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

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

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SYNOPSIS

In recent years there has been pressure exerted on the feed industry in Southern Africa to produce enough animal feed to meet the region's nutritional requirements. The increase in the cost of animal feed eventually affects the affordability and availability of high quality food to low income communities. However, the overall national production of protein feed can easily be surpassed by the amount of protein that could be extracted from sludge. For example, the amount of protein wasted through sludge in one province alone (Gauteng, South Africa) amounts to 106,763 metric tonnes/yr, and slightly lower than the national protein requirement of approximately 145,000 tonnes/yr. Waste Activated Sludge (WAS) from wastewater treatment plants treating domestic wastewater is shown to contain protein in a ratio of 2:1 against fishmeal. However, some of this protein content could be lost during processing. In this study, the protein content in sludge and fishmeal was evaluated in laboratory analyses conducted as a preliminary step towards designing a protein supplement substitute. A pilot test was conducted with 5 batches (10 chicken per batch), with fishmeal to sludge substitutions of 0%, 25%, 50%, 75%, and 100%. Metal content in the sludge was lowered by a rudimentary leaching process and its impact on the protein content was also evaluated. The initial mass gain rate, mortality rate, initial and operational costs analyses showed that protein from Waste Activated Sludge (WAS) could successfully replace the commercial feed supplements with a significant cost saving without adversely affecting the health of the animals.

DECLARATION

I, **Mokobori Tom Moses Lebitso** declare that the dissertation, which I hereby submit for the degree, **Master of Science: Applied Science (Water Utilisation)** at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

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Mokobori Tom Moses Lebitso

AKNOWLEDGEMENTS

"But thanks be to God! He gives us the victory through our Lord Jesus Christ"
1 Corinthians 15:57

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LIST OF NOMENCLATURES

ADP	adenosine diphosphate
AFMA	animal feed manufactures association
AGP	acid glycoprotein
AMP	adenosine monophosphate
AS	activated sludge
ATP	adenosine triphosphate
BSA	bovine serum albumin
CD4+	cluster of differentiation 4
CD8+	cluster of differentiation 8
DE	digestible energy
DNA	deoxyribonucleic acid
E	gross energy
EtBR	ethidium bromine
FAAS	flame atomic absorption spectroscopy
FCR	food conversion ratio
FS	fixed solids (%)
HCl	hydrochloric acid
HNO_3	nitric acid
HPLC	high-pressure liquid chromatography
ME	metabolizable energy
M_R	mortality rate (%)
NE	net energy
SADC	Southern African Development Community
SCP	single cell proteins
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel-electrophoresis

SRT	solid retention time
RNA	ribonucleic acid
T CELLS	T lymphocytes
TDN	total digestible nutrients
TKN	total kjeldahl nitrogen
UV	ultra violet
VS	volatile solids (%)
WAS	waste activated sludge

CHAPTER 1

INTRODUCTION

1.1 Background

Sub-Saharan Africa is typically characterized by a fast growing population and rampant poverty in large segments of the population (Drechsel *et al*., 2001; Cleaver and Schreiber, 1994). The region has been known to have the largest rate of malnutrition among children (Clover, 2003; Konczacki, 1972). The declining economic conditions in most parts of the region have affected the affordability and availability of high quality food stuffs. Specifically, prices of commercially grown meat products have been on the increase in the past four decades mainly due to rising cost in agricultural products including feed stocks (Briedenhann, 2009; Shipton and Hecht, 2005). South Africa, as one of the leading economies in the SADC region, is dependent on imported fishmeal which is used as a protein supplement in livestock feed (Shipton and Hecht, 2005). The cost of importation of these animal feeds is extremely high. In this study, protein from Waste Activated Sludge (WAS) is evaluated as a possible low cost substitute for the commercial protein feedstock. This is with consideration of the large amounts of single cell protein (SCP) disposed daily from wastewater treatment plants as WAS.

In South Africa, the average wastewater treated in one province (Gauteng Province) alone amounts to approximately 2500 MLD (912500 ML/yr) with a resultant production of 273,750 tonnes of dry sludge annually, the protein content of which amounts to 106,763 tonnes per year (Briedenhann, 2009). 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 conventionally disposed of through soil application as fertilizers, landfilling, combustion and/or ocean dumping at coastal cities (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 as it may result in contamination of groundwater and surface water resources due to leaching of heavy metals and phosphorous (Kasselman, 2004). However, based on information from previous feeding experiments Vriens *et al*., (1989) and others, WAS from sewage treatment plants was demonstrated to contain a large content of a range of important nutrients thus it offers an enormous potential as a possible animal feed supplement. The amounts of mineral elements, vitamins, nucleic acids, and amino acid proteins, reported by Vriens *et al*., (1989) are comparable to amounts present in whole egg, symba yeast sludge, soybean and fishmeal meal. Vriens *et al*., additionally observed a higher content of mineral elements (metals) in sludge than in commercial feedstocks such as fishmeal. Sludge was also found to be a very good source of vitamins, particularly vitamin B_{12} (Vriens *et al*., 1989). This indicates that the sludge has sufficient minerals required by animals.

In spite of the above listed desirable properties of sludge, several studies have also shown that the level of heavy metal content in sludge is usually two orders of magnitude higher than the levels in conventional protein sources (Vriens *et al*., 1989; Yoshizaki and Tomida, 2000; Hwang *et al*., 2008). Additionally, the existence of other recalcitrant organic pollutants is also expected. The presence of organics is especially of significant concern with the recent discovery of emerging pollutants and endocrine disrupting chemicals in wastewater (Barcelo, 2003; Barnhoorn *et al*., 2004; Mueller *et al*., 2008; Schilirò *et al*., 2009). Therefore, it is recommended that the feed sludge be pre-treated to remove heavy metals and other priority organic pollutants. For example, such simple processes as washing of WAS with acids could lower the toxic levels of heavy metals significantly (Yoshizaki and Tomida, 2000).

1.2 Objectives of the Study

The primary objective of the study was to investigate the feasibility of replacing fishmeal in chicken meal with waste activated sludge

The specific aims of the study were:

1. To analyse the protein and heavy metal content of the waste activated sludge and compare it with that of the fishmeal that is used as protein source in chicken feed.
2. To reduce the heavy metal content of the sludge to within allowable limits for the chicken feed
3. To replace fishmeal with waste activated sludge in the chicken feed and evaluate the effect of waste activated sludge on the growth of chicken
4. To compare the costs of raising chicken with waste activated sludge as opposed to fishmeal.

1.3 Methodology

In this study, a comparative analysis was conducted between WAS from local sewage plants and commercial supplement (fishmeal), to determine the protein and heavy metal levels in the two sources. Additionally, a real-time pilot study was conducted on approximately 50 chickens under varying sludge: fishmeal replacement ratios. The mass yield rate and cost of growing the chicken on the two sources and a range of proportional substitutions was also evaluated.

1.4 Summary of Results Obtained

Waste activated sludges from three wastewater treatment plants (Zeekoeigat, Baviaansport and Rooiawal) were analysed for crude protein. Zeekoeigat WAS had the highest protein content (38%), and therefore was chosen for protein isolation studies, nucleic acid isolation, and heavy metals removal. Sludge was found to have high amounts of amino acid when compared to the amounts required in starter, grower and finisher chicken feed formulations and nucleic acids were also extracted in the process. In this study Cu, Mn, Zn were within the allowable limit in the broiler feed after the sludge was treated with 1N HCl, Iron was above the allowable limit and the effect thereof needs to be investigated and the mineral tested in the study (Ca, K, Mg, Na and P) were within limits of the broiler feed after the sludge was treated with 1 N HCl. A pilot test was conducted with 5 batches (10 chickens per batch), with fishmeal to sludge substitutions of 0%, 25%, 75%, and 100% and it was concluded that Waste Activated Sludge (WAS) could successfully replace the commercial feed supplements with a significant cost saving without adversely affecting the health of the animals.

CHAPTER 2

LITERATURE REVIEW

2.1. Animal Feed and Raw Material Production in South Africa

South Africa is a developing country and as a result, pressure is exerted on feed industry to produce more animal feed. In his chairman's report at the 62nd Annual General Meeting in September 2009, Dr Erhard Briedenhann, chairman of Animal Feed Manufacturers Association (AFMA), reported on the total feed production in South Africa, Lesotho and Namibia. According to the calculations (based on AFMA's figures, livestock numbers and information supplied by the role players in all the livestock industries), the total national feed production currently is 9.6 million tonnes. The formal feed sector (AFMA members), as shown in Figure 2-1, was responsible for 5.3 million tonnes (55%) during the period 2008/09 (Briedenhann, 2009). AFMA also reported on the major raw materials used by AFMA members in the production of animal feed in South Africa during 2008/2009 (Figure 2-2). It must however, be noted that not all raw materials are being used in all compound feeds. The inclusion rates of different raw materials differ from formulation to formulation as well as between different species (Briedenhann, 2009).

2.2 Fish Meal Production in South Africa

The fishmeal production, after having dropped to 87,000 tonnes in 2006/2007, showed a recovery totaling 91,700 tonnes in 2007/2008 but again slipped back to 82,500 tonnes in 2008/2009 (Figure 2-3). There is however, an estimated fish meal shortage of about 50,000 tonnes (Figure 2-4) in South Africa (Briedenhann, 2009). Despite favorable fishmeal quotas, fish catches in 2009/2010 have been poor and therefore the availability of fishmeal was reduced (Briedenhann, 2009).

2.3 Oilcakes and Fishmeal Utilization by AFMA Members.

Oilcakes are masses of compressed linseed or other plant material left after oil has been extracted and can be used as a fodder. The use of fishmeal by AFMA members has recovered during the past 3 reporting periods (Figure 2-5). The use of fish meal is determined by availability, product mix and price in relation to other protein sources available (Briedenhann, 2009). Cotton oilcake usage continued its decreasing trend (Figure 2-5). This drop in usage is mainly due to the ratio between cotton oilcake and sunflower oilcake prices. Soya oilcake has for the first time in six years dropped in volume as a protein source during 2008/2009 (Figure 2-6) (Briedenhann, 2009). This drop in soya oilcake usage was however covered by a 48% increased usage of sunflower oilcake from 169,291 tonnes in 2007/2008 to 248,884 tonnes in 2008/2009 (Figure 2-6). This could be attributed mainly to the combination of high international Soya prices as well as increased availability of sunflower oilcake and fishmeal (Briedenhann, 2009).

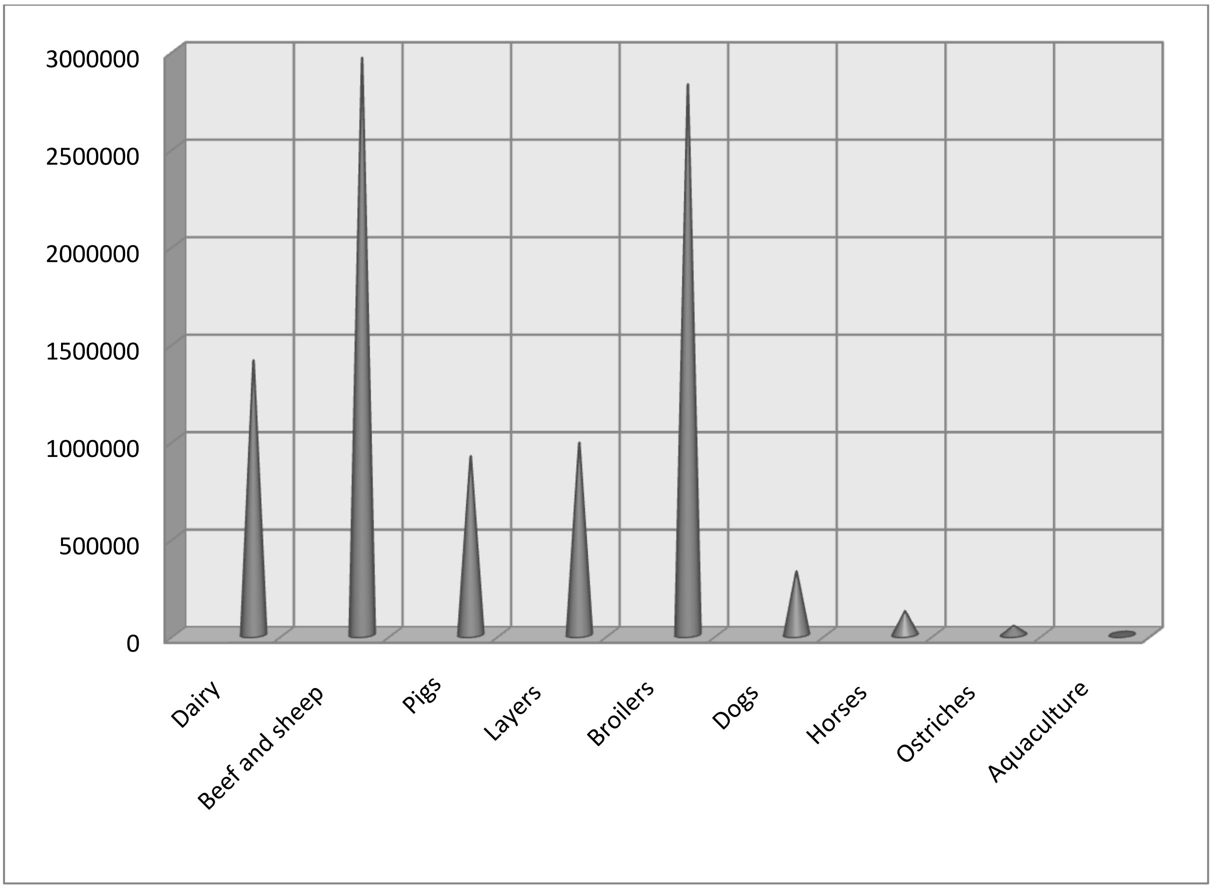

Figure 2- 1. National Animal Feed Utilisation (Tonnes) during 2008/09

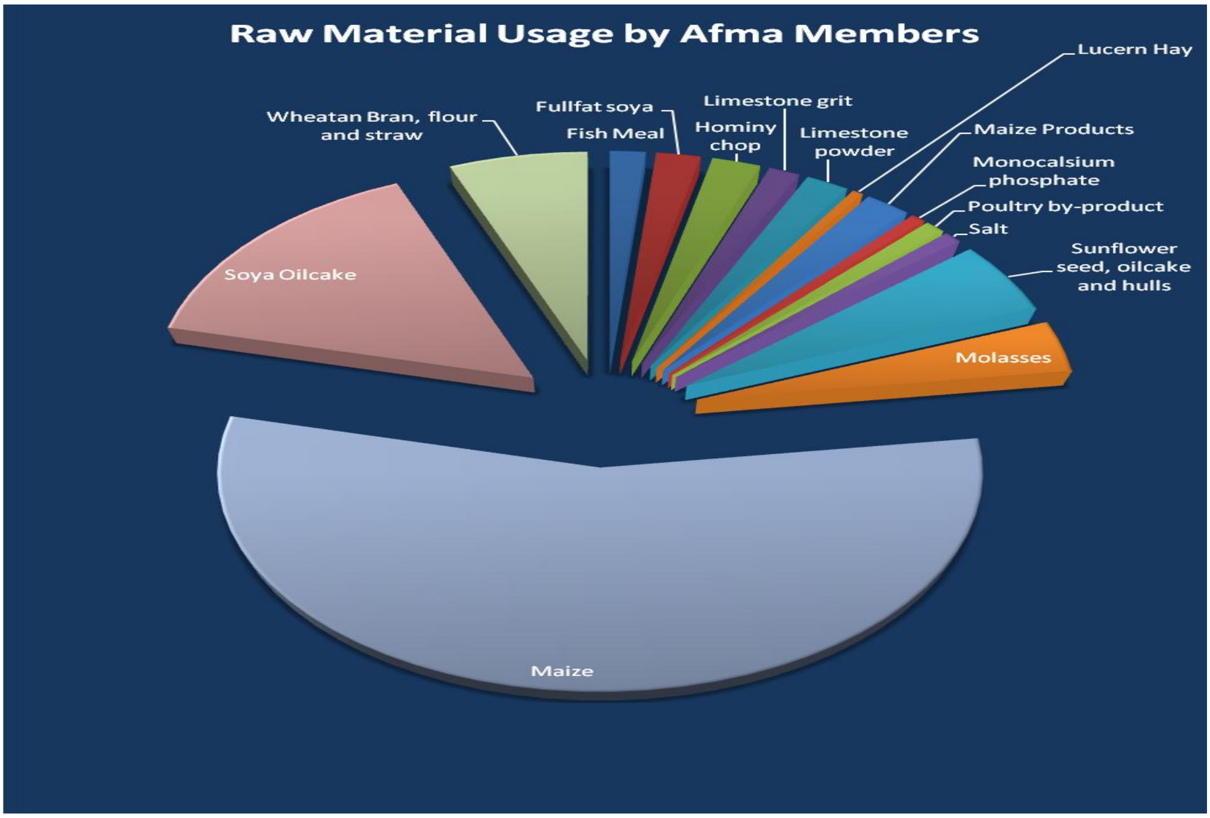

Figure 2-2. Major Raw Materials (%) Used by AFMA Members during 2008/09

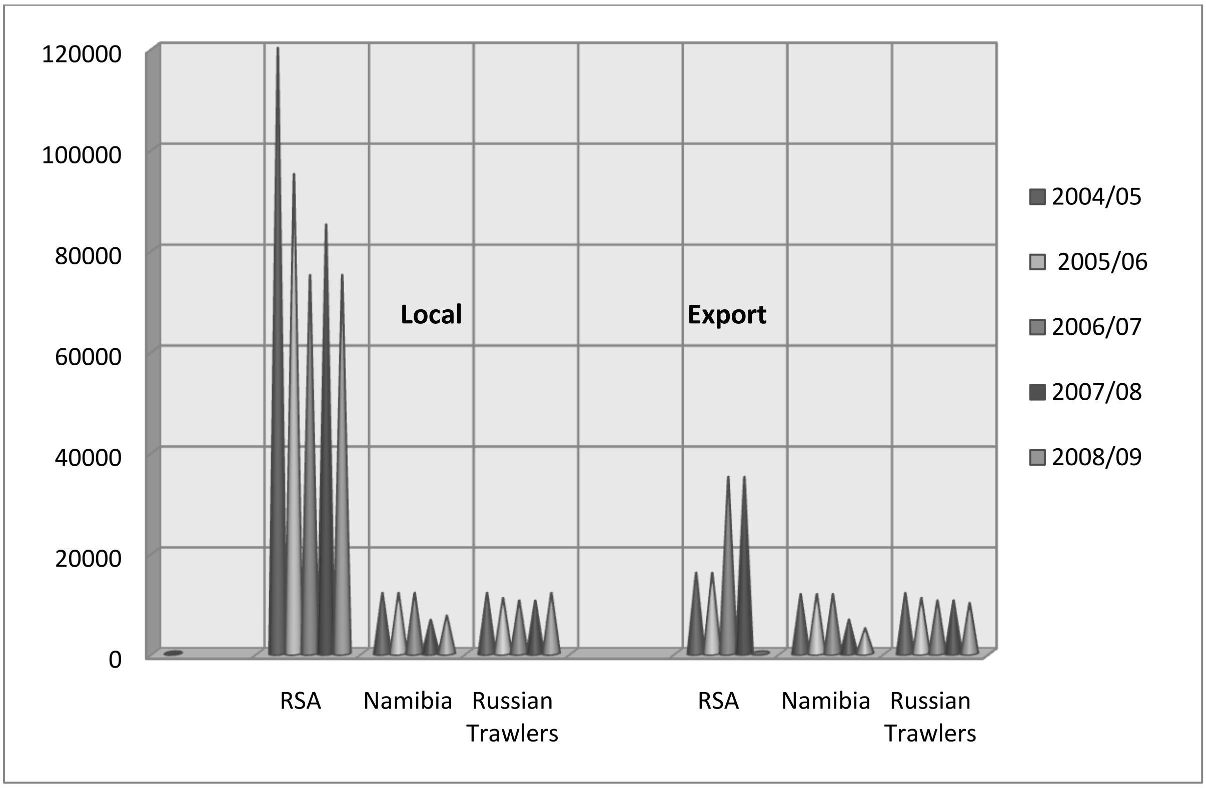

Figure 2-3. Local and Exported Fish Meal (Tonnes)

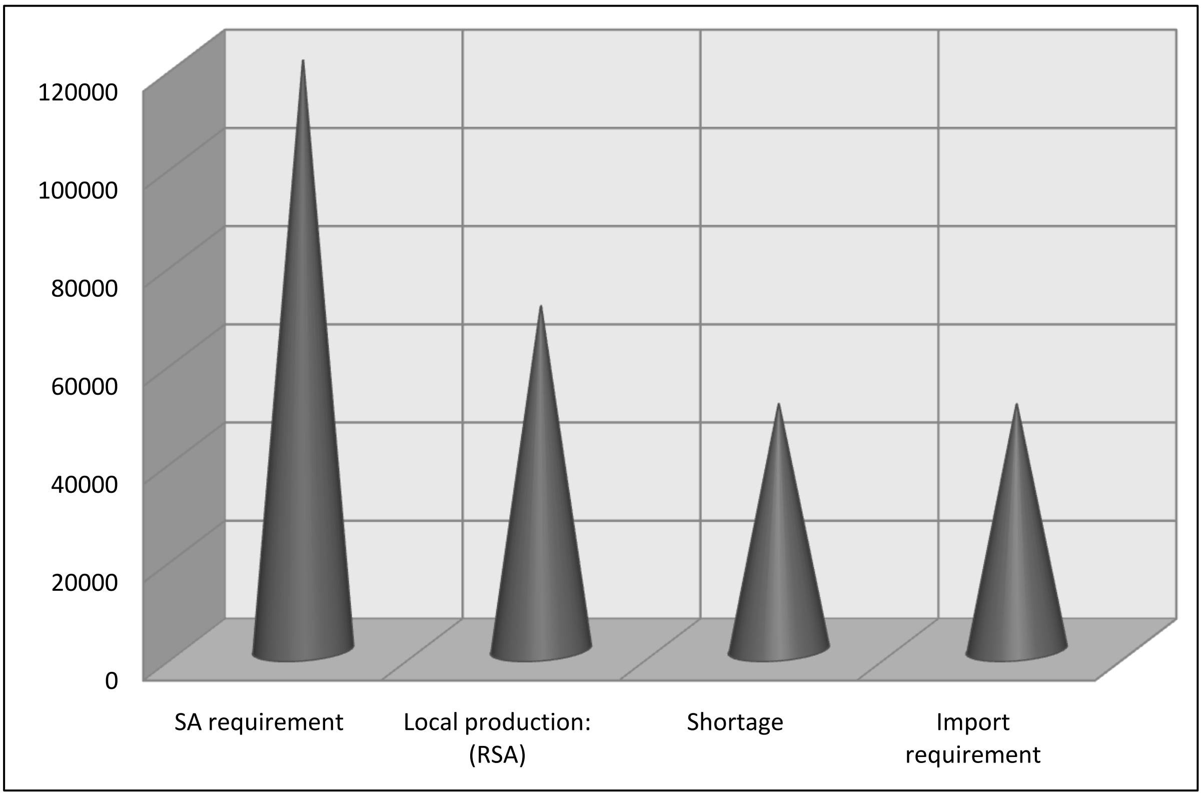

Figure 2-4. Estimated Fishmeal Production, Requirement and Import (Tonnes) -2009/2010.

