Protein requirements of juvenile Nile crocodiles (*Crocodylus niloticus)* in an intensive production system

By

Patrick Marcel Beyeler

Submitted in partial fulfillment of the requirements for the degree

M.Sc. (Agric): Animal Nutrition

In the Faculty of Natural and Agricultural Sciences

Department of Animal and Wildlife Sciences

University of Pretoria

Pretoria

South Africa

July 2011

TABLE OF CONTENTS

LIST OF TABLES

Page

ACKNOWLEGDEMENTS

I am very honoured to have been given the opportunity of carrying out this trial that would improve the understanding of nutritional requirements of the Nile crocodile, and to contribute to science. The information gathered during this trial will hopefully give members of the industry a better understanding of this reptile as well as improving and producing more economically feasible diets. I have gained immense experience during my stay on the crocodile farm, not only in crocodile behaviour and husbandry but also life experiences that will always remain with me.

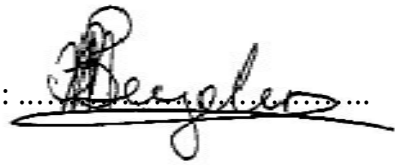
I would firstly like to thank Stefan van As, the owner and director of Le Croc crocodile breeding farm and tannery, for putting up with me for the two years that I worked and did my trial, as well as financing the trial by providing the research crocodiles, feed and staff for the duration of my trial. I would also like to extend my appreciation to all the staff at Le Croc for helping and guiding me during my time at the farm.

My sincere appreciation and gratitude goes out to my promoter Dr Christine Jansen van Rensburg, for her guidance, support, creative criticism and understanding. I am truly honoured to have been supervised by her and also for the assignment of this trial to me. I am also grateful to “Oom” Roelf Coertze (UP experimental farm) for his assistance with the statistical analysis.

Finally I would like to thank my mom, dad and brother, my close friends and co-workers for all the encouragement, guidance and moral support that they have given to me over the two years that I resided at Le Croc, as it would have been difficult to cope without your presence and words of wisdom.

DECLARATION

I, Patrick Marcel Beyeler declare that the thesis/dissertation, which I hereby submit for the degree MSc (Agric) Animal Science : Nutrition Science 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.

SIGNATURE: ..... 

DATE: ……2011/07/13

ABSTRACT

The objective of this study was to determine the dietary protein requirements of the Nile crocodile (*Crocodylus niloticus*) between the ages of 5 – 8 months, and to compare the results with documented protein requirements of the American alligator (*Alligator mississippiensis*). This was achieved by feeding the crocodiles 4 diets with varying amounts of crude protein (CP), including 62%, 56.6%, 51.6% and 46%. All four diets were iso-energetic with a metabolisable energy to protein ratio of 25.85KJ/g. The highest protein diet contained 44% raw minced chicken and 46.9% fish meal with minimal contribution (of 6.4%) made from vegetable protein sources (full fat soya). The lowest protein diet was made up of a majority of vegetable protein (30% soya bean oilcake, 9.8% full fat soya and 17.2% maize meal) and some contribution from protein of animal origin (4.3% carcass meal and 35% fish meal).

This study was carried out for 12 weeks during the crocodiles first year of life. Body mass, total body length, head length and snout to vent length were measured five times at 3 weekly intervals. Twenty representative crocodiles in a pen of 200 were individually tagged for the duration of the trial. As there were 3 replicates for each of the 4 treatment diets, 240 crocodiles in 12 pens were tagged.

During the early phase of the study, it was observed that crocodiles on the 46% CP treatment diet were not performing well, and that most of the crocodiles on this diet were losing mass. This treatment diet was discontinued at 9 weeks into the trial for both financial and ethical reasons. The remaining three diets were tested for the full 12 weeks.

Chromium oxide was mixed into the diets at two time periods during the trial to determine the protein, energy and dry matter digestibility. It was determined that the 46%, the 51.6%, the 56.6% and the 62% CP diets had a digestible protein (DP) content of 246.44 g/kg, 294.80 g/kg, 381.32 g/kg and 468.65 g/kg and a digestibility coefficient of 53.50%, 57.00%, 69.15% and 75.65% respectively.

Performance of crocodiles on the 46% CP treatment diet was found to be lower in all measurement categories than crocodiles on the three higher protein diets. However, crocodiles on the 62% CP treatment diet outperformed all the crocodiles (on all measurement criteria) on the lower protein diets. The poor performance of crocodiles on the lowest protein

diet correlates with previous research indicating that crocodilians are unable to perform optimally when the majority of the diet's protein is made up of vegetable protein sources. It was determined that juvenile American alligators would grow at optimal levels when the diet contained a DP content of 450 g/kg. As the Nile crocodiles in this study performed the best when the diet contained a DP value of 468.65 g/kg, it was concluded that juvenile Nile crocodiles have the same range of protein requirements as that determined for juvenile American alligators.

ABBREVIATIONS, ACRONYMS AND UNITS

AA	Amino acids
BW	Body weight
Ca	Calcium
CF	Crude fibre
CITES	Convention on International trade in Endangered Species of Wild Fauna and
Flora CO_2	Carbon Dioxide
CP	Crude Protein
Cr	Chromium
Cr_2O_3	Chromium Oxide
DC_{energy}	Digestibility coefficient for energy
$DC_{protein}$	Digestibility coefficient for protein
DE	Digestible energy
DM	Dry Matter
DP	Digestible protein
EE	Crude fat
FCR	Feed Conversion Rate
GE	Gross Energy (MJ/kg)
H_2O	Water
H_2SO_4	Sulphuric acid
$HClO_4$	Perchloric acid
HNO_3	Nitric Acid
NO_x	Nitrogen oxide derivatives
NaOH	Sodium hydroxide
rBST	recombinant bovine somatotropin
P	Phosphorous
HCP	High crude protein – 62% CP
MHCP	Medium high crude protein – 56.6% CP
MLCP	Medium low crude protein – 51.6% CP
LCP	Low crude protein – 46% CP

Units

°C	degrees Celsius
cm	centimetres
g/kg	gram per kilogram
kcal	kilocalorie
kg	kilogram
kJ/g	kilojoules per gram
m	metre
min	minutes
Mj/kg	megajoule per kilogram
mm	millimetres
ω	omega
R	Rand (South African currency)

CHAPTER 1. INTRODUCTION

Crocodilians have been harvested for many decades in the past, where until recently all crocodilian skins came from the wild (MacGregor, 2006). Protection from over harvesting was first granted to crocodiles in northern Australia in the late 1960's and early 1970's and CITES came into force around the same time, in 1975 (MacGregor, 2006). CITES refers to the Convention on International Trade of Endangered Wild Fauna and Flora which is aimed at preserving the wild stock and preventing the endangerment of fauna and flora if exploited for profit. Farming and ranching became more favourable over wild harvesting as there was unpredictability and inconsistency of supplies associated with obtaining skins from wild crocodilians (MacGregor, 2006). Crocodiles have been ranched in Zimbabwe since 1965 (Luxmoore, 1992), leading to an upward trend in the number of farms, especially during the late 1980's (MacGregor, 2006). With restrictions imposed by CITES, as well as the fashion industries strict demand for flawless skins, captive breeding became more favourable over wild harvesting.

The primary motivation for farming with crocodiles is the harvesting of crocodilian skins, while meat, live animals and teeth are important by-products (Brazaitis, 1987 and Van Jaarsveldt, 1987). Crocodilian skins are a highly prized commodity in the fashion industry, and people pay a large premium for owning any accessory that is made out of crocodilian leather. Crocodilian skins undergo a strict grading system from a scale of 1 – 5, where a 1st grade skin (the most highly prized skin) has absolutely no marks, scratches, or any other defect on it. A 5th grade skin has so many defects that the farmer would make a loss from selling the skin. There are three main cuts of skin: the belly, the back-strap and the horn-back skin, which are used in the manufacture of belts, wallets, purses, handbags, shoes, watch straps, and even clothing.

Crocodiles are monogastric, exothermic, carnivorous reptiles. This implies that they have a high demand for protein and require optimum temperatures for their metabolisms to function at optimal levels necessary for growth. As protein is the most expensive component of formulated rations, feeding high protein diets can be costly to the farmer as well as to the crocodile, and therefore the energy supplied by protein is more expensive than that from carbohydrates and fat (Staton *et al*. 1986). Organisms are able to convert protein to energy via a metabolic pathway known as gluconeogenesis, which uses more energy than that being

produced. Energy and protein are required by the organism for maintenance, growth and/or reproduction (Staton *et al*. 1986), where energy is derived from carbohydrates, protein and fats. To prevent protein from being used as an energy source, sufficient energy should be supplied in the diet in the form of carbohydrates and fat.

Finding any information on the nutrient requirements of the Nile crocodile is a daunting task. This is due to a lack of research on Nile crocodile nutrition and because some research done as farm trials has not been published. From personal experience by the researcher, the Nile crocodile industry is very secretive with regards to providing any information on improved performance.

Current nutrient requirements of Nile crocodiles are based on research performed on the American Alligator (*Alligator mississippiensis*) and the Australian salt water crocodile (*Crocodylus porosus*). This research is not only on different species of crocodilian but some of the data is outdated as trials were carried out approximately 20 years ago. The most relevant research related to this study, is that done by Staton *et al.* (1990). In this study it was noted that alligators, during their first year of life (between 377g – 857g), performed optimally when the diet contained a crude protein level of 51.9% and a digestible protein (DP) level of 45% (assuming 86.7% protein digestibility).

It was noted in an experiment performed by Coulson *et al.* (1987) that protein from vegetable sources was digested slowly and incompletely. However it was noted that as long as vegetable protein was part of a balanced diet, alligators would perform well under optimal growing conditions (Staton & Edwards, 1987).

Many farmers do not feed their crocodiles a balanced diet, as most crocodilian farmers feed any type of animal protein source (like whole chicken or carcass remnants from the abattoir), resulting in poor growth performance and poor skin quality. Therefore, to better understand the nutritional requirements of the species and to meet the requirements through feed formulations, the determination of the protein requirements of juvenile Nile crocodiles (*Crocodylus niloticus*) would be critical to understanding optimal growth requirements.

The ultimate aim of this study was to determine the dietary CP/ DP requirements of the Nile crocodile between the ages of 5 – 8 months for optimum growth performance. Another aim

was to compare the digestibility of protein in the Nile crocodile from both animal and plant origin.

Four diets with varying amounts of crude protein were fed to juvenile crocodiles. The level of CP included 62%, 56.6%, 51.6% and 46%. All four diets were iso-energetic with an energy : protein ratio of 25.85 kJ/g of protein. Various growth parameters were measured at regular intervals for a period of 12 weeks.

The null hypothesis was that juvenile Nile crocodiles would perform optimally at a CP/DP level close to that required by the American alligator (*A. mississippiensis*), which is close to 51.9%, 45%, and that the protein from animal and plant origin is digested with the same efficiency. The alternative hypothesis was that the Nile crocodile has a different CP/DP requirement to the American alligator and that the digestibility of the animal protein is higher than that of the plant protein in the Nile crocodile.

CHAPTER 2. LITERATURE REVIEW

Crocodile Farming and Nutrition: A Commercial view in a Southern African context

2.1. ABSTRACT

There are 3 basic ways of harvesting crocodiles for the skin industry: Wild harvest, ranching and farming. The key role players in the crocodilian trade industry in Southern Africa are Zimbabwe and South Africa. Botswana, Mozambique, Namibia and Zambia also produce crocodile skins but to a lesser extent than the two first mentioned countries. South Africa exported around 40 000 skins and 222 000 kg of meat in 2003, whereas Zimbabwe exported 93 000 skins and 257 000 kg of meat in 2003. Most of the skins that these countries produce are exported to the European market. In order to preserve species on our planet, the Convention on International trade in Endangered species of Wild Fauna and Flora (CITES) was created. An international agreement between over 166 countries was signed to ensure the survival of threatened and endangered species (including all crocodilian species).

The geographical location of the crocodilian farm or ranch plays an important role in the type of housing system employed in terms of rearing the animal. For example, farmers that are closer to the tropics do not require the expenses of an elaborate heating system in order to maintain the crocodilian ideal temperature for feed consumption and growth. Insulation is a factor that could influence the cost of running the house as well as influencing the temperature control of the house. The insulating material must be able to maintain the internal temperature and humidity as well as prevent external temperatures from affecting the internal temperature.

Generally, there are two choices when it comes to breeding stock and obtaining eggs. There is wild collection (where eggs are collected in the natural environment) and wild caught or using captive bred stock. Stocking density of a dam or pond should be determined by the size and mass of the crocodile to ensure that there is sufficient land area and water in the pen, allowing for normal behaviour.

Biosecurity is necessary to prevent the introduction and spread of pathogenic organisms to the crocodiles and from pen to pen. Another reason for biosecurity is that when an animal contracts a disease, the nutrients of the feed and body are directed towards the functioning of

the immune system and the eradication of the pathogen from the body, and thus away from growth.

In the wild, crocodiles prey on just about every living creature in their natural environment, from insects (aquatic and terrestrial), amphibians (tadpole and frogs) and crustaceans and small fish when they are hatchlings, to large fish, rodents, birds and large game (like wildebeest and zebra), during adulthood.

All crocodilians are carnivorous, and the intake of carbohydrates occurs incidentally when their prey are attached to vegetation or undigested vegetation within the prey. The preconceived idea that crocodilians are unable to utilise plant carbohydrates and proteins, led to the prevention of the use of grain and other feed from plant origin when formulating a crocodilians diet. Crocodilians utilise protein not only for maintenance, growth and reproduction, but it also serves as a glucose source, where the protein or amino acid carbon skeleton is metabolically broken down and converted into glucose by a process called gluconeogenesis, which is either used directly or stored as lipids.

Staton & Edwards (1987) formulated a controlled diet with a calculated analysis of crude protein of 73.7% and total lipids of 3.6%. Energy (in the form of maize) was supplemented to this basal diet and the growth performance (mass and length gains, dry matter consumption and feed:gain) was measured. It was found that, despite the fact that alligators are carnivores, the high protein content of a meat diet was probably not essential as a protein requirement, but that some protein was being utilised to meet energy demands.

Young alligator feeds should contain 45% digestible protein, an energy density between 34.3– 45.6 kJ DE per gram of CP and a total of 11% readily digestible carbohydrate. To reach this, the addition of dietary fat would be essential. Assuming a protein digestibility of 86.7%, the feed would have to have a minimum of 51.9% CP.

2.2. INTRODUCTION

The harvesting of crocodilians has been carried out for many years. The skins are used for clothing and accessories whereby the meat is used as a food source. It is mainly the use of the skins that prompted the harvesting of these semi aquatic reptiles. The skins undergo a tanning

process and are used in the manufacture of belts, watchstraps, wallets, handbags and boots. The exclusiveness of these products in terms of fashion allowed this industry to flourish, however, leading to the demise of some of the crocodilian species, especially during the last half of the 20th century. CITES (the Convention on International trade in Endangered Species of Wild Fauna and Flora) was introduced to govern the harvest of crocodilians and other species. This is an international agreement between 166 countries, to ensure that trade of natural fauna and flora does not endanger wild stock, according to the wildlife trade fact sheet supplied by the Australian Government's Department of Environment and Heritage. As stated by the Wildlife trade fact sheet, "CITES has established a global system of controls on international trade in threatened wildlife and wildlife products by stipulating that government permits are required for such trade". Crocodilians belong to the subfamilies Crocodylinae, Alligatorinae and Gavialinae which includes all crocodiles, alligators, caiman and gharials. The harvesting, farming and ranching of some of the species occurs throughout the world, from alligators in America, to caiman in South America, to Nile crocodile in Africa and salt water crocodiles in Australia.

Little research has been carried out on the nutritional requirements of crocodiles from a commercial stand point, as it is a very secretive and lucrative industry, and farmers are not willing to share nutritional information. This is to ensure that they have an edge over the other farmer in terms of growth rates and skin sizes which ultimately results in greater profits to the farmer. No research was found on the nutritional demands for the growth of the Nile crocodile (*Crocodylus niloticus*) in a commercial farming sense.

2.3. BACKGROUND

In the past wild crocodilians (which include the crocodiles, alligators and caiman) were harvested straight from the river systems of the five continents on which they are found.
There are presently 3 basic ways of harvesting crocodiles for the skin industry;

(i) Wild harvest – Crocodilians are captured in the river systems either by hand or other capture methods (trap door cage traps or sling trap), the crocodilians would live their entire life cycle in the environment until harvest (Macgregor, 2006). Some countries still practice this method.

(ii) Ranching – Crocodilians are taken as eggs or as hatchlings from the wild, where the eggs are incubated in large incubation rooms and the hatchlings are placed into temperature controlled environments. These young crocodilians grow up on the farm until they reach slaughter size. The disadvantage of this method is the inconsistencies and unpredictability of supplies from the wild (Macgregor, 2006).

(iii) Captive breeding/farming – Adult crocodilians are kept in an enclosure in a controlled environment. The eggs of the adults are harvested from the nests on the farm and placed into incubators, in other words the crocodilians were hatched in captivity.

The key role players in the crocodilian trade industry in Southern Africa are Zimbabwe and South Africa. Botswana, Mozambique, Namibia and Zambia also produce crocodile skins but to a lesser extent than the two previously mentioned countries. South Africa exported around 40 000 skins and 222 000 kg of meat in 2003 whereas Zimbabwe exported 93 000 skins and 257 000 kg of meat in 2003 according to Jenkins *et al*. 2004 (conducted for CITES). Most of the skins that these countries produce are exported to the European market, where they end up in countries like France and Italy as well as in the Asian markets.

There are 3 basic cuts of skins available, which are the belly skin, the back strap and the horn backs (du P. Bothma and van Rooyen, 2005). The belly skins are the most highly prized and priced cut skins, due to their smooth texture of the belly scales. These skins are used mainly for the manufacture of shoes and hand bags. The back straps are separated from the belly skins during skinning. These skins are the horny back (± 10cm in width, extending the length of the crocodilian's back) and fetch a lower price than belly skins as they are more ridged (containing bone - osteoderms), and are mainly used in the manufacture of belts. Horn backs on the other hand are produced when the belly skin is not worth much due to anomalies on the skins (like scratches or other deformities), basically the belly skin is cut down the middle where the horny back is the focus of the skin.

Belly skins of crocodiles are priced according to their width and grade, where the width is measured from the third inside osteoderm across to the other side (du P. Bothma and van Rooyen, 2005). Grading of the skins is very subjective and differs from one person to another. The grading scale ranges from 1 – 5, where 1 (the first grade skins, being the best

skins) include skins with no imperfections such as scratches, scars, pattern deformities or pix (an imperfection thought to be caused by virus or bacterium, which causes a pin hole in the leather hide), and 5 (the fifth grade, being the worst skins) are skins that cannot be used due to severe imperfections, these skins are usually sold at a low price, or discarded (du P. Bothma and van Rooyen, 2005).

The extensive use of crocodilian products has lead to the endangerment of some wild populations of crocodilians. This resulted in all crocodilians being listed in the Appendices of CITES

According to Lafleur *et al*. (1995), Appendix I represents all the animals (including crocodilians) that are rare or endangered, and trade in these species for commercial use is prohibited. Therefore, species (live animals and products) on Appendix I must have a CITES export permit issued by the exporting country and an import CITES permit issued by the importing country. Appendix II represents all the animals that are not rare or endangered, but may become if their trade is not managed correctly. Species on Appendix II must be issued with a CITES export permit before being imported.

Appendices of CITES (according to the Australian Government's Department of the Environment and Heritage wildlife trade fact sheet, 2008)

Appendix I species

Species name	**Common name**
Crocodylus acutus	*American Crocodile*
Crocodylus cataphractus	*African Slender-snouted Crocodile*
Crocodylus intermedius	Orinoco Crocodile
Crocodylus moreletti	Morelet's Crocodile
Crocodylus niloticus	Nile Crocodile
(except populations in Appendix II)	
Crocodylus novaeguineae mindorensis	Philippine Crocodile
Crocodylus palustris	Broad-snouted Ormugger Crocodile
Crocodlyus porosus	Saltwater Crocodile

(except populations in Appendix II see below)	
Crocodylus rhombifer	Cuban Crocodile
Crocodylus siamensis	Siamese Crocodile
Osteolaemus tetraspis	(African) Dwarf Crocodile
Osteolaemus tertraspis osborni	Dwarf Crocodile
Osteolaemus tertraspis terraspis	Dwarf Crocodile
Tomistoma schlegelii Tomistoma,	False Gavial
Gavialis gangeticus	(Indian) Gavial, Gharial

Appendix II species

Crocodylidae spp	All crocodiles
(All species in family except those in App.I)	
Crocodylus crododilus yacare	Yacare
Crocodylus johnsoni	Johnson's Crocodile
Crocodylus novaeguineae	New Guinea Freshwater Crocodile
Crocodylus porosus	Saltwater Crocodile
(Australia, Papua New Guinea, and Indonesia)	
Gavialidae spp	
(all species in family except those in Appendix I)	

2.4. ASPECT OF CROCODILIAN FARMING OR RANCHING

2.4.1) Area/ Topography

The geographical location of the crocodilian farm or ranch plays an important role in the type of housing system employed in terms of rearing the animal. For example: farmers that are closer to the tropics do not require the expense of an elaborative heating system, in order to maintain the crocodilians ideal temperature for feed consumption and growth, where those farmers that are more in temperate regions require some sort of heating system such as electricity or hot water pipes (heated from coal or paraffin boilers) circulating under the floor

of the houses to maintain the crocodilians comfort temperature (to ensure ideal feed consumption and growth rates) throughout the year, especially during the cold winter months. The farmers in the tropics can take advantage of the ambient temperatures, and use open pen or housing systems. These structures have no roofs but have pools in the centre and may have some sort of shade in the form of strips of tin roof or shade netting to help the crocodilians to regulate its own temperature. Hot water pipes may be laid under the concrete to aid in temperature regulation during the cooler months. Because additional heat is provided, excessive cooling of the water during the night is prevented, however there is no protective measure against overheating (Huchzermeyer, 2003). Overheating can be just as severe as under heating in that it causes stress, which results in a loss of appetite and a depression in growth rates. Having an environmentally controlled house reduces stress, but requires high maintenance and may be costly.

