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Producing Single Cell Protein from Poultry Manure and Evaluation for Broiler Chickens Diets

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Abstract: An experiment was run to study the possibility of elimination of uric acid from poultry manure and increasing its nutritive value by using it as a medium for single cell protein (SCP, yeast) production. Dried Poultry Manure (DPM) was collected freshly from battery brooders of broiler chicken house, dried in an oven at 80°C for 48 h, crushed with mill and stored in plastic bags. Seven strains of yeast (*Candida utilis*, *Candida tropicalis*, 3 strains of *Saccharomyces cerevisiae*, *S. uvarum* and *Rhodotorula rubra*) were tested to hydrolyze uric acid and produce protein when grown on DPM containing medium. In addition, forty five one-day old commercial broiler chicks were used to determine Total Protein Efficiency (TPE) of raw and treated DPM. The chickens were randomly divided among 3 equal groups of approximately similar initial body weight of three replicates each containing 5 chicks. Another, one hundred and sixty two broiler chickens were allotted to 9 dietary treatments of 3 replicates of 6 chickens each in a randomized complete block design. This experiment aimed to evaluate the nutritive value of the treated DPM. The experimental diets were corn-soybean meal diet in which 3, 6, 9 and 12% treated DPM and untreated DPM were added on the expense of soybean meal protein. *Candida utilis* offered the highest protein yield (12.7%) and the highest efficiency to hydrolyze uric acid; therefore, it was selected for further study. Shorter growth periods (< 5 days) favor protein yields while longer growth times (>5 days) were concomitant to higher uric acid hydrolysis. The optimum amount for protein production and uric acid hydrolysis under the specified fermentation condition were 2 g /50 ml medium and pH range between 6-7. Inoculum size from 2-8% and medium should be consists only 40 g DPM /l without any salt increased the protein yield and high utilized of uric acid. The chemical analysis of the DPM showed 19.1% Crude Protein (CP), 18.2% ash, 7.9% Crude Fiber (CF), 1.7% Ether Extract (EE) and relatively high uric acid content, 7.2%. Fermentation of DPM increased the CP content from 19.1 to 24.9%, the NPN content decreased from 9.6 to 8.7%, uric acid content decreased from 7.2 to 0.3% and the EE increased from 1.7 to 2.4%. Amino acids of fermented DPM were greatly increased than those of the DPM except for glycine, histidine and tyrosine. Results of TPE assay indicated that chickens fed diet containing treated DPM gained significantly more weight than those given diets containing untreated DPM. Chickens fed treated DPM gained significantly more weight than those given the untreated DPM, throughout the experimental period. In conclusion, yeast treatment for DPM improved its nutritive value, thus it could be included up to 9% in broiler diets without adverse effect on growth performance of broiler chickens up to 4 wks of age.

Key words: Dried poultry manure, yeast, uric acid, single cell protein, protein quality, broiler performance

INTRODUCTION

Single cell protein production is a possible step to increase protein supply. SCP is the protein extracted from cultivated microbial biomass. It can be used for protein supplementation of poultry diet by replacing costly conventional sources like soybean meal and fishmeal to alleviate shortage of protein supply after BSE (Attia *et al.*, 2003). Several research reports have shown that animal excreta have nutritional value. Therefore, utilizing animal excreta as a feedstuff could provide an additional feed ingredient plus a reduction of environmental pollution. Dehydrated poultry waste or poultry anaphage which are defined as the dehydrated cage layer excreta, has been successfully fed to ruminants and poultry.

Compositing, anaerobic digestion, combustion, oxidation and drying are all possible ways for use poultry manure as a protein source in poultry diets. DPM has about one third of its total nitrogen as protein (Hodgetts, 1971). DPM is composed of undigested food residues, mainly structural carbohydrates and unabsorbed food constituents (Ca and P) together with metabolic faecal and urinary components. It also contains spilled food, dead microorganisms and external body waste such as lost feathers. The composition of DPM is highly variable, depending upon the ingredients of the diet, the age of the birds, fresh moisture content, methods of storage, physiological status of the birds, the environmental conditions under which the droppings are kept and during temperature. Published data for N, P and K

content are highly variable and can largely be attributed to ambient temperature differences (Coufal *et al.*, 2006). Malone *et al.* (1992), Patterson *et al.* (1998), Bowers *et al.* (2002) and Chamblee and Todd (2002) reported an average of 2.85-3.73, 1.45-3.22 and 2.18-2.95% for N, P₂O₅ and K₂O, respectively, from several sources in USA. Average of total litter (litter plus cake) production varied considerably due to many different factors and averaged 228.2 g of dry litter material per kg of live broiler weight (g/kg) per flock (Coufal *et al.*, 2006). The production of 1.0 dry metric ton per 1000 broilers per flock with a range of 0.7-2.0 metric tons (Malone *et al.*, 1992), 1.6 tons per 1000 broilers if the houses were cleaned out completely on an annual basis and a rate of 1 ton per 1000 broilers if houses were cleaned out completely at the end of 2 yr (Chamblee and Todd, 2002), 1.25 ton per 1000 birds with cake production at 0.4 ton per 1,000 birds (Natural Resource, Agriculture and Engineering Service (NRAES), 1999).

Feeding birds diets containing 50 gm DPM/kg yielded meat of comparable quality to those fed on the control diet, while those fed on diets containing more DPM yielded meat containing more fat and less water (Ogunmodede and Aninge, 1978). El-Deek and Raya (1983) indicated significant difference in Body Weight (BW), Body Weight Gain (BWG), Feed Intake (FI) and Feed Conversion Ratio (FCR) between broiler fed diet had 15% DPM and those fed the control diet. Tibin and Koko (1989) found that sun-DPM significantly decreased broiler FI, growth and FCR. With increasing sun-DPM level, empty carcass weight, dressing percentage, thigh, breast and drumstick significantly decreased. Furthermore, the weight of head, shank and total viscera increased with increasing level of DPM. Liver, gizzard and heart weights were not influenced. CP decreased, but ash and EE of carcass increased with increasing dietary DPM level, although moisture was not affected.

The limited available energy and uric acid toxicity of DPM are the two main problems limiting its use as a feed ingredient (Blair, 1972; Lee and Blair, 1972; Blair and Lee, 1973). Various cultures of organisms can be used for improving utilization and conversion of the manure nitrogenous materials to protein. Bare *et al.* (1974) noted that uric acid acted as a gut irritant, either depressing nutrient absorption or inhibiting the microbiological synthesis of essential nutrients and thus uric acid should be converted into a non-toxic form if poultry manure is to be fed. One possibility is to ferment manure with the aid of microbes which use uric acid and produce cell mass (Jackson *et al.*, 1970; El-Deek, 1979). Also, Vuori and Nasi (1977) used microbial strains for the efficient elimination of uric acid by fermenting poultry manure.

Stanley *et al.* (2004) and Kargi *et al.* (2005) suggested that the operating conditions of 25°C, pH 7.5, 1.5% solids concentration in the feed (media composition), and a residence time (time for the nearly complete

utilization of total uric acid and ammonia nitrogen) of 8.1 h were found to be the most appropriate conditions maximizing the "profit" function for the conversion of DPM (supplemented by molasses) into single-cell protein.

