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ASSESSMENT OF NUTRITIONAL VALUE OF SINGLE CELL PROTEIN FROM WASTE ACTIVATE SLUDGE

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With the fast growing population of Southern Africa, pressure is exerted on the feed industry to produce enough animal feed to meet the region's nutritional requirements. Due to the rising cost of meat, people in poor communities of Southern Africa - especially children - suffer from severe malnutrition. The national requirement of protein supplement for South Africa is 90,000 metric tonnes per year. On the other hand, the protein value of sludge produced in treatment plants in Gauteng province alone (1/8 of the countries population) is approximated at 95,000 metric tonnes per year, enough to replace the commercial supply for the whole country. Sludge from wastewater treatment plants treating domestic wastewater is shown to contain protein in a ratio of 1:2 against fishmeal – the common protein supplement for livestock. The protein content in sludge and fishmeal is validated in this study. A method for extracting toxic heavy metals and its impact on the concentration of amino acids is also investigated.

1. INTRODUCTION

The South Africa is a developing country with a fast growing population. Therefore more animal feed is required to serve the escalating population. The current local animal feed production, however, is insufficient to cater for the demands. Therefore the country is dependent on importation of Fishmeal and other nutrients sources in order to meet the required needs. The cost of importation of these animal feeds is extremely high. The purpose of this project is to study the nutritional potential of Waste Activated Sludge (WAS) as a cheaper replacement of Fishmeal in animal feed.

The average wastewater treated in Gauteng Province (South Africa) alone amounts to approximately 210 MLD (766,500 ML/yr) with a resultant production of 229,950 tonnes of dry sludge annually, the protein content of which amounts to 95,000 tonnes per year. This exceeds the import requirements of fishmeal by approximately two times, which represents a worth of untapped nutritional and economic potential.

The final tailings of the sewage sludge are dealt with by soil application as fertilizers or buried in the ground, landfill, combustion and ocean dumping (Hwang et al., 2008). These methods require huge capital investments more than any other part of waste water treatment (Vriens et al., 1989; Hwang et al., 2008). Soil application of sludge is also rendered environmental unfriendly (Kasselman, 2004). However, WAS from sewage treatment plants depict enormous potential to be used for animal feed due to its large content of a range of important nutrients. This is supported by feeding experiments conducted earlier by others such as Vriens et al. (1989).

The sludge contains significant amount of mineral elements, vitamins, nucleic acids, and amino acid proteins, comparable to amounts present in whole egg, symba yeast sludge, soybean and fishmeal meal (Vriens et al., 1989). The ratio of mineral elements in sludge to that in the feedstock was also reported to be significantly higher (Vriens et al., 1989). This indicates that the sludge has sufficient minerals required by animals. Sludge was also found to be a very good source of vitamins, particularly vitamin B12 (Vriens et al., 1989).

However, the level of heavy metal content is reported to be more than two orders of magnitude higher than the levels in conventional protein sources Vriens et al., 1989; Yoshizaki and Tomida, 2000; Hwang et al., 2008).

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Therefore it is recommended that the feed sludge be pre-processed to reduce heavy metal content to acceptable level. It was reported that washing of WAS with acids can lowers the toxic levels of heavy metals significantly (Yoshizaki and Tomida, 2000).
In this study, protein and heavy metal analysis is conducted on both the WAS from local sewage plants and Fishmeal to determine the comparability of the two protein sources. Additionally, a real-time pilot study was conducted under varying sludge:fishmeal replacement ratios. Growth rate data and cost implications are evaluated.

2. MATERIALS AND METHODS

2.1 Crude Protein Determination

Activated sludge samples were collected from Bavianspoort, Zeekoeigat and Rooiwal sewage works in Gauteng Province, South Africa, over a period of six months. The samples were collected once per week to measure the consistency of the crude protein content of the sludge with Nitrogen Gas Analyzer utilizing induction furnace and thermal conductivity (LECO FP-528) This method quantitatively determines the amount of nitrogen in all forms (ammonium, nitrate, protein and heterocyclic nitrogen) in botanical materials using an induction furnace and a thermal conductivity detector. Samples were ignited in an induction furnace at approximately 900°C in helium and oxygen environment in a quartz combustion tube. An aliquot of the combustion gases was passed through a copper catalyst to remove oxygen and convert nitrous oxides to N2, scrubbed of moisture and carbon dioxide, and nitrogen content determined by thermal conductivity. Total crude protein was calculated from the nitrogen content of the feed material, based on sample type. The method has a detection limit of 0.1% protein (dry basis) and is generally reproducible within 5 %.