Another important aspect with regards to topography is the requirement of a constant source of water, as no crocodilian operation can run without this, as it is very important in temperature regulation for the crocodilians. It is also needed to swallow feed, for drinking water and for hygiene, which will be discussed later.

2.4.2) Climate controlled houses

The optimum temperature for crocodiles to grow ranges from 30 to 34°C, with a relative humidity of 60 to 90% (Davis, 2001). Due to the large variation in temperature in South Africa, bulk insulation, ventilation, heating, cooling and air tightness (Davis, 2001) are all important factors to consider when building a climate controlled house. Insulation is important to control the temperature of the house. The insulating material must be able to maintain the internal temperature and humidity, while at the same time prevent external temperatures from affecting the internal temperatures. Some insulating materials include shredded paper, sandwich panels and fibreglass wool over foil sheets as mentioned by Davis (2001).

Ventilation of the houses may be provided by doors only, but more efficient systems use large extractor fans that are switched on once or twice a day to remove the ammonia fumes produced from the urine of the crocodiles (Huchzermeyer, 2003). According to Davis (2001) there are three types of ventilation systems; hot weather, good weather and cold weather

ventilation systems. Hot weather ventilation systems remove hot air and moisture from the house and it is used when the ambient temperature is higher than the temperature required. The outside ambient temperature can be cooled using evaporative cooling pads or ventilating the house only a few times a day, by removing the ammonia and providing fresh oxygen. Good weather ventilation system is used when the outside temperature is similar to that needed by the crocodiles. The number of fans and rate of usage will vary according to the ambient temperature. Cool weather ventilation systems are used when the outside temperature is lower than that required by the crocodiles. Here, as little ventilation is used as possible to maintain the internal temperature. In this system the air quality is maintained by removing some heat, ammonia, carbon dioxide and moisture.

The advantage of having a well constructed climate controlled house is the absence of flies and rats and the prevention of escapes (Huchzermeyer, 2003).

2.4.3) Breeding stock and egg collection

There are generally two choices when it comes to breeding stock and a supply of eggs. There is wild collection (where eggs are collected in the natural environment) and wild caught or captive bred stock. Wild collection of eggs has its advantages and disadvantages in that there is a lower expense in terms of building an enclosure and feeding the breeding stock, however the age of the female and the quality of the eggs (which is determined by the age, size and diet) cannot be guaranteed (Huchzermeyer, 2003). Collecting eggs from a young female usually results in eggs being small, producing smaller sized hatchlings as well as a generally low fertility and poor hatchability (Maree & Casey, 1993). Wild collection is also very time consuming and laborious as collectors need to travel around the water systems to find nests from which the eggs can be collected. The collection of eggs from the wild also requires the need to release a certain percentage of juveniles back into the water system from where they came, which is a farming method of choice from a conservation point of view (Huchzermeyer, 2003).

Captive bred stock is usually found in countries where the collection of eggs and hatchlings from the wild is prohibited, especially for commercial use. The captive stocks may either be caught wild or reared on the farm. These crocodiles may be penned in a large enclosure (with

a land to water ratio of 33 : 66) (Maree & Casey, 1993) that may house up to 500 animals or more, and has a male to female ratio of 1 : 5 – 6 females. When selecting breeding crocodiles as captive bred stock, the following considerations need to be taken: Captured crocodiles (wild female crocodiles) produce eggs that are larger and have a higher level of fertility and hatchability than farm-reared captive stock (Maree & Casey, 1993). Wild captured crocodiles, however are more territorial and more orientated to fight than farm-reared stock (Huchzermeyer, 2003).

Nesting sites for breeding stock should also be monitored. Nesting sites should be walled off from each other as this reduces fighting between females. The nesting sites should be 1m above the water line with a soil sand mixture about 60 cm deep (Maree & Casey, 1993). All the nesting sites should be at the same level as the one which is at the highest point is the most desirable.

Egg collection should be done within 24 hours after laying, as the embryo has not yet attached to the inner shell wall, making the handling of the egg safer. As soon as the embryo attaches to the inner shell wall, any turning of the egg will result in the death of the embryo (Huchzermeyer, 2003). If eggs are to be collected after 24 hours they should then only be collected after 4 weeks. These eggs should be marked with a pencil indicating their original position and when placed into the transporting box, the egg should be placed in the same position for development (Huchzermeyer, 2003).

2.4.4) Stress and densities

Certain managerial aspects are important to note in order to reduce as much stress as possible to reach optimum growth potentials. Crocodiles in an intensive farming situation are highly strung and the presence of a person, strange (loud) sounds or stress calls from a neighbouring dam increases the stress levels of the animals. Signs of stress can be seen when the animals run into the water and pile on top of each other in pens, which may lead to suffocation and death (Huchzermeyer, 2003) or they stop eating which ultimately affects their growth. Therefore, stress should be minimised as much as possible by allowing the crocodiles to become accustomed to human presence. The keepers should talk to the crocodiles and a radio may be used in the background, which has a quietening effect on the crocodiles

(Huchzermeyer, 2003). Hide boards have also proven useful, where a crocodile can hide underneath when it feels threatened, which prevents the incidence of pilling to some degree.

Stocking density of a dam or pond should be determined by the size and mass of the crocodile. Sufficient area should be available for the animal to use all parts of the pen, including both the land area and the water to feed (Huchzermeyer, 2003). A low stocking density is costly but preferred above a high stocking density which leads to fighting, injury, stress and malnutrition.

2.4.5) Temperature

Temperature is the most important aspect of crocodilian ranching or farming, as the incubation temperature determines the sex of the hatchling and the comfort zone of the crocodilians for the remainder of its life. Crocodiles are poikilothermic, therefore their body temperature is determined by the environmental temperature. Digestion, metabolic rate and growth rate are at their best when the crocodile's body temperature is between 32 to 33°C (Huchzermeyer, 2003). Anything above 35ºC leads to stress which may lead to death, whereas below 28ºC reduces appetite as well as metabolic rate and therefore growth. Thermoregulation of crocodiles is more efficient if the water or floor of the pen is regulated rather than the air. Infrared radiation from the sun in outside rearing pens is absorbed quite effectively and crocodiles are able to regulate their temperature to some extent by evaporative cooling. Care needs to be taken to ensure that the critical temperature (the temperature required for optimal growth) is maintained during hot as well as cold days in order to minimise the incidence of stress and feed refusals (Huchzermeyer, 2003).

2.4.6) Biosecurity

This is a part of farm management that is not always properly adhered to. In the wild, crocodiles and crocodilians alike have a very impressive immune system and many scientists are baffled by the ability of these animals to survive after experiencing a tremendous injury even though they live in microorganism infested waters. Crocodiles may have had amputated legs from fights where they heal well and normally. It was found antibodies in the crocodile's blood can eradicate bacteria including *Staphylococcus aureus* according to Reuters (2005). However, when crocodiles are placed in an intensive farming situation they seem to be more

susceptible to diseases as they are living in a situation which has a high stocking density, maintenance of a constant temperature and minimal interaction with the outside world (where they would come into contact with pathogenic agents that stimulate the formation of their immune systems). When these crocodiles come into contact with pathogenic organisms, most of them become affected by the disease which results in reduced feed intake and may lead to death. The disease may also easily spread and affects other crocodiles. Therefore proper biosecurity is essential to prevent the introduction and spread of pathogenic organisms to the crocodiles and from pen to pen. Another reason for biosecurity is that when any animal contracts a disease, the nutrients of the feed are directed towards the functioning of the immune system and the eradication of pathogens from the body rather than towards growth, which no farmer can afford. Controlling diseases can become expensive because of the cost of the medicines and length of treatment.

Pathogens affecting crocodiles can be specific or non specific. According to Huchzermeyer (2003) crocodile specific pathogens include the caiman-crocodile pox virus, adenovirus, Chlamydia, mycoplasmas, coccidia and roundworms, which are carried by wild (introduced) crocodiles and water where wild crocodiles are found. Worms can only be introduced by the feeding of raw freshwater fish, where the fish serves as the intermediate host. There are many means of ensuring biosecurity: (i) no unauthorised person should be allowed to enter the crocodile housing facilities (erecting a perimeter fence may help), (ii) workers should receive overalls and gum boots that are used exclusively in the crocodile facilities and (iii) a foot bath must be provided at the entrance of the housing facilities to get rid of any pathogens present on the gum boots of anybody entering the facility (Huchzermeyer, 2003).

2.4.7) Disinfection and cleaning

One way to prevent diseases from occurring on the crocodile farm/ranch is to ensure that there is a proper cleaning schedule in place. Crocodile faeces contain pathogenic bacteria and other parasites which will multiply in the warm, moist environment of the house. Another source of bacteria is the feed refusals that crocodiles leave behind on occasion. These two sources of microbial contamination need to be dealt with to prevent bacterial diseases. It is recommended by Huchzermeyer (2003) that the pens are washed out every day, preferably with a pressure hose, which is more effective than a normal hose. Disinfecting detergent should be used on occasions to remove the fat layer on the floor surface that is leached from

the undigested feed and undigested fat in the faeces. The water should be changed on a daily basis, which will reduce the chance of bacterial build up. In the event of excessive build up of fat (on the animals and in the pens), a detergent should be administered onto the crocodiles and rinsed off. A disinfectant should then be sprayed over the crocodiles to kill bacteria on the skin of the crocodile.

2.4.8) Harvesting crocodiles for slaughter

Once the crocodiles have reached a certain age usually, between 2 - 3 years, they are potentially ready for slaughter. The age of the crocodiles for slaughter varies as crocodiles do not grow uniformly, and therefore do not reach slaughter size at the same time (Huchzermeyer, 2003), uniform skins are preferable when marketing. Buyers prefer to buy unblemished skins at a tannery. The skins should not have any form of scratch or scar or any other type of imperfection that is undesired by the leather manufacturing companies or that is unwanted by the fashion industry. To prevent any imperfections the animal's teeth should be clipped after the animal was electrically stunned. The crocodiles are then left for three weeks which gives sufficient time for the animal's belly and other parts to heal (if there was a scratch or scar). However crocodiles are able to regenerate their teeth within 3 weeks (potentially leading to further scarring), so their teeth are clipped again after the third week. The crocodiles that have no defect on their belly skin after the third week are selected for slaughter. This practice is labour intensive, as well as requiring a good degree of management to ensure the well being of the crocodiles. However, the chances of getting a good grade for the skins are increased and a higher price can be received for each skin that does not contain any imperfections.

There are many ways of humanely culling a crocodile. The use of a captive bolt or a gun pointed at the brain results in the immediate loss of consciousness; crocodiles killed in this way do not move or struggle after death (Huchzermeyer, 2003). Another method is where crocodiles are electrically stunned before the spinal cord is severed at the neck using a filleting knife. Loss of consciousness is achieved by pithing a stainless steel rod down the brain and the length of the spinal cord, resulting in the immediate death of the crocodile (Huchzermeyer, 2003).

2.5. FEEDING/NUTRITION

2.5.1) Hatchlings

The feeding of crocodilians has become more specialised in terms of the energy and protein needs of the animal as is the case with other livestock. Farmers and ranchers are seeing the benefits of supplementing energy, minerals and vitamins as well as providing an ideal protein source to the crocodiles.

In the wild, crocodiles prey on just about every living creature in their natural environment, from insects (aquatic and terrestrial), amphibians (tadpole and frogs), crustaceans and small fish when they are hatchlings, to large fish, rodents and large game (like wildebeest and zebra) when they reach adulthood (Huchzermeyer, 2003).

The greatest challenge in the feeding of crocodiles is at the hatchling stage. Newly hatched crocodiles instinctively snap at anything that moves. All the living invertebrates and small vertebrates move around as a part of their daily lives, and the movement of these creatures (like the hopping of a grasshopper or the swimming of a tadpole) stimulates the hatchlings to lunge and snap at their potential prey. However, in a farming environment hatchlings are fed pellets, which move when the feed is initially placed (which stimulates the hatchlings to eat) but come to a halt on the feeding surface. The majority of the hatchlings then take no further interest in the feed.

According to Peucker *et al.* (2005), the feed initiation is a critical step in the introduction of manufactured crocodile diets. Certain attractants have been used to gain acceptance of manufactured feed by crocodiles, including texture, odour and taste (Peucker *et al.*, 2005). Peucker *et al.* (2005) conducted a few trials on different attractants and flavourants, these included chicken head digest, beef liver digest, kangaroo meat, beef liver powder, prawn digest and hen egg yolk. None of the attractants used in the study caused an increase in feeding response or acceptance of feed. Another trial conducted by the same researchers compared the addition of 100% sheep blood to pelleted feed with 100% meat. Using the 100% meat as their control, they noted that fresh blood addition had minimal effects on feed intake of young hatchlings.

It has been suggested that hatchlings are genetically programmed to recognise certain items like smell, taste and the movements of living creatures within their environment (Davis, 2001). It has been said that newly hatched crocodiles have to be trained to eat their pelleted (manufactured) feed. A Zimbabwean researcher did this by rolling pellets in front of the hatchlings, which responded by running after the pellets (Davis, 2001). It has also been observed (by the researcher) that crocodile hatchlings (*Crocodylus niloticus*) responded to a red laser light when hatchlings were housed indoors. By the response of the hatchlings to the laser beam, the hatchlings could be enticed to follow the laser beam and then by pointing the beam on a pellet, the hatchling would then "attack" the pellet and consume it. This is a labour intensive but very successful training method.

2.5.2) Energy and protein

Nutrients required by all species include carbohydrates (energy), proteins, lipids (fats), vitamins and minerals. The feed that animals consume must provide them with these vital nutrient groups, which they require for maintenance, growth and reproduction. Energy is derived from carbohydrates, protein and lipids where nutrients contributing to the structural integrity of the organism include proteins, lipids, vitamins and minerals.

Carbohydrates:
All crocodilians are carnivorous, and the consumption of carbohydrates occurs only when their prey are attached to vegetation or undigested vegetation within the prey. It has been reported by Coulson & Hernandez (1983) that out of a number of carbohydrates fed to caimans (*Caiman crocodilus crocodilus*) only glucose was absorbed, while other monosaccharides, disaccharides and polysaccharides were not absorbed.

The preconceived idea that crocodilians are unable to utilise plant carbohydrates and proteins, led to the prevention of the use of grain and other feed from plant origin when formulating a crocodilian diet. Carbohydrates are the least expensive energy source available in livestock rations, and possesses binding properties which make them highly desirable in the manufacture of various pelletised rations (Staton *et al*. 1986). This is quite important for crocodilians as their jaw structure make them unable to efficiently use a meal/powdered ration. Another important trait of carbohydrates as a binder is that crocodilians drag their feed

into the water and a good binder would prevent the feed from dissolving when the feed enters the water. It would be desirable if the binder served as a nutrient source (Staton *et al.* 1986).

Heat treatment increases the utilisation of carbohydrates by carnivorous species, this process may enhance the nutrient content of the ingredients within the carbohydrate sources (Staton et al. 1986). Increasing the complexity of carbohydrates (by means of increasing the degree of polymerisation) may reduce its availability to the crocodilians (Spannhof & Plantikow. 1983). Spannhof & Plantikow (1983) stated that monosaccharides (like glucose) are absorbed completely, whereas the digestibility values of various crude starches (like gelatine and potato starch) are very low. Care needs to be considered when including the source of carbohydrates in feed rations.

Lipids:
According to Coulson & Hernandez (1983) dietary fat is readily digested by alligators. It may be possible that crocodilians have a basic need for lipids as a source of glycerol and essential fatty acids (Staton *et al*. 1986). It has been said that alligators grow well on a low fat diet (Coulson & Hernandez. 1983), of between 14 – 24%, but from a management point of view, it is important to have low fat diets as the fat may make cleaning of the pens/tanks difficult when the feed is dragged through the water.

It would be advantageous to add as much fat in the diet as possible, within the constraints of the above mentioned, as a higher fat content leads to increased retention time within the gastrointestinal passage. This leads to an increased rate of digestion of feed and more nutrients are available for absorption by the animal. On the other hand, the inclusion of higher levels of lipids in the diet as a source of energy instead of more expensive proteins, will reduce the cost of the ration (Staton *et al.* 1986).

The composition rather than the amount of lipids fed to the animals may be important, especially in carnivores. Carnivores receive adequate amounts of essential fatty acids from their natural diet. Deficiencies of essential fatty acids are most likely to occur when animals are maintained over long periods of time (Staton et al. 1986). Garnett (1985) suggested that *Crocodylus porosus* requires a dietary source of long chain polyunsaturated ω3 (omega 3) fatty acid as cited by Staton *et al.* (1986). Garnett (1985) fed pork meat to crocodiles over a

long period of time. The crocodiles developed a type of dermatitis, possibly due to an essential fatty acid deficiency.

Protein:

Crocodilians utilise protein not only for maintenance, growth and reproduction, but it also serves as a glucose source through the process of gluconeogenesis. Protein or amino acid carbon skeletons are metabolically broken down and converted into glucose, which is either used directly for energy or stored as lipids. Overfeeding proteins, above maintenance, growth and reproduction requirements, is wasteful and energetically expensive, as the animal has to utilise more energy to rid the body of excess amino acids in the form of uric acid, placing a major strain on the liver. This is of particular interest when formulating feed, as protein is the most expensive portion of the diet, and therefore the energy supplied by protein is more expensive than when it is supplied by carbohydrates or fats.

Plant proteins are generally less expensive and are often easier to handle and could be more readily available than proteins from animal origin. Therefore, the inclusion of plant protein sources could save on feed costs and simplify formulations and mixing of diets. However, it has been documented by Coulson & Hernandez (1983) that alligators have a limited capacity to digest proteins of plant origin. On the contrary, Staton and Edwards (1987) found that alligators perform well on a diet where 40% of the dietary protein was made up of vegetable protein.

Vitamins and Minerals:

Crocodilians, as with any other animals require vitamins and minerals for essential bodily functions and therefore it needs to be supplemented into their diet. Animals raised indoors require a source of vitamin D, because of the lack of exposure to sunlight necessary for vitamin D production and normal bone formation. As cited by Staton *et al.* (1986), Lance *et al.* (1983) discussed the importance of vitamin E in alligator reproductive performance, which would be assumed similar for other crocodilians.

Minerals are also an important component of diet formulation as performance of the crocodilians may be affected by a deficiency. According to Huchzermeyer (2003), there is no need to supplement macrominerals if they are fed meat with bones. However supplementation may be required when feeding broiler poultry to crocodiles, as the mineral

content of the birds may be low. A nutrient analysis of the protein (meat) source should be performed, so that an accurate decision can be made as to whether the addition of minerals and vitamins to the diet is required.

Most vitamins and minerals are added into the diet as a premix which is a mixture of the vitamins or minerals in specific proportions to one another. This makes handling and mixing minerals and vitamins more manageable and prevents overfeeding or underfeeding of a specific vitamin or mineral.

Staton *et al.* (1986) reported on the effects of dietary calcium (Ca) and phosphorous (P) on the growth of alligators and reported that adding Ca and P to a diet already high in these minerals resulted in poorer growth. These researchers found that alligators performed the best on a diet containing 1% Ca and 0.5% P.

2.5.3) Growth promoters

Various promoters or stimulants have been tried on crocodilian species, in an effort to improve their growth performance. It was cited by Huchzermeyer (2003) that Leon Ojeda *et al.* (1998) injected Laurobolin (an anabolic steroid) intramuscularly into Morelet's crocodiles. A marked improvement in growth and mass was reported which lasted for 21 days. Kanui *et al.* (1993) injected juvenile Nile crocodiles with the growth hormone, recombinant bovine somatotropin (rBST), and observed that this hormone stimulated the crocodile's appetite and growth. Staton *et al.* (1992) added taurine (an extract of ox heart) to the diet of alligators, which resulted in an improved fat digestibility and mass gain.