The inclusion of dried yeast of brewery or distillery origin in poultry diets in particular is a long-standing practice, but the levels used have not been sufficient to warrant serious consideration of the material as a protein source. Machalek *et al.* (1990) reported that the replacement of 5 and 10% soybean meal in the finisher broiler diets with a similar amount of yeast protein concentrate vitex (48% crude protein) increased growth, while FCR, dressing percentage and carcass quality were similar. Samanta and Monda (1990) indicated that growth, FI, FCR and carcass dressing percentage were not different among broiler chickens (0-6 wks) fed diets containing fish meal replaced by 0, 25, 50 or 75% yeast (a distillery by-product). Alvarez (1990) found there were no significant differences between chicken groups, fed diet contained 15% torula yeast without or supplemented with 0.2% DL-methionine, 0.1% DL-methionine and 0.10% Na₂SO₄ or 0.1% DL-methionine and 0.16% CuSO₄ · 5H₂O, in BWG, FI and FCR, except that FI at the earlier growth stage which was slightly decreased with 0.2% methionine. Also, Ravindran (1995) demonstrated that it is possible to formulate layer diets using non-conventional feedstuffs as DPM, achieve acceptable production and lower the feeding costs. Along the same line, Ignacio (1995) and Onifade *et al.* (1998) reported that feeding yeast to chicks improves BWG and FCR. Also, Kanat and Calialar (1996) reported that active dry yeast effectively increases BWG without affecting FCR of broiler chicks. Supplementation of yeast to broiler diets improved FCR but not growth rate (Onifade *et al.*, 1998). Moreover, Spring (2002) and Santin *et al.* (2003) revealed that yeast can improve immune status of birds and reduce the toxic effects of aflatoxin. Tollba and El-Nagar (2008) indicated that supplementing the laying diet with live yeast considerably improved BWG, mortality rate, FI and FCR. Furthermore, yeast can be an alternative to antibiotic in broiler diets on new litter (Hooge *et al.*, 2003) or on recycled litter. Zhang *et al.* (2005) concluded that dietary yeast components, such as Whole Yeast (WY) or Yeast Cell Wall (YCW) supplementation improved growth performance and meat tenderness and yeast had oxidation-reducing effects. In addition, YCW may improve ileal villus development, thus improve absorptive capacity of the gut. Farhoomand and Dadvend (2007) demonstrated that *Saccharomyces Cerviciae* (SC) supplementation at 100 g SC/kg diet significantly improved FCR, decreased abdominal fat, intestine length and increased BWG, dressing yield, liver and spleen weights compared to control diet or the other SC levels. Gao *et al.* (2008) indicate that dietary supplemental YC at 2.5 g/kg improved growth performance. Dietary YC affected immune functions,

digestibility of Ca and P and intestinal mucosal morphology of broilers. Chen *et al.* (2009) showed that Bac+Sac fermented feed increased chickens growth, FI and increased gross energy availability of the diet.

The present research was directed toward the elimination of uric acid and increasing the nutritive value of DPM by using yeasts and production of single cell protein using DPM. In addition, feeding experiments were conducted to evaluate the nutritive value of treated DPM in poultry diets.

MATERIALS AND METHODS

Poultry Manure (PM) was collected freshly from the broiler chicks house in which they were reared in battery brooders. The manure was placed in trays and dried in an oven at 80°C for 48 h, crushed with mill and stored in plastic bags until used, sample was chemically analysis according to A.O.A.C. (1985) (Table 1).

Elimination of uric acid and increasing the nutritive value of poultry manure:

This study aimed to explore the potentiality of the experimental yeasts to hydrolyze uric acid and to produce single cell protein. The study was made on 30 different yeasts isolates cultivated in a shaken uric acid containing medium (Vuori and Nasi, 1977) to specify uric acid hydrolyzing isolates. The uric acid utilizing strains were then cultivated, under shared conditions, in the medium of Vuori and Nasi (1977) in which the uric acid content was replaced with 20 g/l of DPM. The fermentation period was continued for 7 days, thereafter the necessary analysis were carried out.

Fermentation process: Microorganisms: The identities as well as the sources of the different yeasts used throughout the screening experiments are presented in Table 2.

Maintenance of stock cultures: Each experimental yeast used is a descendant of a pure single slant culture. The stock cultures of each of the experimental yeasts were maintained on glucose-peptone agar slants of the following composition (g/l): Glucose, 20; peptone, 5; KH₂PO₄, 1; MgSO₄·7H₂O, 0.5; FeSO₄·7H₂O, 0.01; agar, 20. The inoculated slants were stored at room temperature with transfers at monthly intervals.

Fermentation process: The tested yeasts succeeded to grow on uric acid (as the sole source of carbon and nitrogen) containing medium of the following composition (mg/l): CaCl₂·2H₂O, 1793; Na₂HPC₄·H₂O, 640.8; KH₂PO₄, 427.2; MgSO₄·7H₂O, 492; FeCl₃·6H₂O, 29; CuSO₄·5H₂O, 2; MnSO₄·4H₂O, 9; ZnSO₄·7H₂O, 11; uric acid, 1107 (Vuori and Nasi, 1977); pH 6. Cultivation was carried out on the latter medium in which uric acid was

Table 1: Composition and comparative analysis of chemical analysis of poultry manure and the treated poultry manure

Composition (%)	Poultry manure (g % to dry weight)	Treated poultry manure (g % to dry weight)
Moisture	10.20	10.40
Crude protein	19.10	24.90
Non-protein nitrogen	9.50	8.70
True protein	9.60	16.20
Ether extract	1.70	2.40
Crude fiber	7.90	7.80
Nitrogen free extract	35.70	35.60
Uric acid	7.20	0.30
Crude ash	18.2	18.60
Element content of total ash (%)		
Copper	7.50	---
Calcium	3.40	---
Phosphorus	1.50	---
Magnesium	0.57	---
Iron	0.14	---
Sodium	0.09	---
Zinc	0.09	---
Potassium	0.08	---
Manganese	0.03	---

Table 2: The composition of experimental diets used for total protein efficiency experiment

Ingredient, %	Basal Diet (%)	Diet (1) (%)	Diet (2) (%)
Wheat	48.00	48.00	48.00
Soybean meal	27.30	13.70	13.70
Yeast	0.40	0.40	0.40
Raw manure	0.00	31.00	0.00
Treated manure	0.00	0.00	24.40
Vegetable oil	9.30	3.30	5.30
Bon meal	2.90	0.00	0.50
Lime stone	0.70	0.00	0.00
Premix*	0.50	2.10	1.00
Sodium chloride	0.50	0.00	0.00
Sand	10.40	0.50	5.70
Total	100.00	100.00	100.00
Calculated analysis			
Crude protein (%)	18.40	18.40	18.40
ME (kcal/kg)	3003	3001	3002
C/P ratio	166.90	166.8	166.8
Calcium (%)	1.59	1.42	1.36
Avi. Phosphorus (%)	0.79	0.70	0.69

*Vitamin-mineral premix supplied per 1 Kg. of diet: Vit.A, 12000 IU; Vit. D3, 2200 ICU; Vit. E, 10 mg; Vit. K3, 2mg; Vit. B1, 1 mg; Vit. B2, 4 mg; Vit. B6, 1.5 mg; Vit. B12, 10 Ug; Nicotinic acid, 20 mg; Folic acid, 1 mg; Pantothenic acid, 10 mg; Biotin 50 Ug; Choline chloride, 500 mg; Copper, 10 mg; Iron 30 mg; Manganese, 55 mg; Zink, 50 mg; Iodine, 1 mg; Selenium, 0.1 mg

replaced with 20 g DPM. The composition of the basal medium was however modified and this will be specified in the text.

The organisms were allowed to grow in 50 ml portions of the basal medium disposed in 250 ml Erlenmeyer flasks. The flasks were sterilized by autoclaving for 15 mm at 121°C. The sterilized media were inoculated with 2 ml of yeast suspension prepared by adding 10 ml sterile distilled water to a 48 h old tube culture and agitating the growth with the aid of a sterilized inoculating needle.

The culture flasks were incubated at 30±2°C for 7 days under shaken conditions (200 shakes/mm, amplitude 7 cm). Thereafter, the necessary analysis were made.

Each treatment was carried out in triplicates and the results obtained were the arithmetic mean of at least two experiments.