2.4 Waste Activated Sludge as an Alternative Raw Material for Animal Feed

Waste activated sludge (WAS) has been suggested as the possible alternative raw material for animal feed. Many authors have reported on the high protein content of activated sludge (Garcia *et al*., 2005 and Shier and Purwono, 1994). Protein is one of the most important constituents in animal feed, furnishing energy and nitrogen (Hwang *et al*., 2008). Practically all essential amino acids are present in sludge (Pillai *et al*., 1953; Wuhrman, 1953; and Hoshino, 1969).

Activated sludge compares well with fish and soyabean meal (Table 2-1) (Vriens *et al*., 1989). The ratio of lysine to methionine, an important nutritional factor, compares well with fishmeal at a value of 2.4:1.

Some problems however, stand between the theoretical and the actual utilization of activated sludge in animal feeding. Objections over the use of sludge as protein supplement have been raised due to problems such as;

(a) the presence of pathogenic bacteria,

(b) the presence of toxic substances, especially carcinogens,

(c) the presence of potentially toxic heavy metals,

(d) the tendency towards constant composition, and

(e) the need for proper dewatering, drying and storage.

In addition to the above problems, microbial protein is usually high in nucleic acid content (Shan *et al*., 2008). Thus the levels of SCP need to be limited in the diets of monogastric animals. Some organisms can also produce mycotoxins. Microorganisms produce antibiotics in the quest for survival among predators and other competing organisms.

Of the problems listed above, the existence of carcinogenic compounds (Zhai *et al*., 2008) and toxic metal accumulation are the most difficult to remedy. Fortunately, sludge coming from predominantly domestic wastewater sources tends to have low recalcitrant organic pollutants (Poffe *et al*., 1981; Clegg *et al*.,1986 and Alcaide *et al*.,1987).

2.5 Production of Waste Activated Sludge in Gauteng Province in 2009

The average wastewater treated in Gauteng province is 2,500 MLD. This amounts to 912,500 ML of wastewater being treated per year, producing 273,750 tonnes of dry sludge annually of which approximately 106,763 tonnes are in the form of proteins (Monique *et al*., 2008) which exceeds the import requirements of fishmeal by more than two times.

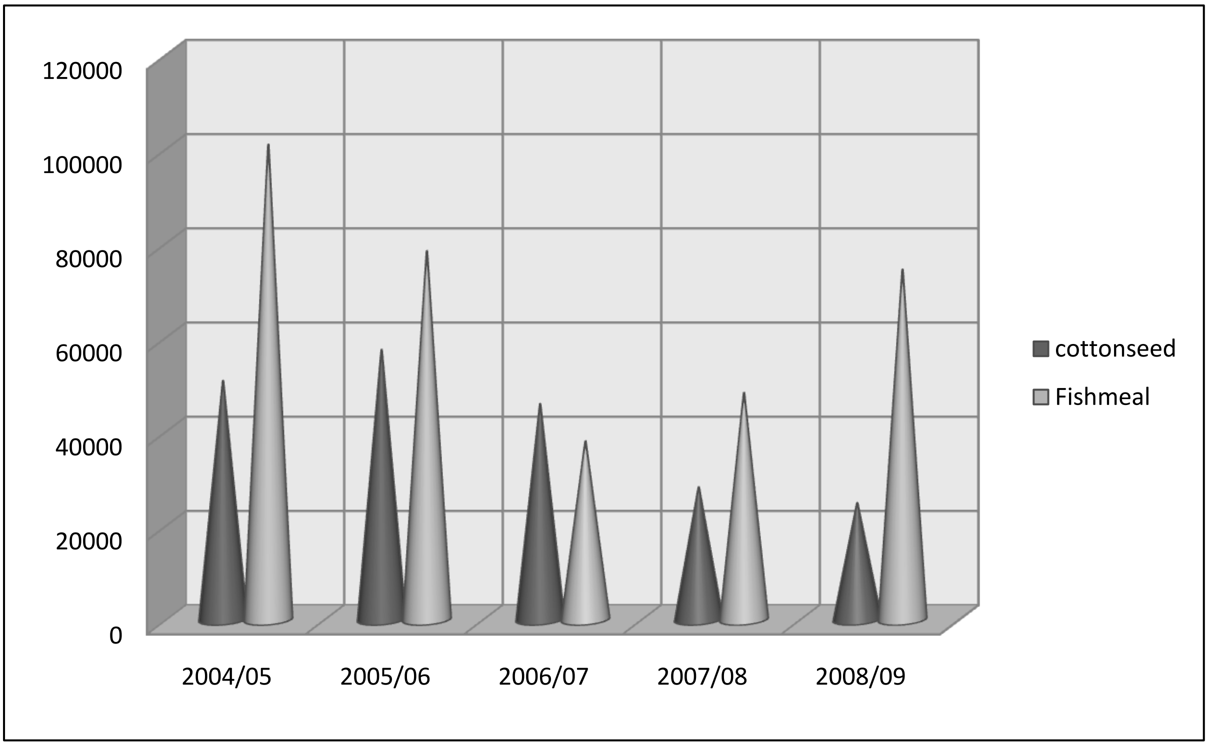

Figure 2-5. Cotton and Fishmeal (Tonnes) Usage

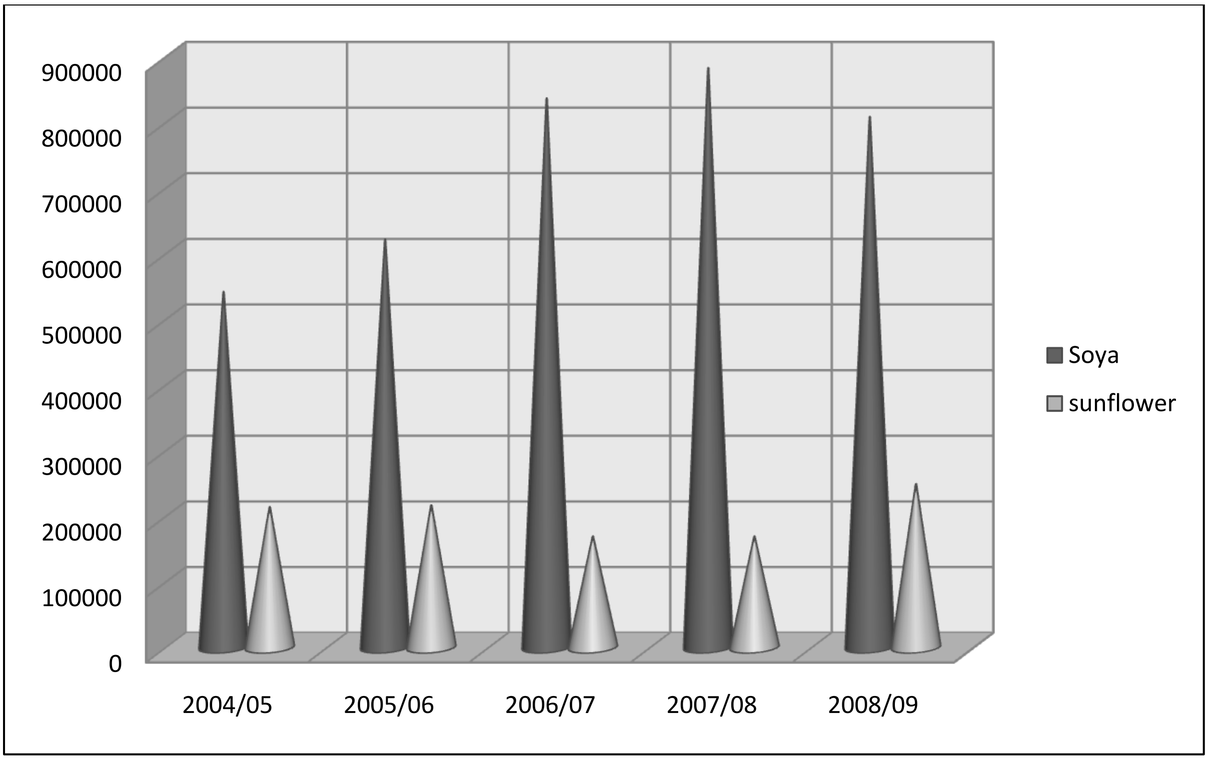

Figure 2-6. Soya and Sunflower Usage (Tonnes)

2.6 Characteristics of Activated Sludge Process

2.6.1 Chemical Composition of Waste Activated Sludge

During conventional municipal wastewater treatment, several dissolved components both organic and inorganic are adsorbed and converted to biomass. This results in a diversified composition formed and simultaneously, other compounds, both organic and inorganic, are adsorbed by various mechanisms. For that reason the resulting biomass has a diversified composition and different chemical composition to pure cultures.

Compared with a feedlot diet, sewage activated sludge is a very rich source of variety of trace elements (Morais *et al*., 2006). The concentration of the macronutrient elements of activated sludge is about five-fold higher than the feedlot diet, and the micronutrient, toxic and non-essential elements are even higher (Capar *et al*., 1978). Thus, the potential for accumulation of trace elements in animal tissues exists when sewage sludge is used as a diet ingredient (Capar *et al*., 1978).

Table 2-1. Amino Acid Content of Activated Sludges from Different Sources Compared to Other SCP-Proteins and Conventional Protein (Percentage in Crude Protein (N x 6.25)). (Adopted from Vriens *et al*., 1989).

Amino Acid	Paper processing[a]	Sewage[b]	Sewage[c]	Abattoir[c]	Brewery[d]	Industrial[e]	Symba Yeast[f,k]	Soyabean meal[g]	White fish meal[g]	FAO ref.protein[h]	Wheat[i]	Whole egg[j]
Alanine	7.07	-	7.3	7	7.9	6.97	-	-	-	-	-	-
Glycine	4.48	4.55-5.13	4.9	4.6	7.1	13.43	-	-	-	-	-	-
Valine	4.3	3.54-5.13	4.1	3.8	5.8	6.29	4.2	5.2	4.7	4.2	4.4	7.3
Threonine	3.72	3.45-6.60	4.2	4.2	5.4	4.25	5.4	4.4	3.8	2.8	2.9	5.1
Serine	4.8	-	3.4	3.3	5.1	13.43	-	-	-	-	-	-
Leucine	8.73	4.74-6.09	5.6	5.8	6.6	9.01	7.5	7.6	6.5	4.8	6.7	8.9
Isoleucine	4.24	2.73-6.60	2.7	2.5	3.7	9.01	4.3	5.8	3.9	4.2	3.3	6.7
Proline	3.89	-	3.1	3.2	4.3	-	-	-	-	-	-	-
Methionine	3.06	1.35-1.95	-	-	1.5	6.29	1.5	1.3	2.9	2.2	1.5	3.2
Aspartic acid	8.04	-	8.3	8.5	9.9	13.43	10.3	-	-	-	-	-
Phenylalanine	5.64	3.60-6.0	3.1	2.4	4.4	3.57	5.4	5.3	3.5	2.8	4.5	5.8
Glutamic acid	11.82	8.67	8.1	8.9	12.2	13.43	13.8	-	-	-	-	-
Lysine	7.92	2.76-3.99	3.3	3.9	3.9	3.4	6.3	6.6	7.6	4.2	2.8	6.5
Tyrosine	8.44	2.1	2.4	2.1	3.4	1.7	4.8	4.1	3	2.8	-	-
Arginine	4.72	3.12-3.78	2.9	3.4	4.8	6.12	4.6	7.3	6.8	-	-	-
Histidine	2.48	1.23-1.50	0.6	0.9	1.7	2.04	2	2.7	2	-	-	-
Cystine	-	0.54	2.1	3.6	2.7	6.29	1	1.2	0.7	2	2.5	2.4
Tryptophan	-	0.66-1.02	-	-	0.7	3.23	1.3	1.3	0.9	1.4	1.1	1.6

[a]Lo (1978); [b]Hurwitz (1957); [c]Kavanagh & Moodie (1978); [d]Goto &Masuda (1974); [e]Leibnitz & Behrens (1960); [f]Skogmann (1976); [g]Cowey *et al*. (1971); [h]Who (1965); [i]FAO(1965); [j]FAO (1970)); [k]Symba yeast: the Symba process converts starch and other carbohydrates into yeast by the symbiotic growth of two yeasts, *Endomycopsis fibuliger and Candida utilis, on these materials*

The levels of the micronutrients Ca, Cl, Mg, P, K and Na and of the macronutrients Co, Cu, Fe, Mn, Se and Zn (Table 2-2) in sewage sludge are considerably above the levels of these elements reported in the literature as being necessary for normal growth of cattle, horses, sheep, swine, rats and dogs (Hafez and Dyer 1969). The levels of Al, As, Cd, Ca, Cr, Cu, Eu, Fe, Mg, Pb, Sb, Sn, Ti and Zn are at least a factor of 100 or higher (Table 2-2) in sewage sludge than in the feedlot diet (Vriens *et al*., 1989). Toxicity can therefore be expected to occur in some animals with levels of Cd, Cr, Cu, Fe, Mn, PB, Se and Zn occurring in sewage sludge (Vriens *et al*., 1989).

2.6.2 Vitamins

Activated sludge is suggested to be one of the richest sources of vitamin B_{12} and the form present in sludge is very efficient in diets for chicks low in animal protein (Kirchgessner and Giessler, 1960) (Figure 2-7), in addition to vitamin B_{12} there are other forms of vitamins (vitamins A, C and E) that are found in waste activated sludge (Obek *et al*., 2005).

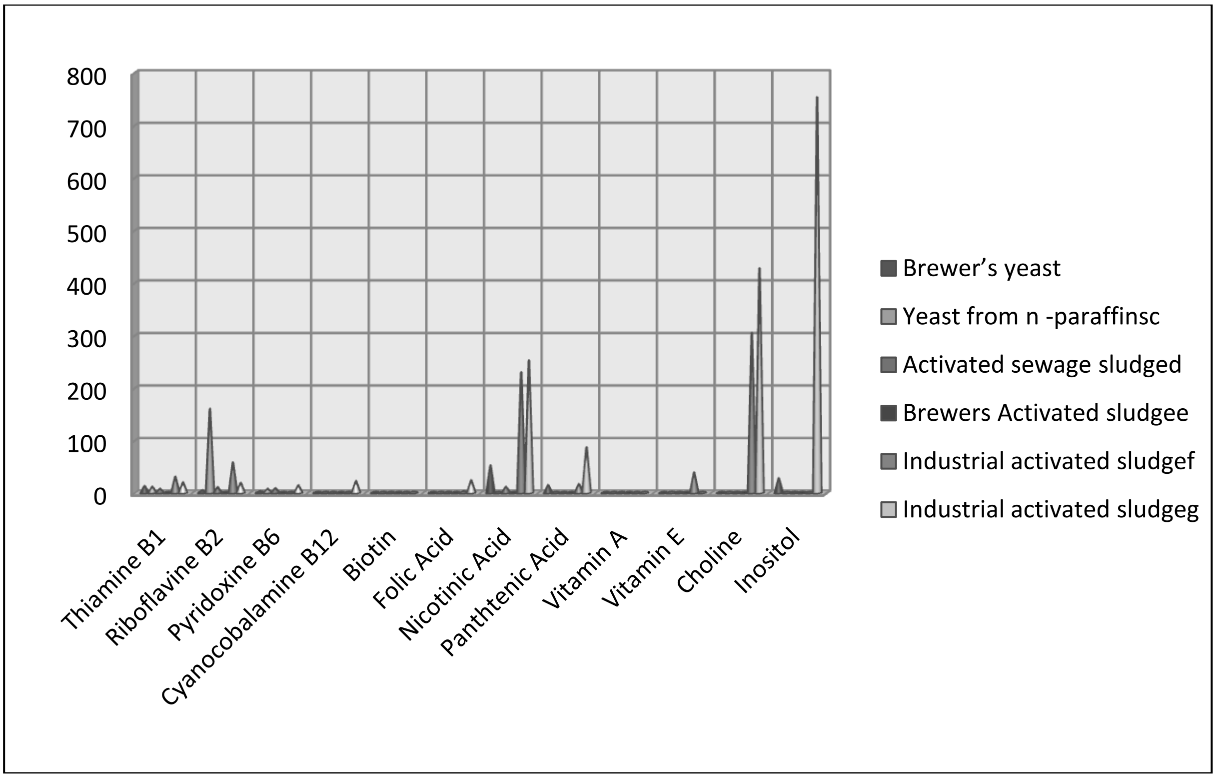

Figure 2-7. Vitamin Content of Activated Sludge (ppm) from Different Sources Compared to Brewer's Yeast from n-Paraffins. (Vriens *et al*., 1989)

Table 2-2: Element Content Of Activated Sewage Sludge (Adopted from Capar et al., 1978)

	Feedlot diet	Processed cattle waste	Cities average sewage
Micronutrients (ppm)			
Ca	0.46	2.32	3.62
Cl	0.17	0.71	0.38
K	0.8	2.8	1.22
Mg	0.156	0.5	0.6
Na	0.088	0.47	0.44
P	0.29	0.41	1.56
Micronutrients (ppm)			
Fe	230	2000	30600
Mn	17	92	194
Co	0.1	1.1	9.6
Cr	0.75	5	1441
Cu	3	18.7	1346
Mo	<2.5	15.6	14.3
Se	0.19	0.36	3.1
Sn	<0.8	3.74	216
V	0.57	3	40.6
Zn	70	76.7	2132
Toxic elements (ppm)			
As	0.1	0.6	14.3
Cd	0.05	0.14	104
Hg	<0.01	<0.09	8.6
Pb	0.36	3.29	1832
Sb	<0.03	0.1	10.6
Non-essential elements (ppm)			
Al	230	1800	18300
Ba	18	70	621
Br	10	66	45.5
Eu	0.012	0.06	37.7
La	0.5	3.2	35.7
Rb	2.3	15	32.6
Sc	0.065	0.44	2.5
Ti	8.1	50	2331

2.7 Nutritional Value of Municipal Waste

Garcia *et al*, (2005), indicted in their research that most of the waste fractions studied had a high nutritional value (Figure 2-8). Household waste and restaurant waste presented a more balanced composition, whereas in the other fractions (meat waste, fish waste and fruit and vegetation waste) one nutrient was dominant. Household and restaurant wastes tend to be good sources of protein. The presence of fruits and vegetables contribute to carbohydrate and vitamin content.