The use of antibacterials as growth promoters has also been widely used, not only in the crocodilian industry but in other livestock production systems. Oxytetracycline and virginiamycin have been used in combination in the diet of crocodiles (Avendaño *et al.* 1992). However, in recent years there have been concerns over the use of antibacterials, as they could cause the resistance of the bacteria to these antibiotics. As consumers became more aware of animal production, and the products used to improve growth, there has been increasing demand for the prevention of the use of antibacterials, especially where animal products are intended for human consumption. The EU banned the use of any antibacterial

for enhanced growth performance as well as the importation of any products that contains traces of these growth stimulants.

2.6. RECENT RESEARCH ON CROCODILIAN PROTEIN AND ENERGY REQUIREMENTS

Only a few trials performed on the *C. niloticus* in terms of nutrition have been published. Most publications refer to trials performed on the American alligator (*.A. mississippiensis*) and the Australian Salt water crocodile (*C. porosus*). Because *C. niloticus* farming is a very lucrative and competitive industry, trials were possibly done but not published.

Work done by Staton & Edwards (1987) on the American alligator focused on the formulation of a controlled diet for research purposes. They used a number of feed ingredients to formulate a basal diet with a 73.7% crude protein and 3.6% total lipids, an energy source of maize was also included. The researchers then proceeded with two experiments. Experiment one was to determine the influence of dietary lipids on alligators, by feeding alligators diets containing graded levels of lipids. The second experiment tested the effects on alligators when varying levels of extruded maize and lipids were included in the diet. Results of experiment one showed that high lipid rations supported greater mass gains and improved feed conversions more than low-lipid diets. Although the increase in gain and feed conversion was due in fact to greater carcass fat deposition, protein deposition was not reduced. Results from experiment two showed that the substitution of extruded maize for a protein source at fat levels of 6.4, 12.0 and 17.6% were varied. In the initial stages of the experiment, the substitution of maize for animal by-products resulted in lower mass gains. However, a shift towards greater mass gains with higher maize inclusions was seen in the second half of the experiment. It was found that, despite the fact that alligators are carnivores, some protein of a meat diet is utilised as a source of energy (Staton and Edwards, 1987). It was reported by Coulson & Hernandez (1987) that alligators were unable to utilise dietary carbohydrates other than glucose but grow maximally on diets containing modest amounts of lipids. They reported that alligators were unable to utilise vegetable protein.

Experiments performed by Staton & Edwards (1987), demonstrated that lipid levels up to 20% were not harmful. It was stated by Staton & Edwards (1987) that alligators receiving

high levels of dietary lipids converted feed more efficiently, had greater mass gains and had better length growth than alligators fed on a low lipid diet. The greater mass gain was attributed to a higher body fat deposition, although the carcass protein content also increased with diets higher in lipid concentration.

The inclusion of dietary carbohydrates was said to be unavailable to alligators (Coulson & Hernandez, 1987). Staton & Edwards (1987) reported that a response surface analysis predicted an optimum feed conversion when the diet contained 19.7% maize and 11.2% lipid over the first half of the experiment and 17.0% maize and 14.6% lipid over the last portion of the experiment. The values for maize are very close to the predicted optimum value for growth. Studying the growth performance, the utilisation of dietary maize increased with time (Staton & Edwards, 1987).

It therefore appears that restricted amounts of dietary carbohydrates and lipids, are acceptable sources of energy for alligators. It was stated by Staton & Edwards (1987) that the maize used in these studies underwent extrusion cooking, and it seems that such processing of the carbohydrates makes them more available to crocodilians.

The inability of alligators to utilise vegetable protein requires further research. Coulson *et al.* (1987) demonstrated that force feeding alligators with isolated soybean protein and maize gluten meal resulted in lower protein digestibility and protein uptake from the gastrointestinal tract than for animal proteins. Unpublished work by Staton & Edwards, cited by Staton & Edwards (1987), showed that feeding alligators a diet of which 40% of the total dietary protein came from vegetable protein, resulted in good growth. It might therefore be possible that the addition of vegetable protein, to well balanced diets, be acceptable to alligators as well as other crocodilians, when maintained under good growing conditions.

Coulson *et al.* (1987) tested the plasma peak times of amino acids from various protein sources within the American alligator, to determine amino acid deficiency within the protein sources. They achieved this by feeding 14 different protein containing diets. These diets ranged from pure sources like fresh nutria (an American rodent), dry non-fat and fat containing nutria, dry non-fat fish, dry non-fat and fat containing chicken, the Staton mix, the Staton mix with methionine, casein, gelatin, edesin, ghadin, corn gluten and soy. The dry preparations were calculated to provide 10g of protein per kg body mass. The powders were

suspended in a water slurry and poured into the stomach through a long stem funnel. The fresh nutria was wetted and pushed down the oesophagus using a long glass stirring rod. The ghadin and gelatin were given in large enclosed gelatin capsules with 50ml of water poured into the stomach to ensure that the capsule dissolved. Blood was collected from the tip of the tail where the plasma was separated using a centrifuge.

Coulson *et al.* (1987) stated that if the rate at which non-essential amino acids are incorporated into the body protein after a meal, exceeds the rate of gain in plasma, the amino acid would be essential for maximal growth. However, if that rate is slower than the maximal rate of incorporation into body protein, a deficiency in amino acids would limit growth.

Peak time is the time required for each free amino acid to reach maximum concentration in the plasma (Coulson *et al*, 1987). The peak time is determined by the rate of protein digestion and the rate of removal of component amino acids from the plasma. Slow digested proteins results in free amino acid levels to increase at a low rate in plasma. However, if any one amino acid is proportionally low in the absorbed mixture, the incorporation of that amino acid into the body protein may be removed as fast as it was absorbed and its peak will occur before those of the others in more plentiful supply (Coulson *et al*, 1987), *i.e.* a protein source that digests slowly could be adequate for maintenance but not for maximum growth promotion. Based solely on peak times, the prediction on whether an animal's diet is adequate or deficient in one or more amino acids can be made.

Results from the Coulson *et al.* (1987) trial indicated that the average peak time for dried defatted, powdered nutria, fish and chicken were not only digested more rapidly than the other diets, but the rate of removal of each amino acid was more uniform. It was also reported that casein was slow to digest and nutritionally complete but was deficient in arginine and glycine. Gelatin was shown to be the poorest protein source even though it was easily digested, it lacked several amino acids as well as rendered protein synthesis impossible due to the improper amino acid ratio. Isolated soybean protein was shown to be deficient in methionine, lysine and histidine. It was slow to digest and a considerable amount of amino acids was found in the faeces. This vegetable protein is not a satisfactory substitute for animal protein when concerning alligators (Coulson *et al*, 1987).

Staton *et al* (1990) researched the protein and energy relationship in the diet of the American alligator. They used hatchling alligators which were fed diets containing various levels of protein, fat and carbohydrates. A total of 9 diets were tested in their first experiment, which lasted 15 weeks, where the protein : carbohydrate : fat ratio differed for each diet. Alligator total length, gains in body mass, dry matter consumption and feed efficiency were measured to determine alligator performance. According to their results, all these performance parameters were significantly responsive to dietary maize and lipid levels. The predicted maximum, from the response surface analysis, for these response variables ranged from 6.3 – 18.8% maize and 15.8 – 27.4% lipid (which consisted of 40% lard, 25% fish oil, 20% linseed oil and 15% safflower oil). According to the response surface analysis, the maximum predicted response of gain in body mass was found at 15.2 % maize and 18.5% lipid, whereas the minimum response in mass gain was observed with the low fat, maize free experimental diets. The response to dietary maize depended on the lipid level of the diet. It was reported that increasing the dietary maize level above 16-25% of the diet, resulted in lower mass gains, depending on the lipid content of the diet. When the diet that was fed contained a lipid level of 20%, maize could be increased to 27% without causing decreased growth rates. The body mass gains of this diet were superior or equal to those where alligators were fed maize-free diets. At the lower and middle levels of dietary lipid (4-12%), the maize content was increased to 36% of the diet. Reported body mass gains were equal to or greater than the higher protein, maize free diets of similar lipid content.

Results from the response surface analysis predicted the final total length of alligators at lower levels of maize (6.3%) and higher levels of lipid (27.4%), where dry matter (DM) consumption was predicted to be at maximum at levels of 18.8% maize and 21.5 % lipid of the diet. Replacing protein sources with maize, up to 36% of the diet resulted in improved consumption over maize free diets with corresponding fat levels. However, feed efficiency was maximised at lower levels of maize (11.4%) and lipid (15.8%) than feed consumption, according to Staton *et al.* (1990). It was also reported that both feed efficiency and total length were reduced by increasing dietary maize above 20-25%.

Protein digestibility tended to decrease with the presence of maize, but the reduction was only slight and protein digestibility was high for all diets. The decrease in protein digestibility appeared to be a function of the carbohydrate content of the diet (Staton *et al*, 1990).

Generally any increases in the dietary lipids will result in slight increases in protein digestibility when diets contain lipid levels of between 4% and 16% and crude protein levels of between 40% and 60 %. Energy digestibility was around 84.3% and was not significantly influenced by maize or fat dietary contents (Staton *et al.*, 1990). According to Staton *et al.* (1990), analysis of various production performance parameters in terms of digestible energy and protein, indicated that a digestible protein requirement between 42 – 49% of the diet, coupled with an energy requirement of 18301 – 18497 kJ DE per kg of feed (digestible energy) are required for optimum growth.

Staton *et al.* (1990) performed a second and third experiment (which lasted 5 and 4 weeks, respectively) where the effects of different feeding strategies were tested. In the experiments a number of animals were fasted and their mass monitored. Animals in Experiment 2 lost on average 32g over the 5 week period and animals in Experiment 3 lost an average of 61g over the 4 week period.

Regression analysis of the results from Experiment 2 indicated that daily maintenance requirements for energy and protein were 23.9 kJ/kg body mass (BW) and 0.49g/kg BW, respectively (Staton *et al.*, 1990). Regression of the mass specific daily body mass gain compared against the protein intake indicated that maximum daily growth rate was 16.9 g/kg BW.

Regression analysis of body mass gains supported by the protein diet in Experiment 3 predicted daily protein and energy maintenance requirements of 0.89 g/kg BW and 35.15 kJ/kg BW, respectively (Staton *et al*, 1990). In the case of the diets containing carbohydrates the daily protein and energy requirements were 0.68 g/kg BW and 30.13 kcal/kg BW.

Staton *et al.* (1990) showed that alligators can utilise and benefit from carbohydrates and fats within their diets, however with some limitations. Minimum responses to the different performance criteria generally occurred with the low fat, carbohydrate free diets. It was observed that energy digestibility did not vary with the diet. This showed that responses were not an indication of the alligators' ability to digest and assimilate energy sources (Staton *et al.*, 1990). A twofold substitution of carbohydrate for fat were predicted by the response surface analysis to result in equivalent body mass gain, when maize was within the range of 0 – 20 % (0 – 14 % carbohydrates) and lipids within 4 – 12%. At higher levels (above 15 –

20%) of dietary carbohydrates, response to these levels became neutral, and eventually negative, with respect to body mass gain.

The physiological demand for glucose for maintenance and maximum growth in an exothermic carnivore may be expected to be relatively low, as there is no dietary energy demand for heat production to maintain elevated body temperature (Staton *et al.*, 1990). It appeared that 11% dietary carbohydrate can satisfy the limited requirements for glucose and thus spare the use of protein as a gluconeogenic substrate. Feeding energy above maintenance and growth results in fat deposition, where carcass lipid would be more efficiently derived from dietary lipid than carbohydrates, as the energetic cost of lipogenesis is quite high. According to Staton *et al.* (1990), at low levels of dietary lipids, the projected 3% decrease in protein digestibility over the range of carbohydrates fed in their study was numerically similar to the 3.2% dietary protein contributed by maize included at 36% of the diet.

Results of Experiment 1 by Staton *et al.* (1990) showed that the maximum response in growth (mass and length) of digestible energy (DE) to protein (DP) was at 40.6:1 (kJ/g protein), and using an average protein digestibility coefficient of 86.7%, an optimal DE: CP (crude protein) ratio of 35.2 : 1 can be calculated. From the second Experiment, the predicted maximum daily requirements for gross energy (GE) and crude protein (CP) could be used to calculate a required GE : CP ratio of 54.0 : 1, then in Experiment 3, the optimum ratio is estimated to be 44.4 : 1 with the carbohydrate diet and 40.6 : 1 with the carbohydrate free diet (Staton *et* al, 1990). Using an average energy digestibility coefficient of 86.7%, the DE: CP ratio could be calculated as 45.6, 32.2 and 34.3:1 kJ/g respectively for each experiment.

Data from Staton *et al.* (1990) suggests that young alligators feed should contain 45% digestible protein, a caloric density between 34.3 – 45.6 kilojoules of DE per gram of CP and a total of 11% readily digestible carbohydrates. To reach this, the addition of dietary fat would be essential. Assuming a protein digestibility of 86.7%, the feed would have to have a minimum of 51.9% CP.

2.7) CONCLUDING REMARKS

Very little research has been published on *Crocodylus niloticus* (in Southern Africa), in terms of nutritional requirements in a productive system. The research preformed by Staton *et al*

(1990) on alligators (*Alligator mississippiensis*) possibly gives a base line as to what the Nile crocodile nutritional requirements, in terms of energy and protein, would be in a productive system. Most farmers do not feed a formulated diet to their crocodiles, as it is believed that crocodiles (like alligators) are unable to utilise vegetable carbohydrates and protein. There is a serious lack of scientific work on the nutritional requirements of *C. niloticus*, and any investigation on nutrition would add to the improvement of growth performances of this animal.

By determining the energy and protein requirements, as well as the ratio between energy and protein needed for Nile crocodiles, Southern African farmers would be able to formulate diets according to available raw materials, to reach the maximum growth potential of their crocodiles. This may in turn lead to greater monetary value of their crocodiles, as the larger the skins at slaughter age, the greater the profit made. As this industry is largely determined by the end consumer and fashion trends, reaching a maximum growth potential not only benefits the farmer, but also the consumer as there will be a greater variety of products to choose from.

CHAPTER 3. MATERIALS AND METHODS

3.1 ANIMALS AND HOUSING

Nile crocodiles (*Crocodylus niloticus*) used in this experiment were all artificially hatched at Le Croc Breeding farm (Brits, North West Province, South Africa). The incubator was maintained at a constant temperature of 32°C and a humidity of 80-90%. After an incubation period of 75-80 days, the hatchlings were transferred to an environmentally controlled grower house. The floor and ambient temperature of the house were maintained as close to 32°C as possible. Floor temperature was maintained by means of heated water pipes embedded in the concrete floor. The air temperature and quality in the house were regulated by means of vents drawing fresh air into the house after passing through a heating system.

The house was divided into 18 pens, of which 12 were used for this trial. The 12 pens were randomly divided into 4 treatments with 3 replicates per treatment. The dimensions of all the pens were 5.2m x 5.2m and 1.5m deep with a centre dam of 5.2m x 1.4m and 20cm deep giving a 2/3rd dry : 1/3rd water ratio.

The trial commenced when the crocodiles were between 5 and 6 months of age (hatched between 16th December 2008 and 31st January 2009). Each pen contained 205 hatchlings. Twenty crocodiles per pen were randomly chosen, although outliers were avoided, and tagged. A total of 240 hatchlings were thus tagged. For tagging, a hole was made in a tail base scute. A cable tie with an identity number (Plate 1 and 2) was tied through the hole. Once the cable tie had been tied, the cable tie's tail was cut off and an antiseptic wound spray was sprayed into and onto the cable tie and hole to prevent infection (Plate 3 and 4).

3.2) CROCODILE MEASUREMENT

The trial started on 25 May 2009 and was terminated on 17 August 2009. During this 12 week experimental period, the representatives of each pen were measured at the start of the trial and thereafter every three weeks. The hatchlings' mass, length (Staton *et al*, 1990), head length and snout-vent length (determined by researchers to observe other parameters) were determined on each of the measuring days.

The 20 crocodiles from each pen were hand caught, placed into holding buckets and transported to a measuring station (Plate 5). Once at the measurement station, crocodiles were re-caught from the bucket where their mouths were closed using rubber bands to protect the researchers from potential injury by the crocodiles (Plate 6). It also made restraining of the animal easier during the measuring procedure. The crocodiles were individually placed into a basket attached to a hang scale fixed on a stand to measure body mass (Plate 7). Thereafter it was placed onto a table fitted with a body length ruler, where the total body lengths were measured (Plate 8).

The head length was measured using a calliper and each crocodile was turned on its back to measure snout to vent length (Plate 9). All the measurement data was captured next to the identity number of the crocodile.

The quantity of the feed placed and the amount of left-over feed retrieved the following day were used to determine feed consumption (Plate 10 & 11). As there were 205 crocodiles in each pen, an average feed consumption per crocodile per pen was calculated daily. These feed consumption values together with the body mass values were used for calculating the feed conversion ratio (FCR).

3.3) DIET FORMULATION AND MIXING

Four treatment diets with varying concentrations of crude protein (CP) were fed during this trial. Two diets were formulated, one with the lowest (46%) and the other with the highest (62%) CP level (Table 3.1). Two additional treatment diets were produced by mixing the 46% CP diet (LCP – low CP) with the 62% CP (HCP – high CP) diet at different ratios. A diet containing 51.3% CP (MLCP – medium low CP) was created by mixing 2/3 of the LCP diet with 1/3 of the HCP diet. Likewise, a diet of 56.6% CP (MHCP – medium high CP) was produced by mixing 1/3 of the LCP diet with 2/3 of the HCP diet. The four CP treatment levels were determined by the previous trial of Staton *et al* (1990. The 51.3% CP would serve as a direct comparison of crocodile to alligator requirements as determined by Staton *et al* (1990), and the other three served to see the performance of crocodiles on a lower and higher CP diets. All diets were kept at an energy to protein ratio as close as possible to 25.85 kJ/g of protein. This ensued that the only variable in the trial was protein.

Table 3.1 Calculated diet composition and nutrient levels

Ingredients	62% CP treatment	46% CP treatment
	(%)	(%)
Fish meal	46.877	35.000
Chicken mince	43.996	-
Soybean oilcake meal (46%)	-	30.000
Full fat soya	6.361	9.778
Carcass meal	-	4.348
Maize meal	1.048	17.159
Premix	1.050	1.050
L-lysine HCl	0.069	0.754
Salmon Oil powder	0.500	0.500
DL methionine	0.099	0.288
Limestone	-	0.519
Monocalcium phosphate	-	0.604
Calculated Nutrient levels (g/kg) on a dry matter basis		
Crude protein	620.0	460.0
Crude fat	241.3	76.2
Ash	82.9	100.1
Lysine	39.5	34.3
Crude fibre	24.1	31.6
Calcium	23.2	20.0
Phosphorous	17.1	15.0
AMEn_adult (Poultry) MJ/kg	16.0	11.9
Methionine	15.5	12.7
Energy: Protein (kJ/g)	25.9	25.9
Ca: P	1.4	1.3

*Premix (Feedmix, Johannesburg, South Africa) contained vitamins at 0.25%, minerals at 0.5% and a growth promotant - Hatch Booster (Intofeed, Midrand, South Africa) at 0.3% of the total ration, respectively.

The dry ingredients for both the HCP and LCP diets were mixed together using an industrial feed mixer. Each ingredient for the respective diet was weighed off in accordance to the formulation and mixed for half an hour, after which the mixed dry portion of the diet was retrieved and temporarily stored into 50kg feed bags. The dry potions for the HCP and LCP diets were then transported to the feed preparation unit (where they were transferred into a 250 L holding drum – Plate 12 & 13).

Final feed preparation involved the plucking of chicken (Plate 15) (mortalities collected from surrounding chicken farms) and mincing the whole defeathered chicken with a meat mincer

(the mincer had a plate with 9mm holes). The minced chicken and dry mixed portion were then weighed out in accordance with the amount represented in the formulation for the 62% protein treatment diet. The minced chicken was spread out onto the table portion of the meat mincer and the dry mix portion was spread on top of the minced chicken. The two ingredients were then thoroughly mixed for about 10min, until the mixture was well mixed. The dry mix – minced chicken mixture was then passed through the meat mincer to form long thin sausages (9mm in diameter for the first 4 weeks of the trial and 12mm in diameter thereafter). These sausages broke into pellets size portions when fed to the crocodiles.