Analysis of the fermentation products: The fermentation residual (DPM and yeast growth) was separated by centrifugation, washed with distilled water followed each time by centrifugation. The washed fermentation residue dried at 60°C for constant weight-referred to as the marc dry weight. The dried residue was analyzed for its content of true protein, while the residue uric acid was determined in the filtrate.

Factors affecting the optimum yield of fermentation products

Effect of incubation period: In an attempt to investigate the hydrolysis of uric acid and protein production by *C. utilis* during the different periods of incubation the following experiment was carried out. Aliquots (50 ml containing 1 g DPM) of the basal medium were diffused in 250 ml Erlenmeyer flasks, sterilized and mixed with standard inoculate (2 ml suspension) of the yeast. The culture flasks were then shaken at 30±2°C for different time intervals. Samples were collected every 24 h for determination of the marc dry weight, the protein percentage and the residual uric acid as well as the final pH value of the fermentation beer (medium).

Effect of level of dried poultry manure (solid/liquid): As a fact, substrate concentration has a considerable effect on the rate of various enzymatic reactions and consequently the different metabolic activities. Different weight of DPM ranging from 0.5-5 g were dispensed, one at a time in 50 ml of the nutrient solution, sterilized inoculated and incubated for 6 days under shaken conditions, where by the necessary analysis were carried out.

Effect of inoculum size: In the previous experiments, 2 ml of 48 h old cultures of *Candida utilis* was used as inoculum in the fermentation process. In an attempt to improve the protein yield and the activity of uric acid hydrolysis, yeast suspension inoculate of different sizes (2-12%) were tested. In this experiment, the fermentation flasks (each containing 2 g DPM/50 ml medium) received different volume (1-6 ml/flask) of 48 h old seed cultures each at a time. The inoculated flask was incubated for 6 days under shaken culture conditions, whereby the necessary analysis were carried out.

Effect of pH: The influence of different starting pH values on protein production and uric acid utilization by the experimental yeast was investigated. Aliquots of the basal medium were initially pH adjustments were carried out, before sterilization of the medium by 1 N NaOH or HCl and Pye-Unicam pH meter was used for

pH measurements. The necessary analyses were carried out after 6 days of inoculation.

Effect of medium size: The effect various medium volumes of 10-125 ml per 250 ml Erlenmeyer flask were tested in this trial in order elucidate the interaction between the volume of air/flask (aeration) and uric acid hydrolysis activity as well as protein production by the tested yeast.

Effect of the mineral salts: The impact of the components of the mineral salts of the fermentation medium of CaCl₂.2H₂O, Na₂HPO₄.H₂O, K H₂ PO₄, MgSO₄.7H₂O, FeCl₃.6H₂O, CuSO₄.5H₂O, MnSO₄.4H₂O and Zn SO₄.7H₂O on the uric acid utilization and protein production by *C. utilis* was elucidated by elimination each salt from the fermentation medium one at a time. In another treatment, the influence of the omission of all mineral salts was tested.

Effect of cane-molasses as a carbon source: Impressed by the fact that available energy of DPM is one of the difficulties in its use in poultry diets (Blair, 1972; Lee and Blair, 1972; Blair and Lee, 1973), different individually supplemented to the modified basal medium consisting of 3 g DPM in 75 ml tap water/250 ml Erlenmeyer flask. The inoculated flasks were shaken at 200 rpm for 6 days, whereby the necessary analyses carried out.

Time course uric acid hydrolysis and protein production by *C. utilis* cultivated under the optimal fermentation conditions: The previous studies outlined the optimal culture conditions which rendered the DPM almost free of uric acid and with enriched protein content by the tested yeast. These suitable conditions involved the shaken cultivation of *C. utilis* in the following mediums 3 g DPM, 75 ml tap water and with initial pH adjusted to 6.0. The autoclaved medium received 4% inoculum of 48 h old yeast culture and incubated at 30±2°C under shaken conditions. However, the pattern actually obtained under these conditions represents the activities of the tested yeast with respect to uric acid hydrolysis and capacity and protein biosynthesis after 6 days incubation only. Therefore, it was essential to test the time course for hydrolysis of uric acid and protein production under these physiological conditions enhancing the formation of uric acid and proteins by the experimental yeast. The methods used for cultivation were previously described, while the analyses of the fermentation process were carried out after 2, 4, 5, 6, 8 and 9 days.

Chemical composition of the fermentation products produced at the optimal conditions: In the previous trials emphasis has been given to outline the important

aspects of uric acid hydrolysis and protein production by the experimental yeast which was found to be able to utilize uric acid as sole carbon and nitrogen source. The present study revealed the optimal fermentation conditions leading to maximal hydrolysis of uric acid and true protein yields. The results of these investigations paved the way to determine the chemical constituents of the fermentation products left after cultivating of the yeast, under the optimized culture conditions previously elucidated.

Aliquots of the simplified basal medium of the following composition: 3 g DPM/ 75 ml water at initial pH 6, were dispensed in 250 ml Erlenmeyer flasks, sterilized by autoclaving and mixed with the standard yeast inoculum (4%). The culture flasks were agitated on a rotary shaker at 200 rpm at 30°C for 6 days. At the end of the incubation period, the fermentation marc (residual DPM + yeast biomass) was separated by centrifugation at 4000 rpm for 15 min, washed and dried at 60°C. Samples of the obtained marc were analyzed for estimations of moisture, true protein, lipid, fiber and ash. The investigation was also extended to analyze the amino acids contents. On the other hand, uric acid content was determined in the supernatant.

Analytical methods: Dry Matter (DM), ash, CP, EE, CF and Nitrogen Free Extract (NFE) was determined in all tested samples according to A.O.A.C. (1985). Also, pH value of the reaction media, before and after growth was measured using digital pH meter. True protein and Non Protein Nitrogen (NPN) estimation according to (Ekman and Ransson, 1949). Uric acid was determined according to Bose and Ghosh (1945). The total and free amino acid, except tryptophan were estimated by the method described Moore (1958) using a Beckman amino acid analyzer model 118/119 CL.

The production of single cell protein using dried poultry manure as a medium: Batch culture systems was carried out continuously for preparing products for poultry feed. The initial used medium contained dried DPM (3 g/l), tap water (75 mg/l). A general production of treated DPM is shown in the following steps -

- Ground DPM wastes (3 g/l).
- Adding tap water (75 ml/250 ml Erlenmeyer flask).
- Adjustment the pH media (6.9 pH).
- Sterilization 121°C for 20 min.
- Inoculation with *Candida utilis*.
- Incubation 6 days 30±2% and shaken at 200 rpm.
- Drying, grinding and storage.

Biological evaluation of raw and treated poultry manure

Total protein efficiency method: The method of Woodham (1968) with modification of the method by Woodham *et al.* (1972) was employed in this study. The composition of the basal ration was modified using local

feeding stuffs. The method is a type of growth trial employing diets which provide more than the quantity of protein needed for maintenance but less than that required for optimum performance. This restriction in the protein level identifies the differences between qualities of proteins and makes it easier to distinguish among samples.

Forty five one-day old commercial broiler chickens were used in this experiment. Chickens were kept in wire-floored battery brooders and placed in controlled-heating room. For two weeks, they were given a normal chicken's starter diet. At 14 days of age, the birds were individually weighed to the nearest gram. The chickens were randomly divided into 3 groups equal in number and approximately similar in the average initial weight. Chickens in each group were subdivided into three replicates each containing five chicks. Diet containing 18.4% protein was formulated from wheat providing 6.0 parts of dietary protein, protein concentrates providing 12 parts and dried yeast providing 0.4. The ration was isocaloric containing 3000 Kcal ME/kg. The composition of the basal rations and tested diets are shown in Table 2.