2.2 Protein Determination Studies

Proteins can be extracted from the waste activated sludge using different method depending on what they were going to be utilised for. Different extraction methods were compared and the extraction method that was employed in this project was the one by Shier and Purwono (1994). This method solubilised proteins thermally at 120 ^{o}C (in an autoclave) or at 155 ^{o}C in a mineral oil heated on a hot plate. Proteins were extracted and then assayed using the Coomassie protein assay reagent (Sedmak and Grossberg, 1977).

2.3 Amino Acid Analysis

To compare the extracted proteins with the proteins from more conventional sources used to supplement nutrition of animals, amino acids analysis was performed on the extracted proteins using the Pico-tag method (Bidlingmeyer et al., 1984a). This method involved hydrolysis of the protein to yield free amino acids, pre-column derivatization of the sample and analysis by reverse phase HPLC.

2.4 Heavy Metal Analysis

The heavy metal content (Zn, Cu, Cd, Mn, and Pb) of the sludge was determined using Inductive Coupled Plasma (ICP). The samples 2g were digested for 30 min with concentrated nitric and perchloric acid, the sample was then cooled to room temperature and then diluted to 200 ml with distilled water and the samples were analyzed with ICP.

2.6 Heavy Metal Extraction

In this project two heavy metal extraction methods were compared. The first method involved using 1 N HCl, and the other method was extraction using organic acids (citric and oxalic acid). Both methods only involved shaking the sludge sample on the horizontal shaker with the appropriate acid (1 N HCl, 0.1 M citric or 0.1 M oxalic acid) for specified time, followed by filtering the samples. The supernatants and the residues were separately dried overnight at 110 ^{o}C and then burnt off at 550 ^{o}C. The samples were then digested in aqua regia,

and then diluted accordingly (see results). The heavy metal content in the supernatant and the residue sludge was determined by FAAS as indicated in the operating manual (Varian, 1979).

2.7 Amino Acid Analysis after Heavy Metal Extraction

To verify whether, when extracting heavy metals from WAS, proteins are extracted together with the heavy metals or not, amino acid analysis was also performed in the supernatant using the Pico-tag method (Bidlingmeyer et al., 1984b).

2.8 Pilot Project- Boiler Feeding

Feeding experiments were performed over a period of thirty five (35) days using five (5) sets of feed formulations. The broilers were weighed daily to measure the weight gained on daily basis. Coefficient of variation was then calculated and mortality rates were also recorded on daily basis.
House preparation was completed prior to chick arrival and that enabled placement of chicks into the brooding area immediately. The chicks were gently placed into the brooding area as soon as possible after arrival, being placed quickly and evenly on to paper and feed over the brooding area. Chicks were then weighed individually and the CV calculated at placement, this then gave a good indication of chick condition
Feed and water were made available immediately to the chicks at placement as at this time it is essential that there is enough feed and water space. To ensure this, supplementary feeders and drinkers were provided. Achieving the correct light intensity in the brooding area will help chicks to find the feed and water and stay active; 30-40 lux was used for the first 7days.
Small amounts of feeds continued to be distributed onto the paper frequently (every 2-3 hours), during the first 24 hours. Supplementary feeding stimulated and encouraged the chicks' instinctive pecking behaviour, by creating noise and movement as the chicks walk on the paper and the feed. After 3 days the birds started eating from the pans feeding system and the paper was removed. Feeders were emptied daily to prevent the build up of any fines/dust. During the first 7 days additional supplementary drinkers were provided and positioned to ensure that chicks do not have to travel more than 1m for access to water in the first 24 hours. The birds had unrestricted access to a supply of fresh, good quality clean water at all times.

2.9 Feed Formulations

When formulating feed rations, sludge volumes were made to be three times so as to have the same effect as fishmeal. This was prompted by the fact that when treating sludge with HCl, some of the intact proteins are denatured in the process.

2.10 Stocking Density

The experiments were designed for ten chicks per group and rearing chicks in overcrowded conditions does not deliver optimal biological or economical results. Initial stocking densities were made up of 10 chicks per 1 m^2 until approximately 4 days of age. After this, space can be progressively increased and access to the whole house was given from 14 days. The full area designated for 10 chicks was 20 m^2

2.11 Litter Management

Before chicks arrived, the floor was covered to an even depth of 5-10 cm with clean, dry litter material (wood shavings).