The LCP diet underwent a different process (Plate 16). The dry mix was spread onto the meat mincer's table where water was added to it to form a hard to medium paste. This paste was then pushed through the meat mincer to produce the long thin sausages and eventually the pellets. The meat mincer was cleaned between each treatment diet. The MHCP and MLCP diets were a combination of HCP and LCP diets (Plate 17 – 21). The HCP and LCP diets were weighed out to 2/3rd of the HCP diet and 1/3rd of the LCP diet to create the MHCP diet. The MLCP was made up of 1/3rd of the HCP diet and 2/3rd of the LCP diet.

The treatment diets were fed in a pellet form to the hatchlings. Feeding buckets used to transport feed to the pens were assigned to a specific treatment and pen (Plate 22). Pens were checked every morning to observe whether left over feed (orts) were present, the orts would then be collected and weighed to determine daily feed consumption.

After mixing and before the commencement of the trial, feed samples were collected and analysed for nutrient content at Nutrilab (University of Pretoria). Feed return (orts) samples were also collected during the trial, as the crocodiles do lie in the feed trays resulting in the feed returns having greater water content than when placed. Because of the variability of dry matter content between the different feeds and also between orts, all the values were first converted to a dry matter basis before calculating feed intake.

3.4 FEEDING SCHEDULES AND PRACTICES

Crocodiles were conditioned 2 weeks prior to the start of the trial, allowing them to become accustomed to the new smell and taste of the trial feed. Hatchlings were fed once every day at 16:00. Feed and feed refusals were weighed out on an electronic scale and hand captured onto

a daily feeding sheet. All crocodiles were fed *ad lib* which gave them the freedom of consuming feed at their leisure for 8 hours.

Once all the feed buckets were weighed with the treatment diets, the buckets were carried out to the grower house and placed on the respective pen's wall (Plate 22). When all buckets were placed inside the grower house, feeding commenced.

The feed was thrown into feeding trays, 1m long by 20cm wide (Plate 23). Each pen had 6 feed trays, 3 on each side of the central pond within the pen. As the crocodiles were used to being fed on the floor prior to the placement of the feed trays, all the hatchling crocodiles that were part of the trial underwent a conditioning phase of one month to familiarise themselves to receiving and consuming feed from the feed trays (Plate 24 & 25). Crocodiles were encouraged to eat out of the trays by creating movement inside the trays. This was done by means of a moving red laser and the placement of disinfected maggots during the conditioning phase.

In the initial months before the start of the trial, the keeper would climb into the pen and spread out the pellets on the trays. This practice had a negative impact on feed intakes. The hatchlings became frightened when the keeper entered their pen to place feed. It was then decided that the feed should rather be thrown than placed because the crocodiles tend to react better that some sort of visual stimulation. This resulted in excellent feed intakes. As in the wild, hatchling crocodiles tend to prey on moving living creatures, and therefore the rolling movement of the feed after throwing the feed in the trays, elicited a more intensive feeding response. Some pellets were lost to the water, but the amount was minimal.

At the start of the trial the hatchling crocodiles were fed pellets of 9mm in diameter. This size was large enough to allow the crocodiles to pick up the pellets with ease. However 4 weeks into the trial it was noticed that the hatchlings were not eating well, as they had difficulty attaining pellets. There had been some problems with temperatures before this period but these were corrected. It was identified that the pellet size was too small for the hatchlings to grasp, so the pellet size was increased to 12mm in diameter. This had a marked response on feed intake. The 12 mm pellet was then fed to the hatchlings for the remainder of the trial.

3.5 FEED COSTING

All feed ingredients were fully sponsored by Le Croc Breeding farm for the duration of the trial.

Feed was supplied from five main feed sources:

- Feedmix, who supplied: the carcass meal, minerals, vitamins, lysine & methionine
- Intofeed, who supplied: the growth promoters - salmon oil powder, hatch booster
- Kanhym, who supplied: the fish meal, maize pop meal and full fat soya meal.
- Obaro the local cooperation, supplied the soya oilcake meal.
- And the chicken mortalities were collected from the surrounding poultry operations

A total amount of 8952.19kg of feed was fed to the crocodiles during the trial amounting to R59,434.89 (Table 3.2)

Table 3.2 Trial feed cost from May - August 2009

Ingredients	**Consumption over trial (kg)**	**Price(R)/kg**	**Cost of Feed**
Carcass Meal	178.91	R 4.65	R 831.92
Fish Meal	3707.81	R 9.33	R 34,593.89
Chicken Mince	2128.29	R 1.20	R 2,553.95
Full Fat Soya	710.05	R 4.65	R 3,301.73
Salmon oil Powder	44.76	R 17.00	R 760.94
Maize pop	729.89	R 2.90	R 2,116.68
Soya oilcake meal (46%)	1261.27	R 6.83	R 8,614.48
Lysine	34.36	R 24.50	R 841.89
Methionine	16.64	R 53.00	R 881.89
Vitamin	22.38	R 105.00	R 2,349.95
Mineral	44.76	R 13.00	R 581.89
Hatch Booster	26.86	R 62.98	R 1,691.43
Lime stone	21.36	R 0.75	R 16.02
Monocalcium P	24.85	R 12.00	R 298.24
Total	**8952.19**		**R 59,434.89**

3.6 CLEANING AND HYGIENE

Each pen was washed daily (Plate 26). This became a normal routine for the hatchlings. The main reason for the daily cleaning was to prevent the growth of pathogenic microorganisms, as the grower house provides an ideal environment for bacterial growth. The house was

maintained at 30 – 32 °C with a very high relative humidity. This coupled with feed and faecal matter present on all surfaces, could result in mass growth of organisms and eventually to infection of the crocodiles. The constant interaction with the crocodiles during washing of the pens not only served a hygienic purpose but also allowed the crocodiles to become accustomed to human presence.

Floors and walls of the pen were cleaned with antibacterial soaps twice a week and the cleaning agents (F10 and Virkon, Immunovet, South Africa) were used interchangeably, to prevent bacteria becoming resistant to the chemicals.

Crocodiles in a production system prefer routine over erratic cleaning procedures. This means that the keeper must follow the same routine every day. Changing a cleaning routine (especially in hatchlings) may lead to stress and reduced feed intake (Plate 27).

The keeper was equipped with an industrial large broom and a hose pipe. The keeper's routine started by removing any feed left behind on the feed tray and placing it onto a transporting tray. Once all the pens feed refusals were collected, the feed refusals were weighed and recorded. Washing of the pens started by first draining the water in the pen's centre pond. The keeper used some of the dirty water of the centre pond to wash off faecal matter from the land surface. As the water reached a low level within the centre pond, the keeper brushed the remainder of the dirty water down the drain, after thoroughly scrubbing the floor with the broom and rinsing it with fresh water from the hose pipe. When soap was used, the entire floor surface received a solution of soap water from a premixed bucket, where it was spread over the floor and scrubbed with the broom. Once the pen was clean, the keeper rinsed off the soap residue as well as any other faecal and feed matter from the pen surface down the drain and filled the centre pond with fresh clean chlorinated water.

The canal water (from Hartebeestpoort dam, North West, South Africa) used on the farm underwent a chlorination process through the farm's chlorinator. This ensured that all water entering the pens was clean. Water used in the cleaning process was heated to 30°C, to prevent the hatchling crocodiles from experiencing cold shock when their pens were being cleaned.

3.7 TEMPERATURE

As temperature is vitally important for crocodiles to feed and grow maximally, floor and ambient temperature readings were taken twice daily using a temperature gun. This was to ensure that all the heating systems were working, and any problem that was encountered could be dealt with immediately.

The grower house that housed the trial crocodiles did experience some temperature problems in the beginning of the trial. This was due to a leaking pipe as well as the use of faulty coal. However, these problems were corrected within 2 weeks into the trial and feeding resumed normally.

3.8 TIME LINE

As mentioned previously, this trial was planned for 12 weeks. However the 48% protein treatment diet was only fed for 9 weeks, as these crocodiles were losing mass. As these animals were part of the production stock, their feed was changed to the farm's pre-formulated diet to regain the growth that was lost and to prevent further growth loss (see Results).

Time line for trial:

- 2009/04/05 – Crocodiles introduced to feed trays
- 2009/05/11 – Trial diet introduced and crocodiles conditioned to trial diet
- 2009/05/25 – First measurements taken
- 2009/06/10-11 – Chromium oxide added into the feed and fed to the crocodiles for 2 days (feed samples were also collected for these 2 days)
- 2009/06/12-14 – Crocodile faeces collected from each trial pen
- 2009/06/15 – Second measurement taken
- 2009/06/19-27 – Feed refusals samples were taken
- 2009/07/07 – Third measurement taken
- 2009/07/21-22 – Chromium oxide was added into the feed and fed to the crocodiles for 2 days (feed samples were also collected for these 2 days)
- 2009/07/23-25 – Crocodile faeces were collected from each trial pen

- 2009/07/27 – Fourth measurement taken
- 2009/08/17 – Fifth measurement taken and experiment terminated

Time line for the laboratory analysis:

- 2009/10/ 19 – 23 Initial DM, ash and crude protein of feed as well as DM analysis faeces
- 2009/10/26 – Fat analysis of feed samples
- 2009/10/29-30 – DM analysis of feed refusals
- 2009/11/04-06 – Crude fibre analysis of feed samples
- 2009/11/13 – Gross energy analysis of feed and faecal samples
- 2009/11/16-20 – Calcium, phosphorous and chromium analysis of feed and chromium analysis of the faecal samples

3.9 LABORATORY ANALYSIS

Nutrient analysis was performed on all the feed and faecal samples to determine the accuracy of the diet formulation, and to determine the digestibility of protein, energy and dry matter of the trial diets.

Two samples per treatment diet were taken over the 12 week trial. One sample per treatment was taken at the beginning of the trial and one sample per treatment diet was taken towards the end of the trial.

All the feed samples were analysed for dry matter (DM), ash, crude protein (CP), crude fibre (CF), crude fat (EE), gross energy (GE), amino acid composition (AA), calcium (Ca), phosphorous (P) and chromium (Cr).

Two faecal samples per pen were taken during the trial. One sample per pen at the beginning of the trial (taken after the first feed samples) and one sample per pen towards the end of the trial (taken after the second feed samples).

The faecal samples were analysed for a DM, CP, GE and Cr. DM, CP and energy digestibility was calculated after the analysis of the Cr concentration in the feed and faecal samples.

108 feed refusal samples were collected over 9 consecutive days from the 19th to the 27th of June 2009. This was done to determine whether the DM content of the refusals was the same as the feed that was fed to the crocodiles. Only DM was determined from these samples. Further analysis was not needed as it was not possible for the crocodiles to feed selectively.

All samples were frozen shortly after collection and only defrosted at the commencement of the laboratory analysis.

3.9.1) Dry matter analysis

Dry matter was determined using the AOAC (2000), Official Method of Analysis 934.01.

5 grams of the feed sample was placed into aluminium crucibles. The DM for each sample was done in duplicate.

The crucibles were placed into an oven for 16 hours (overnight) at 105ºC and weighed. The rest of the sample was placed into larger aluminium containers and dried in an oven at 55ºC over night.

The samples in the larger aluminium containers were removed from the oven the next day and allowed to cool before transferring the samples into zip lock bags.

These samples were then milled into a fine powder using a sieve with a 2mm hole diameter for further analysis.

3.9.2) Ash analysis

Ash analysis of the feed was determined using the AOAC (2000), Official Method of Analysis 942.05.

After drying the samples for DM determination, the samples were transferred to a furnace and incinerated at 200ºC for 1 hour and then at 600ºC for four hours.

After the furnace was allowed to cool over night the ceramic crucibles were weighed to determine the ash content.

3.9.3) Crude Protein (CP) analysis (Dumas/Leco)

Crude protein was determined using the AOAC (2000), Official Method of Analysis 968.06.

The CP analysis was performed on the Leco FP- 428 (Leco Corporation, Michigan, USA) using the Nitrogen and Protein method.

The Leco FP- 428 is a microprocessor based software controlled instrument that determines nitrogen in a variety of materials.

0.2 g of each sample was measured in duplicate into foil cups.

There are 3 phases during the analyze cycle, these include:

- The sample drop purge phase
- The burn phase
- The analyze phase.

For the sample drop purge phase, the encapsulated sample was placed in the loading head, where it was sealed and purged of any atmospheric gases.

During the burn phase, the sample was dropped into a hot furnace (950ºC) and flushed with pure oxygen for a very rapid combustion. The products of combustion, mainly CO_2, H_2O, NO_x, and N_2 are passed through the thermoelectric cooler to remove the water, and the gases collected in the ballast volume. All the gas products in the ballast volume were allowed to become a homogenous mixture at a pressure of approximately 975 mm at a constant temperature.

In the analysis phase, the piston was forced down and a 10 cc aliquot of the sample mixture was collected. The sample aliquot was swept through hot copper to remove and convert NO_x to N_2, as well as passed through Lecosorb and Anhydrone to remove the carbon dioxide and

water, respectively. The remaining combustion product, nitrogen, was then measured by the thermal conductivity cell.

The final product is displayed as a percent nitrogen (or protein if selected)

Protein content of samples were calculated using a conversion factor from sample nitrogen content. Protein conversion factors (AOAC 2000 Official Methods of Analysis):

- For meat, tea and grain (other than wheat) samples, CP is determined by % nitrogen x 6.25.
- For dairy products, cheese, butter, milk samples, CP is determined by % nitrogen x 6.38
- For wheat and wheat products samples, CP is determined by %N x 5.70

3.9.4) Crude Fibre (CF) analysis

CF in feed was determined using the filter bag technique, (AOAC 2000, Official Method of Analysis 962.09).

This method determines crude fibre which is the organic residue remaining after digesting with 0.255N H_2SO_4 and 0.313N NaOH. The compounds removed are predominantly protein, sugar, starch, lipids and portions of both the structural carbohydrates and lignin.

- Apparatus
    1. Analytical balance – which can be weighed down to 0.1mg
    2. Oven – can maintain a temperature of 102ºC
    3. Digestion instrument (ANKOM^{2000}, ANKOM Technology, USA) – that is capable of digestions at 100ºC and maintaining a pressure of 10-25 psi
    4. Filter bags created from chemically inert and heat resistant filter media, capable of being heat sealed and retaining 25micron particles while permitting rapid solution penetration
    5. Heat sealer for sealing of the filter bags
    6. Desiccator pouch – a collapsible sealed pouch with desiccant inside that removes moisture from the air around the filter papers
    7. Marking pen that is solvent and acid resistant

- Reagents
    1. Sulphuric acid solution – 0.255N, 1.25 g H_2SO_4/100ml
    2. Sodium hydroxide solution – 0.313N, 1.25 g NaOH/100ml

- Preparation of sample

  Samples were ground through a centrifugal mill with a 2mm screen or cutter type mill with a 1mm screen. Samples ground finer may show particle loss from the filter bags and result in low values.

- Procedure
    1. Filter bags were labelled with a solvent and an acid resistant marker. Filter bags were weighed (W_1) and the scale zeroed.
    2. 0.95 – 1.00 g of prepared sample (W_2) were weighed directly into the filter bag. Avoiding the placement of sample on the upper 4mm of the bag.
    3. By using the heat sealer, the upper edge of the filter bag within 4mm of the top, is sealed.
    4. One blank bag was weighed and included in the run to determine the blank bag correction (C_1).
    5. Fat was extracted from samples by placing the bags into a 250 ml container, where petroleum ether was added to the container covering the bags. The bags were soaked for 10 min. The solvent was then poured out and the bags were allowed to air dry. Samples were then spread uniformly inside the filter bag by shaking or flicking the bag to eliminate clumps.
    6. A maximum of 24 bags were placed into the Bag Suspender. All nine trays were used regardless of the number of bags being processed. Each tray could only hold a maximum of three bags and the trays were stacked on the centre post with each level rotated at 120 degrees. The Bag Suspender was then inserted with the bags into the fibre analyzer vessel and the Bag Suspender mass was placed on top of the empty 9^{th} tray to keep it submerged.
    7. Instructions on the ANKOM^{2000} display screen were then followed.
    8. When the Crude Fibre extraction and rinsing process was complete, the sample bags were removed. Excess water from the bags was removed by

gently squeezing the bags. The bags were then placed in a 250ml beaker and acetone was added to cover bags, which were soaked for 3-5 min.

9. The bags were removed from the acetone and placed on a wire screen to air dry. The bags were then completely dried in an oven at 102ºC for 2-4 hours.
10. The bags were removed from the oven, placed into a collapsible desiccant pouch. Once the bags were cooled to ambient temperature, the bags were weighed.

- Calculations to determine % Crude Fibre

% Crude Fibre = $100 \times [(W_3 – (W_1 \times C_1))/ W_2]$

Where:

W_1 = Bag tare mass

W_2 = Sample mass

W_3 = Dried mass of bag with fibre after extraction

C_1 = Blank bag correction (running of final oven-dried mass, divided by the original blank bag mass).

3.9.5) Crude Fat (EE) Analysis

Crude fat was determined using the AOAC 2000 Official Method of Analysis 920.39.

A number one Whatman filter paper was torn on the mass balance. A 3 g sample was then weighed, after which a clean and dry Büchi beaker was weighed.

The sample was then placed on a filter paper (folded up) in an extraction thimble. The number of the beaker and thimble were recorded.

The extraction thimble was then placed in the soxhlet extraction tube. Petroleum-ether (40 – 60°C boiling point) was poured into the Büchi beaker, up to the brim. The flat-bottomed flask and extraction tube were connected to the apparatus.

The water taps were then opened and the tap of the steam generator was turned to the horizontal. The power was then switched on, ensuring that the ether boiled slowly. If the ether condenses at a rate of 4 – 6 drops/second, the extraction will last 4 hours (for feed samples).

After the extraction, the ether was evaporated and collected in the soxhlet tube and filtered into a waste bottle.

The Büchi beaker was then placed in an oven at 60 °C (or for at least 1 hour) and cooled in a desiccator. The mass of the Büchi beaker was then determined.

The difference between the mass of the flask before and after the extraction, was the mass of crude fat of the sample.

Example

Sample mass	2.1063 g
Mass crude fat	0.0901 g
% Fat	$\frac{0.0901}{2.1063} \times \frac{100}{1} = 4.28\%$

3.9.6) Gross Energy (GE) analysis

Gross energy was determined by the MC – 1000 Modular Calorimeter.

3.9.7) Mineral Analysis

Samples for mineral analysis were prepared using the AOAC 2000 Official Method of Analysis 935.13.

Acid digestion of samples for mineral analysis was carried out as follows:

The heating block was switched on and set to a temperature of 240 ^{0}C.

A duplicate sample of 0,500 g was weighed out, recorded and transferred into the digestion tube. 25 ml nitric acid (HNO_3) was then added to each sample. The samples were then placed on the pre-heated block and boiled for 15 minutes. The samples were then removed from the block and allowed to cool for 5 minutes.

10 ml perchloric acid ($HClO_4$) was then added to each sample which was then returned to the block. The samples were allowed to boil for 40 minutes (to ensure even heating, the rack containing the samples was rotated 180^0 at the 20 minute mark). Samples should be orange-yellow in colour. If they appear green, return to the block for a few more minutes.

Samples were then removed from the block and allowed to cool in a fume cupboard until no more fumes were released.

Deionised water was added to each sample, but not up to the 50 ml mark, as heat would be generated. The samples were allowed to cool down.

Once the samples were cooled, deionised water was used to make up the 50 ml mark. The samples were shaken to mix and transferred to a clean 50 ml medicine bottle.

The following minerals were analysed from the mineral digestion solutions.

- Calcium (Ca)
    - Calcium concentrations were determined by the Giron, H.C.,1973 Atomic Absorption Newsletter 12, 28. Perkin Elmer Atomic Spectrophotometer.
- Phosphorous (P)
    - Phosphorous concentrations were determined using the AOAC 2000. Official Method of Analysis 965.17 (17^{th} Edition) Volume 1.
    - Samples for phosphorous analysis were not digested as described above due to the presence of Chromium in the sample.
    - The chromium would lead to colour interferences with the P in the sample, and due to this the Milestone microwave method was used to digest the samples for P analysis.
    - The milestone method includes:
        - A 0.5g sample
        - 5ml Nitric acid (65%)
        - 1ml Peroxide
        - These were added into a vial, closed and placed into the Milestone microwave solvent extraction lab-station for 1 hour

- Phosphorous was determined using the AOAC 965.17 photometric method, AOAC 2000, CAS – 7723 – 14 – 0 (Phosphorous)

- Chromium
  - Chromium oxide was analysed using the ICP – Spectro Genesis which is an optical emission spectrometer with inductively–coupled plasma excitation.

3.9.8) Amino acid (AA) analysis

Amino acid composition of the trial diets was determined by the Department of Biochemistry (University of Pretoria) using the AccqTag UPLC method.

