroksuz, *et al*., 2001; Kurtoglu, et al., 2005; Odunsi, *et al.*, 1999), drugs (Zaman, *et al.* 1995) shelter systems (Gunes,*et al.*, 2002) temperature (Vecerek, *et al*., 2002) and water limitations (Iheukwumere & Herbert, 2003) The immune system of the bird can be affected due to higher concentration of zinc (Kidd, *et al.*, 1996) in the feed.

The aim of present work is to study the effect of single cell protein biomass of *Penicillium expansum* on biochemical and morphological change of blood and the tissue cells of the major organs of the chicks during their growth. Single cell protein biomass produced by *Penicillium expansum* grown on acid hydrolysate (6N H_2SO_4) rice husk mineral medium, which was mixed with feeding diet during trial period (Khan, *et al*., 2000)

MATERIALS AND METHODS

Experimental diets and Chicks: Mycelial biomass of *Penicillium expansum* (Khan, *et al*., 2000) was pasteurized at 65 °C for 30 minutes in a water bath. The mycelia was removed by filtration through Whatman No: 01 filter paper and washed twice with sterile distilled water .The biomass on the filter paper was dried at 100 – 110 °C in a hot air oven (Memmert England), until a constant weight was obtained. Protein determination of the biomass was carried out by the microkjeldahl method (Harold, 1950). The crude protein values were obtained by multiplying the nitrogen content by 6.25. The lipid content (A.O.A.C, 1970) DNA analysis (A.O.A.C, 1970) patullin and Aflatoxins (A.O.A.C, 1980), of *Penicillium expansum* biomass were also estimated by reported method. Dry matter and total ash content of biomass were determined using the standard methods outlined in (A.O.A.C, 1970). The mineral content of the ash was determined by Atomic absorption Spectrophotometer (UV HS-360 Germany). The amino acids analysis of the biomass has carried out by automatic amino acid analyzer (Hitachi 835 with 150 X 2.6 mm C-18 column)

after hydrolysis of the protein sample by 6N HCl for 24 hours (Algur & Gokalp, 1991).

Forty chicks of 7 days old were purchased from poultry farm and 20 chicks were used in each experimental dietary group .One group was feeded on basal or commercial poultry feed and another fed basal diet supplemented with 10 % SCP. Feed, water and uniform light was provided round the clock during study period.

The chicks were fed single cell protein biomass of *Penicillium expansum* were sacrificed by decapitation (Libby, 1975), after four weeks of feeding trial, the carcass yield and percentage of meat, dressing, liver and heart were determined. The blood samples were collected into two well-labeled set of sample bottles. One set contained ethylenediamine tetra acetate of potassium salt (K_2EDTA, 1.0 mg/ml of blood) as an anticoagulant while other set of tubes without anticoagulant. The blood samples without anti-coagulant were kept in refrigerator at about 4°C to aid sedimentation. The samples were later spun in a centrifuge machine at 3,000 rpm for 10 minutes and the serum was separated out of blood and stored at – 10°C for analysis. The serum was later used for the analysis of dufferent metabolites. The major body organs (liver, heart, kidney and pancreas) were removed and preserved in 40% formalin for histological examination. The hemato-logical parameters were obtained using whole blood (with anticoagulant).

Haematological examination: The whole blood samples (with anticoagulant) were used for the estimation of hemoglobin, red blood cells count and total leukocyte count (Ross, *et al.*, 1976).

Biochemical examination: The serum was separated into another tube as soon as possible by centrifugation and the serum was used for the estimation of biochemical constituents. Microlab-200 (Germany) was used for the estimation of waste metabolites, enzymes and other nutrients. The Flame photometer (Corning) was used for the estimation of electrolytes.

Estimation of serum enzymes: The serum amylase, alkaline phosphatase, glutamate pyruvate transaminase, glutamate pyruvate oxaloacetate, lactate dehydrogenase creatinine phosphokinase and γ-glutamyl transferase were analysed by Merck kits methods.

Estimation of waste metabolites: The chick's serum was used for the estimation of waste metabolites like uric acid, creatinine by Merck kit methods and urea by Biomerioux Kit methods.