2.12 Measurement of Success

A good measure of successful chick start is crop fill. The objective was to have chicks with a full crop as soon as possible after placement. The aim was to have 80% of chicks with a full crop 8 hours after delivery and more than 95% of chicks with a full crop 24 hours after delivery. This ensures good early uniform body weight achievement and maintenance of uniformity. To assess crop fill, samples of 10 chicks were collected in the house

to establish whether chicks are finding food and water throughout the brooding area. Each chick was handled and the crop felt gently. In chicks that have found food and water, the crop will be full, soft and rounded. If the crop is full, but the original texture of the crumb is still apparent, the bird has not yet consumed enough water.

3. RESULTS AND DISCUSSION

3.1 Heavy Metal Content

Heavy metals found in sewage sludge include zinc, copper, manganese, lead, cadmium and few others (Vriens et al., 1989). Some of these have undesirable health effects such as carcinogenesis and toxicity to animal tissue. The drop in mass of the retained sample in the acid leaching process is indicative that heavy were indeed extracted from the sample. The results of the samples assay are summarized in Table 3 below.
As can be clearly seen, the extraction ratio of the metals was very low. This could be attributed to a number of factors. Firstly, the time allowed for the acid to react with the elements in the sludge sample was insufficient. This may have seen the mixed sample being isolated before the reaction goes to completion. The second factor that could have contributed to low extraction is the difference in densities of the metals in the powdered sample. Although extreme care was exercised to ensure that the powder is thoroughly mixed, heavier metals may have concentrated in one portion of the sample with others being found in the other. This can affect the distribution of the samples to the storage containers. The last factor could have been the higher content of acid insoluble metals. This would have meant that most of the samples were not dissolved in hydrochloric acid. It is also reported that in practice extraction in solid samples is lower than extraction in liquid samples (Kasselman, 2004).

Table 1. Quantities of heavy metals found in samples

Element (as Oxide)	Average Standard error	Dry Sludge (g/100g)	Fish meal (g/100g)	Leached Sludge* (g/100g)
TiO_2	0.03	0.85	0.06	1.12
Al_2O_3	0.10	3.79	3.98	4.02
Fe_2O_3	0.22	9.77	0.69	8.97
MnO	0.00	0.16	0.02	0.08
MgO	0.10	1.80	0.36	0.90
CaO	0.29	4.88	22.60	2.34
Cr_2O_3	0.00	0.13	0.00	0.14
NiO	0.00	0.04	0.00	0.02
ZrO_2	0.00	0.03	0.00	0.04
BaO	0.00	0.11	0.00	0.12
CuO	0.01	0.16	0.01	0.19
ZnO	0.05	3.14	0.07	1.88
PbO	0.00	0.03	0.01	0.03

* Acid leached sludge

The quantity of heavy metals in the sludge was extremely high. This could cause health problems in animals and accumulation in animal tissue making the meat unsuitable for human consumption. And very little heavy metals were removed by leaching with 1N hydrochloric acid. Better methods for removing the heavy metals are thus required in order to apply this technology to animals targeted for meat and dairy.

3.2 Amino Acid Distribution

The results of the amino acid analysis of dry sludge, fish meal and acid-leached sludge is indicated in Figures 1A to C. Looking at Figure 1A, it can be clearly seen that all essential amino acids are present in WAS from sewage. This clearly indicates that sludge from sewage has the required nutritional value. Further analysis of the results indicates that the ratio of lysine to methionine, an important nutritional factor (Vriens et al., 1989), is nearly equal to 2, which compares well with that of Fishmeal (which is nearly 2.1).
Looking at Figures 1A to C, it is noticed that the loss in amino acids during leaching occurred. It can also be noticed that the degree of amino acid loss is nearly the same for all essential amino acids. Therefore the degree of this loss appears to be having no prominent effect in the nutritional factors.
However, data in Figures 1A and B demonstrate that the content of amino acids in Fishmeal was always higher than the content in WAS. The same can be seen for acid-leached dry sludge, as compared to Fishmeal (Figure 1C). In actual fact, the ratios of amino acids in Fishmeal to dry sludge and acid-leached sludge are nearly uniform at 2 and 3, respectively.
The acid leaching process was also seen to have washed out a certain quantity of amino acids (data not shown). Greater quantities of phenylalanine and aspartic acid, being at 35 and 40 percent respectively, were lost. The quantity of cysteine, however, was not washed out. Overall, the effect of the amino acid washed out is insignificant as the ratio is smoothened for all proteins.
When considering the nutritive value of the acid-leached sludge, it becomes evident that WAS from sewage is a rich source of proteins. The comparison of proteins in WAS and Fishmeal, though, sideline the use of WAS as the replacement animal feed for Fishmeal.