3.10 STATISTICAL ANALYSIS

Data was analysed statistically as a randomized block design with the GLM model (Statistical Analysis System, 2009) for the average treatment effect over time. Repeated Measures Analyses of Variance with the GLM model were used for periods. Means and standard deviations were calculated and the significance of difference ($P < 0.05$) between means were determined by Fischer's test (Sameuls, 1989)

The linear model used is described by the following equation:

$$Y = \mu + T + B + e$$

Where Y = variable studied during the period

μ = overall mean of population

T = effect of the i treatment

B = effect of the j block

e = error associated with each Y

Two sets of data were analysed statistically. The one set contained all measurements taken at set intervals (weeks 0, 3, 6, 9, 12) and the other set contained only the data over the trial period where data from the initial measurements was compared to the last.

3.11 DIGESTIBILITY

Digestibility values for crude protein, energy and dry matter were determined for the different treatment diets. The only digestibility values currently available for the Nile crocodile are based on values determined in the American alligator. Digestibility was determined using 0.1% of chromium oxide (Cr_2O_3) (Merck's Chemicals Division, Halfway House, South Africa) in the feed (on DM basis) as an indigestible marker. Chromium was added into the HCP and LCP diets, it was therefore also present in the MHCP and MLCP diets at the same concentration. Digestibility tests were performed twice during the trial, each testing period lasting five days. Cr_2O_3 containing feeds were fed for two consecutive days, after which feed samples were taken, and faecal samples were collected for three consecutive days after feeding. Two feed samples per treatment diet were taken during the trial for chromium oxide analysis. Protein, energy and dry matter digestibility coefficients were determined using a standard equation (Staton *et al.*, 1990):

$$\text{Digestibility coefficients} = 1 - \frac{(\%\ Cr_2O_3 \text{ in food})(\%\ \text{nutrient in faeces})}{(\%\ Cr_2O_3 \text{ in faeces})(\%\ \text{nutrient in feed})}$$

CHAPTER 4. RESULTS

4.1. THREE WEEKLY REPETITIVE MEASUREMENT DATA

Data was analysed over two time periods, i.e. the first 9 weeks of the trial and the total 12 weeks trial period. As the 46% protein treatment (LCP – low crude protein) level had to be discontinued from week 9, the four treatment levels could only be compared to each other for 9 weeks. However the remaining three treatments were compared with each other for the total duration of the trial (12 weeks).

4.1.1. Three weekly repetitive measurements (week 0 – 9)

4.1.1.1) Body mass

As shown in Table 4.2, there were no significant differences for body mass between the treatment levels over 9 weeks of measurement.

4.1.1.2) Total body length

No significant difference was found between treatment levels for total body length, over 9 weeks of measurements (Table 4.3).

4.1.1.3) Head length

No significant difference was found between treatment levels for head length over 9 weeks of measurements (Table 4.4).

4.1.1.4) Snout – vent length

No significant difference was found between treatment levels for snout – vent length over 9 weeks of measurement (Table 4.5).

4.1.1.5) Body length difference

Crocodiles on the 60% protein treatment (HCP) were found to be significantly longer than crocodiles on the LCP. There was no significant difference found between crocodiles for all measurement weeks between the 56.6% (MHCP) and the 51.3% (MLCP) protein treatments

at weeks 3 and 9. Crocodiles on the MHCP and the MLCP were found to be significantly longer than crocodiles on the LCP at week 6 (Table 4.6).

4.1.1.6) Head length difference

Crocodiles on the HCP were significantly longer in head length than crocodiles on the LCP at weeks 3 and 9, where crocodiles on the MHCP and the MLCP diets were not significantly different in head length compared to crocodiles on the HCP or the LCP diets (Table 4.7).

4.1.1.7) Snout-vent length difference

Crocodiles on the HCP were significantly longer than crocodiles on the LCP diet at week 3, but crocodiles on the MHCP and the MLCP diets showed no significant difference in snout to vent length difference. However, at week 6 crocodiles on the HCP and the MLCP diet were significantly longer than crocodiles on the LCP, where there were no differences between the snout-vent lengths of crocodiles on the MHCP compared to the other three protein treatment diets (Table 4.8).

4.1.1.8) FCR

As shown in Table 4.9, crocodiles on the HCP were found to have a significantly lower FCR than crocodiles on the LCP at week 3 ($P<0.05$). No significant difference was found between treatment levels for weeks 6 and 9.

4.1.1.9) Feed intake

No significant difference was found between treatment levels for feed intake, over 9 weeks of measurement (Table 4.10).

4.1.1.10) Mass change

As shown in Table 4.11, the crocodiles that consumed the highest protein level in their diets gained significantly more mass over the first 3 weeks of the trial than the crocodiles that consumed the MHCP and the LCP diets. The crocodiles that received the three highest protein levels, gained significantly more mass at week 6 than the crocodiles that received the LCP. Crocodiles that received the HCP and MHCP diets respectively, gained significantly more mass at week 9 compared to crocodiles on the MLCP and the LCP diets. It was also noted at week 9 that the crocodiles on the LCP diets actually lost mass, and this was the reason for the discontinuation of this treatment

4.1.2. Three weekly repetitive measurements (week 0 – 12)

4.1.2.1) Body mass

No significant difference was found between the three treatment levels for body mass, over 12 weeks of measurement (Table 4.12).

4.1.2.2) Total body length

No significant difference was found between the three treatment levels for total body length, over 12 weeks of measurements (Table 4.13).

4.1.2.3) Head length

No significant difference was found between the three treatment levels for head length, over 12 weeks of measurement (Table 4.14).

4.1.2.4) Snout – vent length

No significant difference was found between the three treatment levels for snout – vent length over 12 weeks of measurements (Table 4.15).

4.1.2.5) Body length difference

No significant difference was found between the three treatment levels for body length difference over 12 weeks of measurements (Table 4.16)

4.1.2.6) Head length difference

At week 9, crocodiles on the HCP diets were significantly longer in head length than the two lower protein treatment diets, as shown in Table 4.17. However, there was no significant difference in head length for crocodiles on the HCP, MHCP and the MLCP diets at weeks 3, 6 and 12.

4.1.2.7) Snout-vent length difference

At week 12 (Table 4.18), crocodiles on the HCP were significantly longer than crocodiles on the MLCP. There was no significant difference between crocodiles on the three protein diets at weeks 3, 6 and 9.

4.1.2.8) FCR

As shown in Table 4.19, crocodiles that were fed the two highest protein levels had a significantly lower FCR than crocodiles that were fed the MLCP at week 9. No significant difference was found for the other measurement weeks for all the treatment levels.

4.1.2.9) Feed intake

No significant difference was found between the three treatment levels for mass change, over 12 weeks of measurements, as shown in Table 4.20

4.1.2.10) Mass change

As shown in Table 4.21, crocodiles that were fed the HCP and the MLCP gained significantly more mass at week 3 than crocodiles fed the MHCP. However, crocodiles that were on the two higher protein levels (the HCP and the MHCP) gained significantly more mass than crocodiles that received the MLCP at both weeks 9 and 12.

Table 4.1 Analysed nutrient values (g/kg) of different treatment diets (on a DM basis)

	Protein Treatment Levels			
Nutrient	**HCP (62%CP)**	**MHCP (56.60%CP)**	**MLCP (51.30%)**	**LCP (46%CP)**
GE (MJ/kg)	20.44	19.98	19.67	19.28
DC_{energy} [1]	0.7015	0.6415	0.5035	0.5100
DE (MJ/kg)	14.34	12.82	9.90	9.83
Crude Protein	619.50	565.90	517.21	461.08
$DC_{protein}$ [2]	0.7565	0.6915	0.5700	0.5350
Digestible Protein	468.65	381.32	294.80	246.64
Crude Fibre	61.06	54.45	61.08	46.26
Crude Fat	126.78	107.92	93.39	77.40
Calcium (Ca)	45.63	39.86	36.26	28.61
Phosphorous (P)	27.52	23.87	21.15	17.48
Ca:P	1.66	1.67	1.71	1.64
Ash	180.01	160.31	143.74	121.11
Aspartic acid	56.39	52.78	46.23	42.32
Glutamic acid	89.82	84.88	76.47	70.22
Serine	24.86	23.14	20.56	19.03
Glycine	40.15	34.68	30.46	26.86
Histidine	15.61	13.01	11.35	9.80
Arginine	36.64	33.84	30.40	28.42
Threonine	25.22	22.98	19.37	16.96
Alanine	37.52	33.42	28.11	24.48
Proline	27.75	25.29	23.27	21.11
Tyrosine	20.05	18.57	15.98	14.57
Valine	30.34	27.75	23.63	20.90
Methionine	16.64	14.85	12.70	11.20
Isoleucine	26.15	24.29	20.77	18.66
Leucine	44.45	41.39	35.71	31.94
Phenylalanine	24.29	22.98	20.09	18.46
Lysine	43.47	39.35	34.09	32.98

[1] Digestibility coefficient for energy
[2] Digestibility coefficient for protein

Table 4.2 Mean body mass (grams) of crocodiles that received different levels of dietary protein over a period of 9 weeks (± standard deviation)

Measurement weeks	Protein Treatment Levels			
	HCP	MHCP	MLCP	LCP
0	1214 (± 171)	1390 (± 116)	1308 (± 234)	1391 (± 134)
3	1355(± 170)	1470 (± 125)	1410 (± 229)	1452 (± 124)
6	1466 (± 146)	1577 (± 103)	1509 (± 291)	1475 (± 155)
9	1671 (± 172)	1684 (± 028)	1548 (± 269)	1444 (± 100)

HCP : High Crude Protein (diet containing 62% crude protein)
MHCP : Medium High Crude Protein (diet containing 56.6% crude protein)
MLCP : Medium Low Crude Protein (diet containing 51.3% crude protein)
LCP : Low Crude Protein (diet containing 46% crude protein)

Table 4.3 Mean body length (cm) of crocodiles that received different levels of dietary protein over a period of 9 weeks (± standard deviation)

Measurement weeks	Protein Treatment Levels			
	HCP	MHCP	MLCP	LCP
0	70.97 (± 2.78)	74.30 (±2.21)	72.80 (±3.45)	75.23 (± 2.45)
3	74.20 (± 2.71)	76.80 (± 1.76)	75.27 (± 3.65)	77.27 (± 2.49)
6	77.07 (± 2.05)	79.10 (±2.08)	78.20 (± 3.56)	78.63 (± 2.48)
9	79.43 (± 2.23)	80.97 (± 1.05)	79.67 (± 4.10)	79.07 (± 2.42)

HCP : High Crude Protein (diet containing 62% crude protein)
MHCP : Medium High Crude Protein (diet containing 56.6% crude protein)
MLCP : Medium Low Crude Protein (diet containing 51.3% crude protein)
LCP : Low Crude Protein (diet containing 46% crude protein)

Table 4.4 Mean head length (cm) of crocodiles that received different levels of dietary protein over a period of 9 weeks (± standard deviation)

Measurement weeks	Protein Treatment Levels			
	HCP	MHCP	MLCP	LCP
0	9.07 (± 0.32)	9.37 (± 0.32)	9.17 (± 0.35)	9.57 (± 0.23)
3	9.57 (± 0.32)	9.83 (± 0.29)	9.60 (± 0.40)	9.93 (± 0.21)
6	9.97 (± 0.23)	10.17 (± 0.32)	10.00 (± 0.46)	10.23 (± 0.31)
9	10.43 (± 0.25)	10.53 (± 0.21)	10.37 (± 0.50)	10.43 (± 0.29)

HCP : High Crude Protein (diet containing 62% crude protein)
MHCP : Medium High Crude Protein (diet containing 56.6% crude protein)
MLCP : Medium Low Crude Protein (diet containing 51.3% crude protein)
LCP : Low Crude Protein (diet containing 46% crude protein)

Table 4.5 Mean snout to vent length (cm) of crocodiles that received different levels of dietary protein over a period of 9 weeks (± standard deviation)

Measurement weeks	Protein Treatment Levels			
	HCP	MHCP	MLCP	LCP
0	35.07 (± 1.46)	36.53 (± 1.08)	35.70 (± 1.80)	37.10 (± 1.13)
3	36.83 (± 1.32)	38.03 (± 1.06)	37.00 (± 1.60)	38.33 (± 1.08)
6	38.80 (± 1.30)	39.73 (± 1.17)	39.07 (± 1.86)	39.67 (± 1.37)
9	40.23 (± 1.14)	41.03 (± 0.73)	40.07 (± 2.14)	40.27 (± 1.18)

HCP : High Crude Protein (diet containing 62% crude protein)
MHCP : Medium High Crude Protein (diet containing 56.6% crude protein)
MLCP : Medium Low Crude Protein (diet containing 51.3% crude protein)
LCP : Low Crude Protein (diet containing 46% crude protein)

Table 4.6 Mean body length differences (cm) of crocodiles that received different levels of dietary protein over a period of 9 weeks (± standard deviation)

Measurement weeks	Protein Treatment Levels			
	HCP	MHCP	MLCP	LCP
3	3.27 (± 0.84)^{a}	2.50 (± 0.46)^{ab}	2.50 (± 0.20)^{ab}	2.03 (± 0.25)^{b}
6	2.90 (± 0.66)^{a}	2.30 (± 0.53)^{a}	2.93 (± 0.31)^{a}	1.37 (± 0.55)^{b}
9	2.27 (± 1.01)^{a}	1.80 (± 1.25)^{ab}	1.43 (± 0.68)^{ab}	0.43 (± 0.06)^{b}

^{ab} Row means with the same superscript do not differ significantly (P<0.05)
HCP : High Crude Protein (diet containing 62% crude protein)
MHCP : Medium High Crude Protein (diet containing 56.6% crude protein)
MLCP : Medium Low Crude Protein (diet containing 51.3% crude protein)
LCP : Low Crude Protein (diet containing 46% crude protein)

Table 4.7 Mean head length differences (cm) of crocodiles that received different levels of dietary protein over a period of 9 weeks (± standard deviation)

Measurement weeks	Protein Treatment Levels			
	HCP	MHCP	MLCP	LCP
3	0.50 (± 0.10)^{a}	0.47 (± 0.06)^{ab}	0.47 (± 0.06)^{ab}	0.37 (± 0.06)^{b}
6	0.43 (± 0.06)	0.33 (± 0.06)	0.43 (± 0.06)	0.33 (± 0.06)
9	0.47 (± 0.06)^{a}	0.33 (± 0.06)^{ab}	0.33 (± 0.06)^{ab}	0.20 (± 0.10)^{b}

^{ab} Row means with the same superscript do not differ significantly (P<0.05)
HCP : High Crude Protein (diet containing 62% crude protein)
MHCP : Medium High Crude Protein (diet containing 56.6% crude protein)
MLCP : Medium Low Crude Protein (diet containing 51.3% crude protein)
LCP : Low Crude Protein (diet containing 46% crude protein)

Table 4.8 Mean snout-vent length differences (cm) of crocodiles that received different levels of dietary protein over a period of 9 weeks (± standard deviation)

Measurement weeks	Protein Treatment Levels			
	HCP	MHCP	MLCP	LCP
3	1.77 (± 0.32)[a]	1.50 (± 0.26)[ab]	1.37 (± 0.21)[ab]	1.23 (± 0.12)[b]
6	1.97 (± 0.23)[a]	1.70 (± 0.26)[abc]	2.00 (± 0.26)[ab]	1.37 (± 0.47)[c]
9	1.43 (± 0.35)	1.30 (± 0.50)	1.03 (± 0.42)	0.60 (± 0.20)

[ab] Row means with the same superscript do not differ significantly (P<0.05)
HCP : High Crude Protein (diet containing 62% crude protein)
MHCP : Medium High Crude Protein (diet containing 56.6% crude protein)
MLCP : Medium Low Crude Protein (diet containing 51.3% crude protein)
LCP : Low Crude Protein (diet containing 46% crude protein)

Table 4.9 Mean FCR (body mass gained / feed intake) of crocodiles that received different levels of dietary protein over a period of 9 weeks (± standard deviation) on a DM basis

Measurement weeks	Protein Treatment Levels			
	HCP	MHCP	MLCP	LCP
3	2.72 (± 0.93)[a]	4.83 (± 0.69)[ab]	3.71 (± 1.76)[ab]	6.34 (± 1.77)[b]
6	3.67 (± 1.22)	3.97 (± 1.35)	5.01 (± 3.07)	4.97 (± 51.09)
9	2.23 (± 1.08)	5.27 (± 3.07)	13.42 (± 7.73)	-1.88 (± 16.87)

[ab] Row means with the same superscript do not differ significantly (P<0.05)
HCP : High Crude Protein (diet containing 62% crude protein)
MHCP : Medium High Crude Protein (diet containing 56.6% crude protein)
MLCP : Medium Low Crude Protein (diet containing 51.3% crude protein)
LCP : Low Crude Protein (diet containing 46% crude protein)

Table 4.10 Mean feed intake (grams) of crocodiles that received different treatment levels of dietary protein, over a period of 9 weeks (± standard deviation) on a DM basis

Measurement weeks	Protein Treatment Levels			
	HCP	MHCP	MLCP	LCP
3	371 (± 65)	377 (± 55)	346 (± 87)	374 (± 28)
6	386 (± 66)	403 (± 70)	367 (± 103)	358 (± 29)
9	407 (± 26)	401 (± 31)	379 (± 52)	375 (± 21)

HCP : High Crude Protein (diet containing 62% crude protein)
MHCP : Medium High Crude Protein (diet containing 56.6% crude protein)
MLCP : Medium Low Crude Protein (diet containing 51.3% crude protein)
LCP : Low Crude Protein (diet containing 46% crude protein)

Table 4.11 Mean mass change (grams) of crocodiles that received different levels of dietary protein over a period of 9 weeks (± standard deviation)

Measurement weeks	Protein Treatment Levels			
	HCP	MHCP	MLCP	LCP
3	142 (± 26)[a]	80 (± 23)[b]	102 (± 28)[ab]	62 (± 15)[b]
6	111 (± 25)[a]	106 (± 22)[a]	99 (± 65)[a]	22 (± 41)[b]
9	205 (± 72)[a]	107 (± 77)[ab]	39 (± 28)[bc]	-31 (± 62)[c]

[abc] Row means with the same superscript do not differ significantly (P<0.05)
HCP : High Crude Protein (diet containing 62% crude protein)
MHCP : Medium High Crude Protein (diet containing 56.6% crude protein)
MLCP : Medium Low Crude Protein (diet containing 51.3% crude protein)
LCP : Low Crude Protein (diet containing 46% crude protein)

Table 4.12 Mean body mass (grams) of crocodiles that received different treatment levels of dietary protein over a period of 12 weeks (± standard deviation)

Measurement weeks	Protein Treatment Levels		
	HCP	MHCP	MLCP
0	1214 (± 170)	1390 (± 116)	1308 (± 233)
3	1355 (± 170)	1470 (± 125)	1410 (± 229)
6	1466 (± 146)	1577 (± 103)	1509 (± 291)
9	1671 (± 172)	1684 (± 028)	1548 (± 269)
12	1931 (± 198)	1922 (± 016)	1699 (± 302)

HCP : High Crude Protein (diet containing 62% crude protein)
MHCP : Medium High Crude Protein (diet containing 56.6% crude protein)
MLCP : Medium Low Crude Protein (diet containing 51.3% crude protein)

Table 4.13 Mean body length (cm) of crocodiles that received different levels of dietary protein over a period of 12 weeks (± standard deviation)

Measurement weeks	Protein Treatment Levels		
	HCP	MHCP	MLCP
0	70.97 (± 2.78)	74.30 (± 2.21)	72.80 (± 3.45)
3	74.20 (± 2.71)	76.80 (± 1.76)	75.27 (± 3.65)
6	77.07 (± 2.05)	79.10 (± 2.08)	78.20 (± 3.55)
9	79.43 (± 2.23)	80.97 (± 1.05)	79.67 (± 4.10)
12	82.57 (± 2.20)	83.97 (± 0.59)	81.80 (± 3.81)

HCP : High Crude Protein (diet containing 62% crude protein)
MHCP : Medium High Crude Protein (diet containing 56.6% crude protein)
MLCP : Medium Low Crude Protein (diet containing 51.3% crude protein)

Table 4.14 Mean head length (cm) of crocodiles that received different levels of dietary protein over a period of 12 weeks (± standard deviation)

Measurement weeks	Protein Treatment Levels		
	HCP	MHCP	MLCP
0	9.07 (± 0.32)	9.37 (± 0.32)	9.17 (± 0.35)
3	9.57 (± 0.32)	9.83 (± 0.29)	9.60 (± 0.40)
6	9.97 (± 0.23)	10.17 (± 0.32)	10.00 (± 0.46)
9	10.43 (± 0.25)	10.53 (± 0.21)	10.37 (± 0.50)
12	10.93 (± 025)	10.97 (± 0.15)	10.70 (± 0.53)

HCP : High Crude Protein (diet containing 62% crude protein)
MHCP : Medium High Crude Protein (diet containing 56.6% crude protein)
MLCP : Medium Low Crude Protein (diet containing 51.3% crude protein)

Table 4.15 Mean snout to vent length (cm) of crocodiles that received different levels of dietary protein over a period of 12 weeks (± standard deviation)