The experimental rations were given *ad libitum* to the 3 group of 5 birds each from 14-28 days of age. At the end, the chickens in each group were weighed and FI was recorded during the experimental period (14-28 days). The values of FI, BWG, FCR and TPE were calculated. TPE is the BWG of all birds in each group divided by the protein consumed by the same group.

Feeding trial: This experiment was conducted to evaluate the nutritional potential of the dried DPM which was treated with an adaptive strain of *Candida utilis*. One hundred and sixty two broiler chickens were wing banded for individual identification, equalized as to body weight and allotted in groups of 18 to 9 dietary treatments, each replicated 3 times in a randomized complete block design. They were raised in electrically heated battery brooder in a temperature controlled room. They were given the experimental diets *ad libitum* for 4 weeks. The experimental diets were composed of a typical corn-soybean meal diet to which 3, 6, 9 and 12% treated DPM and untreated DPM were included on the expense of corresponding quantity of soybean meal protein. Variable amount of corn oil or supplemented DL-methionine were added to maintain the same level of ME (2990) in the experimental diets and the same level of crude protein (Table 3). A diet with no DPM and supplied similar levels of ME and protein was used as a control. Performance of the chicks was assumed in terms BWG, FI and FCR at weekly intervals up to 4 wks of age.

Statistical analysis: The data was analyzed by analysis of variance as described by Snedecor (1961) and multiple range test comparisons were made according to Duncan (1955).

Table 3: Composition of the experimental diets containing treated dried poultry manure and dried poultry manure as a nutrient ingredients fed to broiler chick at various levels

Ingredients (%)	Diets								
	1	2	3	4	5	6	7	8	9
Yellow corn	65.0	62.0	59.0	56.0	53.0	59.2	53.4	47.6	41.8
Soybean meal	24.8	23.8	22.8	21.8	20.8	23.6	24.4	24.2	24.0
Concentration*	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Treated DPM	0.0	3.0	6.0	9.0	12.0	0.0	0.0	0.0	0.0
Row DPM	0.0	0.0	0.0	0.0	0.0	3.0	6.0	9.0	12.0
Vegetable oil	0.0	1.0	2.0	3.0	4.0	2.0	4.0	6.0	8.0
Sand	0.0	0.0	0.0	0.0	0.0	1.0	2.0	3.0	4.0
Di. Methionine	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Calculated analysis									
Crude protein (%)	21.8	21.8	21.8	21.8	21.8	21.8	21.8	21.8	21.8
ME (kcal/kg)	2990	2990	2990	2990	2990	2990	2990	2990	2990
C/P ratio	137.2	137.2	137.2	137.2	137.2	137.2	137.2	137.2	137.2
Ether extract (%)	2.8	2.75	2.69	2.64	2.58	2.62	2.34	2.26	2.09
Crude fiber (%)	3.05	3.16	3.28	3.40	3.51	3.16	3.27	3.38	3.49
Calcium (%)	0.94	1.05	1.16	1.26	1.37	1.05	1.16	1.27	1.38
Avi. Phosphorus (%)	0.45	0.49	0.53	0.57	0.61	0.49	0.52	0.56	0.60
Methionine (%)	0.46	0.48	0.50	0.52	0.54	0.48	0.49	0.50	0.51
Lysine (%)	1.16	1.32	1.46	1.60	1.74	1.30	1.45	1.60	1.75

Concentrate: Crude protein (%) 52.00, ME (Kcal/kg) 2440, Ether Extract (%) 2.00, Crude fiber (%) 3.00, Calcium (%) 7.50, Avi. Phosphorus (%) 3.50

RESULTS AND DISCUSSION

Chemical composition of poultry dropping and the fermentation products produced at the optimal conditions:

The chemical analysis of the raw DPM indicated that the moisture content and the crude protein were 10.2 and 19.10%, respectively. True protein and NPN are of comparable values and each contributed about 50% of the CP. In addition, DPM contained 18.2% ash, 7.9% CF, 1.7% EE and relatively high uric acid content (7.2%). The elements content of the total ash (sulphated) are proved that Cu is the major element (about, 7.5%) followed by Ca and P which formed about 3.7 and 1.5%, respectively. Na, Zn, K and Mn were found in trace amounts (less than 0.1%). However, Mn was the only detected trace elements (0.03%) in DPM ash (Table 1).

The data given in Table 1 intend to compare between the chemical composition of the original DPM and the fermented sample. It is clear that comparable contents were detected in both CF and ash contents of samples, DPM and the fermentation marc (unfermented DPM+ yeast growth). On the other hand, the CP content increased from 19.1 for DPM to 24.9% for fermentation marc. The increase in CP content of the marc resides mainly in the true protein yield which increased by about 69%, while, NPN showed about 8.4% decrease. The EE content of the fermentation marc showed about 41.0% increase as compared to DPM. However, the uric acid content decreased to be as little as 0.3%, i.e. about 96.0% of the uric acid content of DPM was hydrolyzed as a result to the fermentation process.

The index of protein quality is often expressed in term of the EAAs content of the protein. Table 4 presents AAs

contents of DPM and fermentation marc proteins as compared with the Food Agricultural Organization (FAO) EAAs reference values.

As shown in the data, the amounts of AAs of the fermentation marc proteins were greatly higher than those of the DPM, except for glycine, histidine and tyrosine which showed lower values. The fermentation marc (unfermented DPM + yeast growth) has a profile that compares favorably with the FAO values or higher as regards isoleucine, leucine, lysine, phenylalanine, threonine, tyrosine and valine, except for methionine which showed lower values. However, the fermentation marc proved to lack cystine. As far as AAs of the soybean meal and the fermentation marc proteins are concerned, comparable amounts or higher of histidine, isoleucine, lysine, methionine, phenylalanine and threonine, tyrosine and valine. However, relatively higher arginine and leucine as well as cystine contents were present in soybean meal.

Several research reports that composition of DPM is highly variable, depending upon the diet composition, bird's age, DM content, storage methods, physiological status of the birds, the environmental conditions under which the droppings are kept and drying temperature (Hodgetts, 1971; El-Deek and Raya, 1983; Malone, 1992; Patterson *et al.*, 1998; Chamblee and Todd, 2002 and Coufal *et al.*, 2006).

Uric acid hydrolysis by the experimental yeasts:

Screening studies were made on 30 different yeasts to test their ability to utilize uric acid as the sole source of carbon and nitrogen. Only seven strains of the tested

Table 4: Amino acid content (g/100g true protein) of the poultry dropping and the fermentation marc as compared with the FAO reference and soybean meal protein

Amino acid	Poultry dropping	Fermentation marc	FAO	Soybean Meal
Alanine	5.09	7.02		
Arginine	2.01	3.55		7.20
Aspartic acid	7.36	9.24		
Cystine	0.00	0.00	2.00	1.40
Glutamic acid	11.15	14.55		
Glycine	5.49	4.79		
Histidine	3.018	2.59		2.50
Isoleucine	3.99	4.59	4.20	5.70
Leucine	2.04	5.50	4.80	7.70
Lysine	5.49	6.20	4.20	6.50
Methionine	0.86	1.08	2.20	1.40
Phenylalanine	3.17	6.02	2.80	5.10
Proline	5.57	7.70		
Serine	3.68	3.70		
Threonine	3.91	3.47	2.80	4.00
Tyrosine	3.52	2.27	2.80	2.70
Valine	3.92	7.02	4.20	5.00

Table 5: List of the tested yeasts and their sources

Yeast	Source
<i>Candida tropicalis</i> Y-21	NRRL (Northern Regional Research Laboratory, Peoria, III, USA)
<i>Candida utilis</i> Y-900	NRRL
<i>Rhodotorula rubra</i> 70403	DSM (Deutsche Sammlung Von Mikroorganismen)
<i>Saccharomyces cerevisiae</i> 1848	DSM
<i>Saccharomyces cerevisiae</i> Y-2034	NRRL
<i>Saccharomyces cerevisiae</i> Y-2235	NRRL
<i>Saccharomyces cerevisiae</i> Y-1347	NRRL