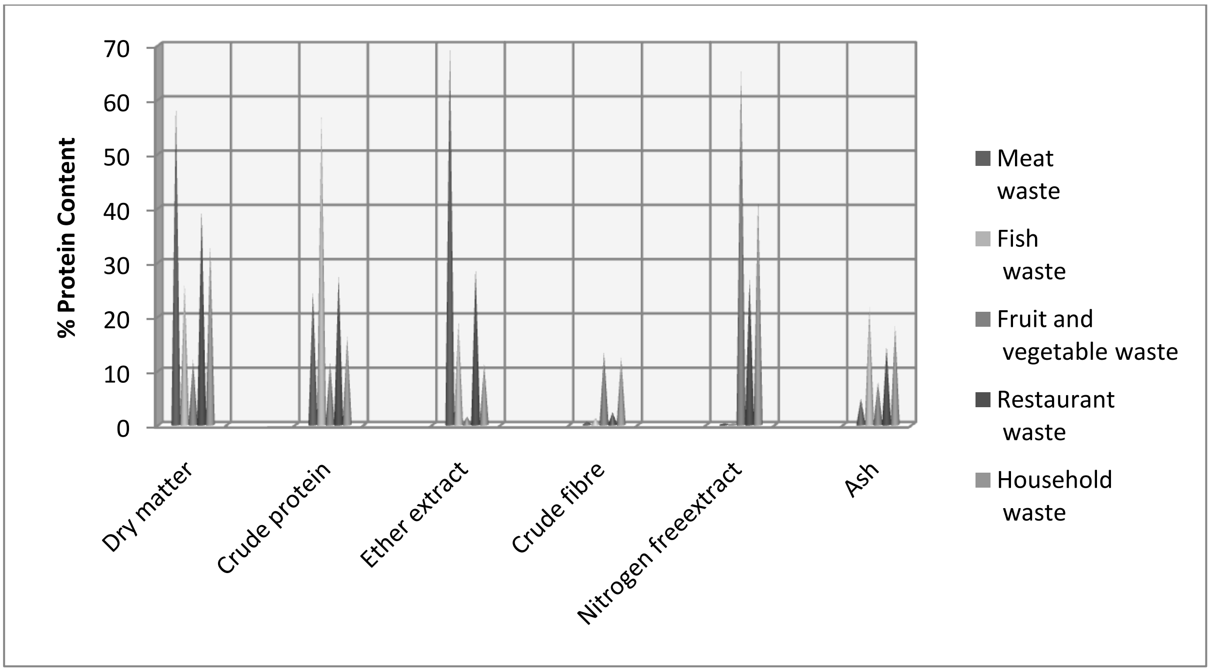

Figure 2-8. Nutritional Composition of Biodegradable Municipal Wastes of Different Sources (% on a dry matter basis) Adopted from Garcia *et al*, (2005).

2.8 Mineral Tolerance of Domestic Animals

The mineral tolerances of animals have been investigated in a variety of studies in which graded levels of the element were offered and specific effects examined. Ideally, long-term feeding of 1 year or more should be done with domestic animals, with additional studies in laboratory animals involving two or more generations (Mineral tolerance of domestic animals, National Research council. Subcommittee on Mineral Toxicity in Animals, 1980) Extensive studies of this type generally have not been made and, depending on the element, may not be necessary.

Studies of much shorter duration have been conducted in which the criteria included feed intake, growth rate, biochemical or morphological lesions, mortality, and deposition of the element in meat, milk, or eggs. The form of element, length of study, criteria for response and species of test animal have all been considered in developing the suggested maximum tolerable levels of dietary minerals for domestic animals.

Maximum tolerable level is defined as that dietary level that, when fed for a limited period, will not impair animal performance and should not produce unsafe residues in human food derived from the animal. The maximum tolerable levels shown in Table A-1 (Appendix A), are expressed in terms of either parts per million (ppm) or percent (%) in the total diet. Good nutritional practice recommends maintaining the mineral intake at required levels, which generally are well below the maximum tolerable levels. Greater sensitivity to high mineral levels can be expected in animals that are young, pregnant, lactating, malnourished, or diseased. The amounts of certain elements, such as cadmium, lead, and mercury, should always be maintained as far below the maximum tolerable level as feasible to minimise the contributions to the human diet

2.9 Nutrients Requirements of Domestic Animals

This section will discuss the nutrient requirements of domestic animals. It will give an indication of substances that should be removed (those that are above the tolerable levels/ concentrations, and may be toxic to animals), and those that must be increased/ enriched (these are substances that may be nutritionally important to animals and are found in low amounts in the sludge).

2.9.1 Monogastric Animals

When diets are formulated to meet the recommended nutrient requirements of swine, it is necessary to know the nutrient composition of and if possible, the bioavailability of nutrients in each ingredient used (Nutrient Requirements of Swine, 1998). The nutrient values given in Figures 2-9 and 2-10 are averages, reflecting the concentrations of nutrients most likely to be present in feeds that are commonly used in swine diets.

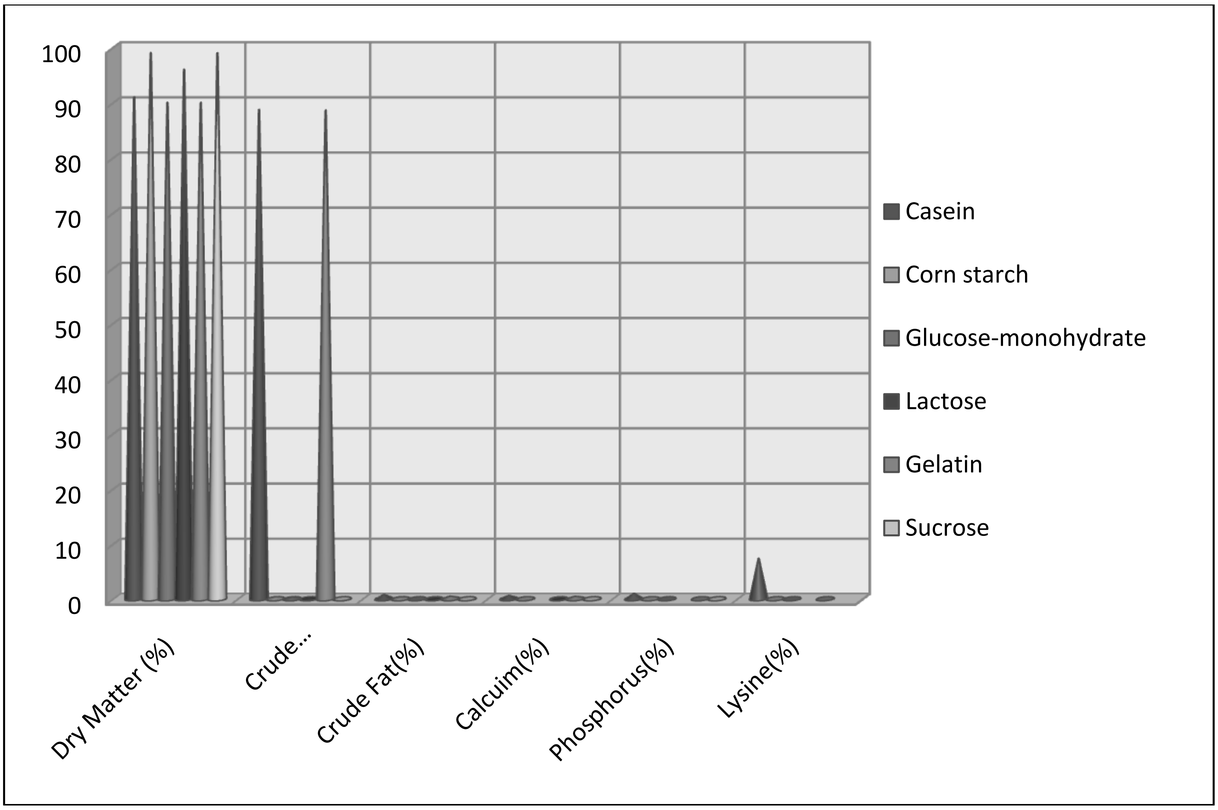

Figure 2-9. Chemical Composition of Feed Ingredients Used in Swine Research

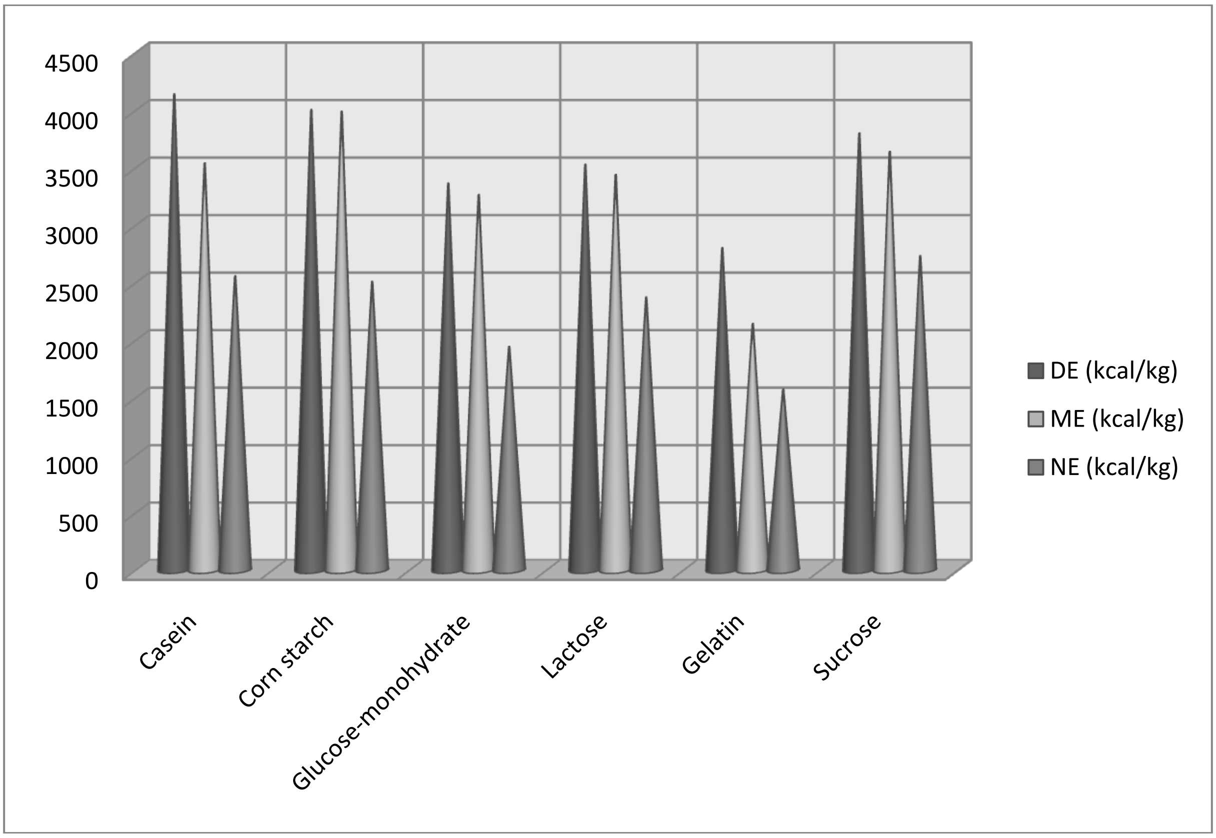

Figure 2-10. Chemical Composition of Feed Ingredients Used in Swine Research

2.9.1.1 Chickens

Chickens differ according to the purpose for which they are bred (Nutrient Requirements of Poultry 9th Revised Edition, 1994). Those intended for the production of eggs for human consumption (leg-horn-type) have a small body size and are prolific layers, whereas those used as broilers or broiler breeders (meat type) have rapid growth rates and a large body size. They are less efficient egg layers. The nutrients requirements differ for these two kinds of chickens, as indicated in Table 2-3.

Table 2-3: Nutrients Requirements of Broilers and Leghorn-Type Chickens (Brown Egg-Laying Strain) as Percentages or Units per Kilogram of Diet

		Brown-Egg- Laying Strain				Broilers		
		0-6 weeks,	6-12 weeks,	12-18 weeks	18 - first Egg stage	0 - 3 Weeks	3 - 6 Weeks	6 – 8 Weeks
Nutrients	units							
Crude protein	%	17.00	15.00	14.00	16.00	23.00	20.00	18.00
Arginine	%	0.94	0.78	0.62	0.72	1.25	1.10	1.00
Glysine + serine	%	0.66	0.54	0.44	0.50	1.25	1.14	0.97
Histidine	%	0.25	0.21	0.16	0.18	0.35	0.32	0.27
Isoleucine	%	0.57	0.47	0.37	0.42	0.80	0.73	0.62
Leucine	%	1.00	0.80	0.65	0.75	1.20	1.09	0.93
Lysine	%	0.80	0.56	0.42	0.49	1.10	1.00	0.85
Methionine	%	0.28	0.23	0.19	0.21	0.50	0.38	0.32
Met + cystine	%	0.59	0.49	0.39	0.44	0.90	0.72	0.60
Phenylalanine	%	0.51	0.42	0.34	0.38	0.72	0.65	0.56
Phe + tyrosine	%	0.94	0.78	0.63	0.70	1.34	1.22	1.04
Proline	%	-	-	-	-	0.60	0.55	0.46
Threonine	%	0.64	0.53	0.35	0.44	0.80	0.74	0.68
Tryptophan	%	0.16	0.13	0.10	0.11	0.20	0.18	0.16
Valine	%	0.59	0.49	0.38	0.43	0.90	0.82	0.70

2.9.2. Polygastric Animals

There are approximately forty-seven (47) types of nutrients used to feed dairy cattle including, alfalfa, almond, apple etc (Nutrient Requirements of Dairy Cattle: Seventh Revised Edition, 2001). Only 15 mineral elements have been demonstrated to be essential for sheep. Seven are major mineral constituents: sodium, chlorine, calcium, phosphorus, magnesium, potassium, and sulfur. The other eight are trace elements: iodine, iron, molybdenum, copper, cobalt, manganese, zinc and selenium (Nutrient Requirements of Sheep Sixth Revised Edition, 1985).

2.10 Summary

From this literature survey, it can be concluded that there is a shortage of fishmeal meal in South Africa and waste activated sludge could be used to substitute fishmeal in animal feed in future.

Many authors have reported on high protein content of waste activated sludge and the fact that waste activated sludge compares well with fishmeal. Production of waste activated sludge in one of the nine provinces (Gauteng, South Africa) exceeds the whole of the South African fishmeal demand by more than two times. However, there were concerns about the occurrences of carcinogenic compounds in waste activated sludge but the literature demonstrated that domestic wastewater tend to have low to insignificant levels of carcinogenic compounds. Compared with feedlot diet, WAS is a very rich source of variety of trace elements and thus the potential for accumulation of trace elements in animal tissue when sewage sludge is used as a diet ingredient. The following chapters reports on results on metal analysis in sludge and efficiency of heavy metal removal by proposed pre-treatment method. Feed ration formulations are tested in a pilot feed study on broiler chicken.

CHAPTER 3

EXPERIMENTAL METHODS

3.1 Sludge Characterization

3.1.1 Dry Matter, Volatile and Non-Volatile Fractions

A measured volume (500 ml) of activated sludge (from Rooiwal, Zeekoeigat, and Bavianspoort) was dried overnight in an oven at temperature of 103 - 105°C. Mass of the dried sludge was recorded and the sludge was then burnt off at 550°C for 2.5 hours, to get the volatile and non-volatile fractions of the sludge

3.1.2 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 from the above mentioned sewage works using Nitrogen Gas Analyzer utilizing induction furnace and thermal conductivity (LECO FP-528, Ho Chi Minh City, Vietnam). 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 are 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 is passed through a copper catalyst to remove oxygen and convert nitrous oxides to N_2, scrubbed of moisture and carbon dioxide, and nitrogen content determined by thermal conductivity. Total crude protein is 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% (AOAC Official Method 990.03, in Official Methods of Analysis of AOAC International, 16th edition, Volume I Chapter 4, pp 18-19).

3.2 Protein Determination Studies

Protein from sludge was analyzed within four hours after collection from the wastewater treatment plants. This was to avoid loss of single cell proteins through cellular endogenesis. Proteins was firstly extracted from the waste activated sludge using different methods in order to choose the most effective method. The extraction methods from Learch *et al*. (1993), Ogunseitan (1997), Ehlers and Cloete (1999), and Shier and Purwono (1994) were tested and compared for use on the sludge. The extraction method by Shier and Purwono yielded the best results thus was chosen for this study. This method solubilized 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 reagent (Sedmak and Grossberg, 1977). The amount of protein was estimated by interpolation from a standard curve prepared with bovine serum albumin (BSA). Assays were carried out in quadruplicate to generate a mean and standard error per analysis.

3.2.1 Amino Acid Analysis

To compare the extracted proteins with the proteins from more conventional sources (fishmeal) used to supplement nutrition of animals, amino acids analysis was performed on the extracted proteins using the Pico-tag method (Bidlingmeyer *et al.*, 1984). This method involved hydrolysis of the protein to yield free amino acids, pre-column derivatization of the sample and analysis by Reverse Phase HPLC (Snyder *et al*., 1997).

3.3 Nucleic Acid Assay

The sample was analyzed on 1.5% (*w/v*) agarose (promega, Wisconsin, USA) / TAE (0.04 M tris-acetate, 1mM EDTA) gels by electrophoresis in 1 x TAE at 78 V (5.2 V/cm) in a minicell EC 370 M electrophoretic system (E-C Apparatus Corporation, USA). The sample was loaded in loading dye (30% (*v/v*) glycerol, 0.025 % (*w/v*) bromophenol blue). The gel was subsequently stained in a 10μg/ml EtBr solution and the bands were visualised on a spectroline TC-312A UV transilluminator at 312 nm. Images were captured with a charge-coupled devise (CCD) camera linked to a computer system.

3.4 Reverse Phase Chromatography

The extracted samples at 120°C and 155°C were run separately on the reverse phase column (Amersham Biosciences, 18-1134-16, Edition AA) to give the constituents in the sample. The samples were eluted with 50% 0.1 trifloroacetic acid on a 6.4 column, with the 3ml capacity, and a 100mm height with the maximum flow rate of 10ml/min.

3.5 Heavy Metal Analysis

As previously stated that sludge may contain heavy metals, it was also the aim of the project to remove or reduce heavy metal content of the sludge to within the allowable or tolerable levels when consumed by animals

The heavy metal content (Zn; Cu; Mn; Fe) of the sludge was determined using Inductive Coupled Plasma. (ICP) (EPA Method 200.7, Winsford, Cheshire, CW7 3GA, UK). The samples (2g aliquots) 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. (EPA Method 200.7, Winsford, Cheshire, CW7 3GA, UK)

3.5.1 Heavy Metal Extraction

In this project two heavy metal extraction methods were compared. The first method involved using 1 N HCl (Yoshizaki and Tomida, 2000), and the other method was extraction using organic acids (citric and oxalic acid) (Veeken and Hamelers, 1999). 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°C and then burnt off at 550°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 (Analytical methods for flame spectroscopy, 1979).

3.5.2 Amino Acid Analysis after Heavy Metal Extraction

To verify that, when extracting heavy metals from WAS, no proteins are extracted together with the heavy metals, amino acid analysis was also performed in the supernatant using the Pico-tag method (Bidlingmeyer *et al*., 1984).

3.6 Boiler Pilot Run

Feeding experiments were performed over a period of thirty five (35) days using five (5) sets of feed formulations as indicated in Tables 3-1, 3-2 and 3-3. The broilers were weighed daily to measure the weight gained on daily basis. Weight distribution was then calculated and mortality rates were also recorded on daily basis.

3.6 .1 Chick Supply and Planning

3.6.1.1 Parent Stock Flock Age

Broiler placements were placed so that day old chick sizes were as uniform as possible to help with subsequent performance. Chicks were also placed so that there is only one source flock per house (Arbor acres, 2008)

3.6.2 Brooding

3.6.2.1 House Preparation and Placement

House preparation was completed prior to chick arrival and that enabled placement of chicks placed 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 gives a good indication of chick condition (Arbor acres, 2008)

3.6.2.2 Temperature and Environment

It is important that the house is maintained at the correct temperature if the birds are to be active and develop a good appetite (Arbor acres, 2009). Temperature in the brooding area was considered in two parts, firstly the temperature of the air (measured at chick height and in the vicinity of the feeders and drinkers) and secondly the temperature of the litter. Air temperature was maintained at 30°C and litter temperature 28-30°C when the chicks were placed. House temperature was greatly influenced by local environmental conditions and was correlated to the effective temperature perceived by the chick. Variation in relative humidity (RH) influenced the effective temperature experienced by the chicks. Higher RH reduces evaporative heat loss, increasing the effective temperature; lower RH decreases the effective temperature. RH% ranged between 60-70%, with the help of the addition of surface water.