Measurement weeks	Protein Treatment Levels		
	HCP	MHCP	MLCP
0	35.07 (± 1.46)	36.53 (± 1.08)	35.70 (± 1.80)
3	36.83 (± 1.32)	38.03 (± 1.06)	37.00 (± 1.60)
6	38.80 (± 1.30)	39.73 (± 1.17)	39.07 (± 1.86)
9	40.23 (± 1.14)	41.03 (± 0.72)	40.07 (± 2.13)
12	41.50 (± 1.04)	42.10 (± 0.36)	40.67 (± 2.01)

HCP : High Crude Protein (diet containing 62% crude protein)
MHCP : Medium High Crude Protein (diet containing 56.6% crude protein)
MLCP : Medium Low Crude Protein (diet containing 51.3% crude protein)

Table 4.16 Mean body length difference (cm) of crocodiles that received different levels of dietary protein over a period of 12 weeks (± standard deviation)

Measurement weeks	Protein Treatment Levels		
	HCP	MHCP	MLCP
3	3.37 (± 0.84)	2.50 (± 0.46)	2.50 (± 0.20)
6	2.90 (± 0.66)	2.30 (± 0.53)	2.93 (± 0.31)
9	2.27 (± 1.01)	1.80 (± 1.25)	1.43 (± 0.68)
12	3.17 (± 0.31)	3.03 (± 0.57)	2.13 (± 0.61)

HCP : High Crude Protein (diet containing 62% crude protein)
MHCP : Medium High Crude Protein (diet containing 56.6% crude protein)
MLCP : Medium Low Crude Protein (diet containing 51.3% crude protein)

Table 4.17 Mean head length difference (cm) of crocodiles that received different levels of dietary protein over a period of 12 weeks (± standard deviation)

Measurement weeks	Protein Treatment Levels		
	HCP	MHCP	MLCP
3	0.50 (± 0.10)	0.47 (± 0.06)	0.47 (± 0.06)
6	0.43 (± 0.06)	0.33 (± 0.06)	0.43 (± 0.06)
9	0.47 (± 0.06)[a]	0.33 (± 0.06)[b]	0.33 (± 0.06)[b]
12	0.50 (± 0.00)	0.47 (± 0.12)	0.37 (± 0.06)

[ab] Row means with the same superscript do not differ significantly (P<0.05)
HCP : High Crude Protein (diet containing 62% crude protein)
MHCP : Medium High Crude Protein (diet containing 56.6% crude protein)
MLCP : Medium Low Crude Protein (diet containing 51.3% crude protein)

Table 4.18 Mean snout-vent length difference (cm) of crocodiles that received different levels of dietary protein, over a period of 12 weeks (± standard deviation)

Measurement weeks	Protein Treatment Levels		
	HCP	MHCP	MLCP
3	1.77 (± 0.32)	1.50 (± 0.26)	1.37 (± 0.21)
6	1.97 (± 0.23)	1.70 (± 0.26)	2.00 (± 0.26)
9	1.43 (± 0.35)	1.30 (± 0.50)	1.03 (± 0.42)
12	1.23 (± 0.15)[a]	1.03 (± 0.35)[ab]	0.57 (± 0.20)[b]

[ab] Row means with the same superscript do not differ significantly (P<0.05)
HCP : High Crude Protein (diet containing 62% crude protein)
MHCP : Medium High Crude Protein (diet containing 56.6% crude protein)
MLCP : Medium Low Crude Protein (diet containing 51.3% crude protein)

Table 4.19 Mean FCR (body mass gained/ feed intake) of crocodiles that received different levels of dietary protein over a period of 12 weeks (± standard deviation) on a DM basis

Measurement weeks	Protein Treatment Levels		
	HCP	MHCP	MLCP
3	2.72 (± 0.93)	4.82 (± 0.70)	3.70 (± 1.77)
6	3.66 (± 1.21)	3.97 (± 1.35)	5.01 (± 3.07)
9	2.23 (± 1.08)[a]	5.26 (± 3.37)[ab]	13.41 (± 7.72)[b]
12	2.69 (± 0.31)	2.76 (± 0.61)	4.12 (± 1.28)

[ab] Row means with the same superscript do not differ significantly (P<0.05)
HCP : High Crude Protein (diet containing 62% crude protein)
MHCP : Medium High Crude Protein (diet containing 56.6% crude protein)
MLCP : Medium Low Crude Protein (diet containing 51.3% crude protein)

Table 4.20 Mean feed intake (grams) of crocodiles that received different levels of dietary protein over a period of 12 weeks (± standard deviation) on a DM basis

Measurement weeks	Protein Treatment Levels		
	HCP	MHCP	MLCP
3	371 (± 65)	377 (± 55)	346 (± 87)
6	386 (± 66)	403 (± 70)	367 (± 103)
9	407 (± 26)	401 (± 31)	379 (± 52)
12	695 (± 28)	643 (± 49)	593 (± 73)

HCP : High Crude Protein (diet containing 62% crude protein)
MHCP : Medium High Crude Protein (diet containing 56.6% crude protein)
MLCP : Medium Low Crude Protein (diet containing 51.3% crude protein)

Table 4.21 Mean mass change (kg) of crocodiles that received different levels of dietary protein over a period of 12 weeks (± standard deviation)

Measurement weeks	Protein Treatment Levels		
	HCP	MHCP	MLCP
3	142 (± 26)^{a}	80 (± 23)^{b}	102 (± 28)^{a}
6	111 (± 25)	106 (± 22)	99 (± 65)
9	205 (± 72)^{a}	107 (± 77)^{ab}	39 (± 28)^{b}
12	260 (± 25)^{a}	238 (± 38)^{a}	152 (± 42)^{b}

^{ab} Row means with the same superscript do not differ significantly (P<0.05)
HCP : High Crude Protein (diet containing 62% crude protein)
MHCP : Medium High Crude Protein (diet containing 56.6% crude protein)
MLCP : Medium Low Crude Protein (diet containing 51.3% crude protein)

4.2. DATA ANALYSED OVER TRIAL PERIOD

In the following section, data was analysed over the whole trial period rather than weekly repetition as in the previous section. Analysis of data was compared over a 9 week and a 12 week period. The low protein diet was discontinued after week 9 due to severe negative growth performance.

4.2.1) Measurement parameters taken over 9 weeks

As shown in Table 4.22, no significant difference was found between treatments levels for age at the start of the trial (Age – start), mass at the start of the trial (Mass – start), age at the end of the trial (Age – final) and mass at the end of the trial (Mass – final).

At the end of 9 weeks crocodiles that received the HCP diet gained significantly more mass than the crocodiles receiving the three lower protein levels (Mass – difference : the starting mass subtracted from the final mass). However, the crocodiles that received the MHCP and the MLCP diets gained significantly more mass than the crocodiles that received the LCP diets.

Crocodiles on the three highest protein treatments had a significantly lower FCR than crocodiles on the lowest protein treatment diet.

No significant difference was found between treatment levels for body length at the start (Body Length – start) or body length at the end (Body Length – final) of the trial.

Crocodiles that received the three highest protein levels gained significantly more body length than crocodiles that were fed the lowest protein levels.

No significant difference was found between treatment levels for head length at the start (Head length – start) or head length at the end (Head length – final) of the trial.

Crocodiles that received the three highest protein treatment levels gained significantly more head length compared to crocodiles that were fed the lowest protein level. However, the crocodiles that were fed the MHCP diet gained significantly less in head length compared to crocodiles that received the HCP diets.

No significant difference was found between treatment levels for snout to vent length at the start (Snout to vent length – start) or the snout to vent length at the end (Snout to vent length-Final) of the trial.

All crocodiles that received the three highest protein levels gained significantly more in snout to vent length than crocodiles that were fed the lowest protein level.

4.2.2) Measurements parameters taken over 12 weeks

As shown in Table 4.23, no significant difference was found between treatment levels for all the parameters except mass difference where crocodiles that were fed the HCP diets gained significantly more mass than crocodiles that were fed the MLCP diets.

Table 4.22 Mean values of crocodiles that received different levels of dietary protein over a period of 9 weeks (± standard deviation)

	Protein Treatment Levels			
Parameters	HCP	MHCP	MLCP	LCP
Age – start [1] (days)	141.00 (± 8.18)	146.33 (± 12.34)	140.33 (± 9.07)	148.33 (± 6.43)
Age – final [2] (days)	204.00 (± 8.19)	209.33 (± 12.34)	203.33 (± 9.07)	211.33 (± 6.43)
Mass - start (grams)	1214 (± 170)	1390 (± 116)	1308 (± 233)	1391 (± 134)
Mass - final (grams)	1671 (± 172)	1684 (± 28)	1548 (± 269)	1444 (± 100)
Mass difference[3] (grams)	458 (± 104)[a]	294 (± 88)[b]	240 (± 44)[bc]	53 (± 35)[d]
DM Feed intake (grams)	1164 (± 146)	1181 (± 154)	1092 (± 241)	1107 (± 54)
FCR (DM corrected)	2.70 (± 1.04)[a]	4.35 (± 1.66)[a]	4.61 (± 1.19)[a]	26.47 (± 12.35)[b]
Body length - start (cm)	70.97 (± 2.78)	74.30 (± 2.21)	72.80 (± 3.45)	75.23 (± 2.45)
Body length - final (cm)	79.43 (± 2.23)	80.97 (± 1.05)	79.67 (± 4.10)	79.07 (± 2.42)
Body length difference (cm)	8.47 (± 2.05)[a]	6.60 (± 1.21)[a]	6.87 (± 0.96)[a]	3.83 (± 0.35)[b]
Head - start (cm)	9.07 (± 0.32)	9.37 (± 0.32)	9.17 (± 0.35)	9.57 (± 0.23)
Head - final (cm)	10.43 (± 0.25)	10.53 (± 0.21)	10.37 (± 0.50)	10.43 (± 0.29)
Head difference (cm)	1.37 (± 0.15)[a]	1.17 (± 0.15)[b]	1.20 (± 0.17)[ab]	0.87 (± 0.06)[c]
Snout to Vent - start (cm)	35.07 (± 1.46)	36.53 (± 1.08)	35.70 (± 1.80)	37.03 (± 1.08)
Snout to Vent - final (cm)	40.23 (± 1.14)	41.03 (± 0.72)	40.07 (± 2.13)	40.27 (± 1.18)
Snout to Vent – difference (cm)	5.17 (± 0.87)[a]	4.53 (± 0.35)[a]	4.37 (± 0.50)[a]	3.23 (± 0.23)[b]

[abcd] Row means with the same superscript do not differ significantly (P<0.05)
[1] start: measurement taken at the start of the trial
[2] final: measurement taken at the end of 9 weeks
[3] difference: The difference in measurement between the final and the start measurement value
HCP : High Crude Protein (diet containing 62% crude protein)
MHCP : Medium High Crude Protein (diet containing 56.6% crude protein)
MLCP : Medium Low Crude Protein (diet containing 51.3% crude protein)
LCP : Low Crude Protein (diet containing 46% crude protein)

Table 4.23 Mean measurement parameters of crocodiles that received different levels of dietary protein over a period of 12 weeks (± standard deviation)

Parameters	Protein Treatment Levels		
	HCP	MHCP	MLCP
Age – start [1] (days)	141 (± 8.19)	146 (± 12.34)	140 (± 9.07)
Age – final [2] (days)	225 (± 8.19)	230 (± 12.34)	224 (± 9.07)
Mass – start (grams)	1214 (± 171)	1390 (± 116)	1308 (± 234)
Mass - final (grams)	1931 (± 198)	1922 (± 16)	1699 (± 302)
Mass difference [3] (grams)	717 (± 116)[a]	533 (± 126)[ab]	391 (± 87)[b]
DM feed intake (grams)	1859 (± 168)	1824 (± 189)	1686 (± 314)
FCR (DM corrected)	2.67 (± 0.70)	3.60 (± 1.12)	4.42 (± 1.21)
Length - start (cm)	70.97 (± 2.78)	74.30 (± 2.21)	72.80 (± 3.45)
Length - final (cm)	82.57 (± 2.20)	83.97 (± 0.59)	81.80 (± 3.82)
Length difference (cm)	11.63 (± 2.34)	9.67 (± 1.63)	9.03 (± 1.17)
Head - start (cm)	9.07 (± 0.32)	9.37 (± 0.32)	9.17 (± 0.35)
Head - final (cm)	10.93 (± 0.25)	10.97 (± 0.15)	10.70 (± 0.53)
Head difference (cm)	1.87 (± 0.23)	1.60 (± 0.20)	1.57 (± 0.21)
Snout to Vent - start (cm)	35.07 (± 1.46)	36.53 (± 1.08)	35.70 (± 1.80)
Snout to Vent - final (cm)	41.50 (± 1.04)	42.10 (± 0.36)	40.67 (± 2.01)
Snout to Vent - difference (cm)	6.43 (± 1.06)	5.57 (± 0.72)	4.97 (± 0.61)

[ab] Row means with the same superscript do not differ significantly at P <0.05.
[1] start: measurement taken at the start of the trial
[2] final: measurement taken at the end of 12 weeks
[3] difference: The difference in measurement between the final and the start measurement value
HCP : High Crude Protein (diet containing 62% crude protein)
MHCP : Medium High Crude Protein (diet containing 56.6% crude protein)
MLCP : Medium Low Crude Protein (diet containing 51.3% crude protein)

4.3 DIGESTIBILITY

The digestibility coefficients for protein (CP), energy and dry matter were determined at week 3 and 9 of the trial. Results are shown in Table 4.24.

Crude protein within the HCP and the MHCP diets was significantly more digestible than that within the lower protein diets

Gross energy within the HCP diet was significantly more digestible at week 3 than that within the MLCP and the LCP diets.

Dry matter digestibility was significantly higher for the highest protein level diet at week 3 than for the MLCP diet. No significant difference was found for dry matter digestibility at week 9.

Table 4.24 Digestibility coefficients for crude protein, energy and dry matter of crocodile diets containing different levels of dietary protein (± standard deviation) at week 3 and week 9 of the trial

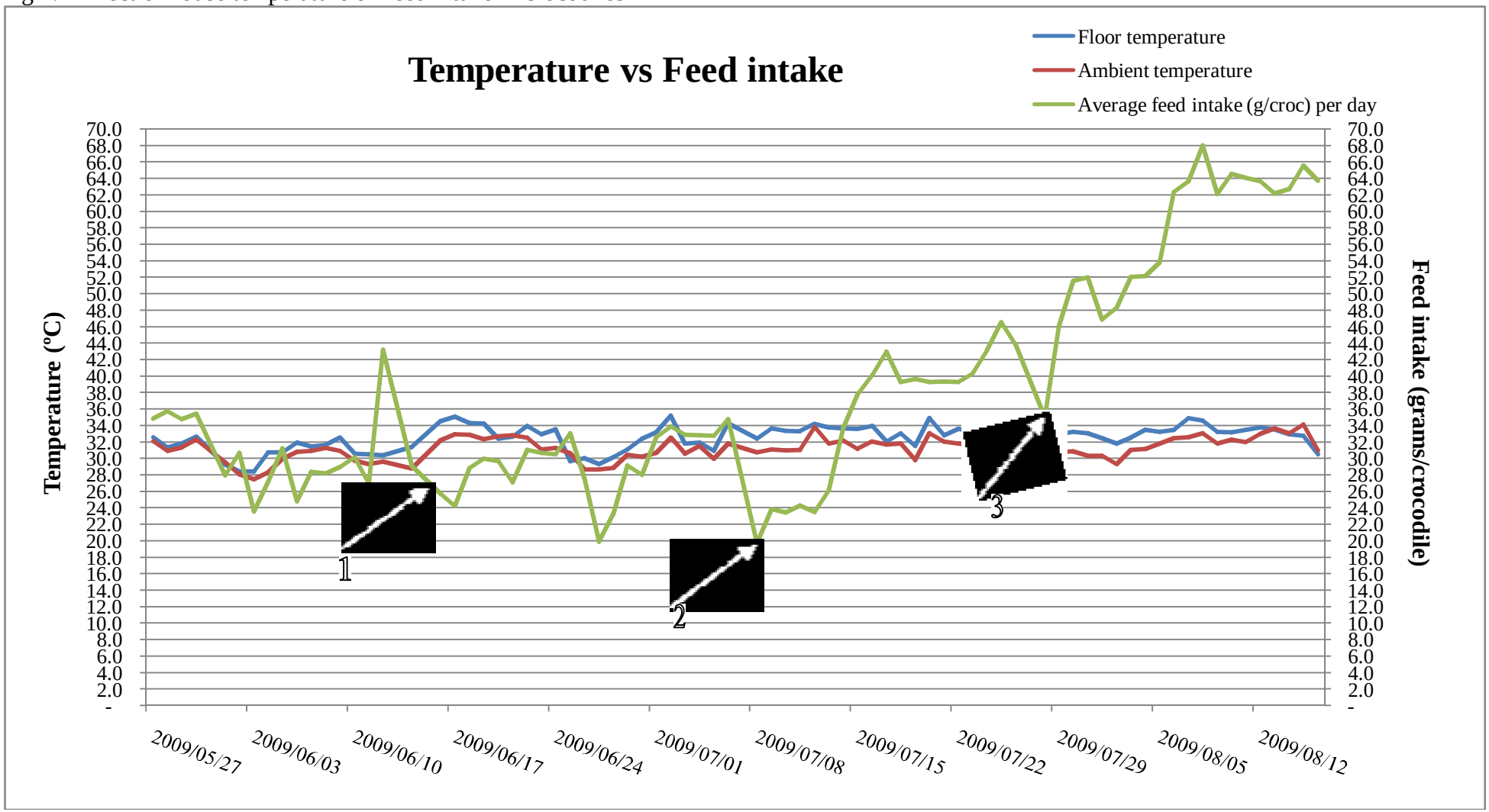
	Protein Treatment Level			
Parameter	HCP	MHCP	MLCP	LCP
Crude Protein – week 3	0.790 (± 0.020)^{a}	0.663 (± 0.045)^{ab}	0.510 (± 0.095)^{bc}	0.460 (± 0.122)^{c}
Crude Protein – week 9	0.723 (± 0.059)^{a}	0.720 (± 0.070)^{ab}	0.630 (± 0.044)^{bc}	0.613 (± 0.021)^{c}
Energy – week 3	0.760 (± 0.017)^{a}	0.630 (± 0.085)^{abc}	0.440 (± 0.155)^{bc}	0.427 (± 0.136)^{c}
Energy – week 9	0.643 (± 0.084)	0.653 (± 0.107)	0.567 (± 0.051)	0.550 (± 0.026)
Dry matter – week 3	0.577 (± 0.021)^{a}	0.457 (± 0.075)^{ab}	0.253 (± 0.215)^{b}	0.380 (± 0.147)^{ab}
Dry matter – week 9	0.407 (± 0.133)	0.520 (± 0.122)	0.463 (± 0.057)	0.500 (± 0.020)

^{abc} Row means with the same superscript do not differ significantly ($P<0.05$)
HCP : High Crude Protein (diet containing 62% crude protein)
MHCP : Medium High Crude Protein (diet containing 56.6% crude protein)
MLCP : Medium Low Crude Protein (diet containing 51.3% crude protein)
LCP : Low Crude Protein (diet containing 46% crude protein)

4.4 TEMPERATURE VERSUS FEED INTAKE

Graph 4.1 shows the relationship between ambient and floor temperature and feed intake. Feed intake was negatively affected when the temperature was 2ºC or more below or 5ºC or more above the comfort temperature of 32ºC. Ambient and floor temperatures were monitored twice a day during the trial.

Fig 4.1 Effect of house temperature on feed intake in crocodiles



* The arrows on the graph represent the day that measurements took place, where feed intake was not affected by temperature but rather the stress of measurement. The arrows from left to right indicate; Week 3, Week 6 and Week 9, respectively. The feed intake is the average per pen, and the floor and ambient temperatures are the daily averages taken over the trial period.

CHAPTER 5. DISCUSSION

It is widely believed that the protein requirements for *Crocodylus niloticus* are similar to *Alligator mississippiensis* as they belong to the same order of reptiles. However, these species exhibit differences in behaviour within their natural environment which influences the farming methods used. For example, *C. niloticus* are farmed using a 2:1 land to water ratio, where *A. mississippiensis* would be farmed using a 2:1 water to land ratio (Staton *et al.*, 1990*)*.

Coulson *et al.* (1987) demonstrated that vegetable protein in the diet of the *A. mississippiensis* was only partially digested and the uptake of the digested protein was slower than protein of animal origin. However, Staton & Edwards (1987) disproved this hypothesis by feeding *A. mississippiensis* diets of which the total dietary protein constituted of 40% protein from vegetable origin, without negatively affecting growth.