Table 6: Uric acid hydrolysis and true protein yield of the tested yeasts

Yeast	Final pH	True protein	Residual Uric acid (mg/100 ml)	Uric acid Recovery (%)
<i>Candida tropicalis</i> Y-21	7.8	12.70	27.00	81.40
<i>Candida utilis</i> Y-900	7.7	11.60	27.80	80.80
<i>Rhodotorula rubra</i> 70403	8.4	9.90	52.60	63.70
<i>Saccharomyces cerevisiae</i> 1848	8.5	9.80	56.80	60.80
<i>Saccharomyces cerevisiae</i> Y-2034	7.6	11.20	65.10	55.10
<i>Saccharomyces cerevisiae</i> Y-2235	7.6	11.20	72.20	50.20
<i>Saccharomyces cerevisiae</i> Y-1347	7.6	12.00	91.80	36.70

yeasts proved to have the ability to utilize uric acid as the sole source of carbon and nitrogen, while the rest failed to grow (Vuori and Nasi, 1977). These yeasts were two species of *Candida* (*Candida utilis* and *Candida tropicalis*), three strains of SC and one isolate of both *S. uvarum* and *Rhodotorula rubra* (Table 5).

From the results given in (Table 6), it is clear that the tested yeasts exhibited different capacities to hydrolyze uric acid and to produce protein when grown in DPM containing medium. The discrepancy of uric acid hydrolysis and of the protein yields was observed among the different species of one genus and even among the different isolates of the same yeast species. The lowest uric acid hydrolyzing capacity (36.7%) was maintained with *Candida tropicalis* and the highest hydrolyzing efficiency (81.4%) was obtained with *Candida utilis*. On the other hand, the two species were of almost equal protein yielding capacity. The tested isolates of SC proved to be with different efficiencies to

hydrolyze uric acid and to produce protein. Thus, SC 1848 gave as low as 9.8% true protein and with uric acid hydrolyzing capacity of about 61%, while, SC Y-2235 (NRRL) produced higher protein yields (11.2%) and moderate uric acid hydrolysis (about 50%).

The uric acid hydrolysis capacity (expressed as residual uric acid and uric acid recovery) by the tested yeasts seems not to be consistently related to the protein yields. *R. rubra* showed relatively high uric acid hydrolyzing activity (about 64%) and lower protein yields (about 9.9%). On the contrary, *C. tropicalis* gave higher protein value (12.0%) and lower uric acid hydrolyzing activity (about 37%). A similar finding can also be traced among the tested species and isolates of *Saccharomyces* of these latter yeasts, *S. uvarum* gave both higher protein yield (11.6%) and uric acid hydrolysis (80.8%), while SC Y-2235 (NRRL) was with higher protein output (11.2%) and lower uric acid hydrolyzing capacity (50.2%). *Candida utilis* Y- 900 (NRRL) offered

the highest protein yield (12.7) and the highest efficiency to hydrolyze uric acid. Therefore, it was selected for the further experimentation involved in the present work. These favorite criteria justify the selection of *C. utilis* as the experimental organism of choice throughout the present investigation, which was directed to eliminate uric acid from DPM together with the achievement of better protein production. In accordance with these findings *C. utilis* was used for uricase production by Sadaji *et al.* (1988). Certain species of yeast appear to be of greater interest and approved for specific use as feed additives, natural flavorings, nutritional ingredients, etc. *C. utilis* is one of the commercially important yeasts for SCP (Coorey *et al.*, 1980). Uric acid was utilized as the sole carbon source of yeast (Middelhoven and Hoogkamerteniet, 1984) and as a sole nitrogen source (Norbert and Heinrich, 1988).

Factors affecting the optimum yield of fermentation products

Effect of incubation period: The biomass (in terms of marc dry weight) was found to be accelerated regularly during the first four days of fermentation. The rate of growth was increased by about 23.2% during the period that lasted from one to 4 days. At the end of the 4th day, the highest protein yield (14.0%) was recorded. Therefore, the growth and the protein yields decreased gradually indicating that the decline phase of growth and ensued (Table 7).

As for the residual acid, a parallel decrease was estimated as the fermentation period extended from one to 6 days. Thus, about 72.6% decreased in the residual uric acid was recorded under the latter conditions, while the efficiency of hydrolyzing uric acid (uric acid recovery) showed about 2.2 fold increase under these conditions. As the fermentation period increase (> 6 days) a detectable decrease in uric acid hydrolysis activity was noticed. The results also revealed that shorter growth periods (<5 days) favor protein yields while longer growth times (>5 days) were concomitant to higher uric acid hydrolysis.

The pH value of the fermentation medium (initially adjusted to pH 6.0) showed a slight change with the extension of the inoculation period. This due to an internal pH regulation brought about by the medium formulation during that period of the fermentation process.

The uric acid hydrolysis activity reached its maximum value at the end of the 6th days. At that period, relatively higher protein yield were also obtained. Thus, the residual uric acid/100 ml medium (24.7 mg), uric acid recovery (83%) and protein yield (13.5%) were recorded after 6 days of incubation.

The present results are in accordance with the findings of other researchers (Litchfield, 1979 and Ghonem *et al.*, 1986) which indicated that maximal protein yields were

obtained at the exponential phase of yeast growth. In addition, Demnerova *et al.* (1986) reported that the inducible property of uricase and the presence of uric acid or some other inducer in the medium are necessary for enzyme formation, i.e. uricase productivity needed lower fermentation periods and only the uric acid hydrolysis increased with the extension of the fermentation period.

Effect of poultry dropping level (solid/liquid): The results of Table 8 indicated that within the DPM levels (1-6%); a parallel biomass increase (expressed in terms of marc dry weight) was in agreement with raising the DPM concentration, followed by a detectable decrease at higher DPM levels (8-10%). The protein yield was regularly increased as the DPM increased from one to 4%. Thus, about 25% increase in protein yield was detected as the DPM rose from one to 4%. A drop in the protein percentage to reach 13% at 10% DPM level followed this. As the hydrolysis of uric acid was considered, increase in the hydrolysis activity were recorded as the DPM concentration increased from 1-4% to reach as high as 88% at 4 DPM level. Higher DPM concentrations (5-10%) induced lower hydrolyzing activity of uric acid by the experimental yeast. At DPM, level of 2 g/50 ml medium, moderate marc dry weight (2.10 g/flask) and highest protein percentage (16.0%) were recorded. At the same conditions, the yeast activities to utilize (hydrolyze) uric acid recorded the highest value (88%). Thus, 2 g/50 ml medium of DPM found was optimum for protein production and uric acid hydrolysis activity by the tested yeast under the specified fermentation conditions.

Effect of inoculum size: Table 9 exhibits the effect of the inoculum size of *Candida utilis* on protein production and uric acid hydrolysis. An increase in the protein yield was noticed on elevating the inoculum size from 2-8%. The protein content of the dried marc was 15.2% with 2% inoculum, which was raised up to 17.3% with 8% inoculum, recording about 13.8% increase. At the latter conditions, as high as 93.3% of the uric acid content of the fermentation medium was utilized by the experimental yeast, indicating higher yeast activities to hydrolyze uric acid and to biosynthesize proteins. Increasing the inoculum size more than 8% was concomitant with lower protein yield and with reduced uric acid hydrolyzing activity. Increasing inoculum volume, as the source of the enzyme system was necessary to obtain higher metabolic activities (Garg and Neelakantan, 1981; Ghonem *et al.*, 1991).