Estimation of electrolytes: The Sodium, potassium and chloride were analyzed by flame photometer while calcium and bicarbonates by Merck kit methods.

Estimation of plasma nutrients: The serum samples were used for the estimation of protein A/G ratio, cholesterol and triglycerides by Merck Kit methods.

Histological examination: The organs (liver, kidney, spleen, and panaceas) required for histological studies were dissected and preserved in 10 % formalin solution. Histological study was carried out according to methods described by (Ker, et al., 1982)

RESULTS AND DISCUISSION:

The proximate composition of *Penicillium expansum* biomass is given in Table–1, contains sufficient amount of protein (38.81 %) and amino acids. The concentration of nucleic acids (1.82%) and lipids (1.05) is lower while aflatoxins and patullin were absent in single cell protein biomass. The single cell protein

biomass was quite favorable fed for chicks.

Table–1: Shows the composition of mycelial biomass of *Penicillium expansum* when grown in 0.6N H_2SO_4 pretreated rice husk mineral medium supplemented with 1% sucrose at pH 6.0 for 240 hours at 35°C in fermenter.

Constituents	Observation	
Weight of biomass	6.56 G/Liters	
Lipid	1.05 %	
DNA	1.82 %	
Aflatoxin	Nil	
Pattulin	Nil	
Ash	2.0 %	
Calcium	3.98 mg/Total ash	
Magnesium	27.40mg/Total ash	
Sodium	94.76mg/Total ash	
Potassium	2.92 mg/Total ash	
Nitrogen	6.21 %	
Protein	38.81 %	
Amino acids	**Micromole/G mycelia**	**Relative %**
Phospho serine	252.4	2.06
Aspartic acid	812.5	6.61
Threonine	494.5	4.02
Glutamic acid	3305.5	26.84
Proline	646.5	5.25
Glycine	978.0	7.94
Valine	1093.5	8.88
Isoleucine	468.5	3.80
Leucine	1020.0	8.29
Phenylalanine	562.5	4.57
Alo-Lysine	553.0	4.50
Halo-Lysine	555.0	4.50
Lysine	922.0	7.48
1-Methyl Histidine	109.0	0.88
Arginine	539.5	4.38

Table-2 shows the weight of meat, dressing, liver and heart of chicks fed on normal diet and normal diet supplemented with 10% *Penicillium expansum* single cell protein. It was observed that percentage yield of meat to body weight, liver and heart is higher in those chicks which were grown on normal diet supplemented with 10% single cell protein biomass of *Penicillium expansum.* However the percentage of dressing to body weight is 16% lower in chicks grown on normal diet supplemented with 10% *Penicillium expansum* biomass in comparison to chicks grown on normal diet.

Table–2: Percentage yield of meat, dressing, liver and heart weight to body weight of chicks grown on commercial poultry feed and commercial poultry feed supplement with 10% *Penicillium expansum* biomass protein.

Study group	Mean weight grams	Components	Yield grams	Body weight %
Control Basal diet.	1227.75±23.2	Meat	670.30±35.8	54.39
		Dressing	440.60±3.74	35.88
		Liver	06.97±0.31	00.57
		Heart	31.09±0.29	02.53
Basal diet supplemented with 10% biomass protein	1441.00±93.5	Meat	780.82±8.74	54.19
		Dressing	489.40±8.96	33.96
		Liver	11.46±0.48	00.79
		Heart	40.60±0.40	02.82

Table–3 shows the results of hematological parameters (Hb, RBC and WBC) of chick's blood fed on basal diet and the diet supplemented with 10% SCP biomass of *Penicillium expansum.* The hematological parameters particularly hemoglobin (8.70 v/s 8.0g/dl) and RBC's (2.3 v/s 2.00m/cmm) were no longer influenced significantly ($p>0.05$) by the diet, while the WBC count (9840 v/s 16400/cmm) is significantly ($p<0.05$) lower in case of diet without single cell protein biomass. The mean values of hematological parameters obtained in the present study fall below the range reported in Nigerian indigenous chickens ((Ikhimioya & Imasuen, 2007; Ladokun, *et al.*, 2008; Simaraks, *et al.* 2004), Fayoumi, Asil and local chickens (Islam, *et al.*, 2000). The hematological parameters are in agreement with the finding of previous workers (Adeyemo, 2008 Aderolu, et al., 2007).