In this study, the HCP diet had a digestibility of 75.65%, giving a digestible protein (DP) value of 468.65 g/kg. This value is in the range of the DP requirements of *A. mississippiensis* proposed by Staton *et al.* (1990) of 450 g/kg. The high digestibility of the 62% CP diet may be due to the inclusion of a high content of chicken mince and lower amounts of vegetable protein. As the CP levels dropped from one treatment diet to the next, the DP value decreased. It was determined that the DP content of the MHCP, the MLCP and the LCP diets were 381.32 g/kg, 294.80 g/kg and 246.44 g/kg and the DCprotein were 69.15%, 57.00% and 53.50% respectively. The reduction of protein digestibility from one diet to another coincided with the reduction in the amount of raw minced chicken. No minced chicken was present in the LCP diet but it did contain 35% fish meal and 4.35% carcass meal which served as the only two sources of animal protein. This diet also contained 9.8% full fat soya and 30 % soya oilcake meal (46%). The high content of vegetable protein and low amount of raw animal protein in this diet could have lead to a lower protein digestibility value than a diet that is made up majorly of raw animal protein. This suggests that *C. niloticus* are less effective in utilising protein from vegetable origin than from animal origin. This was also noted by Coulson *et al.* (1987) in *A. mississippiensis*.

It was observed that crocodiles on the LCP diet had a higher amount of feed wastage compared to crocodiles on the other treatment diets. This may be due to a number of factors.

Due to the lack of raw animal protein source, the LCP diet did not elicit the same intensity of feeding response than observed by the crocodiles that received the other treatment diets. This lack of response may be due to the diet's low palatability and pellet integrity. The LCP diet pellets were firm when initially produced, but once they were placed onto the feed trays, the crocodile's habit to lie on top of the pellets allowed the pellets to be squashed. This prevented the crocodiles from ingesting their feed and resulted in large feed wastage and reduced body growth rate. Of the four treatment diets, crocodiles on the LCP diet had significantly lower body mass changes than the other treatment diets. Over 9 weeks crocodiles on the 46% CP diet gained 53 g compared to crocodiles on the 62% CP diet that had a 458 g gain over the same period of time.

The FCR for the LCP diet had very large variations between measurement weeks. The negative FCR, of the LCP diet, can possibly be explained by the large amount of feed wastage, due to the poor pellet integrity. Other factors contributing to the poor FCR could also have been due to the low palatability of the pellet, therefore leading to low feed intake and poor digestibility of the feed. The negative FCR did not only affect protein digestibility, but energy and DM digestibility. This was shown to be true by the poor performance experienced by crocodiles on the LCP diet.

Over the three weekly repetitive measurement periods, crocodiles on the LCP diet were shown to have poorer performance parameter values in all measurement weeks. This included the mean mass change parameter as well as the body length, head length and the snout – vent length differences parameters. These crocodiles had a substantial amount of growth at the second measurement interval (week 3). However, this may have been due to either the crocodiles using their body reserves (like the fat cell body – a fat reserve organ) or the breakdown of tissue (muscle tissues for example) to fuel length growth. This is evident by examining the results at each week. The crocodiles on the LCP diet were shown to first gain and then loose mass over the 9 week period and were shown to have a reduced rate of length growth throughout the 3 length parameters.

The DP value of the LCP diet was 246.44 g/kg. This diet contained a high content of vegetable protein, originating from soya bean oilcake and full fat soya bean meal. Protein from animal origin was also present (in the form of fish meal and carcass meal) but the vegetable protein portion was slightly larger (refer to Table 2.1). Although a starch binder

was added to this diet to aid the formation of the pellets, the integrity of the pellets did not last long once placed onto the feed trays. It was observed that there was a lack of intensive feeding response with these pellets, which lead us to believe that the palatability of these pellets was poor. It was suggested by Davis (2001) that hatchling crocodiles are genetically programmed to recognise certain items like smell, tastes and movements in the environment representing their food. The lack of a meaty smell would therefore reduce the allure for crocodiles intending to ingest the feed. Trials conducted by Peucker *et al.* (2005) tested to see whether different attractants and flavourants (like chicken head digest, beef liver digest, kangaroo meat etc) would influence feeding response of saltwater crocodile hatchlings (*C. porosus*). They tested the effects of adding fresh sheep blood (as an attractant) in the saltwater crocodile's pelleted diet. By using a control of 100% meat, the effect of the fresh blood (as part of the crocodile's pellets) caused minimal differences in feed intake. As seen with the study by Peucker *et al.* (2005), meat was compared to sheep blood, so the lack of a raw protein source would lead to a lowered feeding response of crocodiles, resulting in a large wastage of feed and poor growth performance. Coulson *et al.* (1987) demonstrated that American alligators (*Alligator mississippiensis*) were unable to utilise vegetable protein. They fed the alligators isolated soya bean protein and corn gluten meal, and found that these two protein sources were partially digested and assimilation was slower through the gastrointestinal tract. This was shown to be true for the crocodiles on the LCP diet regarding their poor performance with all measurement parameters. This would suggest that the small amount of protein absorbed was not sufficient for maximum body growth and that the protein available for muscle and tissues were used to fuel small amounts of skeletal growth. This further proves the fact that this protein level is not recommended for optimal growth in juvenile Nile crocodiles.

The MLCP diet had a DP content of 294.80 g/kg, which was slightly better than the DP of the LCP diet, resulting in slightly better growth performances. By first investigating the three weekly repetitive measurements and measurement over 9 weeks, crocodiles on the MLCP diet had a tendency for lower feed intake and higher FCR compared to crocodiles on the MHCP and HCP diets. Therefore the crocodiles on the MLCP diet weighed significantly less than crocodiles on the two higher CP treatment diet. The MLCP diet consisted of 2/3rd LCP diet and 1/3rd HCP diet. The high inclusion of vegetable protein and the lowered palatability of the LCP diet were carried over to the MLCP diet. Pellet integrity was also affected (due to low amounts of chicken mince) which lead to higher feed wastage than the two higher protein

diets. Crocodiles on the MLCP diet sustained minimal growth. The mass changes for these crocodile increased to a smaller degree than the crocodiles on the higher protein diets. The length difference categories of all measurement periods showed that there were no real significant differences between the three higher protein diets, however there were tendencies for the length differences of crocodiles on the MLCP diet to be lower than the two higher protein diets. It was interesting to note that the head length differences of these crocodiles were found to be significantly lower than crocodiles on the HCP diet over the 9 week period. The same tendency for lowered length growth was also observed over the 12 week period for crocodiles on the MLCP treatment diet. The performance of the crocodiles on the MLCP diet further substantiates Coulson *et al* (1987) finding, of poor performance found in alligators fed diets consisting mainly of vegetable protein.

The DP value of the MHCP diet was shown to be 381.32 g/kg, which is substantially higher than the DP values of the two lower protein diets. This would suggest that the growth performance of crocodiles on the MHCP diet would be greater than crocodiles on the two lower protein diets. By analysing the results, crocodiles on the MHCP diet weighed significantly more than crocodiles on the two lower protein diets. Crocodiles on the MHCP diets were observed having higher feed intake than crocodiles on the LP and MLCP diets. This also confirms Peucker *et al.* (2005) trials where sheep's blood was added to the pelletized crocodile diet. The MHCP diet consisted of 67% of HCP diet. The higher amount of raw chicken mince in the MHCP diet allowed for the increased feed intake compared to crocodiles on the lower protein diets. Over the 9 week period crocodiles on the MHCP diet performed significantly better than crocodiles on the lower protein diets. This was extensively seen when comparing the mass and the head length difference parameters. However, the mass difference performance of the crocodiles on the MHCP diet showed no significant difference to crocodiles on the HCP diet over the 12 week period. Similar results are seen for the length criteria for crocodiles on the MHCP diet when compared to crocodiles on the MLCP diets. The repetitive measurements over the 12 week period showed no significant differences between the three highest protein levels. There was a tendency for crocodiles on the MHCP diet to be longer than crocodiles on the two lower protein diets in all the length difference parameters. Performance of crocodiles on the MHCP diet was shown to be better than crocodile on the two lower protein diets, but not as optimal as crocodiles on the HCP diet. The FCR for MHCP diet showed a tendency to be greater than the 60% CP diet. Staton *et al* (1990) stated that a maximum response in performance criteria would occur when

the diet contained a DP of value 425-487 g/kg. A DP of 381.32 g/kg would be sufficient for growth and was shown to be the lowest DP range for optimal growth for Nile crocodile (*Crocodylus niloticus)*.

Crocodiles that were fed the HCP diet, outperformed all the other crocodiles on lower CP diets. This diet had a DP value of 468.65 g/kg, which is substantially higher than the DP levels of the other treatment diets. This diet contained a higher quantity of raw meat (chicken mince), which elicited a more intensive feed response than crocodiles on the other diets. The smell and taste of the meat encouraged these crocodiles to consume most of their feed. Feed wastage from crocodiles consuming the HCP diet was rare indicating the high palatability of the diet. By comparing their feed intake, crocodiles on the HCP diet had a higher DM intake over the repetitive measurement of 9 weeks, resulting in lower FCR and a corresponding increase in body mass mass. These crocodiles also weighed significantly more than the crocodiles on the lower protein diets at every measurement. It was recorded that they weighed almost double that of crocodiles on the MHCP and MLCP diet over the 9 week measurement period. The improved feed intake together with the higher DP value and increased palatability of the feed allowed these crocodiles to gain 717 grams over the 12 week trial period. This can be compared to the 533g and the 391g gained by the crocodiles on the MHCP and the MLCP diets respectively.

Crocodiles on a HCP diet were seen to grow significantly more in body length over 9 weeks of repetitive measurements than crocodiles on the LCP diet, and also had a tendency to grow more than the MHCP and the MLCP diets respectively. Similar results were seen for head length difference and the snout to vent length differences over the same measurement periods. Crocodiles on the HCP diet were 1.87cm, 1.6cm and 4.64cm longer than the crocodiles on the MHCP, MLCP and the LCP diet respectively, over a 9 week period. Similar results were obtained for the head length and snout to vent length differences. Over the 12 week period a similar trend was noted between all the length measurements parameters, where crocodiles on the HCP diet grew more than the crocodiles on the two lower CP diets.

When investigating the DP requirements of carnivorous fish, some similarities are seen. It was reported by Pirozzi *et al.* (2008) that the juvenile mulloway (*Argyrosomus japonicus*) performed optimally when the diet contained a DP value of 444 – 491 g/kg, which depended on the DE content of the diet and the size of the mulloway. The DP value reported by Wee

and Tacon (1982) for the juvenile snakehead (*Channa micropeltes*) was similar to that found by Pirozzi *et al.* (2008). Wee and Tacon (1982) estimated that a diet containing a CP content of 52%, when herring meal was used as the protein source, would result in the optimal growth of the snakehead. It was also found by Wee and Tacon (1982) that the dietary protein had a digestibility coefficient of 0.91, this would result in the diet having a DP value of 47.32%, which is in the same range as that found by Pirozzi *et al.* (2008) in the mulloway.

Durazo *et al.* (2008) discovered that the DP level for optimal growth of the white sea bass (*Atractoscion nobilis*) was between 541 and 491 g/kg, however using a broken line regression analysis, Durazo *et al.* (2008) reported that the maximum mass gain would be obtained when the diet contained a DP level of 503 ± 23 g/kg. However Lee *et al.* (2001) reported that the DP requirement for optimal growth of the juvenile rockfish (*Sebastes schlegeli*) was 42%, which is lower than that of the white sea bass but more in line with the mulloway and snakehead. These figures compare well with the requirements of the Nile crocodile, as the best growth was seen when crocodiles received a diet containing 468.65 g/kg DP. The DP required for optimal growth in the American alligator was determined to be 450 g/kg (Staton *et al.*, 1990).

Throughout the trial period the protein level of each diet was to be the only variable tested. This was not the case. Each treatment diet was formulated to contain protein levels of 46%, 51.3%, 56.6% and 62% CP respectively. These diets were made up of various protein sources to reach these protein levels. The protein contained in the diets was made up from animal and plant protein, which added more variability as it was shown that crocodiles on the HCP diet performed substantially better than crocodiles on the LCP. The HCP diet owed most of its protein to minced raw chicken, fish meal and carcass meal, where the protein of the LCP was made up primarily from soya meal (full fat soya and soya oilcake) with some contributions from fish meal. The digestibility of these two diets were also shown to be vastly different as the HCP had a higher protein digestibility than the LCP diet. This shows that proteins from animal sources are more digestible for crocodiles than proteins that originate from plant sources.

It was also interesting to note that the HCP with its higher protein content also had the highest fat content. This was attributed to the raw chicken mince. It is known that fat increases the passage time of feed through the digestive tract, which increases the digestion

time of protein, fats and carbohydrates (Mateos *et al* 1982). This lead to greater digestibility values for nutrients. This is seen when comparing the HCP diet to the three lower protein diets.

Energy sources were also shown to be another variable within this trial. Carbohydrate ingredients (such as maize meal) did not play a major role in the HCP diet. The major energy source was from the lipid content of the minced chicken. The LCP diet on the other hand received its energy source from maize meal as well as from soya oilcake with contributions of energy from lipid from full fat soya. The energy yield of lipids from complete oxidation is about twice the energy yield from carbohydrates (McDonald *et al* 2002).

In determining body proportion relationship, the head lengths and snout to vent lengths were measured during the 12 week trial. These were taken not only to compare the growth response between the diets, but also to see whether there was a relationship between head length and total body length and snout to vent length and total body length. At the start of the trial (when crocodiles were 5 months of age) there was a 7.9:1 (cm) ratio and at the end of the trial (when crocodiles were 8 months of age) there was a 7.6:1 (cm) ratio between total body length and head length. However the relationship between total body length and snout-vent length remained a constant 2:1 ratio throughout the trial. This relationship is important when only one measurement can be taken, and an estimated body length needs to be determined.

A relationship between temperature and feed intake was observed and measured for the duration of the trial. The individual pen floor and the house ambient temperatures were monitored twice daily. This was practiced throughout the trial period, to ensure that a constant temperature was maintained within the grower house for optimal feed consumption and growth. Temperature problems were experienced during the initial 3 weeks of the trial. However, the feed intake was also negatively influenced by handling stress that occurred during the trial. It was shown that when the temperature fell below 30°C the feed intake decreased substantially, however when the temperature remained stable (around 32°C) the feed intake increased by an exponential degree. This was observed towards the end of the trial where the crocodiles became used to the handling stress and the temperatures remained constant. It was reported by Coulson *et al.* (1996) cited by Pina and Larriera (2002) that caimans maintained at a temperature lower than 25ºC refused to eat. Similar results were seen in the first 3 weeks of the study where the Nile crocodile hatchlings refused to eat, when they

experienced reduced temperatures. It was then shown in a study by Pina and Larriera (2002), that caimans spending more time at temperatures above 31ºC were able to increase the rate of digestion and process more food. These correlations between feed intake and temperature are very important in crocodilians production system, as shown by Pina and Larriera (2002).

CHAPTER 6. CONCLUSION

This study was aimed at determining the dietary protein requirements of the juvenile Nile crocodile (*Crocodylus niloticus*) between 5 and 6 months of age in an intensive farming situation. By feeding four different levels of protein, the performance of the crocodiles on these diets was closely examined. It was noted from early on in the trial that the LCP diet was negatively influencing growth. It was observed that crocodiles on this specific diet had reduced mass and the rates of length growth were minimal. This diet was therefore discontinued after the 9th week of measurement, due to the fact that these animals were part of the production stock, and any severe loss in growth would have lead to poor economic returns for the producer.

The LCP diet had a CP content of 46% and a DP content of 246.44 g/kg. The main source of protein of this diet was contributed by plant protein. This was shown to be the poorest diet, as crocodiles on the diet performed the worst out of the four treatment diets.

The MLCP diet had a CP content of 51.3% and a DP content of 294.80 g/kg. The main source of protein was of plant origin with small contributions from animal origin. The performance of crocodile on this diet was shown to be better than crocodiles on the LCP, but poorer than crocodiles on the two higher protein diets. This protein level could be suggestive of the protein maintenance requirements for the Nile crocodile.

The MHCP diet had a CP content of 56.6% and a DP content of 381.32 g/kg. The main source of protein was of animal origin with small contributions from plant origin. Crocodiles on this diet had higher performance values than crocodile on the two lower protein diets, however the growth performance of these crocodiles was not as significant as crocodiles on the HCP diet.

The HCP diet had a CP content of 62% and a DP content of 468.65 g/kg. This diet contained protein mainly from animal origin. Crocodiles on the HCP diet outperformed all the crocodile on the lower protein diets. The DP content of this diet was found to be in the same range as the DP value that was determined by Staton *et al.* (1990) for the American alligator. Staton *et al.* (1990) determined that a juvenile American alligator would perform the best if the diet contained a CP value of 51.9 %, and assuming a protein digestibility of 86.7%, the diet would

contain a digestible protein content of 45% (450 g/kg). Staton *et al.* (1990) also predicted that maximum growth performance would occur in a digestible protein range of 425 – 487 g/kg. Protein digestibility in the Nile crocodile was found to be lower than that found in the American alligator. The maximum protein digestibility coefficient was found to be 75.65% of the HCP diet, where those of the MHCP, MLCP and the LCP diets were 69.15%, 57.00% and 53.50% respectively. The protein digestibility coefficient tended to decrease with the decrease in CP content of the other treatment diets, which is quite interesting as the amount of protein from vegetable origin increased, as the CP content decreased. This would suggest that the Nile crocodile do not perform well when the diet contains a large percentage of protein from vegetable origin. This further substantiates the hypothesis of Coulson *et al.* (1987).

In conclusion, this is the first study of this kind on the nutritional requirements of the Nile crocodile. It was observed that the juvenile Nile crocodile (*C. niloticus*) performed optimally in all the performance criteria when the diet contained a DP value of 468.65 g/kg. As the American alligator's DP requirement was determined to be 450 g/kg (Staton *et al.* 1990), this would confirm the first part of the null hypothesis. However, the trial showed that animal protein was digested at a higher efficiency level than protein from plant origin and that crocodiles on the HCP diet out performed crocodiles on the three lower protein levels. This confirms the alternative hypothesis, in which the Nile crocodile has a different CP requirement than the American alligator (*A. mississippiensis*) and that the digestibility of the animal protein is higher than that of plant protein in the Nile crocodile. The DP level for optimum growth was met, however the diet had to contain a CP level of 62%. This was attributed to the poor protein digestibility coefficient of the diet which was found to have a maximum value of 0.79.

CHAPTER 7. RECOMMENDATIONS

I would like to give some recommendations to any researchers that intends to repeat or improve on this study or if any future research is intended to be performed on crocodilians.

First of all, when embarking on a trial of this nature, I would recommend that pens hold a maximum of 10 crocodiles at any one time, as it is a laborious task to find and capture 20 representative crocodiles in a pen containing 200. The tagging system used in this study would be advisable as it would give each crocodile an identification and individual growth measurement can be tracked throughout the study.

Second of all, when considering specific diets, ensure that palatability and pellet integrity are maintained with each diet. Ensure that some meat source is included in each diet to maximise pellet ingestion.

Another important point to note is that the young crocodiles should become acquainted with human presence and handling. Crocodiles should undergo capture and handling procedure every three weeks (or the number of weekly intervals that are determined by the researchers) from the day they are born, not only to gather early measurement statistics but also to allow them to familiarise themselves to the stress of handling. This would decrease the number of days that it takes for the crocodiles to eat at normal levels. The use of a radio, calming feed additives, would also improve the crocodile's ability to handle stress.

Lastly, I would recommend that at least 4 – 6 replicates are used for each diet as it would improve statistical outcomes of the study in hand.

Further research that would be needed to improve the knowledge of Nile crocodile nutrition would be to determine the energy (ME) requirements, the amino acid requirements or the mineral and vitamin requirements of the Nile crocodile. These potential research topics would help farmers better understand the nutrient requirements of the Nile crocodile which would improve on growth performance and reduce feed costs.

CHAPTER 8. REFERENCES

AOAC, 2000. Official method of analysis (17th Edition). Association of Official Analytical Chemists, Inc., Maryland, USA.

Australian Government, Department of Environment and Heritage (2008). Wildlife trade fact sheet. www.environment.gov.au/biodiversity/trade-use/factsheets/crocodiles.html.

Avendaño, G., Báez, L. and Michelangeli, L., 1992. Effects of diets complemented with sodium L-thyroxine, white corn flour and a complement of vitamins and essential amino acids in *Caiman crocodilus* growth. In: Crocodiles. Proceedings of the 11th Working Meeting of the Crocodile Specialist Group. Species Survival Commission of the IUCN – The world Conservation Union, Gland, Switzerland, Vol. 1, pp. 31-41.

Brazaitis, P., 1987. Identification of crocodilian skins and products. In : Webbs, G., Maonlis, C. and Whitehead, P. (eds) Wildlife management: Crocodiles and Alligators. Surrey Beatty and Sons, Chipping Norton, NSW, Australia.