Effect of pH: It is evident, from the results presented in Table 10 that protein production and the hydrolysis of uric acid produced by the experimental yeast responded differently to the reaction of the pH in the fermentation

Table 7: Uric acid hydrolysis and true protein yield of *Candida utilis* during 7 days of incubation

Growth Period (day)	Final pH	Marc dry weight (g)	True protein	Residual Uric acid (mg/100 ml)	Uric acid Recovery (%)
1	7.4	1.98	12.20	104.70	27.80
2	7.5	2.16	12.60	76.00	47.60
3	7.5	2.31	13.50	60.50	58.30
4	7.6	2.44	14.00	48.80	66.30
5	7.6	2.38	13.90	31.30	78.40
6	7.7	2.34	13.50	24.70	83.0
7	7.8	2.21	12.7	27.00	81.40

Table 8: Uric acid hydrolysis and true protein yield of *Candida utilis* as affected with poultry dropping level

Poultry dropping level (%)	Final pH	Marc dry weight (g)	True protein	Residual Uric acid (mg/100 ml)	Uric acid Recovery (%)
1	6.9	1.70	12.80	14.70	79.70
2	7.7	2.70	13.50	24.70	83.00
3	7.7	3.60	14.50	30.20	86.10
4	7.8	4.20	16.00	35.10	88.00
5	7.8	5.30	15.00	52.20	85.60
6	7.8	6.20	14.30	77.80	82.10
8	7.9	7.62	13.60	116.00	80.00
10	7.9	9.50	13.00	153.70	78.80

Table 9: Uric acid hydrolysis and true protein yield of *Candida utilis* as affected with inoculum size

Inoculum size (%)	Final pH	Marc dry weight (g)	True protein	Residual Uric acid (mg/100 ml)	Uric acid Recovery (%)
2	7.8	3.91	15.20	38.60	86.70
4	7.8	4.20	16.00	35.10	88.00
6	7.7	4.30	16.50	28.40	90.20
8	7.7	4.05	17.30	19.40	93.30
10	7.8	3.85	16.30	29.60	89.80
12	7.8	3.80	15.70	34.50	88.10

Initial uric acid = 290 mg/100 ml

Table 10: Uric acid hydrolysis and true protein yield of *Candida utilis* as affected with the initial pH value of the fermentation medium

Initial pH	Final pH	Marc dry weight (g)	True protein	Residual Uric acid (mg/100 ml)	Uric acid recovery (%)
2	0.0	0.0	0.0	0.0	0.0
3	3.7	3.23	13.90	75.50	74.00
4	5.00	3.30	14.60	42.60	85.30
5	6.1	3.76	15.10	32.80	88.7
6	7.7	4.06	17.40	19.20	93.40
7	7.8	3.85	18.00	24.40	91.50
8	8.0	3.80	15.20	36.80	87.30
9	8.2	3.71	13.60	49.90	82.80

medium. Thus, although the adjustment of the medium to pH 7.0 seems to be optimal for protein biosynthesis, yet uric acid utilization reached a maximum at pH 6.0. Thus, at pH 7.0 maximal protein yield (18.0%) was recorded, while the best hydrolysis activity of uric acid was achieved at pH 6.0. At the latter pH (6.0), value the highest growth yield of *C. utilis* was estimated at lower or higher pH less biomass was recorded. However, the tested yeast failed to grow at pH 2.0 as traced by microscopic examinations.

It is note worthy that neutral pH (6-7) are more favorable to the different metabolic activities under investigation than the acidic or alkaline pHs. Thus, a parallel decrease in the fermentation products (growth yields, protein production and uric acid hydrolysis) was

recorded as the pH value lowered from 6-3 or elevated from 7-9. In accordance with our results, it was reported (Vuori and Nasi, 1977) that pH 7 was optimal for higher colonies number of yeast per gram uric acid than pH 4. Uricase stability is higher at neutral and alkaline pHs (Sadaji *et al.*, 1988).

Effect of medium size: The results given in Table 11 indicated that there was a reversible proportional between protein percentage and medium volume/flask. Thus, as the medium volume increases a parallel decrease in the protein yields recorded. Under the experimental conditions, as the size of the medium/250 ml Erlenmeyer flask increased from to 10-25 ml the protein percentage decreased by about 19.4%. On the

other hand, elevating medium volume from 25-75 ml/flask was accompanied by a detectable increase in uric acid hydrolyzing capacity. Thus, the experimental yeast utilized as much as 95.4% of the uric acid content. A negative correlation between protein yields and increased medium volume/flask indicating that protein biosynthesis by *C. utilis* needed high aeration. At only 10 ml medium/flask, the maximal protein yield was recorded (18.6%), whereas at 125 ml medium volume/flask, 15.0% protein was observed. Medium size of 75 ml (containing 3 g DPM)/flask was optimal for maximal uric acid hydrolysis (95.4%) and for moderate true protein yield (16.9%).

In harmony with our finding, it was reported (Reed and Pepler, 1973; Ghonem *et al.*, 1986) that high aeration allowed maximal yeast propagation and protein yields. Where the increase of air (oxygen) supply favors oxidative reactions essential for growth and inhibits the anaerobic assimilation of the medium constituents. However, the uric acid hydrolysis activity needed less aeration. At 75 ml medium/flask, a maximal uricase activity was indicated where about 95.4% of the uric acid was eliminated. This may ascribed to the relatively high oxygen content of uric acid and consequently less oxygen was needed in its breakdown by uricase as an oxidoreductase (Lehejkova *et al.*, 1986).

Effect of the mineral salts: The results presented in Table 12 revealed that none of the ingredients of mineral salts of the fermentation medium has a significant influence on the uric acid utilization (hydrolysis) by the yeast under the tested fermentation conditions. Thus, all

of the tested salts did not effect uric acid assimilation and almost equal uric acid hydrolyzing activities were recorded in all cases. However, the protein yield responded differently to the omitted salt. The protein biosynthesis by *C. utilis* required addition of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, $\text{Zn SO}_4 \cdot 7\text{H}_2\text{O}$ and to a lesser extends $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ in the fermentation medium. The omission of all the mineral salts, i.e. the fermentation medium contained only 3 g DPM/ 75 ml distilled water, decreased protein yields by 4.1%. Thus, it is safely to use a fermentation medium consists only of 40 g DPM/1 distilled water without salt addition, for maximal uric acid assimilation and good protein yields.

Effect of cane-molasses as a carbon source: The results presented in Table 13 indicated that cane molasses addition was accompanied by parallel increase in yeast growth. Thus, about 33% increase in marc dry weight was recorded as cane-molasses concentration increased from 0.0-2%. However, inoculation of the fermentation medium (DPM in tap water) with different levels of cane molasses resulted in a detectable decrease in true protein outputs, where the protein content decreased by about 17.3% as cane molasses increased from 0.0-20 g/l. It was reported that relatively lower C/N favored protein biosynthesis (Litchfield, 1979). Also, El-Refai *et al.* (1985) reported that increased molasses level within certain limits was accompanied with increased yeast growth yields. As the uric acid content of the fermentation medium was considered, a parallel decrease in uric acid hydrolysis capacity was resulted as cane molasses level

Table 11: Uric acid hydrolysis and true protein yield of *Candida utilis* as affected by medium volume/flask

Medium Volume (ml)	Marc dry weight (g)	True protein	Residual Uric acid (mg/100 ml)	Uric acid Recovery (%)
10	0.40	18.60	2.70	90.60
25	1.01	18.20	5.60	92.30
50	2.00	17.30	9.60	93.40
75	3.05	16.90	10.00	95.40
100	4.07	16.20	20.60	92.9
125	5.10	15.00	37.00	89.80
Initial pH = 6.0	Final pH = 7.2			

Table 12: Uric acid hydrolysis and true protein yield of *Candida utilis* as affected with the elimination of medium ingredients of salts one at time