3.3 Comparative Amino Acid Distribution

As indicated above, proteins were isolated according to the method by Shier and Purwono (1994). The sludge samples were autoclaved at 120 ^{o}C and other samples were digested in mineral oil at 155 ^{o}C. Proteins were assayed using Coomassie blue reagent. The amount of protein was estimated by interpolation from a standard curve prepared with bovine serum albumin (BSA). Assays were carried out in quadruplicate, and the results are presented as the mean.
In the supernatant from the sludge that was autoclaved at 120 ^{o}C, the protein concentration was 0.82 mg/mL, and in the supernatant from the sludge that was digested at 155 ^{o}C, the protein concentration was 0.47 mg/mL. The results showed that there were little intact proteins that were found at 15 ^{o}C than at 120 ^{o}C. The most logical explanation to this behaviour is the fact that at high temperatures, intact proteins denature, and they are broken down into constituent amino acids.
This profile was then compared to the chicken feed requirement as depicted in Figure 2. Sludge in general was found to have high amounts of amino acid when compared to the amounts required in starter, grower and finisher feed formulations.

3.4 Broiler Pilot Studies

3.4.1 Feed formulation: 0% sludge; 100% fishmeal versus 100% sludge; 0% fishmeal

There were no significant weight differences in chickens that were fed the conventional feed and the ones that were fed with the feedlot where fishmeal was substituted with sludge. It was however noted that from day 25 until day 35 the chicken that were feed with sludge weighed slightly higher that the chickens that were feed with conventional feedlot (Figure 3).

3.4.2 Feed Formulation: 0% Sludge;100% fishmeal versus 75% Sludge; 25 % fishmeal

No difference in the daily weight of the chicken was observed between the chicken fed with fishmeal:sludge combination feed and pure fishmeal feed until day 9. Thereafter the weight of the chicken that were feed with 75% sludge was slightly higher until day 30 and then picked up again after day 33 (data not shown). These results suggested that the sludge may contain other nutrients not present in the fish meal.