Coulson, R.A., Coulson, T.A., Herbert, J.D., and Staton, M.A., 1987. Protein nutrition in the alligator, Comparative Biochemistry and Physiology, Vol 87A No 2 pp 449 – 459, UK.

Coulson, R.A., Coulson, T.A. and Herbert, J.D., 1996. Metabolic rate, nutrition and growth of the alligator. (unpublished report).

Coulson, R.A. and Hernandez, T., 1983. Alligator metabolism studies on chemical reactions in vivo. Peragamon Press. Oxford, UK.

Coulson, R.A. and Hernandez, T., 1987. Alligator metabolism studies on chemical reactions in vivo. Peragamon Press, New York, USA.

Davis, B.M., 2001. Improved nutrition and management of farmed crocodile: From Hatchlings to Harvest. Rural Industries Research and Development Corporation, Australia, RIRDC Publication No. 01/123, pp 1-95.

Du P.Bothman, J. and van Rooyen, N., 2005. Intensive wildlife production in southern Africa. Van Schaik Publishers, Hatfield, Pretoria.

Durazo, E., Cruz, A.C., Lopez, L.M., Lazo, J.P., Drawbridge, M. and Viana, M.T., 2008. Effects of digestible protein levels in isonitrogenous diets on growth performance and tissue composition of juvenile *Atracoscion nobilis*. Aquaculture Nutrition 2010 16; 54-60.

Garnett, S., 1985. Fatty acid nutrition of estuarine crocodile *Crocodylus porosus.* Comparative Biochemistry and Physiology 81B, 1033 – 1035.

Huchzermeyer, F.W., 2003. Crocodiles: Biology, Husbandry and Diseases. CAB International.

Jenkins, R.W.G., Jeldon, D., Manolis, S.C., Ferguson, R.A., Larriera, A., and Ross, J.P., 2004. Review of crocodile Ranching programs. Crocodile Specialist Group of IUCM/SSC, Australia.

Kanui, T.I., Kimwele, C.N., and Aulie, A., 1993. Influence of recombinant bovine growth hormone on growth and feed intake in juvenile Nile crocodiles *(Crocodylus niloticus)*. Comparative Biochemistry and Physiology 106A, 381 – 384.

Lafleur, Y., Charette, R., Woy, U., King, F.W., and Ross, J.P., 1995, Cites World Identification Guide – Crocodiles. Minister of Supply and service, Canada.

Lance, V., Joanen, T. And McNease, L., 1983. Selenium, Vitamin E and trace elements in the plasma of wild and farm-reared alligators during the reproductive cycle. Canadian Journal of Zoology 61, 1744 – 1751.

Leon Ojeda, F.J., Arrendondo Ramos, P.L. and Robles Monitijo, M.C., 1998. Use of anabolic steroids in the commercial raising of crocodiles. In: *Crocodiles. Proceedings of the 14th Working Meeting of the Crocodile Specialist Group*. IUCN – The World Conservation Union, Gland, Switzerland and Cambridge, UK, pp. 353-359.

Luxmoore, R.A.(ed), 1992. Directory of crocodilian farming operations. 2nd Edition. ICUN, Gland, Switzerland.

MacGregor, J., 2006. The call of the wild: Captive crocodilian production and shaping of conservation incentives. Traffic International, Cambridge, UK.

Maree, C. and Casey, N.H., 1993. Livestock production systems: Principals and Practice. Agri- Development Foundation, Brooklyn, Pretoria, South Africa.

Mateos, G.G., Sell, J.C., and Eastwoods, J.A., 1982. Rate of food passage (transit time) as influenced by level of supplemental fat. Poult Sci 61, 94 – 100.

McDonald, P., Edwards, R.A., Greenhalgh, J.F.D and Morgan, C.A., 2002. Animal Nutrition. Sixth Edition. Pearson Education Limited, Essex, UK.

Peucker, S.K.J., Davis, B.M. and van Barneveld, R.J., 2005. Crocodile farming research: Hatchlings to Harvest. Rural industries Research and Development Corporation, Australia.

Pina, C. and Larriera, A., (2002). *Caiman latirostris* growth: the effect of a management technique on the supplied temperature. Aquaculture 211, 387 – 392.

Pirozzi, I., Booth, M.A. and Allan, G.L., 2008. The interactive effects of dietary protein and energy on feed intake, growth and protein utilization of juvenile mulloway *(Argyrosomus japonicas)*. Aquiculture Nutrition 2010 16; 61-71.

Reuters, 2005. Antibiotics from crocodile blood. www.wired.com/medtech/health/news/2005/08/68553.

Sameuls, M.L., 1989. Statistics for the life sciences. Collier MacMillan Publishers, London.

Staton, M.A, Brisbin, I.L. and Pesti, G.M., 1986. Feed formulation for Alligators: An overview and initial studies. Proceedings 8th working meeting, IUCN crocodile specialists group, pp 84 – 104, Ecuador.

Staton, M.A. and Edwards H.M., 1987. Studies on alligator nutrition. Proceeding of 1987 Georgia nutrition conference for the feed industry. Pp 66 – 76.

Staton, M.A., Edwards, H.M., Brisbin, I.L., Joanen, T. and Mcnease, L., 1990. Protein and energy relationship in the diet of the American alligator *(Alligator mississippiensis*). Journal of Nutrition 120, 775 – 785.

Staton, M.A., Edwards, H.M., Brisbin, I.L., Joanen, T. and Mcnease, L., 1992. The influence of environmental temperature and dietary factors on utilization of dietary energy and protein in purified diets by alligators (*Alligator mississippiensis*). Aquaculture 107, 369 – 381.

Statistical Analysis Systems, 2009. SAS User's guide : Statistics Version, SAS Institute Inc. Cary, NC., USA.

Spannhof, L. and Plantikow, H., 1983. Studies on carbohydrate digestion in rainbow trout. Aquaculture 30, 95-108.

Van Jaarsveldt, K.R., 1987. Flaying, Curing and Measuring Crocodile Skins. In: Webb, G., Grahame, J.W., Manolis, S.C., and Whitehead, P.J. (eds). Wildlife Management: Crocodiles and Alligators. Surrey Beatty & Sons Pty Limited, Australia.

Wee, K.L. and Tacon, A.G.J., 1982. A preliminary study on the dietary protein requirement of juvenile snakehead. Bulletin of the Japanese Society of Scientific Fisheries 48(10), 1463-1468.

ANNEXTURE A (PHOTO PLATE)

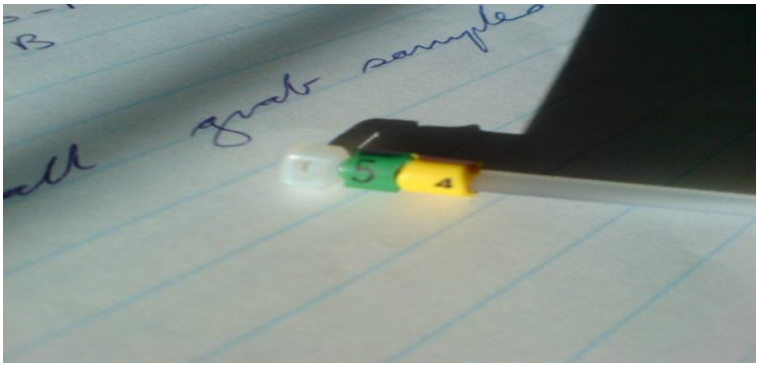


Plate 1: The numbering of a tag used to individually identify the crocodile pen representatives.

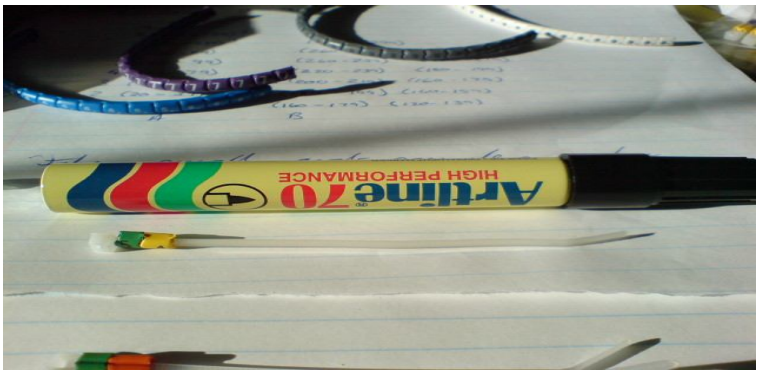


Plate 2: The cable ties used for the tags were quite small, this was to ensure that the tags would not restrict normal crocodile behaviour, nor allow other crocodiles to attack the tags. Tag sequences from 00 – 240 were used.

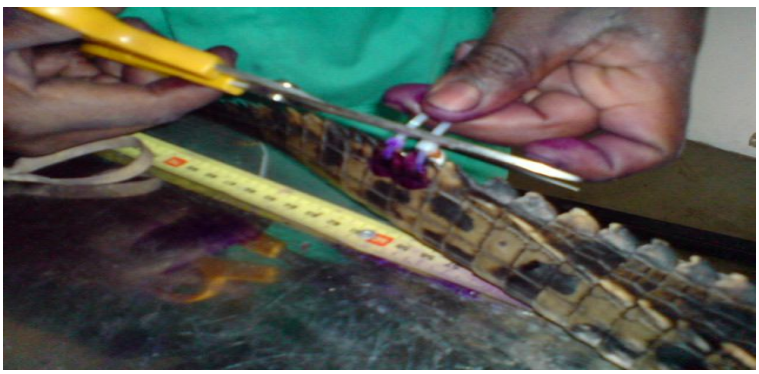


Plate 3: Two holes in two consecutive tail scutes were made with a belt hole maker. The cable ties were tied through the holes and the excess cable tie was cut off, an antiseptic was sprayed on the holes that were made. Two cable ties were used as one cable tie served as crocodiles identification and the second cable tie served as an average marker (in the case that one was bitten off).

Plate 4: This illustrates what the tag looks like on the tail. The tag numbers came in different colours, however each number was associated to a specific colour (as seen here), where black was zero and orange was number 4. This allowed us to identify the crocodile number in the case the number faded.

Plate 5 : The measuring station used to measure all the performance parameters.

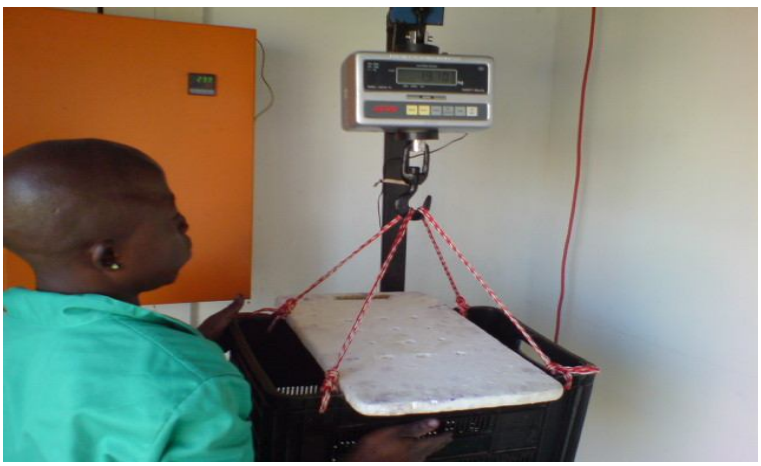


Plate 7: Once the mouths were tied closed, the crocodiles were placed in the crate attached to a hang scale, where the weight was recorded.

Plate 9: The crocodile was flipped onto its back to determine the snout to vent length.

Plate 6: To prevent injury to the crocodile and to the members of the measuring team, all the crocodiles mouths were closed using a rubber band.

Plate 8: The crocodiles were placed on a measuring scale. This scale was custom built for ease of measurement. It consisted of a metre long piece of steal with a lip on the one end. At the base of the lip a strip of measuring tape was secured, ensuring that 0cm was at the lip. The crocodile's snout was then placed at the lip and the body length was measured up to the end of the tail.

Plate 10: Feed wastage (orts), was removed from each pen and placed onto these bucket lids for weighing

Plate 11: Orts were weighed and weight recorded using an electric scale.

Plate 12: All the dry mix was temporarely stored in these 250 L drums

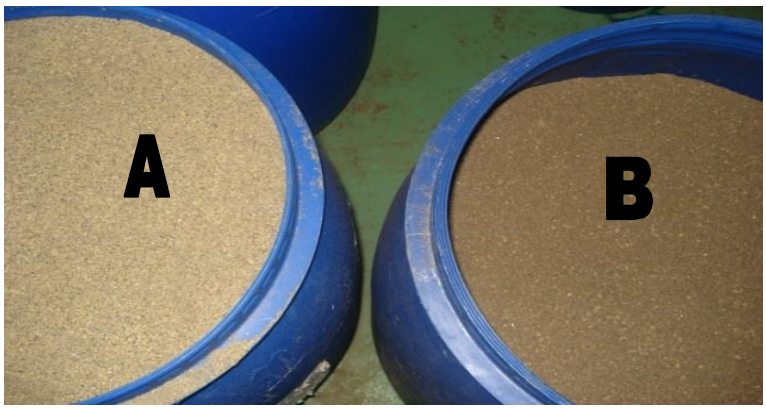


Plate 13 : A – is the 48% CP treatment diet, and B – is the dry portion of the 60% CP treatment diet stored within the drums

Plate 14 : This is a closer view of A – 48% CP treatment diet and B – 60% CP treatement diet. The darker colour of B is from the high amount of fishmeal present in the 60% CP treatment diet.

Plate 15: Staff members preparing the chicken for the 3 higher protein treatment diets

Plate 16: Water is added to the dry mix portion of the treatment diet. This would aid in maintaining the integrity of the pellets.

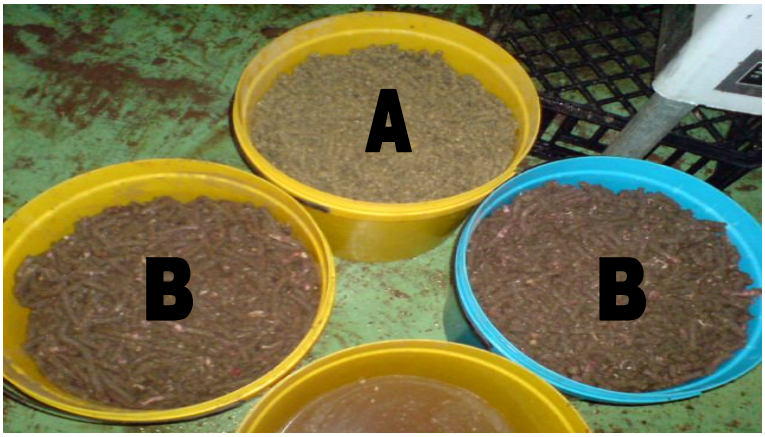


Plate 17: The pellets seen here are the ingredients required to make the 55.6% CP treatment diet, which contain 67% of the 60% CP treatment diet (**B**) and 33% of the 48% CP treatment diet (**A**).

Plate 18: The 48% CP treatment diet (**A**) is added to the 60% CP treatment diet (**B**).

Plate 19: One of the crocodile farm staff mixing the two treatment diets.

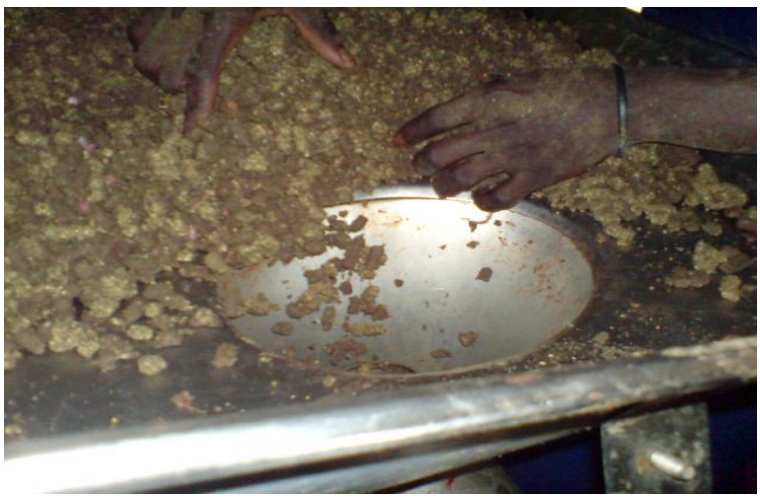


Plate 20: Once the two diets have been thoroughly mixed, the new mixture is passed into the mincer to form the new treatment diet

Plate 21: These thin sausages are the product of the mixing which formed the 55.6% CP treatment diet. These sausages will break up into pellets when fed to the crocodiles.

Plate 22: The buckets for all the pens are first placed on the walls before feeding commences**.** All the feed that is taken out to the crocodiles is weighed in accordance to the previous days feed consumption. Each bucket was individualy marked with the treatment diet and pen to which the bucket was to go.

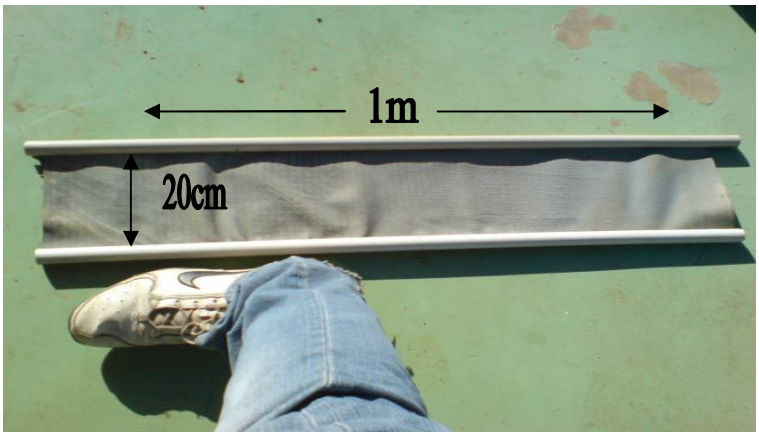


Plate 23: Each tray measured 1m long , 20cm wide and 5 cm deep. The shoe in the photo is to illustrate the size of the tray as the shoe is a UK size 10.

Plate 24: The feed is thrown on the feed trays and the crocodiles are showing a keen interest in the feed.

Plate 25: These crocodiles are showing a very intense feeding behavior, as they could not even wait until all the feed was placed onto the trays. All the crocodiles during this trial were fed ad lib, and there was always wastage the following day. However a 12 hour fast and competition for food results in this response.

Plate 26: The grow house keeper busy cleaning the pen. This was a daily routine as each pen contained over 200 crocodiles and which could lead to severe microbial growth if the feacal and feed waste was not removed. The water from each pen was drained and replaced with fresh water.

Plate 27: Crocodiles enjoying the clean pen. This image illustrates that the crocodiles are calm as they are evenly spread out throughout the pen.

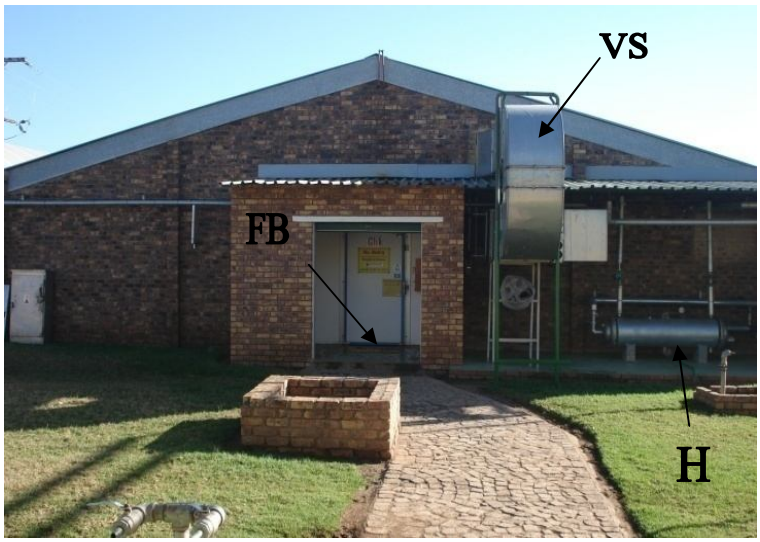


Plate 28: A front view of the grow house, where the vent system (**VS**) and the water heating system (**H**) can be seen. This house also has a foot bath (**FB**) to serve as biosecurity for the crocodiles inside.

Plate 29: An external view of the grow house used during the trial

Plate 30: An internal view of the grow house. The columns from the roof carry warm water pipes for the under floor heating. This house is 70m long and contains 2 rows of 9 pens. The vent system (**VS**) can be seen on the roof which carries fresh (heated) air from outside. A window is present at the back of the house to allow the air pressure to escape.

Plate 31: A radio was placed in the middle of the house. The constant sound of the radio allowed the crocodiles to experience less stress (to overcome stress to a faster degree) when any activity took place inside the grow house. The radio was vital in stress management.