Initial pH	Final pH	Marc dry weight (g)	True protein	Residual Uric acid (mg/100 ml)	Uric acid Recovery (%)
Non (Basal)	7.7	3.05	16.90	10.00	95.40
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	7.5	2.85	16.70	10.80	95.00
$\text{Na}_2\text{HPO}_4 \cdot \text{H}_2\text{O}$	7.6	2.75	15.60	13.40	93.80
KH_2PO_4	7.7	2.95	15.90	10.50	95.20
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	7.7	2.65	16.90	9.90	95.40
$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	7.7	2.55	14.90	13.5	93.80
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	7.7	2.95	15.80	10.70	95.10
$\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$	7.7	2.40	15.40	13.20	93.90
$\text{AZnSO}_4 \cdot 7\text{H}_2\text{O}$	7.7	2.20	14.80	13.00	94.00
All*	7.7	2.20	16.20	10.20	95.30

*The tested salts were removed totally

Initial uric acid = 217.5 mg/100 ml

Table 13: Uric acid hydrolysis and true protein yield of *Candida utilis* as affected with the addition of different concentrations of can molasses

Can Molasse Level (%)	Final pH	Marc dry weight (g)	True protein	Residual Uric acid (mg/100 ml)	Uric acid Recovery (%)
0.0 (Basal)	7.7	2.20	16.20	10.20	95.3
0.2	7.5	2.72	15.60	14.20	93.20
0.4	7.5	2.61	15.0	19.90	90.90
0.6	7.6	2.71	14.70	23.20	89.90
0.8	7.6	2.73	14.30	26.40	87.90
1.0	7.7	2.84	13.70	29.30	86.50
1.5	7.7	2.91	13.50	32.20	85.20
2.0	7.6	2.93	13.40	32.50	85.00

Table 14: Uric acid hydrolysis and true protein yield of *Candida utilis* growth on poultry dropping water medium for different incubation periods

Incubation Periods (day)	Marc dry weight (g)	True protein	Residual Uric acid (mg/100 ml)	Uric acid Recovery (%)
2	1.85	15.80	29.30	86.50
4	2.03	16.00	17.60	91.90
5	2.11	16.10	14.30	93.40
6	2.20	16.20	10.20	95.40
8	2.10	15.70	10.80	95.00
9	2.02	15.00	10.90	95.00

Table 15: Mean Total Protein Efficiency (TPE) value and the performance of chicks at second week of age used in the Total Protein Efficiency (TPE) experiment

Test Protein	TPE value	Performance		
		BWG (g)	FI (g/chick/period)	FCR (g feed/g gain)
Control	2.72±0.09 ^a	537.9±13.2 ^{ab}	1100.1±48.9	2.09±0.10 ^b
Poultry manure	1.73±0.10 ^b	434.1±17.6 ^b	1395.9±24.7	3.24±0.18 ^a
Treated poultry manure	2.80±0.04 ^a	603.5±14.8 ^a	1195.9±5.5	1.98±0.03 ^b
Significant	**	**	NS	**

Means with different superscript differ significantly ($p \leq 0.05$).

NS = Not Significantly

** = highly significant

increased. Thus, about 3.2 fold increased in uric acid content was recorded as cane molasses increased from 0.0-2% and the uric acid recovery decreased from 95.3-85% under the same conditions. Therefore, cane molasses was required for increasing growth yields only and was in conducive to protein biosynthesis as well as uric acid hydrolysis by *Candida utilis* under the tested fermentation conditions.

Time course uric acid hydrolysis and protein production by *C. utilis* cultivated under the optimal fermentation conditions: The result given in Table 14 showed that the growth, expressed as a marc dry weight, reached maximum value after 6 days with a slight decrease with increasing the growth period to 8 or 9 days. Maximal true proteins yield was also attained after 6 days incubation, while decreased as the fermentation period extended to 8 and 9 days. On the other hand, the uric acid content of the culture medium considerably decreased as the incubation period elongated to reach about only 4.7% after 6 days of fermentation. At the higher incubation periods (8 and 9 days) comparable uric acid content were observed.

Biological evaluation of raw and treated poultry manure

Total proteins efficiency method: The raw and treated DPM was incorporated in the rations to contribute 12%

crude protein, (Table 15). The results showed highly significant differences ($p < 0.01$) among treatments on the average of TPE values. However, Duncan's test revealed that TPE values were significant higher than the corresponding values for untreated DPM. The BWG was significantly (603.5 g) heavier of broiler chickens fed the treated DPM and followed by the control group (537.9 g). The results also showed that BWG was lighter of broilers fed raw DPM (434.1 g). The FI ranged from 1395.8 g for ration contained raw DPM to 1100 g for the control group. However, difference was not statistically different.

Dietary treatments had a significant effect on FCR and the poorest FCR was observed for broiler chickens fed ration contained untreated DPM. These results were expected since they consumed the highest amount of feed. The results also showed that the inclusion of treated DPM significantly improved FCR when compared to the corresponding value for the untreated DPM. These results suggested that treated DPM is a good source of protein supplement to growing chicks owing to its high content of protein, essential and non-essential AAs, since it recorded the highest values for TPE, BWG, FCR and lowest value for FI.

Feeding trial: The effect of feeding four levels of untreated and treated DPM with yeast (*Candida utilis*), on

the expense of corresponding levels of soybean meal on growth of chickens from one day to 4 weeks of age is shown in Table 16. The results indicated that treating DPM with yeast had a significant effect on growth of chickens, regardless of the level of these two products. Growth of chickens fed diets containing the treated DPM, over all the tested period, were significantly higher than those given the diet containing the untreated DPM. Moreover, growth of chickens given treated DPM and those of the control birds were not statistically different during all tested growth period (Table 16).

Increasing the level of DPM in the diet, whether the DPM was treated with yeast or untreated above 9% had an adverse effect on growth of the chickens. However, it is interesting to note that at the fourth week of the experimental period, growth of chickens given diet containing treated DPM at 12% level was statistically equal to those given diets with lower levels of treated DPM along with those given the control diet.

The present results are in agreement with the data obtained by Stapleton and Biely (1975) and Gunningham (1976) who demonstrated that increasing level of raw hen manure in the diet of young chicks decreased growth. El-Deek and Raya (1983) also found that increasing the level of manure up to 15% adversely effect growth compared to the control group. The results reported herein demonstrated that the decrease in growth due to feeding untreated DPM could be largely overcome by treating the manure with yeast, Table 16.

Data concerning the nutritive value of manure treated with yeast are not available in the literature. However, the nutritional evaluation of other SCP grown on poultry manure was discussed by Shuler *et al.* (1977) and Austic *et al.* (1978). They showed that SCP could be used satisfactory as a part of the chickens diet substituting for much of the conventional high protein feed supplements such as soybean meal. They also showed that normal growth occurred at 10% level, but

above the 20% and 30% level the growth was depressed. El-Deek (1979) also showed that increasing the level of manure treated with mycelium (5, 10 or 15%) decreased growth and attributed this reduction to poor palatability of the tested product.

Shannon and McNab (1972), working with yeast grown on n-paraffin, found that live weights at 4 weeks of age were reduced when chicks were given 200 g of yeast/kg diet compared with 100 gm/kg or less. They attributed the reduction in growth to the presence of nucleic acids in the yeast, which reduced the true protein content of the yeast. Hewitt and Labib (1978) also concluded that yeast in a diet marginal in total protein decreased growth compared to expected from its composition. The above mentioned findings supported partially the present results which showed that the inclusion of above 9% treated DPM with yeast caused depression in growth. However, 12% DPM did not affect growth at 4 week of age compared to the control diet.

There were no significant differences in FI due to inclusion of untreated or treated DPM at different levels (Table 17). Similarly, El-Deek and Raya (1983) reported no significant differences in FI of chickens given rations containing DPM at a level up to 15%. On the other hand, Tibin and Koko (1989) found that inclusion of 4 or 8% sun- DPM significantly decreased FI. This contradictory in the results could be due to differences in the nature, methods employed in the preparation and/or levels of poultry manure used by those workers.