3.6.2.3 Feed and Water

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 (Arbor acres, 2008). 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 (Arbor acres, 2008); 30-40 lux was used for the first 7 days.

During the first 7 days additional supplementary drinkers were provided and positioned 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.

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 (Arbor acres, service bulletin, www.aviagen.com, 2008). After 3 days the birds started eating from the pans feeding system and the paper was removed. Feeders were emptied daily to prevent the buildup of any fines/dust.

3.6.3 Feed Formulations

Poultry diets are composed primarily of a mixture of several feedstuffs such as cereal grains, soybean meal, animal by-product meals, fats, and vitamin and mineral premixes. These feedstuffs, together with water, provide the energy and nutrients that are essential for the bird's growth, reproduction, and health, namely proteins and amino acids, carbohydrates, fats, minerals, and vitamins (Nutrient Requirements of Poultry 9th Revised Edition, 1994). The energy necessary for maintaining the bird's general metabolism and for producing meat and eggs is provided by the energy-yielding dietary components, primarily carbohydrates and fats, but also protein**.**

3.6.3.1 Carbohydrates

Dietary carbohydrates are important sources of energy for poultry. Cereal grains such as corn, grain sorghum, wheat, and barley contribute most of the carbohydrates to poultry diets. The majority of the carbohydrates of cereal grains occur as starch, which is readily digested by poultry (Moran, 1985a).

3.6.3.2 Proteins and Amino Acids

Dietary requirements for protein are actually requirements for the amino acids contained in the dietary protein. Amino acids obtained from dietary protein are used by poultry to fulfill a diversity of functions. For example, amino acids, as proteins, are primary constituents of structural and protective tissues, such as skin, feathers, bone matrix, and ligaments, as well as of the soft tissues, including organs and muscles (Nutrient Requirements of Poultry 9th Revised Edition, 1994). Also, amino acids and small peptides resulting from digestion-absorption may serve a variety of metabolic functions and as precursors of many important nonprotein body constituents. Because body proteins are in a dynamic state, with synthesis and degradation occurring continuously, an adequate intake of dietary amino acids is required. If dietary protein (amino acids) is inadequate, there is a reduction or cessation of growth or productivity and a withdrawal of protein from less vital body tissues to maintain the functions of more vital tissues (Nutrient Requirements of Poultry 9th Revised Edition, 1994)..

There are 22 amino acids in body proteins, and all are physiologically essential. Nutritionally, these amino acids can be divided into two categories: those that poultry cannot synthesize at all or rapidly enough to meet metabolic requirements (essential) and those than can be synthesized from other amino acids (nonessential). The essential amino acids must be supplied by the diet. If the nonessential amino acids are not supplied by the diet, they must be synthesized by poultry. The presence of adequate amounts of nonessential amino acids in the diet reduces the necessity of synthesizing them from essential amino acids. Thus, stating dietary requirements for both protein and essential amino acids is an appropriate way to ensure that all amino acids needed physiologically are provided (Nutrient Requirements of Poultry 9th Revised Edition, 1994).

3.6.3.2.1 Specific Amino Acid Relationships

Although each amino acid can be metabolized independently of others, relationships between certain amino acids exist. In some instances, the relationship may be beneficial. For example, one amino acid may be converted to another to fulfill a metabolic need. In other instances, a metabolic antagonism may exist with undesirable consequences. A brief description of amino acid relationships that may be of importance in poultry nutrition is given in the following section.

3.6.3.2.1.1 Methionine Plus Cystine

Methionine can donate its methyl group to biological processes, and the resulting sulfur-containing compound, homocysteine, together with serine, can be used to synthesize cysteine via cystathionine. The sulfhydryl groups of two molecules of cysteine are oxidized to form cystine. This conversion cannot be reversed, and two methionine molecules are needed to ultimately supply the two sulfur atoms of cystine (Baker, 1976). The requirement for methionine can be satisfied only by methionine, whereas that for cystine can also be met with methionine.

The catabolism of methionine and cystine largely leads to conversion of the associated sulfur into sulfate. This sulfate may be used in metabolism, particularly as a part of certain connective tissues. Similarly, methyl groups of methionine may be used in transmethylation and the de novo synthesis of sarcosine, betaine, and choline. Choline is a constituent of phospholipids, and its incorporation into membranes is extensive. During rapid growth, when accrual of connective tissue and expansion of membrane surfaces are great, an increased sensitivity to methionine at levels marginal to the requirement may occur if dietary choline and sulfate are not sufficient (Blair *et al*., 1986).

3.6.3.2.1.2 Phenylalanine Plus Tyrosine

Tyrosine is the initial product formed during the biological degradation of phenylalanine. In turn, phenylalanine can be used to meet the bird's need for tyrosine on a mole-for-mole basis (Baker, 1973). Although this conversion may be reversed to a small extent and tyrosine used to form phenylalanine, its contribution is too small to be of practical significance (Ishibashi, 1972).

3.6.3.2.1.3 Glycine Plus Serine

Although glycine can be synthesized by fowl, the rate is not adequate to support maximal growth (Featherston, 1976). Serine can be converted to glycine on an equimolar basis. This reaction is reversible, and glycine can be used to form serine (Sugahara and Kandatsu, 1976).

3.6.3.3 Fats

Fats are usually added to the feed for meat-type poultry to increase overall energy concentration and, in turn, improve productivity and feed efficiency. Oxidation of fat is an efficient means to obtain energy for the cell in large quantity, whereas anabolic use involves direct incorporation into the body as a part of growth (Nutrient Requirements of Poultry 9th Revised Edition, 1994).

3.6.3.4 Vitamin-Mineral Premixes

Vitamin-Mineral Premixes provide a broader range and higher levels of vitamins and minerals than possible by using "old-fashioned" ingredients such as milk, green feed and fish oil. Indeed, many organic certifying organizations will not allow fish oils to be added to poultry diets even though they can be good sources of vitamins A and D (Nutrient Requirements of Poultry 9th Revised Edition, 1994).

3.6.3.5 Minerals

Minerals are the inorganic part of feeds or tissues. They are often divided into two categories, based on the amount that is required in the diet. Requirements for major, or macro, minerals usually are stated as a percentage of the diet, whereas requirements for minor, or trace, minerals are stated as milligrams per kilogram of diet or as parts per million.

Minerals are required for the formation of the skeleton, as components of various compounds with particular functions within the body, as cofactors of enzymes, and for the maintenance of osmotic balance within the body of the bird (Nutrient Requirements of Poultry 9th Revised Edition, 1994). Calcium and phosphorus are essential for the formation and maintenance of the skeleton. Sodium, potassium, magnesium, and chloride function with phosphates and bicarbonate to maintain homeostasis of osmotic relationships and pH throughout the body. Most of the calcium in the diet of the growing bird is used for bone formation, whereas in the mature laying fowl most dietary calcium is used for eggshell formation (Nutrient Requirements of Poultry 9th Revised Edition, 1994). Other functions of calcium include roles in blood clotting and as a second messenger in intracellular communications.

3.6.3.6 Vitamins

Vitamins are generally classified under two headings: fat soluble vitamins, A, D, E, and K, and water-soluble vitamins that include the so-called B-complex and vitamin C (ascorbic acid). Vitamin C is synthesized by poultry and is, accordingly, not considered a required dietary nutrient. There is some evidence, nevertheless, of a favorable response to vitamin C by birds under stress (Pardue et al., 1985).

Dietary supplements frequently contain, as a factor of safety, levels of vitamins in considerable excess of the minimum requirements. Vitamin tolerances have been reviewed by the National Research Council (1987b). Maximum tolerances for vitamins are of the order of 10 to 30 times the minimum requirement for vitamin A, 4 to 10 times for vitamin D3, and 2 to 4 times for choline chloride (possibly because of the chloride). Niacin, riboflavin, and pantothenic acid are generally tolerated at levels as great as 10- to 20-fold their nutritional requirement. Vitamin E is generally tolerated at intakes as great as 100-fold the required level. Vitamins K and C, thiamin, and folic acid are generally tolerated at oral intake levels of at least 1,000-fold the requirement. Pyridoxine may be tolerated at 50 times or more of the requirement (Aboaysha and Kratzer, 1979). High levels of biotin and vitamin B12 have not been tested.

3.6.3.7 Limestone

Limestone is the most common and economical source of calcium for bone development and shell quality (Nutrient Requirements of Poultry 9th Revised Edition, 1994).

3.6.3.8 Dicalcium Phosphate

Dicalcium phosphate is one of the mineral phosphorous sources that is commonly used in organic diets. Because meat and bone meals are not allowed in organic diets, it is essential to provide a mineral source of phosphorous to promote good skeletal health (Nutrient Requirements of Poultry 9th Revised Edition, 1994). The mineral sources used in poultry diets have been acid treated to remove heavy metals that can be toxic to the birds. Untreated rock phosphates that are sometimes used as phosphorous fertilizers in organic crop production can be harmful to birds and should not be used (Nutrient Requirements of Poultry 9th Revised Edition, 1994). The feeds in this factsheet have been formulated assuming that dicalcium phosphate contains 22% calcium and 18.5% phosphorous. Slight alterations in the amount included in the diet may be necessary depending on the actual calcium and phosphorous levels in the dicalcium phosphate.

3.6.3.9 Salt

Salt is essential for growth, production and appetite in poultry (Nutrient Requirements of Poultry 9th Revised Edition, 1994). Nutritionally, common table salt is adequate as a feed ingredient but some organic producers do not like the iodine added to it. Non-iodized salt is sometimes used and kelp is added as an iodine source; care must be taken not to add too much kelp or the birds suffer from iodine toxicity (Nutrient Requirements of Poultry 9th Revised Edition, 1994).

3.6.3.10 Water

Water must be regarded as an essential nutrient, although it is not possible to state precise requirements. The amount needed depends on environmental temperature and relative humidity, the composition of the diet, rate of growth or egg production, and efficiency of kidney resorption of water in individual birds (Medway and Kare, 1959). It has been generally assumed that birds drink approximately twice as much water as the amount of feed consumed on a weight basis, but water intake actually varies greatly.

Several dietary factors influence water intake and water:feed ratios. Increasing crude protein increases water intake and water:feed ratios (Marks and Pesti, 1984). Crumbling or pelleting of diets increases both water and feed intake relative to mash diets, but water:feed ratios stay relatively the same (Marks and Pesti, 1984). Increasing dietary salt increases the water intake (Marks, 1987).

When formulating feed rations (tables 3-1; 3-2 and 3-3), sludge volumes were made to be three (3) times so as to have the same nutritional value as fishmeal. This was prompted by the fact that when treating sludge with HCl, some of the intact proteins are denatured in the process as depicted in figure 4-9 below

Table 3- 1. Feed Formulation for Broiler Starter Ration

Raw Material	Raw Cost per ton	CURRENT RATION (100% Fishmeal, 0% Activated Sludge) kg	New ration inclusive of Waste Activated Sludge (75 % Fishmeal, 25% Activated Sludge) kg	New ration inclusive of Waste Activated Sludge (50 % Fishmeal,50% Activated Sludge) Kg	New ration inclusive of Waste Activated Sludge (25 % Fishmeal 75 % Activated Sludge) kg	New ration inclusive of Waste Activated Sludge (0 % Fishmeal100 % Activated Sludge) kg
Maize 7.5%	R 1 650.00	598.23	598.23	598.23	598.23	598.23
Full Fat Soya Extr	R 4 180.00	125	125	125	125	125
Soya Oil Cake 47%	R 4 935.00	175.67	175.67	175.67	175.67	175.67
Fish Meal 65%	R 9 600.00	42.81	32.11	21.41	10.7	0
Poultry By-Product 55%	R 2 500.00	25	25	25	25	25
Limestone	R 360.00	16.76	16.76	16.76	16.76	16.76
Monocal Phos (21%)	R 5 400.00	6.47	6.47	6.47	6.47	6.47
Salt	R 1 050.00	3.17	3.17	3.17	3.17	3.17
Vitamin Mineral Premix	R 71 220.00	7	7	7	7	7
MagaCal	R 35 000.00	0.5	0.5	0.5	0.5	0.5
Sludge Single Cell Prot	R200.00	0	32.11	64.22	96.32	128.43

Table 3-2. Feed Formulation for Broiler Grower Ration

Raw Material	Raw Cost per ton	CURRENT RATION (100% Fishmeal, 0% Activated Sludge) Kg	New ration inclusive of Waste Activated Sludge (75 % Fishmeal, 25% Activated Sludge) Kg	New ration inclusive of Waste Activated Sludge (50 % Fishmeal,50% Activated Sludge) Kg	New ration inclusive of Waste Activated Sludge (25 % Fishmeal ,75 % Activated Sludge) Kg	New ration inclusive of Waste Activated Sludge (0 % Fishmeal,100 % Activated Sludge) Kg
Maize 7.5%	R 1 650.00	666.15	666.15	666.15	666.15	666.15
Full Fat Soya Extr	R 4 180.00	125	125	125	125	125
Soya Oil Cake 47%	R 4 935.00	70.94	70.94	70.94	70.94	70.94
Fish Meal 65%	R 9 600.00	87.25	65.44	43.63	21.81	0
Poultry By-Product 55%	R 2 500.00	30	30	30	30	30
Limestone	R 360.00	11.04	11.04	11.04	11.04	11.04
Monocal Phos	R 5 400.00					
Salt	R 1 050.00	2.5	2.5	2.5	2.5	2.5
Vitamin Mineral Premix	R 71 220.00	6.6	6.6	6.6	6.6	6.6
MagaCal	R 35 000.00	0.5	0.5	0.5	0.5	0.5
Sludge Single Cell Prot	R200.00	0	64.44	130.88	196.31	261.75

Table 3-3 .Feed Formulation for Broiler Finisher Ration

Raw Material	Raw Cost per ton	CURRENT RATION (100% Fishmeal, 0% Activated Sludge) kg	New ration inclusive of Waste Activated Sludge (75 % Fishmeal, 25% Activated Sludge) kg	New ration inclusive of Waste Activated Sludge (50 % Fishmeal,50% Activated Sludge) kg	New ration inclusive of Waste Activated Sludge (25 % Fishmeal 75 % Activated Sludge) kg	New ration inclusive of Waste Activated Sludge (0 % Fishmeal100 % Activated Sludge) kg
Maize 7.5%	R 1 650.00	684.13	684.13	684.13	684.13	684.13
Full Fat Soya Extr	R 4 180.00	125	125	125	125	125
Soya Oil Cake 47%	R 4 935.00	64.14	64.14	64.14	64.14	64.14
Fish Meal 65%	R 9 600.00	73.09	54.82	36.55	18.27	0
Poultry By-Product 55%	R 2 500.00	30	30	30	30	30
Limestone	R360.00	10.59	10.59	10.59	10.59	10.59
Monocal Phos	R 5 400.00	1.8	1.8	1.8	1.8	1.8
Salt	R 1 050.00	2.2	2.2	2.2	2.2	2.2
Vitamin Mineral Premix	R 71 220.00	5	5	5	5	5
fat		2.8	2.8	2.8	2.8	2.8
MagaCal	R 35 000.00	0.5	0.5	0.5	0.5	0.5
Sludge Single Cell Prot	200.00	0	54.82	109.64	164.45	219.27

3.6.4 Stocking Density

The experiments were designed for ten chicks per group as rearing chicks in overcrowded conditions does not deliver optimal biological or economical results (Arbor acres, service bulletin, www.aviagen.com, 2008). 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.

3.6.5 Litter Management

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

3.6.6 Ventilation

Providing good air quality for the chick is critical (Arbor Acres, 2008). Ventilation practices during the brooding period brought in enough fresh air to provide sufficient oxygen and exhaust excess moisture and harmful gases — without chilling chicks. To achieve this, the house had air inlets evenly distributed along the entire length of the house. In-house air was above the chicks as a rule of thumb.

3.7 Measurement of Success

A good measure of successful chick start is crop fill. (Arbor Acres, 2008). 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.

3.8 Weight Measurement

Chickens were individually weighed every morning using the digital scale. The weight was then recorded on a weight sheet.

3.9 Chicken Mortality Rate

This was discovered by counting the live birds (Awobajo et al., 2007), the dead birds were also easily discovered on the litters. Also when there were sick ones, such were isolated and catered for separately in order not to transfer the illness or sickness to others which may make the mortality rate to increase. The Formula for calculating percentage mortality is given as:

$$M_R = \frac{\text{Number of dead birds}}{\text{Total number of chicken}} \times 100 \qquad (3\text{-}1)$$

Where M_R = Mortality rate (%)

CHAPTER 4.

RESULTS AND DISCUSSIONS

4.1. Dry Matter, Volatile and Non-Volatile Fractions

The ratio of the volatile solids (VS) to non volatile solids or fixed solids (FS) is used to characterize the wastewater with respect to amount of organic matter present. The results in Figure 4-1 represent the average values taken over a six-month period. Waste activated sludge from Zeekoeigat had 4.10% dry matter, followed by Rooival and then Baviansport with 3.20% and 1.30% dry matter, respectively. Zeekoeigat waste activated sludge had 72.70% VS; 27.30% FS, Rooiwal waste activated sludge had 73.30% VS; 26.70% FS and Bavianspoort activated sludge had 55.6% VS and 44.4% FS.

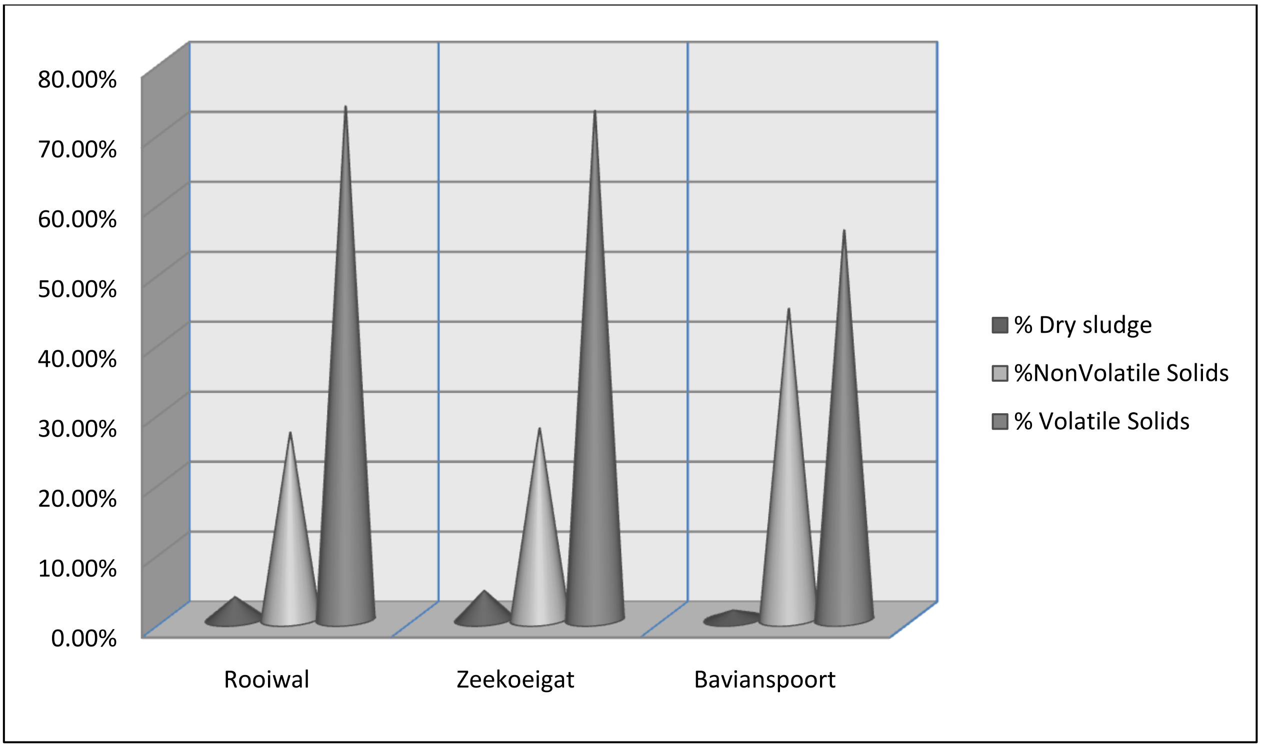

Figure 4-1. Sludge Dry Matter, Volatile and Non Volatile Solids

4.2 Crude Protein Determination

TKN analysis was performed on waste activated sludges from Rooiwal, Zeekoeigat and Baviaansport sewage works over a period of six months to monitor protein concentration consistency in those sludges. The average values are presented in Figure 4-2 . The solutions were distilled with sodium hydroxide (added in small quantities) which converts the ammonium salt to ammonia.

Degradation: **Protein + $H_2SO_4 \rightarrow (NH_4)_2SO_4$ (aq) + CO_2 (g) + SO_2 (g) + H_2O (g)** (4-1)

The amount of ammonia present (hence the amount of nitrogen present in the sample) is determined by back titration.