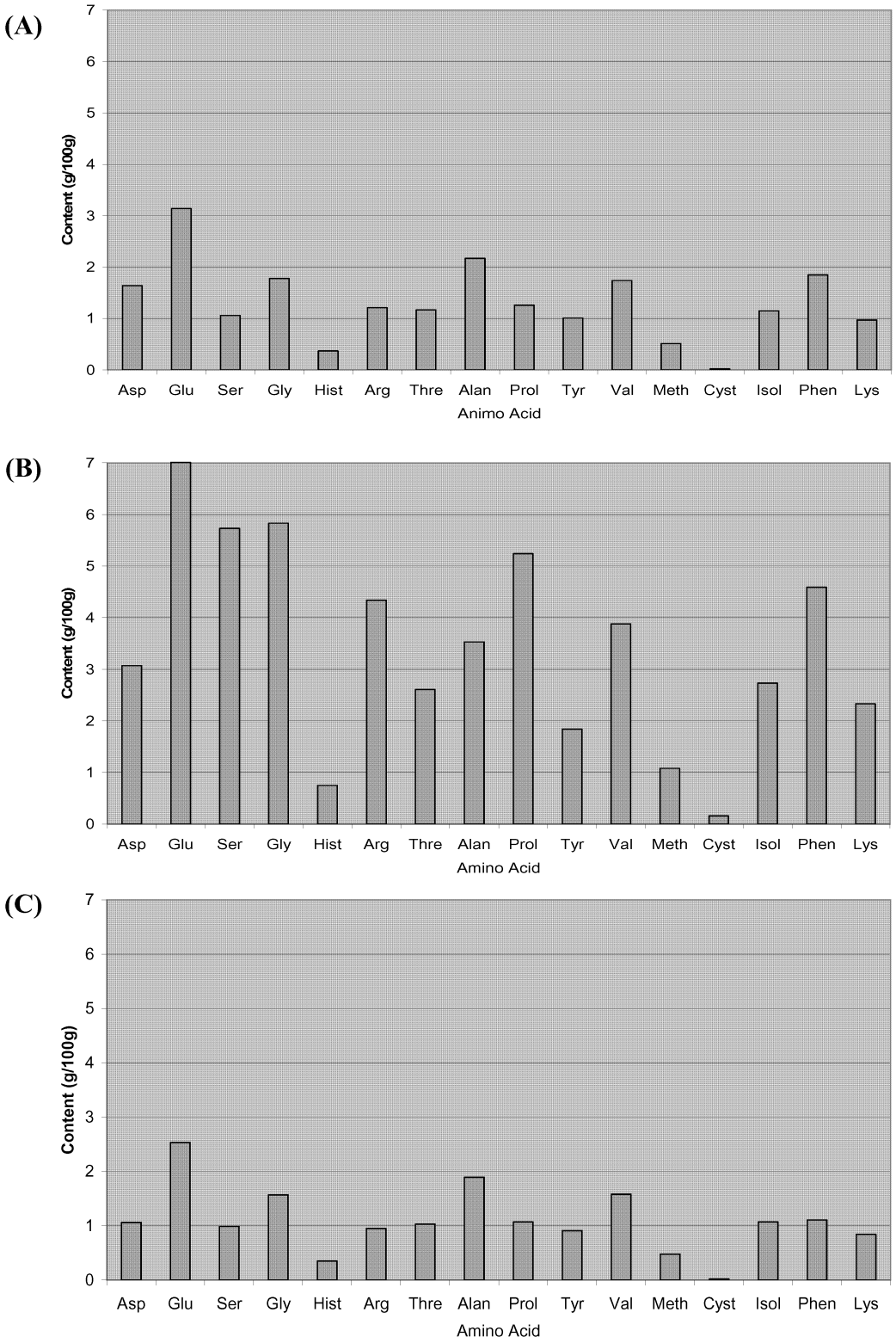

Figure 1. (A) Quantity of Amino Acid in Dry Sludge, (B) Quantity of Amino acids in Fish meal, and (C) Quantity of Amino Acids in Dry Acid-Leached Sludge

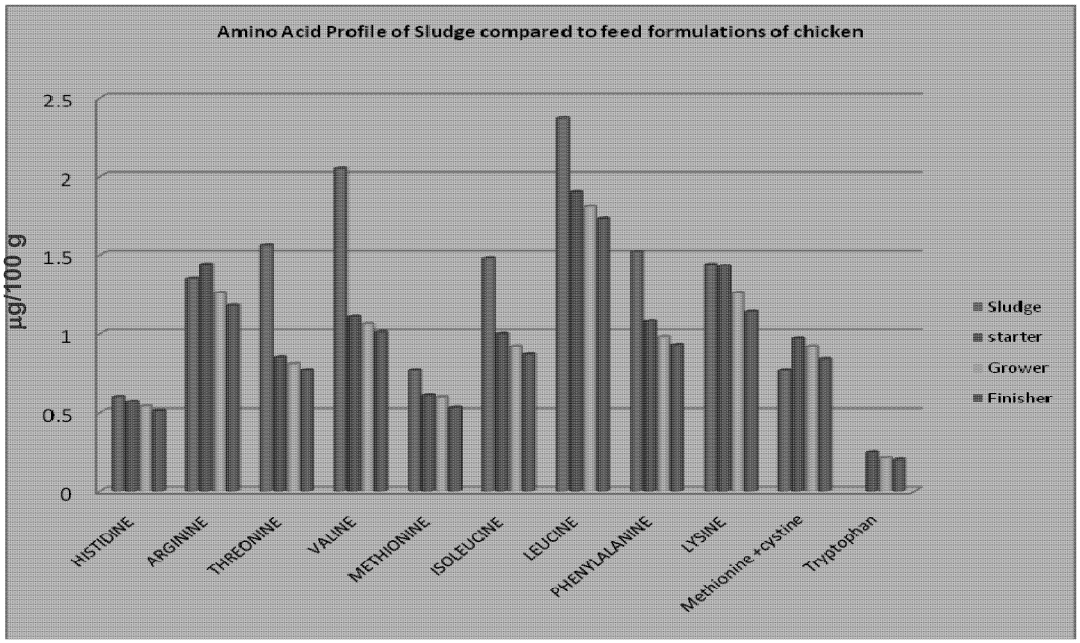

Figure 2: Amino acid profile of sludge compared to feed formulations of chicken.