Feed conversion ratio was significant different due to feeding treated or untreated DPM, regardless of level of inclusion (Table 18). At the first week of the experiment, chickens given the treated or untreated DPM, utilized feed less efficiently than the control ones. At the second week, significant differences in FCR between the groups given treated and untreated DPM showed better utilization of the diets containing treated DPM and this

Table 16: Weekly means of body weight gain (g/bird) for broiler chicks used in the growth experiment

Ration	One day	First week	Second week	Third week	Fourth week
Control diet	40.8±0.9	107.8±3.3 ^a	198.5±7.3 ^a	239.2±11.7 ^a	370.1±16.1 ^{abc}
Treated diets					
Level (%)					
3.0	39.9±0.9	102.5±3.3 ^{ab}	190.6±7.3 ^{ab}	244.7±11.7 ^a	399.6±16.1 ^a
6.0	40.1±0.9	107.8±3.3 ^a	196.3±7.3 ^a	240.0±11.7 ^a	386.2±16.1 ^{ab}
9.0	42.1±0.9	108.3±3.3 ^a	197.6±7.3 ^a	238.1±11.7 ^a	410.6±16.1 ^a
12.0	40.1±0.9	82.6±3.3 ^c	175.2±7.3 ^{bc}	222.1±11.7 ^b	412.3±16.1 ^a
Mean	40.6±0.5	100.3±1.7	189.9±3.7 ^A	236.2±5.9 ^A	402.1±8.9 ^A
Untreated diets					
Level (%)					
3.0	40.6±0.9	100.8±3.3 ^{ab}	158.9±7.3 ^{cd}	178.6±11.7 ^d	334.2±16.1 ^d
6.0	41.8±0.9	104.9±3.3 ^a	174.7±7.3 ^{bc}	203.6±11.7 ^{bc}	362.8±16.1 ^{bcd}
9.0	39.6±0.9	97.4±3.3 ^{bc}	195.7±7.3 ^a	208.7±11.7 ^{bc}	384.8±16.1 ^{ab}
12.0	40.6±0.9	94.6±3.3 ^{bc}	128.2±7.3 ^d	186.4±11.7 ^{cd}	361.1±6.1 ^{bcd}
Mean	40.6±0.5	99.5±1.7	164.4±3.7 ^B	194.3±5.9 ^B	360.7±8.9 ^B
Significant					
Treatments	NS	NS	**	**	**
Levels	NS	**	**	**	**

Means with different superscript, within each column, differ significantly ($p \leq 0.05$). NS = Not Significantly

** = highly significant ($p \leq 0.01$)

Table 17: Weekly means of feed intake (g/bird) for broiler chicks used in the growth experiment

Ration		First week	Second week	Third week	Fourth week
Control diet		205.8±6.7	370.3±28.8	519.9±51.3	845.7±55.9
Treated diets					
Levels (%)	3.0	200.4±6.7	398.9±28.8	504.8±51.3	855.9±55.9
	6.0	203.4±6.7	401.4±28.8	500.2±51.3	869.2±55.9
	9.0	206.4±6.7	406.2±28.8	530.9±51.3	872.8±55.9
	12.0	193.5±6.7	400.3±28.8	515.8±51.3	870.3±55.9
Mean		200.9±3.4	401.7±14.4	512.9±25.6	867.1±27.9
Untreated diets					
Levels (%)	3.0	202.7±6.7	401.3±28.8	430.3±51.3	795.6±55.9
	6.0	204.9±6.7	378.5±28.8	470.7±51.3	803.2±55.9
	9.0	198.3±6.7	394.1±28.8	487.4±51.3	809.7±55.9
	12.0	195.4±6.7	350.4±28.8	491.9±51.3	800.3±55.9
Mean		200.4±3.4	381.1±14.4	470.1±25.6	802.2±27.9
Significant					
Treatments		NS	NS	NS	NS
Levels		NS	NS	NS	NS

Table 18: Weekly means of feed conversion ratio (g feed/ g gain) for broiler chicks used in the growth experiment

Ration		First week	Second week	Third week	Fourth week
Control diet		1.91±0.01 ^c	1.86±0.08 ^d	2.17±0.01 ^{cd}	2.29±0.11
Treated diets					
Levels (%)	3.0	1.96±0.01 ^b	2.09±0.08 ^{cd}	2.06±0.01 ^d	2.24±0.11
	6.0	1.89±0.01 ^c	2.05±0.08 ^{cd}	2.08±0.01 ^d	2.25±0.11
	9.0	1.91±0.01 ^c	2.06±0.08 ^{cd}	2.23±0.01 ^{cd}	2.13±0.11
	12.0	2.35±0.01 ^a	2.29±0.08 ^{bc}	2.32±0.01 ^{bc}	2.11±0.11
Mean		2.02±0.01	2.02±0.04 ^b	2.17±0.05 ^b	2.16±0.05
Untreated diets					
Levels (%)	3.0	2.01±0.01 ^{ab}	2.55±0.08 ^{ab}	2.42±0.01 ^{ab}	2.38±0.11
	6.0	1.95±0.01 ^b	2.16±0.08 ^{bcd}	2.31±0.01 ^{bc}	2.21±0.11
	9.0	2.04±0.01 ^{ab}	2.01±0.08 ^d	2.34±0.01 ^{bc}	2.10±0.11
	12.0	2.06±0.01 ^{ab}	2.73±0.08 ^a	2.64±0.01 ^a	2.22±0.11
Mean		2.02±0.01	2.37±0.04 ^a	2.43±0.05 ^a	2.23±0.05
Significant					
Treatments		NS	**	**	NS
Levels		**	**	**	NS

Means with different superscript, within each column, differ significantly ($p \leq 0.05$). NS = Not Significantly

** = highly significant ($p \leq 0.01$)

was clear only at 3 and 12% levels. However, both groups utilized feed less efficiently than the control ones. At the third week, groups fed untreated DPM utilized feed less efficiently compared to the control group and that fed diets containing treated DPM except for 9% level. In general, increasing the level of inclusion above 9% impaired feed utilization by chicks consuming these diets during the first periods of experimental. At the end of the experiment (fourth week of age), there were no significant differences among different treatments and levels in FCR.

Similarly, Sloan and Harmes (1973) demonstrated that there were a linear negative relationship between growth and FCR due to inclusion different levels of laying hen manure in the young chickens diets. On the other hand, Bhargava and O'Neill (1975) showed that feeding broiler chickens diets containing DPM resulted in an increase in FCR. This discrepancy in the results could be due to using different levels of DPM and/or the nature and method employed in preparing the product.

No documented reports could be located concerning the nutritive value of yeast protein grown on poultry manure. Yet, Hewitt and Labib (1978) evaluated the nutritional potential of yeast grown on n-paraffin. They found that FCR of diet containing yeast was worse than the control group. They attributed this reduction in FCR to the presence of nucleic acid and/or lower true protein content and slightly lower efficiency of utilization of yeast protein. On the other hand, Schwarz (1960) working with yeast grown on n-paraffin indicated that the poor FCR of diets containing yeast may be due to its inadequate amounts of vitamin E and selenium.

Conclusion: The nutritional estimates of the tested two products (raw DPM and treated DPM with yeast) whether based upon the feeding trial or TPE indicated that treating DPM with yeast improved its nutritive value and it could be incorporated up to 9% in broiler diets without adverse effect on growth up to 4 weeks of age. This improvement in nutritive quality of DPM due to yeast

treatment was associated with increase true protein content, showing the ability of *Candida utilis* to convert DPM to high quality protein supplement and eliminate its uric acid content and environmental pollution.

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