Liberation of ammonia: **$(NH4)_2SO_4$ (aq) + 2NaOH $\rightarrow$ Na_2SO_4 (aq) + $2H_2O$ (l) + $2NH_3$ (g)** (4-2)

The end of the condenser is dipped into a solution of boric acid.

Capture of ammonia: **B $(OH)_3$ + H_2O + NH_3 $\rightarrow$ NH^{4+} + B $(OH)^{4-}$** (4-3)

The ammonia reacts with the acid and the remainder of the acid is then titrated with a sodium carbonate solution with a methyl orange pH indicator.

Back-titration: **B $(OH)_3$ + H_2O + Na_2CO_3 $\rightarrow$ $NaHCO_3$(aq) + $NaB(OH)_4$(aq) + CO_2(g) + H_2O** (4-4)

The protein content is then calculated based on the assumption that the protein contains 16% (w/w) nitrogen. The protein content is then calculated according to the formula:

Protein content = **(Kjel-N – NH4-N) x (100/16)** (4-5)

Zeekoeigat WAS had the highest protein content (38 %), and therefore was chosen for protein isolation studies.

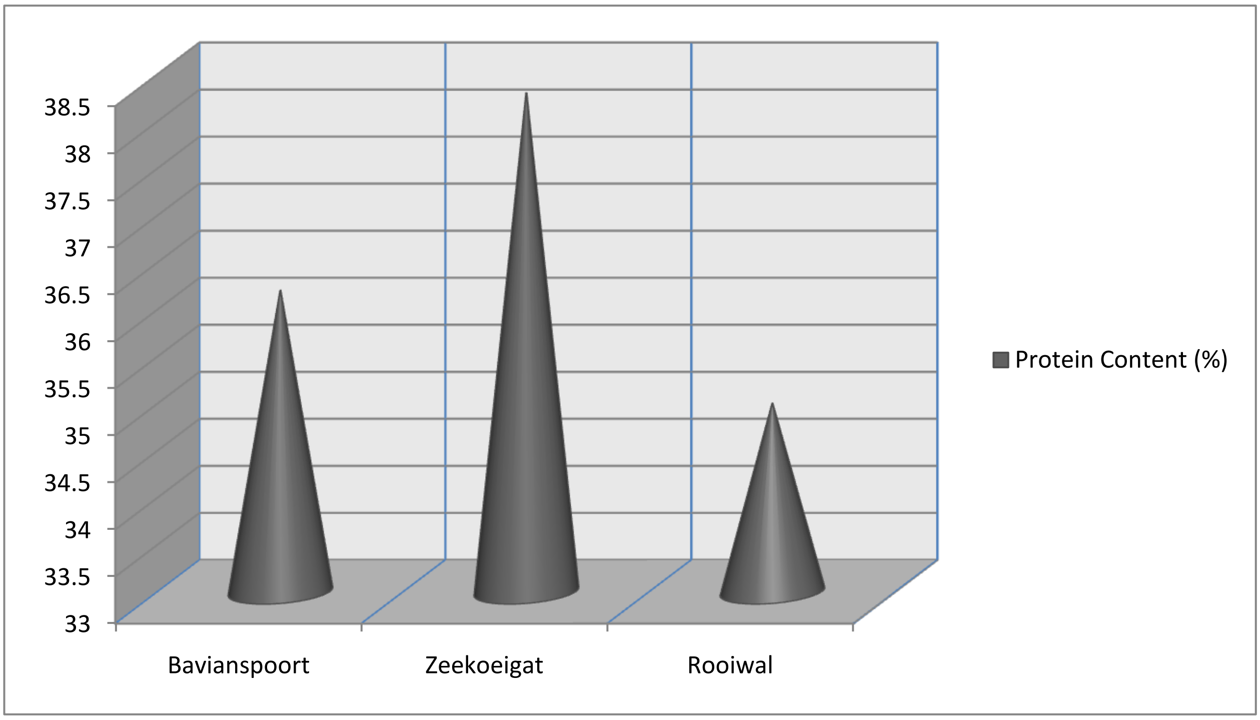

Figure 4-2. Crude Protein Content of Zeekoeigat, Baviaanspoort and Rooiwal Wastewater Treatment Plants.

4.3 Protein Isolation Studies

Proteins were isolated according to the method by Shier and Purwono (1994). The sludge samples were autoclaved at 120°C and other samples were digested in mineral oil at 155°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°C, the protein concentration was 0.82 mg/mL, and in the supernatant from the sludge that was digested at 155°C, the protein concentration was 0.47 mg/mL. The results showed that there were little intact proteins that were found at 150°C than at 120°C, and that was probably due the fact that at high temperatures, intact proteins denature, and they are broken down into constituent amino acids.

4.3.1 Amino Acid Analysis

Amino acid profile of sludge was done using the Pico-tag method (Bidlingmeyer *et al.*, 1984). This profile was then compared to the chicken feed requirement as depicted in Figure 4-3 below. 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 and thus have an added advantage of growing broiler chicken stronger and bigger than the chicken feed with conventional feedlot.

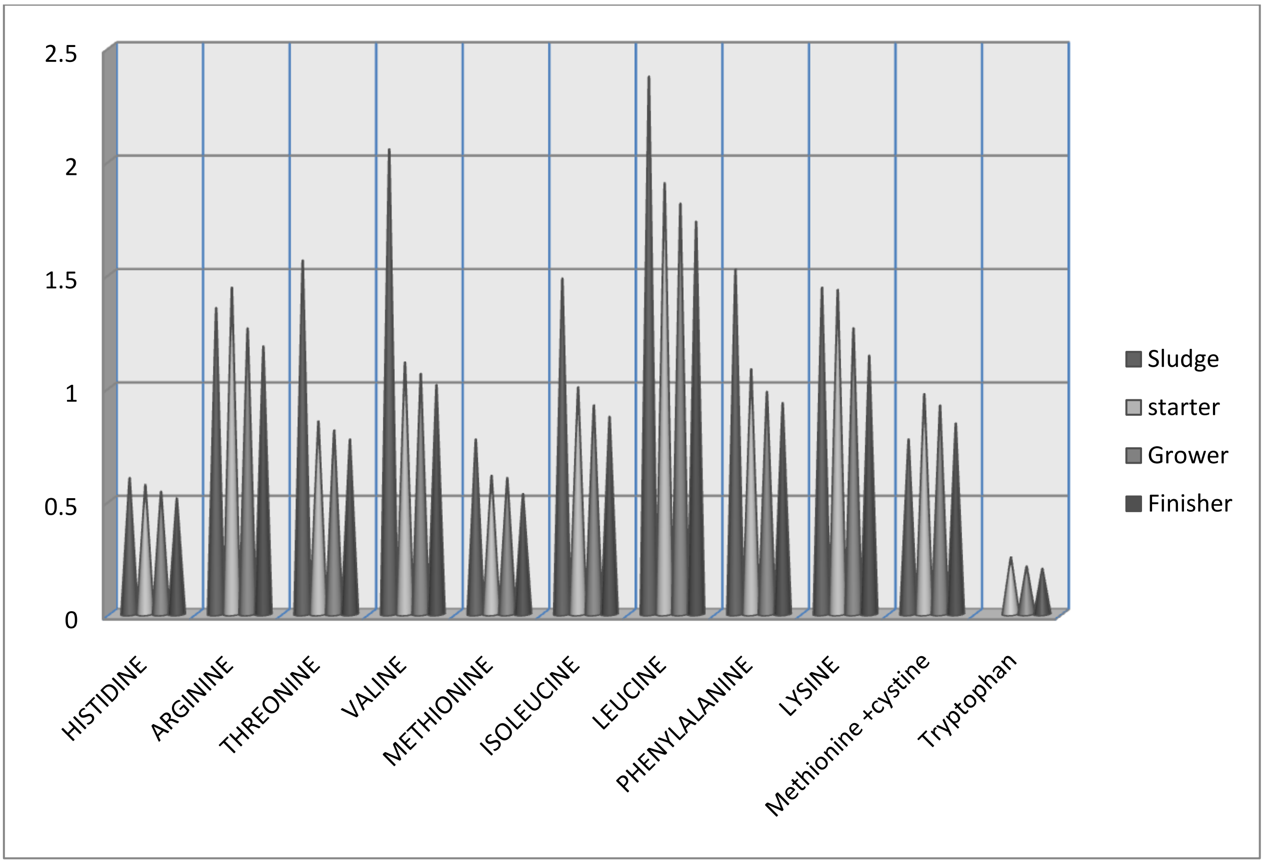

Figure 4-3. Amino Acids Profile (g/100g) of Sludge Compared to the Chicken Feed Requirement

isoleucine, arginine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, histidine, tyrosine and leucine are essential amino acids because the body does not synthesize them and therefore have to be provided in the diets. The sludge however, did not contain tryptophan and tryptophan, has emerged as a regulator of many immunological and physiological processes (Emadi *et al*., 2010). Its plasma concentration declines in animals suffering from different illnesses and inflammations (induced or natural), suggesting an increased utilization of the amino acid in such instances (Le Floc`h *et al*., 2004).

Study by Takahashi *et al*. 1997, has shown that dietary cysteine is not only important for T-cell function and antibody production, but also for macrophage response to lipopolysaccharide in broilers. The methionine:cysteine ratio in the diet is an important factor affecting some immune responses, e.g. interleukin - 1-like activity, acid glycoprotein (AGP) concentration in plasma and mitogenic response of mononuclear cell in spleen.

4.3.2 Nucleic Acid Analysis

The samples were then run on the agarose gel to analyse for nucleic acids. The results however, showed that at 120°C, nucleic acids are also extracted (Figure 4-4). Nucleic acids are the building blocks of proteins and in particular, RNA helps to boost the immune system response and improves wound healing (Gibb, 2009).

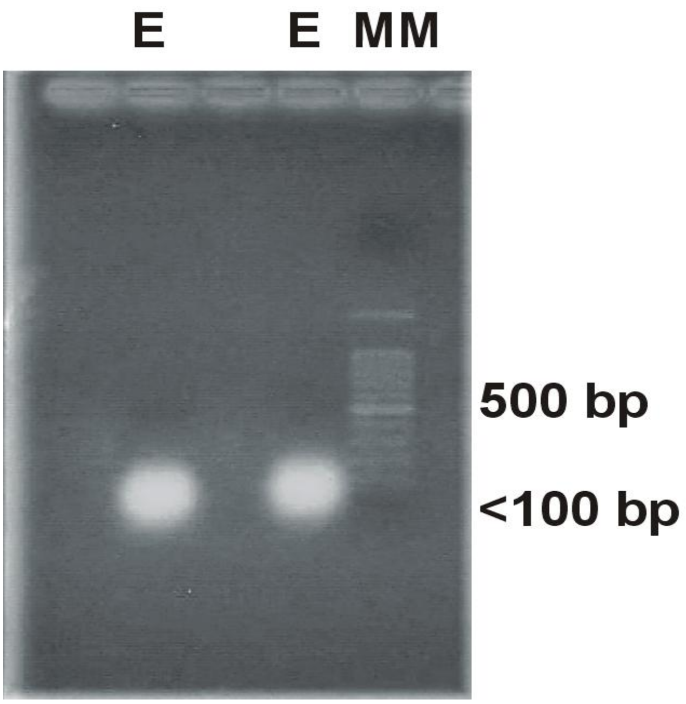

Figure 4-4. Agarose Gel Electrophoresis Showing Sample (E) in Duplicate and DNA Makers (MM)

4.3.3 Reverse Phase Chromatography

These samples (autoclaved at 120°C and digested at 155°C) were separately run on Reverse Phase Chromatography Figures 4-5 and 4-6. Each peak was analyzed on the wavelength spectrum from 200 to 300 nm. The analysis showed that most of the extracted materials were nucleic acids, which had the maximum wavelength at 260 nm, and the modified nucleic acids had their maximum wavelength around 260 nm.

Again each peak was analysed on the wavelength spectrum from 200 to 300 nm. Tryptophan (λmax 218.6) was predominant in peaks 1 and 2. Tyrosine (λmax 223.2) was also found in peak 2. Peak 9 is a characteristic peak of L-Tyr-Gly-Gly(λmax 255) Peak 7 was phenylalanine (λmax 254

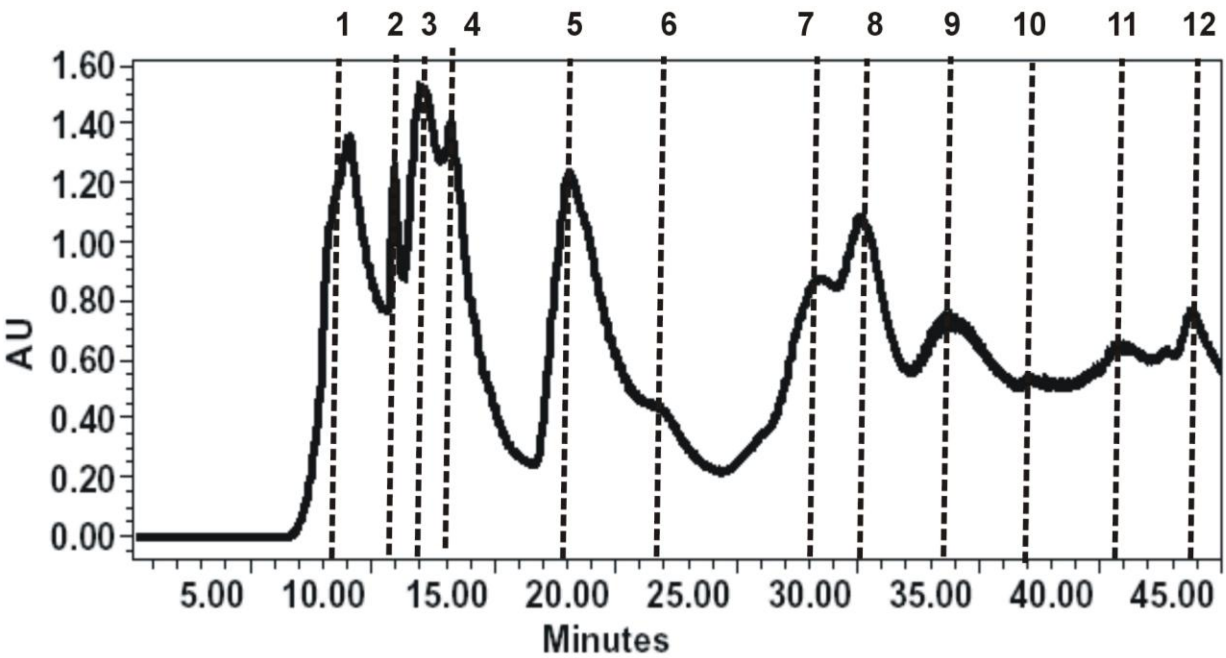

Figure 4-5. RP Chromatogram of Sludge Supernatant After Digestion at 120°C

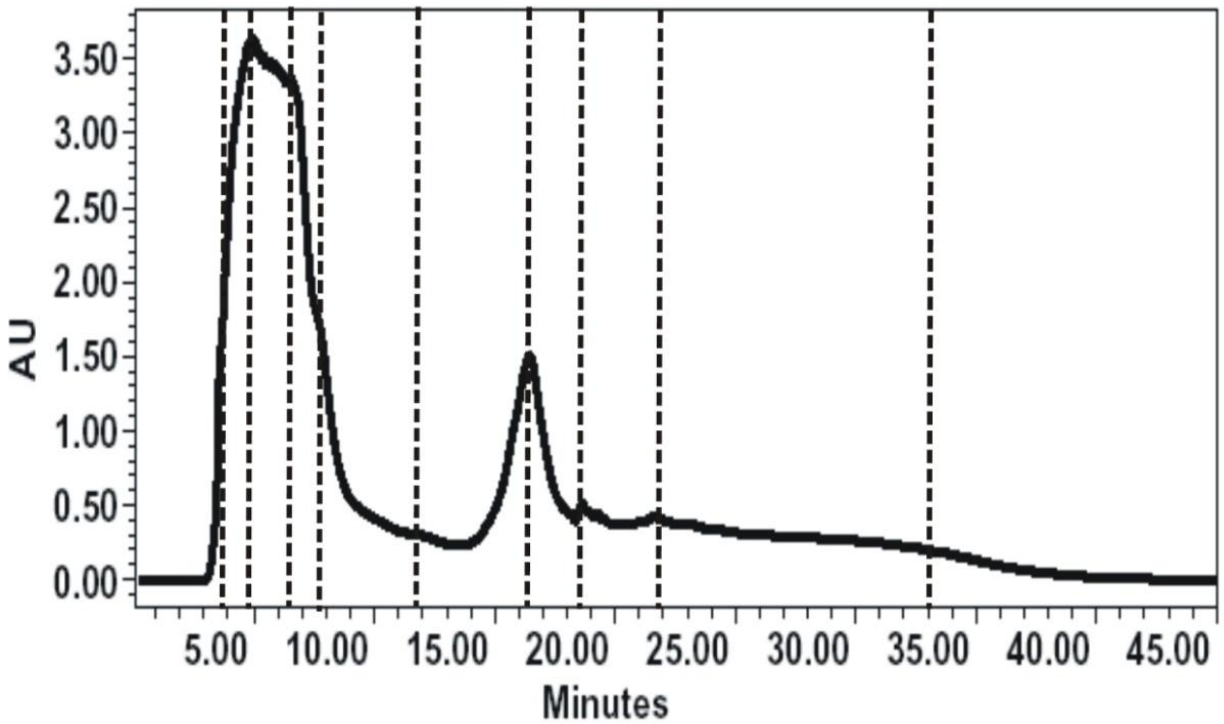

Figure 4-6. RP Chromatogram of Sludge Supernatant After Digestion at 155°

4.4 Metal Removal

The heavy metal (Figure 4-7) and the minerals (figure 4-8), were removed from the Zeekoeigat activated sludge and compared to fishmeal and to allowable limit in Chicken feed.

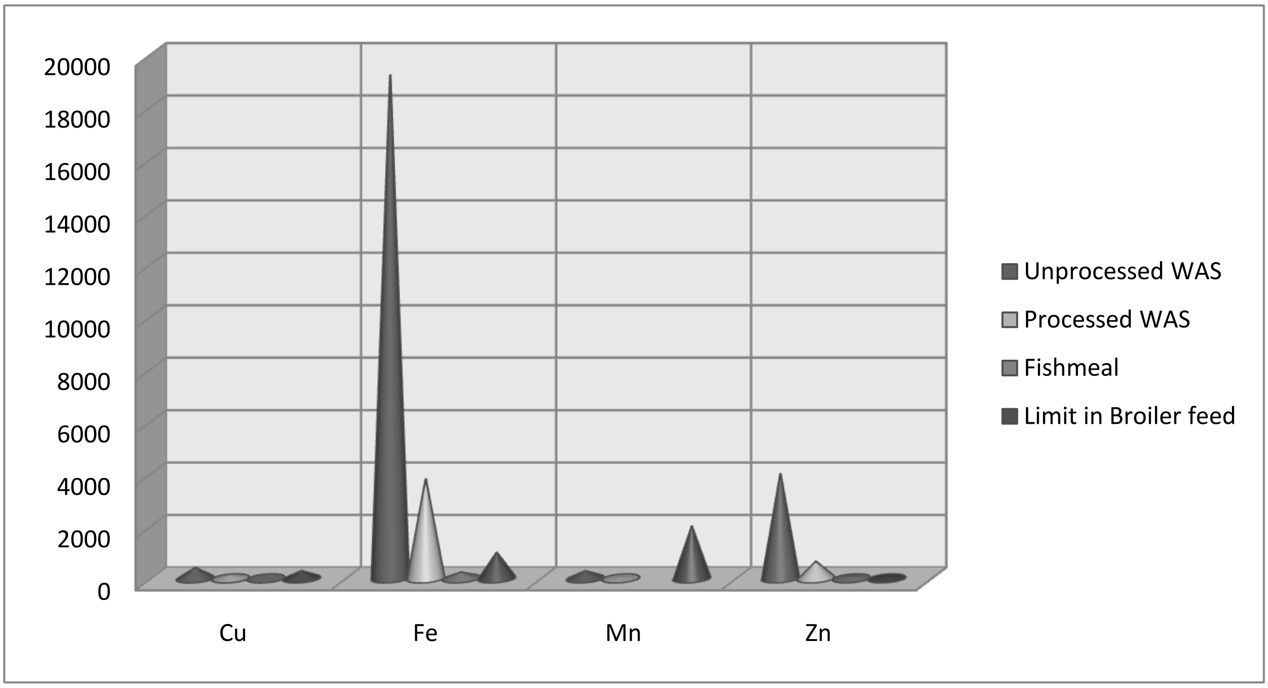

Figure 4-7: Efficiency of Heavy Metal Removal (ppm) by 1N HCl and 0.1 M Citric Acid

A study by Hengmin *et al*., 2008, showed that dietary copper in excess of 300mg / kg suppressed the development of T-lymphocytes and reduced the percentage of CD4+ T cells and the CD4+/CD8+ ratio, and resulted in pathological injury of the thymus in chicken and also impaired the cellular immune function. Sheppard and Dierenfeld, 2006, have suggested that concentration of 100mg/kg Fe may be sensitive to birds and therefore can cause Iron Storage Disease in Birds which is a 'more than expected' deposition of hemosiderin (deposition of iron) in the liver, spleen, marrow and reticulocytes.