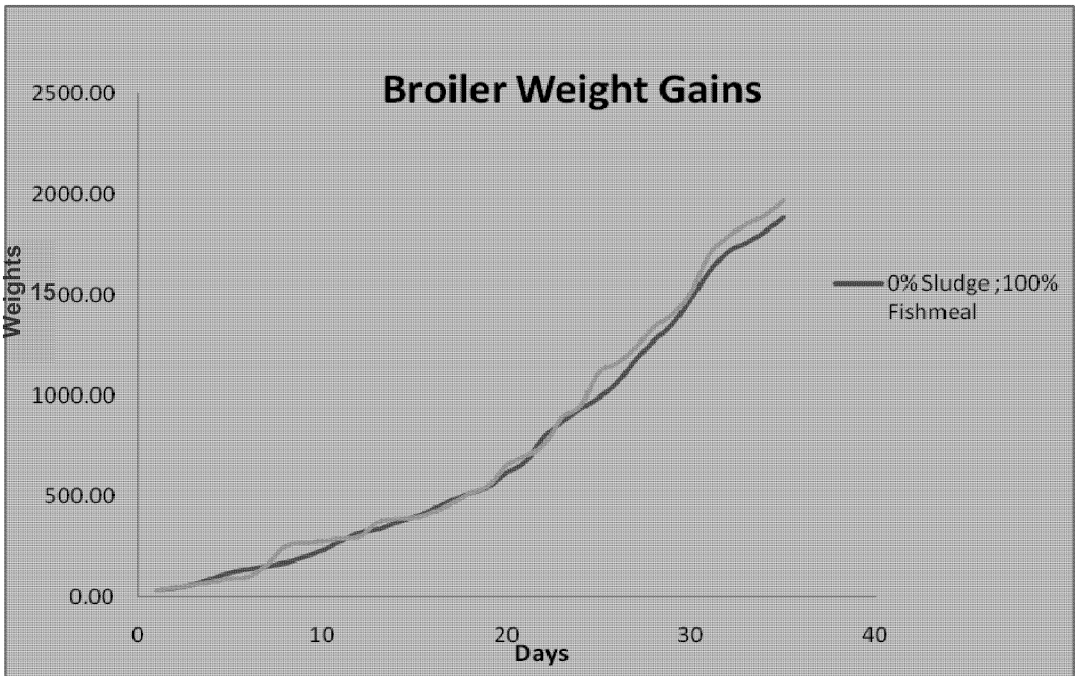

Figure 3: Broiler weight gain of chicken fed with 0% sludge and 100% fishmeal

3.4.3 Mortality

High rate of mortality occurred in the first 10 days of brooding, mostly in the chickens that we feed conventional feedlot (0% sludge and 75 % Sludge). There was on one occasion that a chick was put down because of stretched legs (it was not able to walk) (Figure 4).

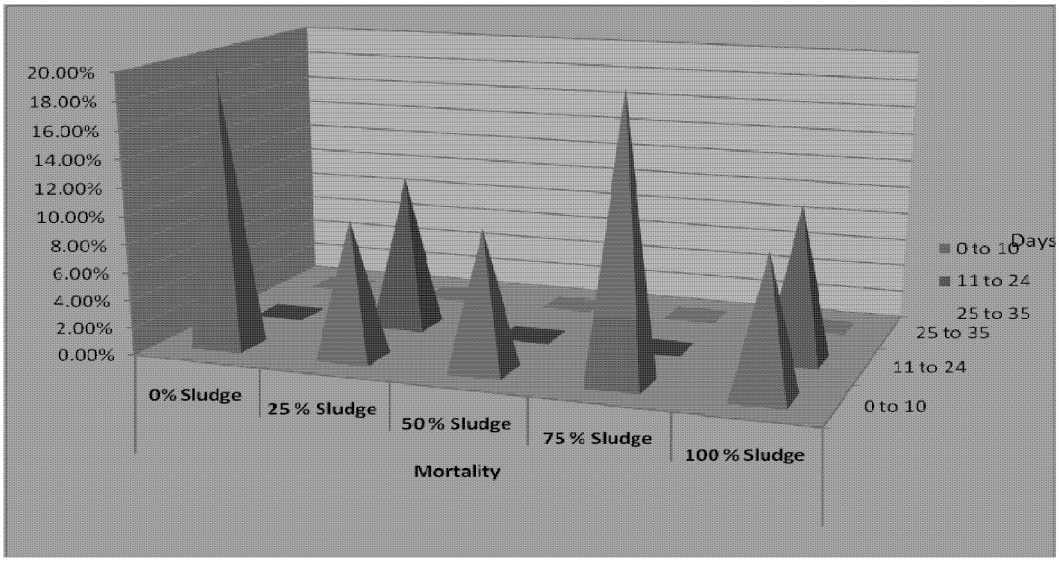

Figure 4. Experiment on Chicken Mortality.