Table –3: Showing the % of Hb, RBC,WBC chick's blood fed on basal diet and the basal diet supplemented with 10 % single cell protein biomass.

Parameters (Hematological)		**Chicks fed on basal diet (n = 20)**	**Chicks fed on basal diet Supplemented with 10 % SCP biomass (n =20)**	**p-Value**
		Mean±S.D.	Mean±S.D.	
Hb	g/dl	8.7 ± 8.69	8.00 ±1.22	0.1273
RBC	m/cmm	2.30 ± 0.33	2.00 ± 0.33	0.9900
WBC	/cmm	3650 ± 3649.20	9840 ± 9839.60	0.0472

Serum enzymes of chicks fed on basal diet and supplemented with 10% single cell protein biomass shown in Table–4. There were no significant ($p>0.05$) difference in the mean concentration of serum amylase (328 v/s 376U/L), ALP level (4176 v/s 4758U/L), GOT (313 v/s 382U/L) and γ-GT (17 v/s 20U/L), and significant ($p<0.05$) difference of GPT (6.0 v/s 5.0 U/L) of chicks fed on basal diet and supplemented with 10 % single cell protein respectively and were no longer influenced with the diet. The result of serum enzymes (amylase, ALP, GPT, GOT and γ- GT) shows normal function of liver, kidney and pancreas. The results of serum enzymes were in agreement reported by (Ikhimioya & Imasuen, 2007). The level of LDH (3168 v/s 3655, $p<0.05$) and CPK (2694 v/s 3100, $p>0.05$) of the

chicks fed on basal diet and diet supplemented with 10% single cell protein, showed the significant difference. Increase in serum LDH and CPK level are therefore specific and occur in diseases of liver, after myocardial infraction, muscular dystrophy, hemolytic and pernicious anemia (Hees, 1962; Elliott & Wilkinson, 1963).

Table – 4: Showing the serum enzymes of chicks fed on basal diet and the basal diet supplemented with 10 % single cell protein biomass.

Parameters (Serum enzymes)	Chicks fed on basal diet (n =20)	Chicks fed on basal diet Supplemented with 10% SCP biomass (n =20)	p-Value
	Mean±S.D.	Mean±S.D.	
Amylase U/l	328.± 53.72	376 ± 59.28	0.6801
ALP U/l	4176.00 ±131.45	4758.±195.55	0.1546
GPT U/l	6.00 ±1.04	5.00 ± 0.73	0.0359
GGT U/l	17.00 ±1.61	20.00 ± 2.11	0.0957
CPK U/l	2694.00 ± 378.40	3100.00 ± 306.46	0.4340
GOT U/l	313.00 ±12.74	282.00 ± 9.48	0.2102
LDH U/l	3168.00 ± 210.56	3655.00 ± 397.66	0.0069

The biochemical composition of serum metabolites of chicks fed on basal diet and diet is supplemented with 10 % single cell protein given in Table – 5. The content of waste metabolites, Urea (13.2 v/s 11.2 mg/dl) and creatinine (0.36 v/s 0.41 mg/dl) showing no significant ($p>0.05$) effect of the diet, while uric acid (4.3 v/s 6.5 mg/dl) level is significantly ($p<0.05$) higher in chicks fed on 10 % single cell protein. The data revealed that the concentration of waste metabolites (urea, creatinine, uric acid) were not affected by the diet in both groups of chicks (on basal diet and the diet supplemented with 10% SCP) and are in agreement with the data reported by (Adeyemo, 2008; Olorede & Longe, 1999), except the concentration of uric acid which is found in chicks fed on 10% single cell protein biomass. Uric acid is the primary catabolic product of protein, non-protein nitrogen and purines is a good indicator of the quality of protein fed to bird. hyperuricemia occur in birds when there is starvation, massive tissue destruction and renal disease (Chandra, et al., 1983). Urea concentration is normal which shows that good quality of protein in diet (Aderolu, et al., 2007).