Manganese is required in diets for poultry because it is important for growth, bone development, feathering, enzyme structure and function (Julean *et al*., 2008) Excess manganese interferes with the absorption of dietary iron. A study by Bao *et al*, (2007), showed that supplementation with 4 mg of Cu and 40 mg each of Fe, Mn, and Zn from organic sources may be sufficient for normal broiler growth to 29 d of age and thus it is possible to use these lower levels of organic trace minerals in broiler diets to avoid high levels of trace mineral excretion (Bao *et al*., 2007).

In this study Cu, Mn, Zn were within the allowable limit in the broiler feed after the sludge was treated with 1N HCl, Iron was above the allowable limit and the effect thereof needs to be investigated.

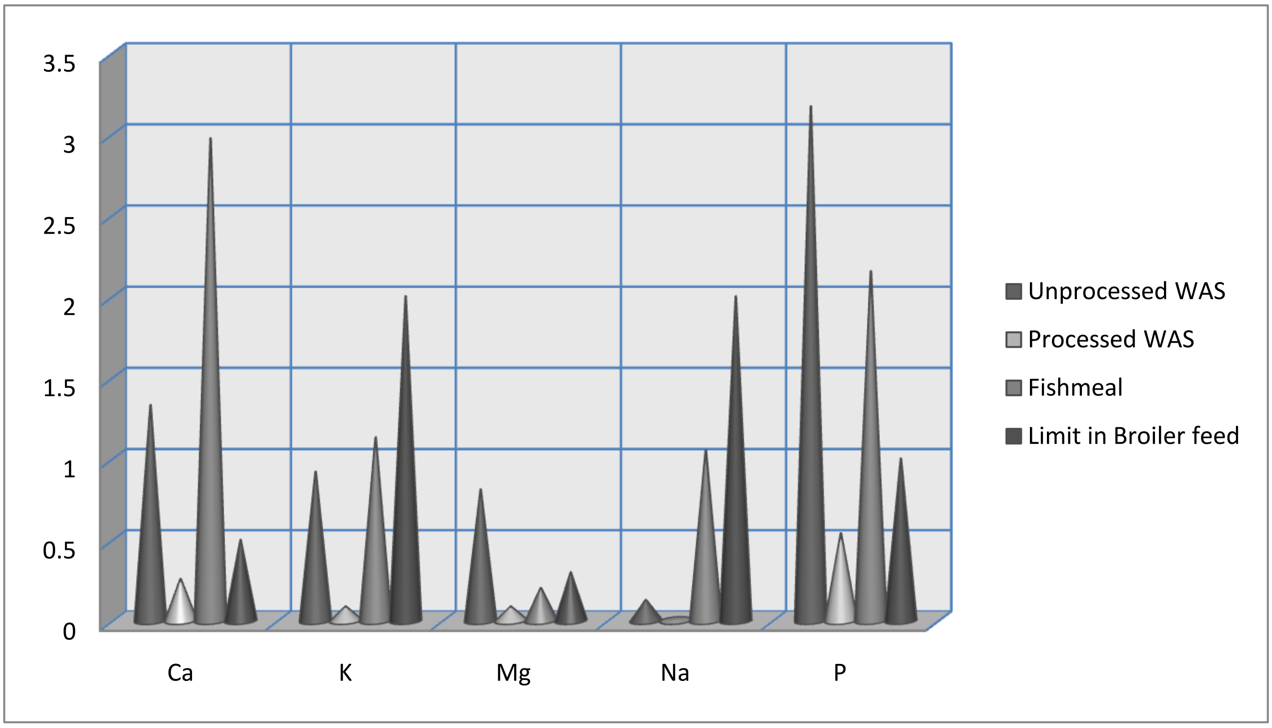

Figure 4-8. Metal Concentration of the Sludge (%) From Zeekoeigat Waste water Treatment Plant Compared with Fishmeal and Allowable Limit in Broiler Feed

Calcium is the primary mineral that makes up eggshells and when not supplied in the diet, the hen does not have the basic materials needed to make the shell. Phosphorus is needed so that the chickens can metabolize calcium. Broiler chickens fed with magnesium supplement result in increased processing yield, feed conversion and body weight. All the mineral tested in the study (Ca,K,Mg,Na and P) were within limit of the broiler feed after the sludge was treated with 1 N HCl.

4.4.1 Amino Acid Analysis / Crude Protein Determination

In order to determine whether proteins are extracted together with the heavy metals using 1 N HCl, the supernatant was analysed for protein using the Pico-tag method (Bidlingmeyer *et al.*, 1984). From the analysis it was shown that approximately 40% the proteins are extracted with the heavy metals (Figure 4-9).

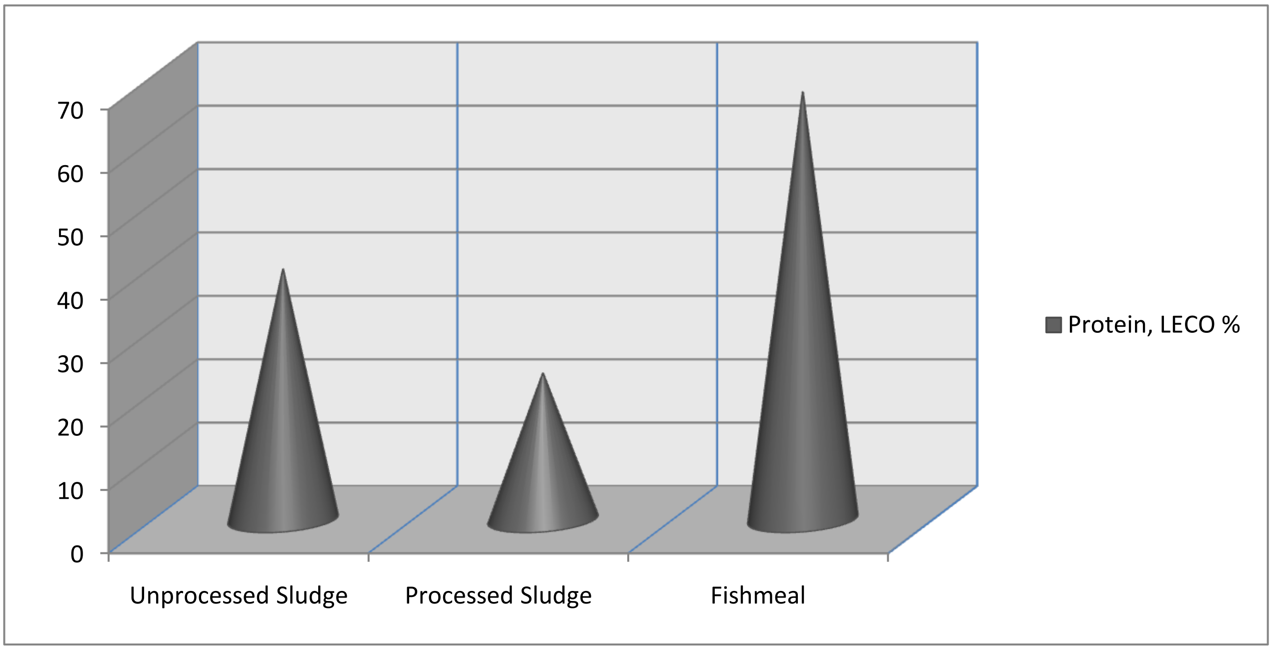

Figure 4-9. Protein Removed with the Heavy Metal in the Supernatant Compared with the Protein Content of Fishmeal.

The protein content of the Unprocessed waste activated sludge was 39.48%. After treating waste activated sludge with HCl to remove heavy metals, protein content of the processed sludge was 23.07%. This was then compared with fishmeal and it was found that protein content of fishmeal was 67.42%. To have the same protein content as fishmeal 3 parts of processed waste activated sludge was the added in feed to have the same protein content as fishmeal.

4.5 Broiler Feeding Pilot Studies

4.5.1 Terminology of Energy Values of Animal Feed

The digestive process is generally not able to make all the energy (E) consumed available to the animal for absorption; thus there is a loss of energy in the faeces. Subtracting the energy excreted (EX) in faeces from the E consumed yields digestible energy (DE).

$$E - EX = DE \quad (4\text{-}6)$$

Digestible energy can be expressed in absolute terms per unit of weight (kcal/g) or as a percentage of gross energy. The term total digestible nutrients (TDN) is also used, but feed energy values are expressed in units of weight of calories (Kilojoules). TDN is determined by summing digestible crude protein, digestible carbohydrates (nitrogen-free extract and crude fiber), and 2.25 x digestible crude fat. Metabolizable energy (ME) is determined by subtracting gaseous and urine energy losses from DE. Net energy (NE) is the most refined expression of the value of energy in animal feed. It represents the amount of energy available to the animal for maintenance and reproductive processes

4.5.2 Broiler Weight Monitoring

Chicken were fed with feed formulations as described in Tables 3-1, 3-2 and 3-3 above. Chicken were then weighed on daily basis, and their average weight gains were the plotted and compared with average weight gains of chicken which were fed with conventional feedlot (Figures 4-10 A – D).

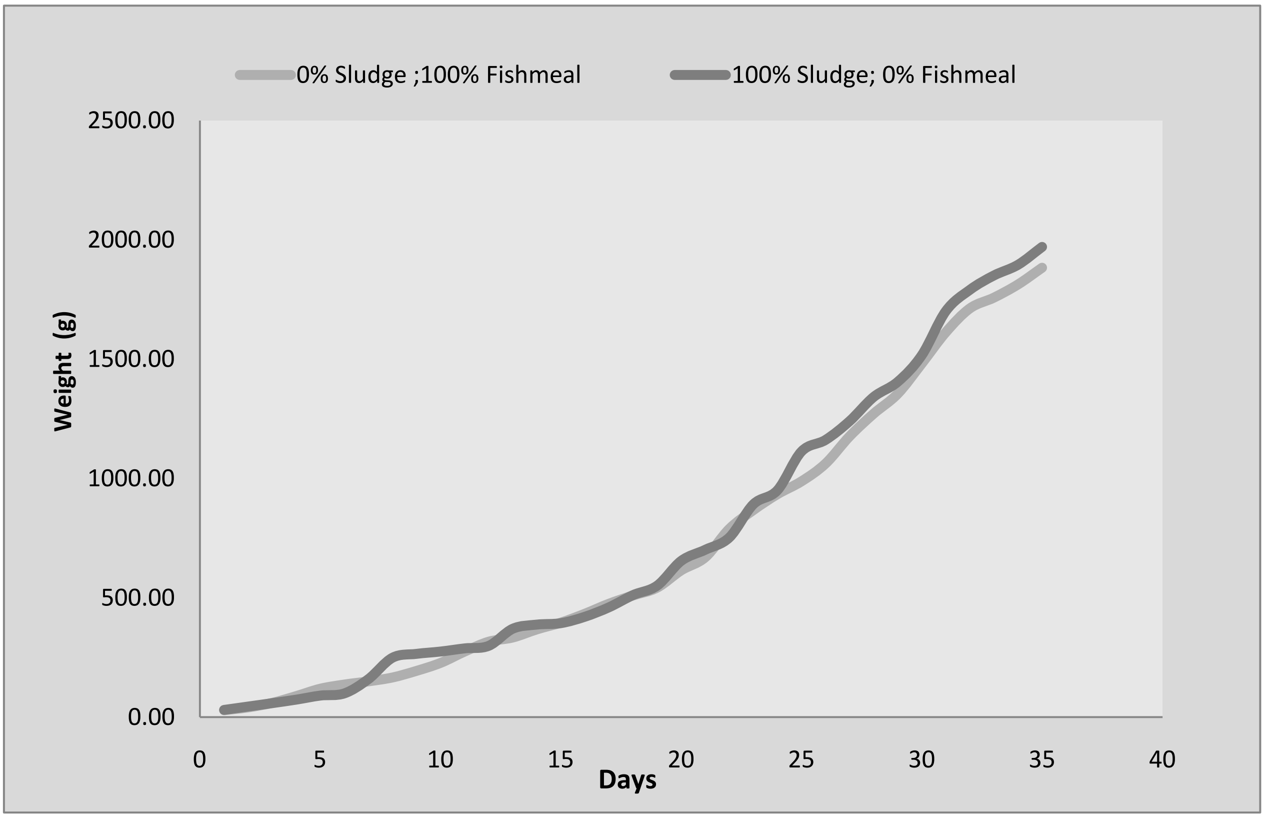

Figure 4-10 (A). Broiler Weight Gain of Chicken Fed with 100% Sludge and 0 % Fishmeal Compared with Chicken Fed with Conventional Feed

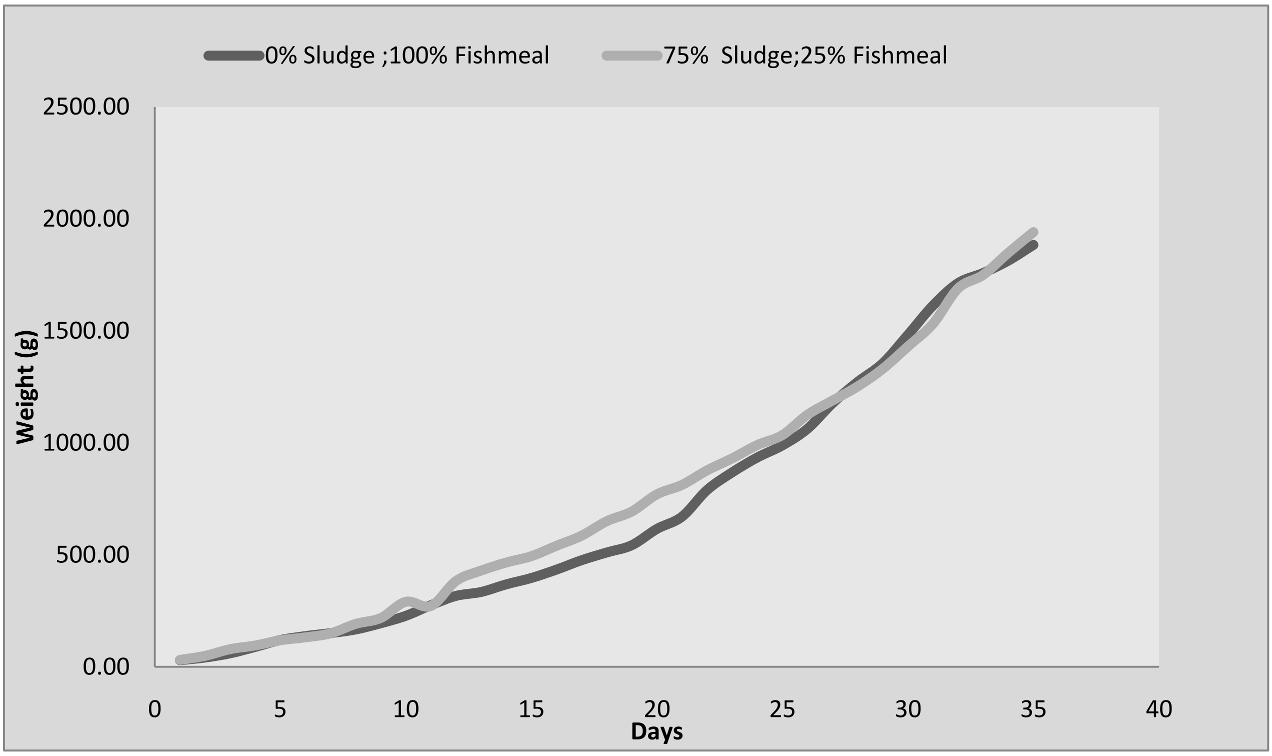

Figure 4-10 (B). Broiler Weight Gain of Chicken Fed with 75%Ssludge and 25% Fishmeal, Compared with Chicken fed with Conventional Feed

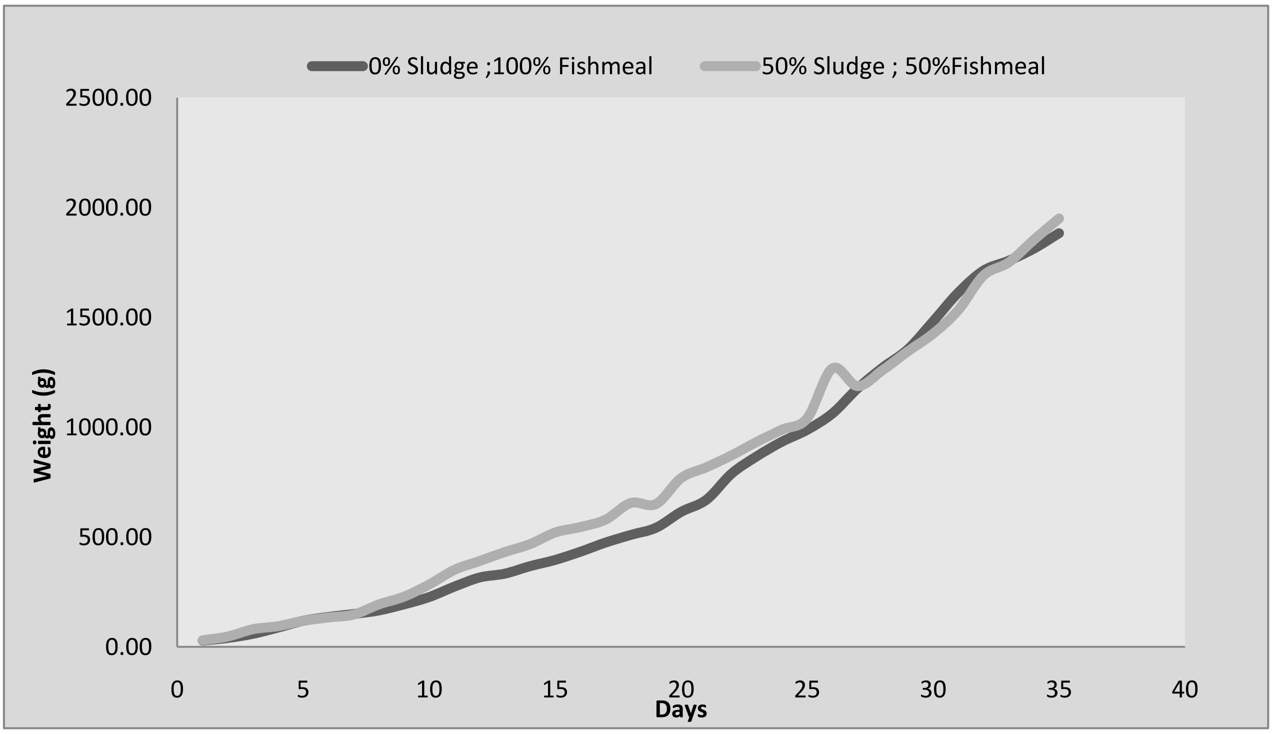

Figure 4-10 (C). Broiler Weight Gain of Chicken Fed with 50% Sludge and 50% Fishmeal Compared with Chicken Fed with Conventional Feed

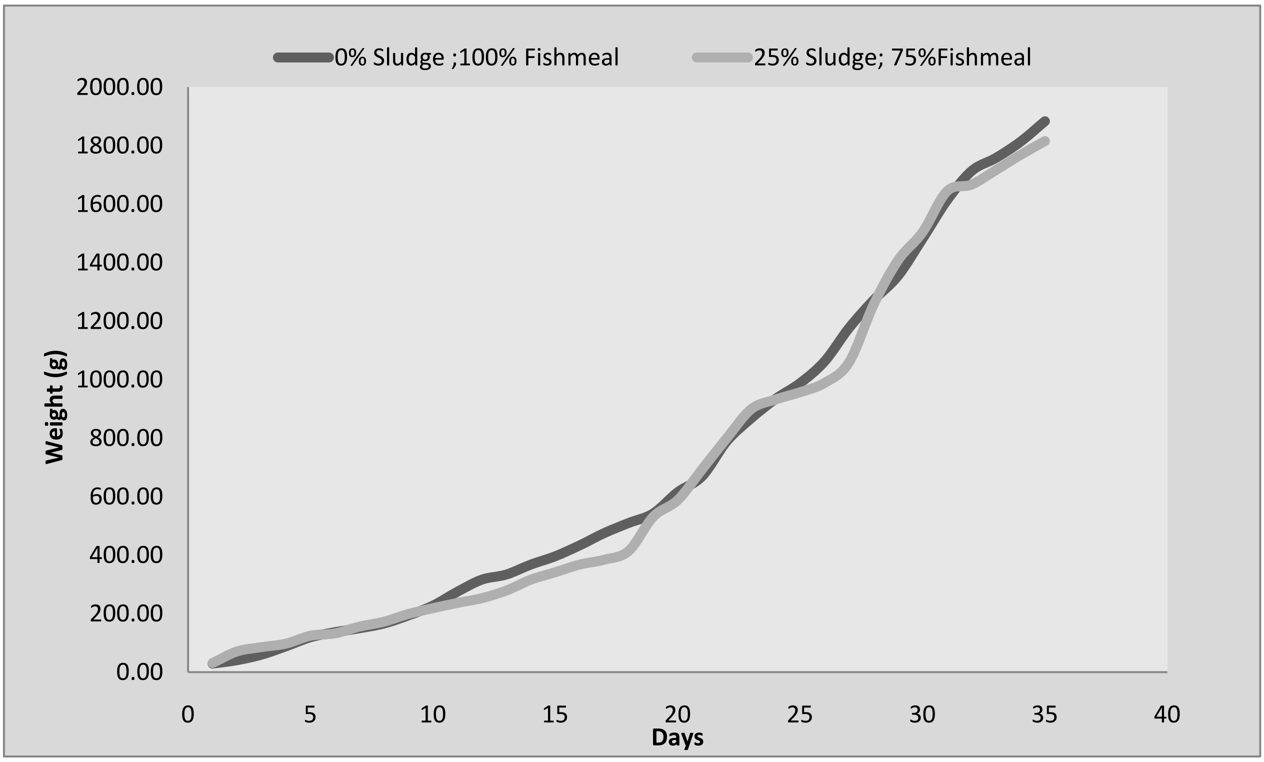

Figure 4-10 (D). Broiler Weight Gain of Chicken Fed with 25 % Sludge and 75% Fishmeal Compared with Chicken Fed with Conventional Feed

4.5.2 (A) Feed Formulation: 0% Sludge; 100% 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 4-10 A)

4.5.2(B) Feed Formulation: 75% Sludge; 25 % Fishmeal

The chicken's weight was roughly the same until day 9, and thereafter the weight of the chicken that were feed with sludge was slightly higher until day 30 and then picked up again after day 33 (Figure 4-10 B).