3.4.4 Food Conversion Ratio

This is a measure of an animal's efficiency in converting feed mass into increased body mass. From the graph below it is indicated that when chickens are feed with conversional feed they consume 22% less feed to produce the same weight as chicken which is feed 100 % sludge (Figure 5).

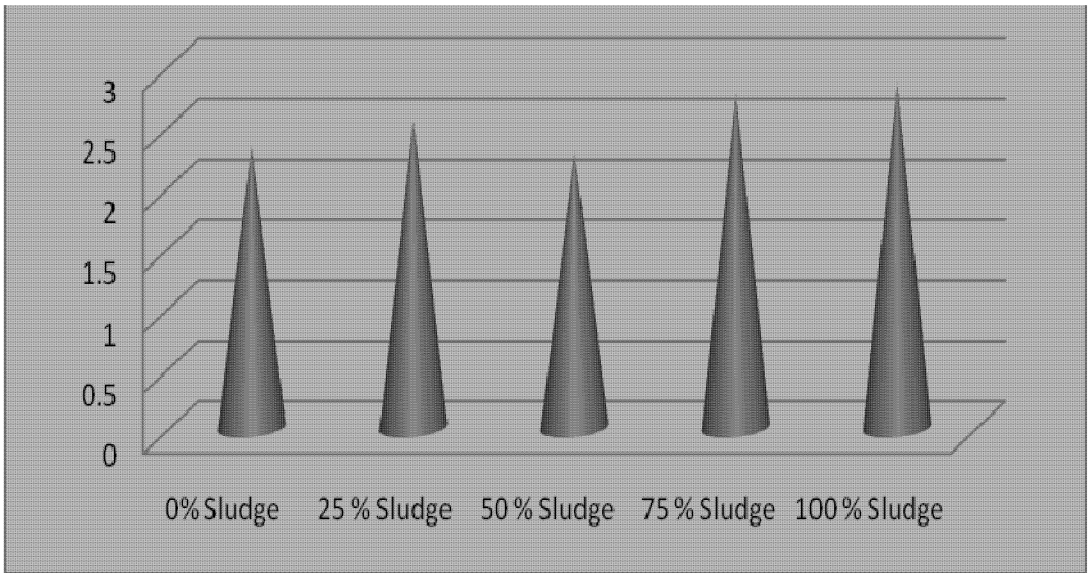

Figure 5: Food conversion ratio as the rate of mass accumulated against mass of feed provided.

3.4.5 Costing

The feeding experiment cost was ZAR 551.05 (US$ 73.47) to raise 41 chicks, and the cost was shared as follows, ZAR 114.95 (US$ 15.33) for chicken fed with conventional feed (100% Fishmeal); ZAR 111.58 (US$ 14.88) for chicken fed with 75% fishmeal + 25 % sludge; ZAR 110.68 (US$ 14.76) for chicken fed with 50 % fishmeal + 50 % sludge; R 109.08 (US$ 14.54) for chicken fed with 25 % fishmeal + 75%sludge; and lastly, ZAR 104.75 (US$ 13.97) for chicken fed with 100% sludge. This analysis shows that the cost of raising the chicken decreases with the amount of sludge used as a supplement. The money conversion is based on the yearly average ZAR to US dollar exchange rate of 2009.

4. CONCLUSION

Chicken fed with sludge weighed on average higher than chicken fed with conventional feed and the cost to raise chicken fed with conventional feed was higher than the chicken fed with sludge. Additionally, the cost of raising the chicken to maturity decreased with the amount used as protein supplement. This establishes the nutritional value of the WAS, however, the high content of metals raise a concern over the impact of long term consumption of animals grown from the sludge supplement. If applied to animals the use of leached dried sludge is strongly recommended. At the current leaching rate, three volumes of leached sludge could need to be used to replace one volume of Fishmeal in order to maintain the amino acid proportion comparable to Fishmeal.

5. ACKNOWLEDGMENT

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