The major nutrients like cholesterol (186.0 v/s 197.0mg/dl) and triglycerides (107.0 v/s 99.0mg/dl) did not significantly ($p>0.05$) differ between the groups of chicks, (on basal diet and the diet supplemented with 10% SCP). The data are in agreement reported in the literature (Aderolu, et al., 2007; Paryad & Mahmoudi, 2008)

The content of proteins including mean of total proteins (5.3 v/s 5.1 G/dl), albumin (3.6 v/s 2.1 g/dl), did not show significant ($p>0.05$) difference, while globulin (1.7 v/s 3.0 G/dl) was significantly ($p<0.05$) higher and A/G ratio (2.1 v/s 0.70) was significantly ($p<0.05$) lower in chicks of blood fed on the diet supplemented with 10 % single cell protein biomass. No major difference in total protein level except the globulin,

which is higher in the chick's blood, fed on diet supplemented with 10% single cell protein biomass. The reduction in globulin is an indication of malnutrition (Aderolu, et al., 2007).The results of proteins (total), albumin, globulin and A/G ratio are in agreement with the data reported in literature (Adeyemo, 2008; Aderolu, et al., 2007; Bolu, et al., 2009; Chandra, et al., 1983; Olorede & Longe, 1999).

Table– 5: Showing the serum metabolic profile (Nutrients, Wastes metabolites, Electrolytes) of chicks fed on basal diet and the basal diet supplemented with 10 % single cell protein biomass.

Parameters (Serum metabolites)	**Chicks fed on basal diet (n =20)**	**Chicks fed on basal diet Supplemented with 10 % SCP biomass (n =20)**	**p-Value**
	Mean±S.D.	Mean±S.D.	
Urea mg/dl	13.2 ±1.14	11.2 ±1.5	0.1950
Creatinine mg/dl	0.36 ± 0.04	0.41 ± 0.058	0.0987
Uric Acid mg/dl	4.3 ± 0.92	6.5 ± 0.75	0.3608
Cholesterol mg/dl	186.0 ± 29.07	197.0 ± 49.9	0.6701
Triglyceride mg/dl	107.0 ±15.38	99.0 ± 10.63	0.0330
Protein g/dl	5.28 ± 5.28	5.14 ± 5.14	0.1437
Albumin g/dl	3.61 ± 3.61	2.14 ± 2.14	0.2276
Globulin g/dl	1.76 ±1.76	3.08 ± 3.08	0.0368
A/G ratio	2.10 ± 2.10	0.69 ± 0.69	0.0000
Sodium meq/l	158.0 ± 8.84	165.0 ±10.44	0.4143
Potassium meq/l	4.9 ± 4.93	3.23 ± 0.73	0.0878
Chloride mmol/l	117.0 ±3.96	123.0 ± 122.87	0.0120
Bicarbonate mmol/l	23.70 ±1.31	24.2 ±1.01	0.1403
Calcium mg/dl	9.9 ± 0.90	10.4 ± 0.84	0.4067

The electrolyte content, sodium (158.0 v/s 165.0meq/L $p>0.05$), potassium (4.9 v/s 5.2meq/L $p>0.05$), chloride (117.0 v/s 123.0meq/L $p<0.05$), bi-carbonate (23.7 v/s 24.3meq/L $p>0.05$) and the calcium (9.9 v/s 10.4mg/dl $p>0.05$) of chicks blood on basal diet and the diet supplemented with 10 % single cell protein biomass showing no major change in the level. The content of serum electrolytes (sodium, potassium, chloride, calcium is in line with the corresponding data reported in the literature (Gokhan, et al., 2005). The tissues of major body organs (Liver, Heart, Kidney and pancreas) were preserved in 40% formalin and send to histopathology laboratory for analysis. The organ's tissues were processed in histological laboratory by using the standard method. The organs tissues were fixed in neutral formalin and were trimmed. The tissues were embedded in paraffin blocks, sectioned and stained with hematoxyline-eosin. The study revealed that no pathological changes have been seen in the organs of chicks grown on basal diet and basal diet supplemented with 10 % single cell protein biomass.

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