4.5.2(C) Feed Formulation: 50% Sludge; 50% Fishmeal

There was a significant weight differences from 10 days to 30 days in chickens that were fed the conventional feed and the ones that were fed with the feedlot where fishmeal was substituted with sludge. The chicken feed with sludge weighed higher. This phenomenon was also observed from day 33 to day 35 (Figure 4-10 C).

4.5.2 (D) Feed Formulation: 25% Sludge;75 % Fishmeal

There was a 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. From day 10, to day 20 chicken feed with conventional feedlot weighed higher (Figure 4-10 D). This was also observed from day 25 to day 30, and from day 32 to day 35

4.5.3 Weight Distribution of Broilers

Standard curve of the weight of Broilers was generated using the Arbor Acres Yield U.S. Standard and Flock Records, 2000 (Figure 4-11). The broilers were weighed on day 7,14,21,28 and 35 and the standard weights were 147g, 368g, 750g, 1248g and 1740g respectively.

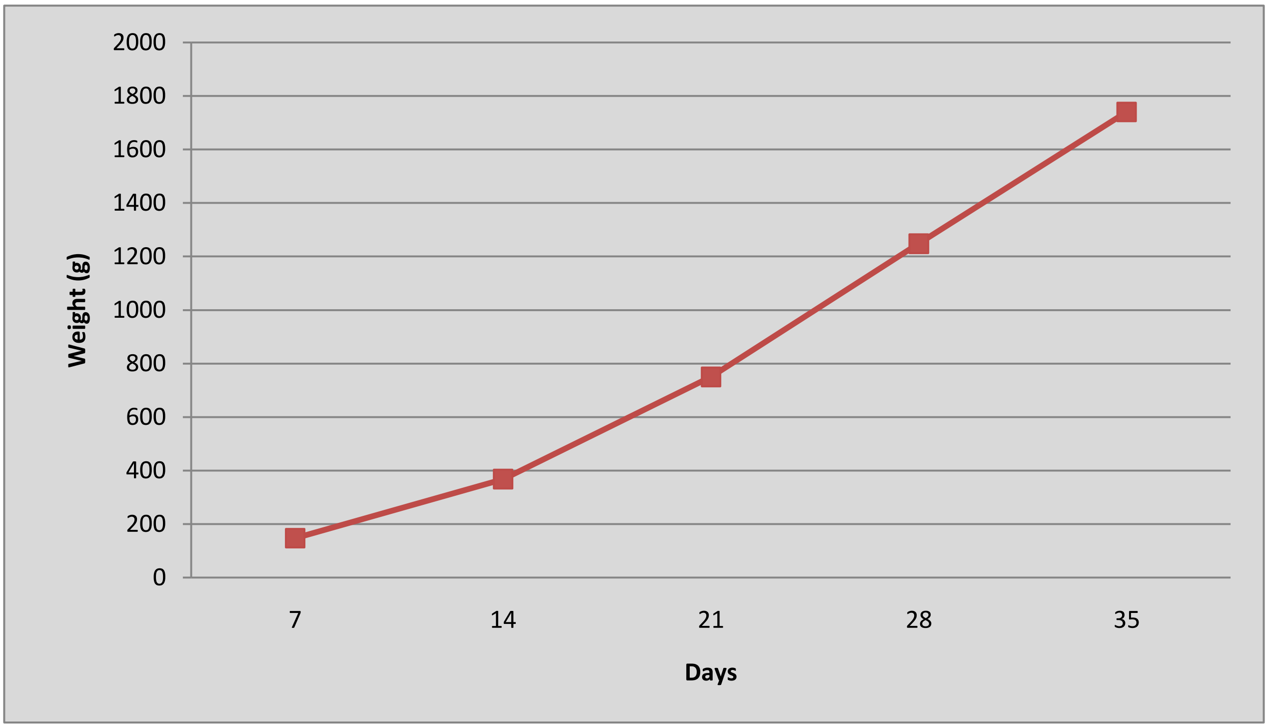

Figure 4-11. Standard Weight of Broilers.

Weight distribution of broilers was then done at day 7 (Figure 4-12.A), day 14 (Figure 4-12.B), day 21 (Figure 4-12.C), day 28 (Figure 4-12.D) and day 35 (Figure 4-12.E).

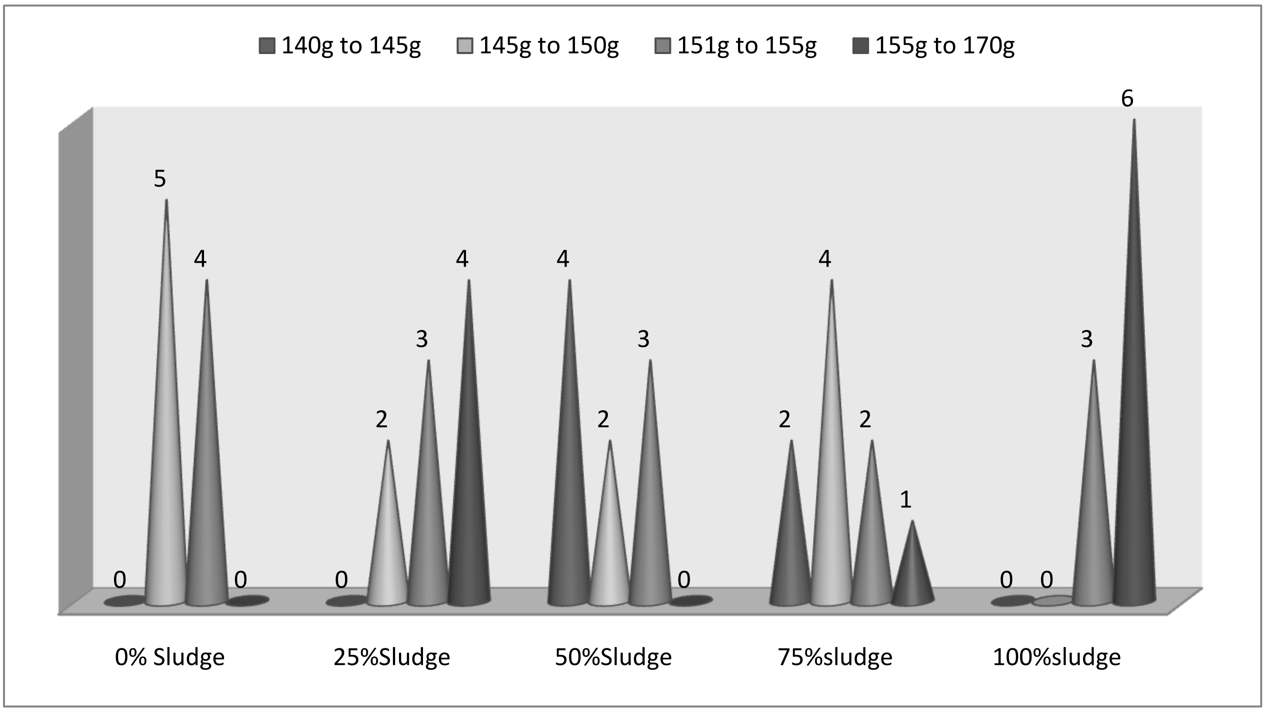

Figure 4-12 A. Distribution of Live Weights of Chicken at Day 7

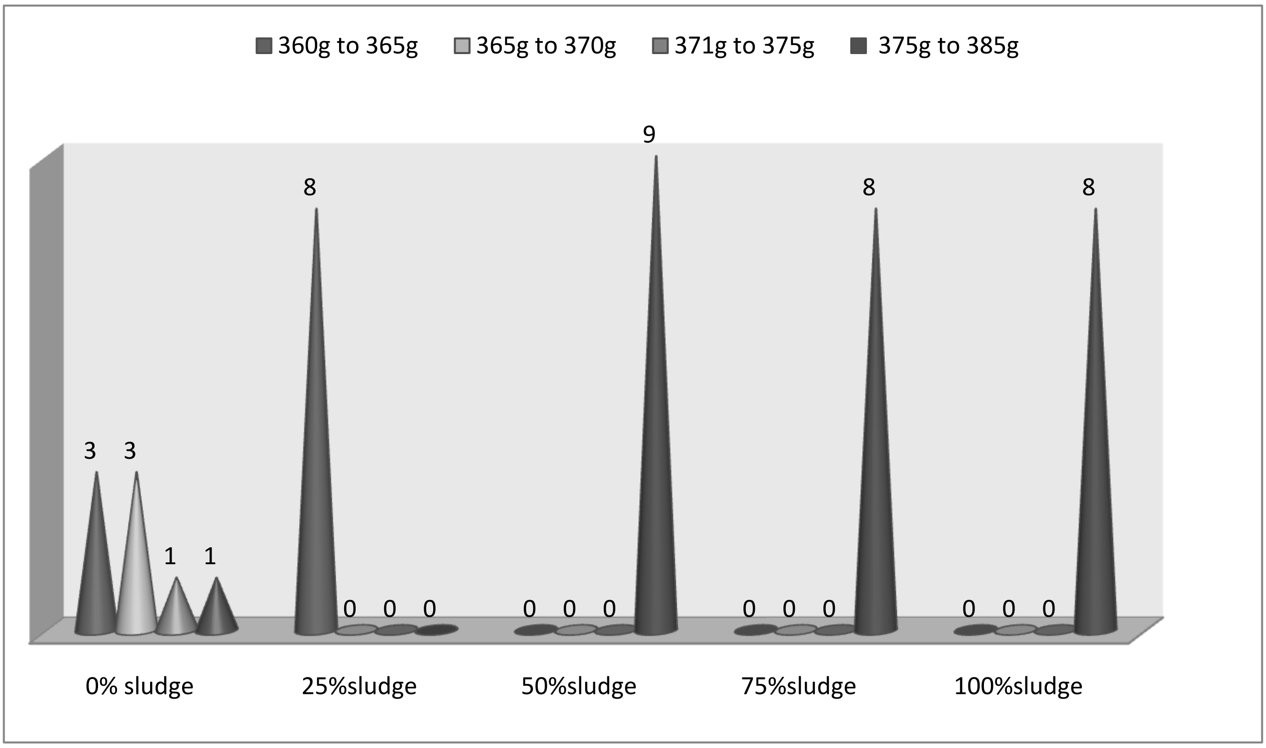

Figure 4-12.B. Distribution of Live Weights of Chicken at Day 14

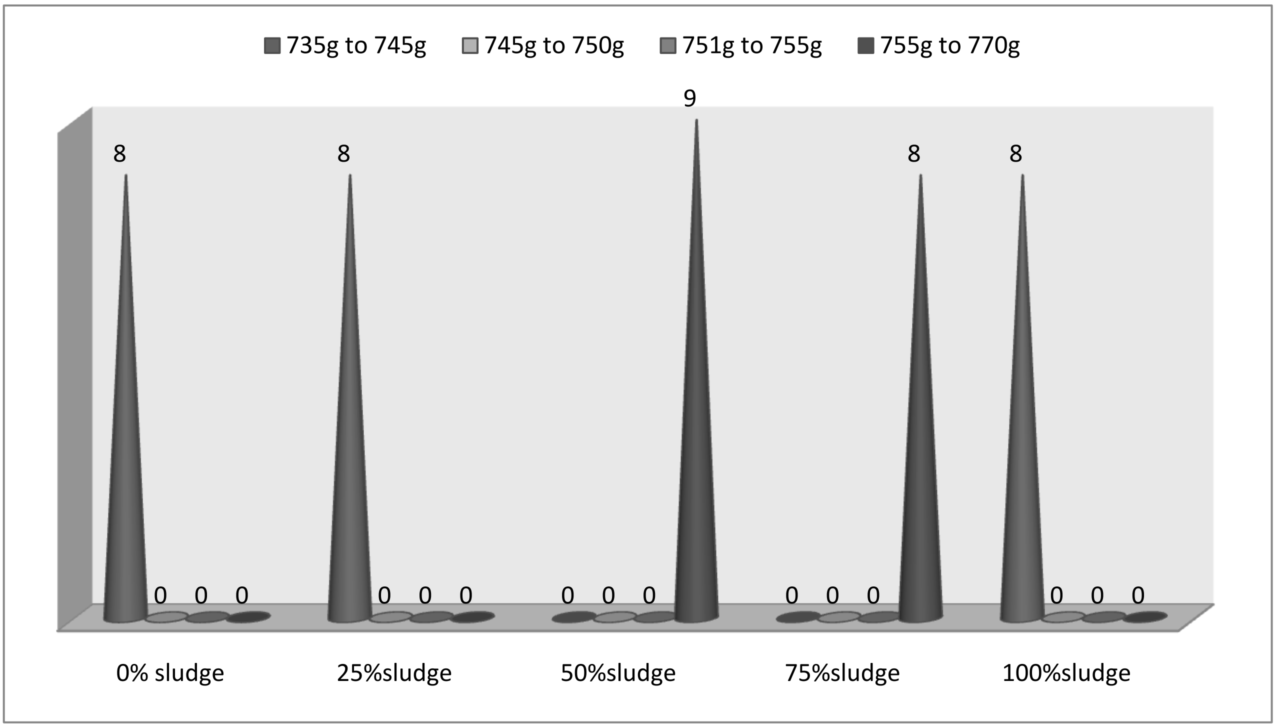

Figure 4-12.C. Distribution of Live Weights of Chicken at Day 21

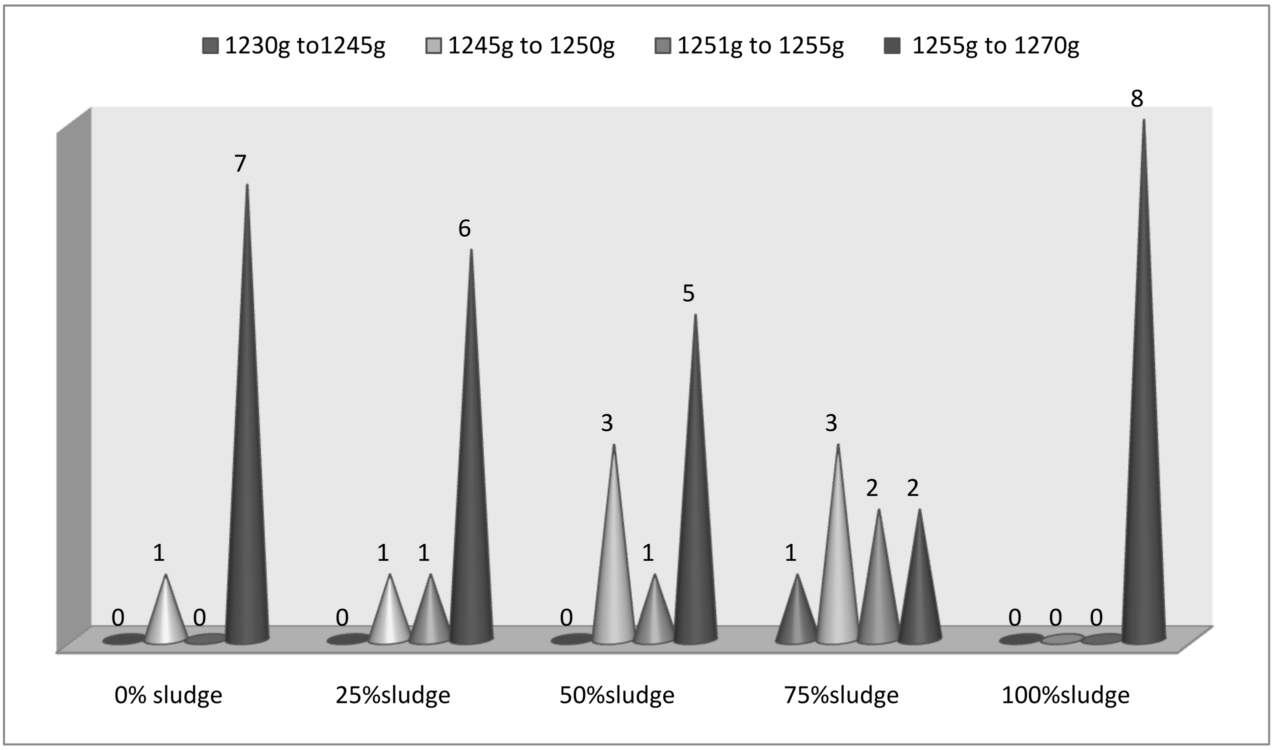

Figure 4-12.D. Distribution of Live Weights of Chicken at Day 28

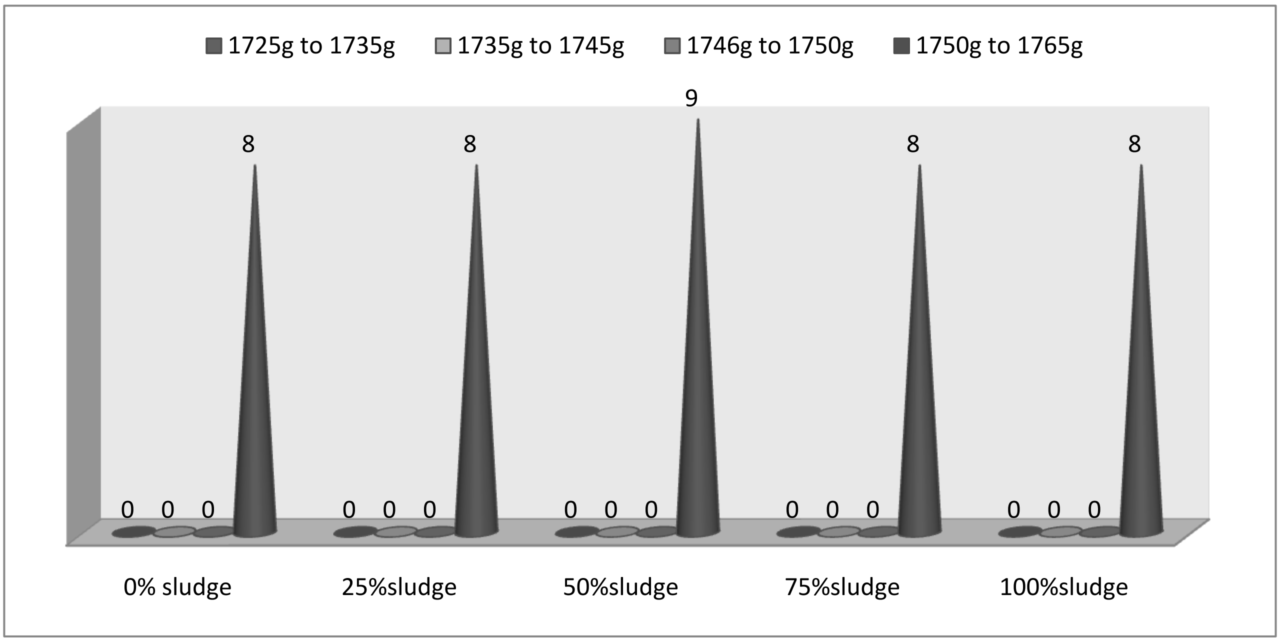

Figure 4-12.E. Distribution of Live Weights of Chicken at Day 35

From the above figures, Figures 4-12 A – E, a summary table was then generated, Table 4-1

Weight Distribution: Day 7

The results showed that 67 % of the chicken which were fed with 100% sludge; 0% fishmeal, weighed above 155g on day 7 whereas chicken which were fed with conventional feed (100% fishemeal;0% sludge) all weighed less than 155g and majority (56%) weighed between 145g – 150g.

Weight Distribution: Day 14

There was a wide distribution of weight in chicken that were fed with conventional feed on day 14, 37.5 % of chicken weighed below 365g; 37.5% weighed between 365g and 370g; 12.5% weighed between 371g and 375g and lastly 12.5% chicken weighed above 375g, and all other chicken except chicken that were fed 25% sludge; 75 % fishmeal (all weighed below 365g), weighed above 375g.

Weight Distribution: Day21

Chicken that were fed with conventional feed (0% sludge;100% fishmeal),25 % sludge;75 % fishmeal and 100% sludge;0 % fishmeal all weighed below 745g on day 14. Chicken that were fed with 50% sludge;50% fishmeal and 75% sludge;25% fishmeal, weighed above 755g.

Weight Distribution: Day 28

All chickens that were fed with 100% sludge; 0% fishmeal weighed above 1255g. There was a wide distribution of weight in all other groups, which was probably due to competition of chicken for food.

Weight Distribution: Day 35

All chicken weighed above 1750g

Table 4-1. Weight Distribution of Broilers

Weighing Day	Weight Range	% chicken per Feed Formulation				
		0% sludge; 100%Fishmeal	25% sludge; 75%Fishmeal	50% sludge; 50% Fishmeal	75% sludge; 25% Fishmeal	100% sludge; 0% Fishmeal
Day 7	< 145g	0%	0%	44%	22%	0%
	145g – 150g	56 %	22%	22%	44%	0%
	151g – 155g	44 %	33%	33%	22%	33%
	>155g	0%	44%	0%	11%	67%
Day 14	<365g	37.5%	100%	0%	0%	0%
	365g – 370 g	37.5%	0%	0%	0%	0%
	371g -375 g	12.5%	0%	0%	0%	0%
	>375g	12.5%	0%	100%	100%	100%
Day 21	<745g	100%	100%	0%	0%	100%
	745g-750g	0%	0%	0%	0%	0%
	751g-755g	0%	0%	0%	0%	0%
	>755g	0%	0%	100%	100%	0%
Day 28	<1245g	0%	0%	0%	12.5%	0%
	1245g-1250g	12.5%	12.5%	33%	37.5%	0%
	1251g-1255g	0%	12.5%	11%	25%	0%
	>1255g	87.5%	75%	55.5%	25%	100%
Day 35	1735g	0%	0%	0%	0%	0%
	1735g-1745g	0%	0%	0%	0%	0%
	1746g-1750g	0%	0%	0%	0%	0%
	1750g	100%	100%	100%	100%	100%

4.6 The Effect of Waste Activated Sludge on Overall Performance of Broiler Chicks

4.6.1 Mortality

In this study Abor acres broilers were used and it was shown elsewhere that the mortality rate pattern of abor acres was higher compared to other types of broilers e.g Anak 2000 (Awobajo *et al.*, 2007). The mortality rate of the experimental broilers was then compared to the standard percentage mortality rate (Figure 4-13) which was generated from Yield U.S. Standard and Flock Records, 2000.

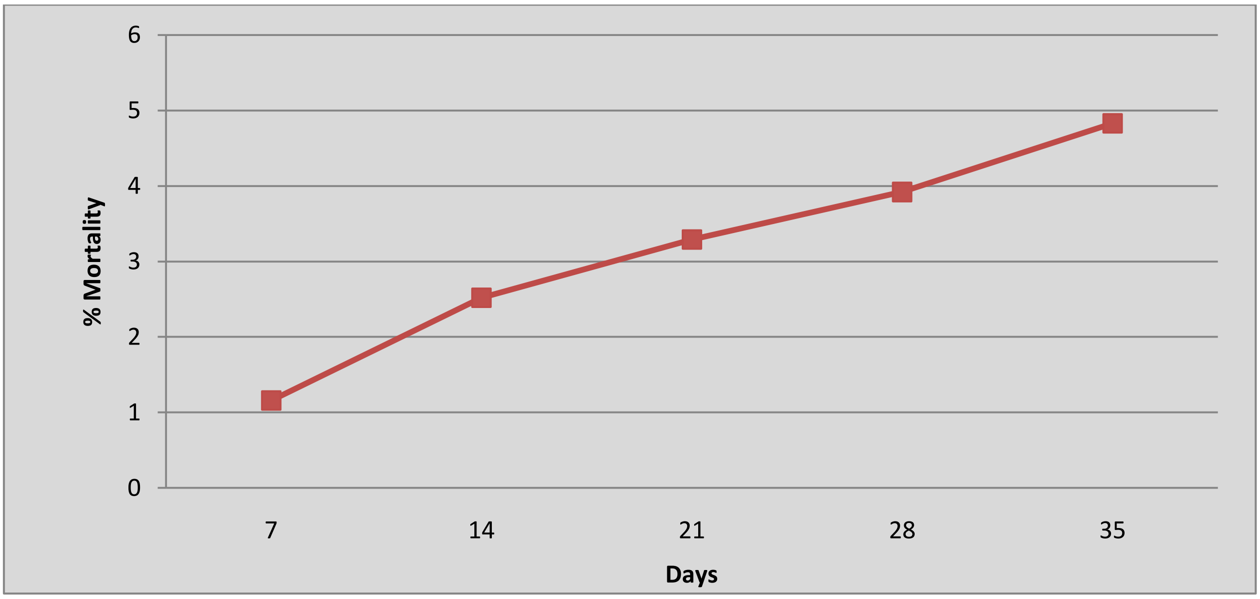

Figure 4-13 Standard Mortality Rate of Abor Acres

Mortality rate is very important because although it does not affect production costs of broilers it drastically affects the net profit. High rate of mortality occurred in the first 10 days of brooding, mostly in the chickens that were fed conventional feed (0% sludge and 75 % Sludge) (Table 4-2.). There was on one occasion that a chick was put down because of stretched legs (it was no able to move).

Broiler mortality usually peaks at approximately 3 to 4 days after placement, declines until approximately day 9 or 10 then stabilizes until approximately day 30 (Tabler *et al*., 2004). After day 30 a gradual increase is seen until approximately day 40 to 45 (Tabler *et al*., 2004). Note however should be taken that a normal colony of birds used to generate a standard percentage mortality rate (Figure 4-13) was 1500 birds (Farooq *et al*., 2001) and in this study only ten (10) broilers were used therefore the results (Table 4-2.), cannot be accurately correlated with the standard percentage mortality rate (Figure 4-13), but represent the actual results of the experiment and was used in calculating the cost of the experiment.

4.6.2 Food Conversion Ratio

This is a measure of an animal's efficiency in converting feed mass into increased body mass. Feed intake and its efficient utilization is one of the major concerns in poultry as feed cost is one of the highest components of total cost of production (Rosário *et al.*, 2007). Feed alone may contribute from 60 to 70% to the total cost of production in broiler chickens (FAO, 2006). From Table 4-2., below it is indicated that when chickens are feed with conversional feed they consume 18 % less feed to produce the same weight as chicken which is feed 100 % sludge. The contributory factor to this phenomenon was probably due to the fact that in preparing the experimental feed rations, one (1) part fishmeal was substituted with three (3) parts waste activated sludge, thereby exposing chicken that were fed with feed rations were activated sludge was included to more feed that chicken which were fed with conventional feed.

Table 4-2. The Effect of Waste Activated Sludge on Overall Performance of Broiler Chicks (0 – 5 weeks)

WAS level (%)	0	25	50	75	100
Mortality (%)					
0 – 10 days	20.00	10.00	10.00	20.00	10.00
11 – 24 days	0.00	11.11	0.00	0.00	11.11
25 – 35 days	0.00	0.00	0.00	0.00	0.00
Overall Mortality	20.00	21.11	10.00	0.00	0.00
Total feed intake) (g/bird)	4375	4625	4444	5373	5625
Total weight gain (g/bird)	1883.25	1815.38	1950.67	1941.25	1970.38
FCR	2.32	2.55	2.28	2.77	2.86

4.7 Costing

The feeding experiment cost was R551.05 to raise 41 chicks (9 chicks died), and the cost was shared as follows, R114.95 for chicken fed with conventional feed, R111.58 for chicken fed with 75% fishmeal; 25% sludge, R110.68 for chicken fed with 50% fishmeal; 50% sludge, R 109.08 for chicken fed with 25% fishmeal; 75% sludge and lastly R104.75 for chicken fed with 100% sludge, (Figure 4-14).

The cost was further broken down per chicken taking into consideration the number of chicken which died per group, and it was found that chicken which were fed conventional feed cost R4.0.5/kg, chicken fed with 75% fishmeal cost R4.22/ kg, chicken fed with 50% fishmeal cost R3.23/kg, chicken fed with 25% fishmeal cost R3.62/ kg and chicken fed with 100% sludge cost R3.37/kg, (Figure 4-15).

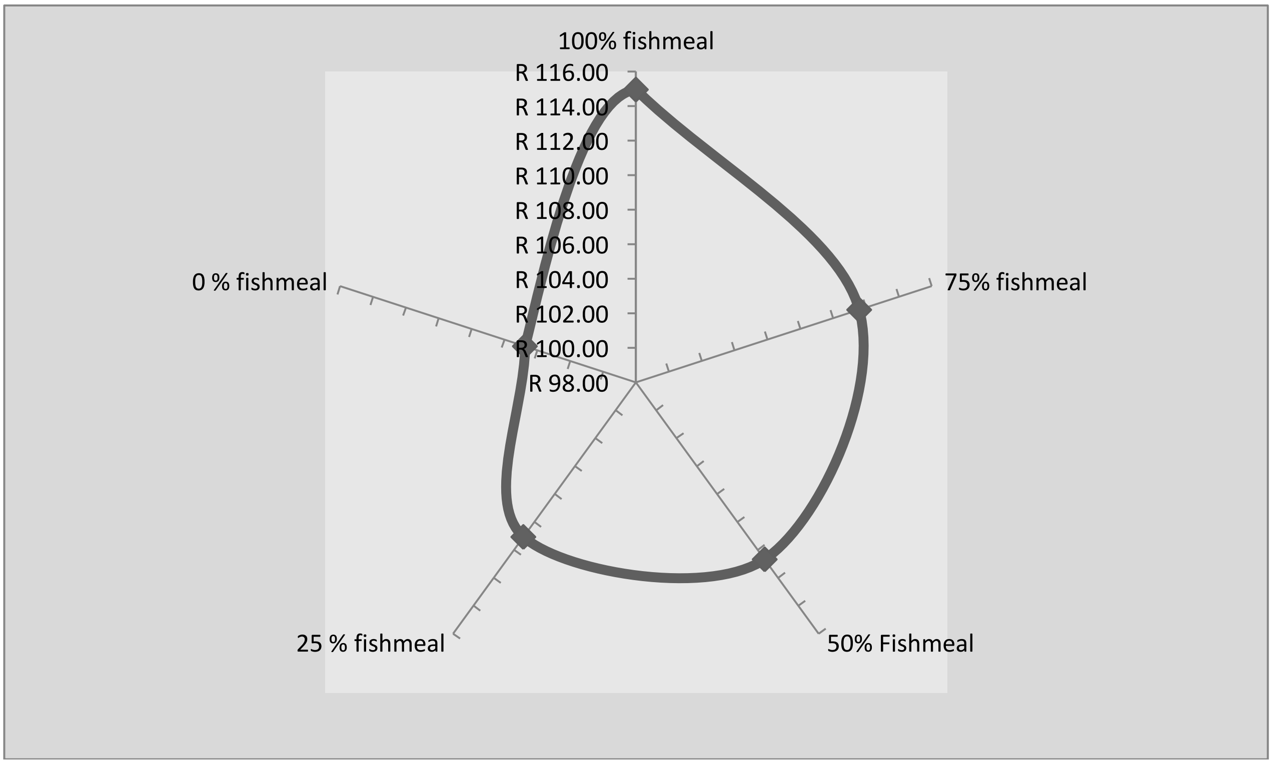

Figure 4-14. Food Cost Incurred to Raise Chickens Over 35 Days (per group)

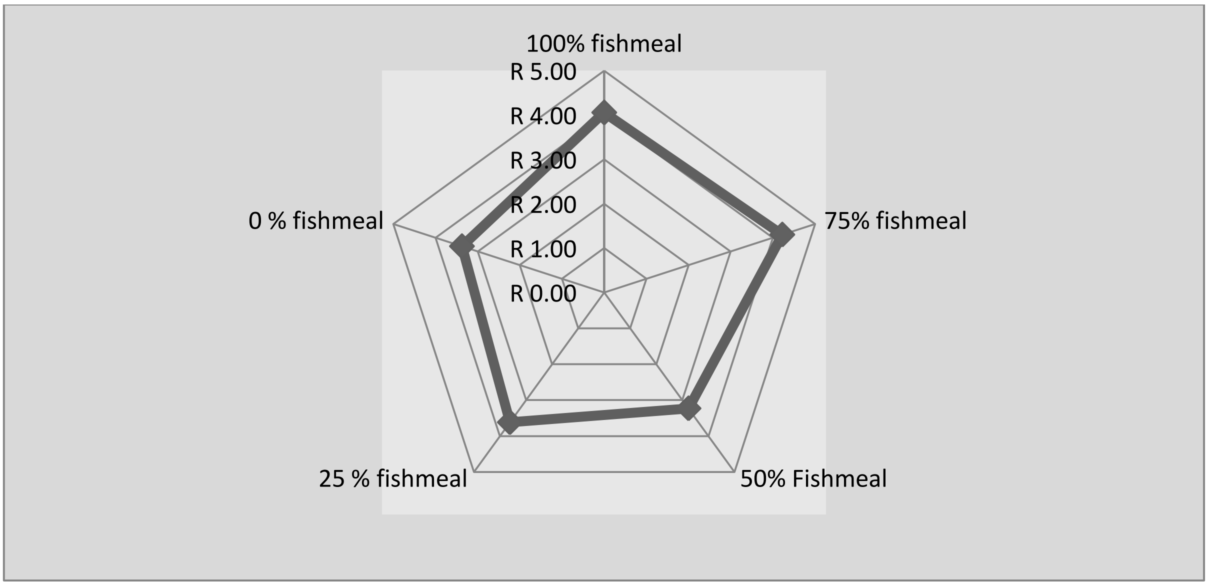

Figure 4-15. Food Cost Incurred Per Kilogram of Chicken

CHAPTER 5.

CONCLUSSION AND RECOMMENDATIONS

The main objective of the study was to analyse waste activated sludge in terms of its constituents, compare WAS with conventional fishmeal, remove heavy metals from the waste activated sludge and then evaluate the effect of waste activated sludge on broiler chicken.

The study has shown that WAS contain all the amino acids which are needed or are essential for the growth of broiler chicken except for tryptophan. Tryptophan is a regulator of many immunologigal and physiological processes in chicken and therefore its effect may need to be evaluated in further experiments, as its immediate effect may be overshadowed by the amino acid combination of methionine and cysteine in sludge. These amino acids are also triggering some immune responses in broilers. It was also demonstrated in the study that WAS contains nucleic acids, and the nucleic acids are desirable as an additional source of dietary nitrogen in animal feed supplements.

Heavy metals were managed to be removed from the sludge and after their removal, the manganese and copper level were within the tolerable levels for broiler chickens Iron and zinc were higher than normal limit in broiler feed, but still fall within the maximum tolerable levels for broilers (Table A-1, Appendix A). After the removal of heavy metals from the sludge, feed formulation were designed with the WAS inclusion of 0%, 25%, 50%, 75%, 100%. The broiler feed is divided into three parts. The starter ration used from one day old to ten days, the grower ration was then used from day eleven to day twenty five and lastly the finisher ration to day thirty five. The importance of the feed constituents have been discussed in section 3.6.3 The effect of feeding broilers with rations containing sludge protein at different percentages is given in Figures 4-10A-D) In the beginning of feeding experiments, there were no significant weight differences in chickens that were fed with the conventional feed and the ones that were fed with the feedlot where fishmeal was substituted with sludge. However, after 25 days(Figure 4-10 A), 9 days (Figure 4-10 C),10 days (Figure 4-10 D), chickens feed with sludge weighed higher that the chickens fed with conventional feed.

The effect of waste activated Sludge on overall performance of broiler chicks was also evaluated through mortality rate and food conversion rate and further tests should be done to test for meat quality in future for instance, skin colour, grilling losses and texture of breast meat, proportion of thigh meat and wings, and fatty acid profiles of breast and thigh meat, and the liver metal content. High rate of mortality occurred in the first 10 days of brooding, mostly in the chickens that were feed conventional feedlot (0% sludge and 75% Sludge)

It terms of feeding costs, chicken which were fed conventional feed cost R4.05/kg, chicken fed with 75% fishmeal cost R4.22/kg, chicken fed with 50% fishmeal cost R3.23/kg, chicken fed with 25% fishmeal cost R3.62/kg and chicken fed with 100% sludge cost R3.37/kg, therefore it was cheaper to raise chicken with sludge even though the chicken have to be given more food.

The study does not at all suggest that sludge can be reliably used as a feed component. However what the study really suggest is the fact that waste activated sludge has a potential of been used as fishmeal replacement in broiler chicken feed.

Recommendations for the Future Experiments

Many follow-up test/experiment should be made to evaluate the effect of WAS on chicken; surely weight alone cannot be the only criteria. Analysis of sludge should be improved to test for pathogens and carcinogens that may have negative effect on the growth and meat quality of chickens. The method of removing the heavy metals as well have to be optimized and other methods of rendering WAS safe have to be investigated.

APPENDIX A

Table A-1 . Maximum Tolerable Levels of Dietary Minerals for Domestic Animals.

	Species			
Element	Cattle	Sheep	Swine	Poultry
Aluminium[b], ppm	1000	1000	(200)	200
Arsenic, ppm				
Inorganic	50	50	50	50
Organic	100	100	100	100
Barium[b], ppm	(20)	(20)	(20)	(20)
Bismuth, ppm	(400)	(400)	(400)	(400)
Boron, ppm	150	(150)	(150)	(150)
Bromide, ppm	200	(200)	200	2500
Cadmium[c], ppm	0.5	0.5	0.5	0.5
Calcium[d], %	2	2	1	Laying hen, 4.0 Other, 1.2
Chromium, ppm				
Chloride	(1000)	(1000)	(1000)	1000
Oxide	(3000)	(3000)	(3000)	3000
Cobalt, ppm	10	10	10	10
Copper, ppm	100	25	250	300
Florine[e], ppm	Young, 40 Mature dairy, 40 Mature beef, 50	Breeding, 60 Finishing, 150	150	Turkey, 150 Chicken, 200
Iodine, ppm	50[f]	50	400	300
Iron, ppm	1000	500	3000	1000
Lead[c], ppm	30	30	30	30
Magnesium, %	0.5	0.5	(0.3)	(0.3)
Manganese, ppm	1000	1000	400	2000
Mercury[c], ppm	2	2	2	2
Molybdenum, ppm	10	10	20	100
Nickel, ppm	50	(50)	(100)	(300)
Phosphorus, %	1	0.6	1.5	Laying hen, 0.8 Other, 1.0
Potassium, %	3	3	(2)	(2)
Selenium, ppm	(2)	(2)	2	2
Silicon[b], %	(0.2)	(0.2)	-	-
Silver, ppm	-	-	(100)	100
Sodium Chloride, %				
Lactating	4	9	8	2
Nonlactating	9	-	-	-
Strontium, ppm	2000	(2000)	3000	(3000)Laying hen, 30000
Sulfur %	(0.4)	(0.4)	-	-
Tin, ppm	-	-	-	-
Titanium[g], ppm	-	-	-	-
Tungsten, ppm	(20)	(20)	(20)	20
Uranium, ppm	-	-	-	-
Vanadium, ppm	50	50	(10)	10
Zinc, ppm	500	300	1000	100

[a] The parantheses were derived by interspecific extrapolation. Dashes indicate that data were insufficient to set a maximum tolerable level.
[b] As soluble salts of high bioavailability. Higher levels of less- soluble forms found in natural substances can be tolerated
[c] Levels based on human food residue considerations
[d] Ratio of calcium to phosphorus is important
[e] As sodium fluorides of similar toxicity
[f] May result in undesirably high iodine levels in milk ; [g] No evidence of oral toxicity has been found

CHAPTER 6.

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