Application of dietary bentonite clay as feed additive on feed quality, water quality and production performance of African catfish (*Clarias gariepinus*)

By

Ayoola Mathew Oluwaseyi

Dissertation presented for the degree of Doctor of Philosophy

Stellenbosch University

Promoter: Dr Khalid Salie
Co-promoter: Mr Lourens de Wet

Faculty of AgriSciences

March 2016

DECLARATION

By submitting this thesis/dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

March 2016

ABSTRACT

Aquaculture remains the fastest growing animal food producing sector and is gradually overtaking capture fisheries as a source of food fish. The challenges of poor feed quality, feed utilisation and water quality are recurring issues that hinder the growth of aquaculture industry.

This study investigated the effects of natural bentonite (NB) and its acid activated form (AB) as feed additives on aquafeeds with reference to the physical quality, growth performance, gut evacuation rate and water quality with African catfish (*Clarias gariepinus*) as the experimental species. Bentonite clay has found application as a feed additive in animal science and aquaculture. It is used as a mycotoxin binder to improve feed utilisation. Application of dietary activated bentonite form is yet to be fully elucidated to the best of our knowledge.

Aquafeeds' physical qualities, including water stability, feed durability, feed bulk density and nutrient leaching, were investigated. Dietary bentonite clay was added to commercial aquafeeds and each treatment were replicated four times. Water stability, feed durability, feed bulk density and nutrient leaching were all significantly affected ($p<0.05$) by treatments. Measured parameters (water stability, feed density and feed durability) increased ($p<0.05$) with the quantity of AB in the clay blend and at high inclusion level as compared to control. Values increased with an increased inclusion level of clay in feed, while nutrient leaching value decreased with quantity of AB and high inclusion. AB had higher values as compared to NB and the control. These results validated the potential utilisation of NB and AB as feed binders. Their ability to improve pellet physical qualities is expected to enhance feed utilisation, maintain good water quality and increase fish growth.

Aquafeeds with bentonite clay blend, and each at different inclusion levels improved ($p<0.05$) growth performance, feed conversion ratio (FCR), specific growth rate (SGR), red blood cell (RBC), and haematocrits (HCT). Parameters improved with an increase in quantity of natural bentonite and at low inclusion level. Growth performance increased ($p<0.05$) as compared to control with bentonite inclusion up to 1500 mg/kg and decline at 3000 mg/kg. The contents of crude protein (CP), fat, moisture, ash and abdominal fat in the whole body composition were significantly affected ($p<0.05$) by the treatment diets. Abdominal fat and tissue fat content increased with bentonite inclusion.

To evaluate the effect of NB and AB on gut evacuation rate of *Clarias gariepinus*, fish were randomly allocated to treatment diets and fed *ad libitum*. Four (4) fish were randomly selected per treatment to measure post feeding stomach content (SC) and intestinal filling (IC) content at (5, 30, 60 and 120 min). The control diet had higher values for SC at 5 min which differed significantly ($p<0.05$) as compared to clay diets. SC decreased ($p<0.05$) with time in all diets and clay diets had higher value ($p<0.05$) between (30 – 120 min) as compared to control. In clay diets, SC decreased faster over time with reduced quantity of AB and low inclusion level. The IC of control diets had higher values ($p<0.05$) over time as compared to clay diets.

The water quality parameters (pH, $N0_2$-, NH_3-N and TSS) were evaluated in a static aerated tank. Aquafeeds were fed to adult fish at 3% body ratio twice daily. Each treatment diet was replicated four (4) times, and 10 fish were randomly allotted to each tank. The water temperature was 25 ± 2°C and dissolved oxygen (DO) was 3-9 mg/L. Water samples were collected daily from each tank. In phase I, water quality was evaluated over seven (7) days, and in phase II over five (5) days. No mortality was recorded during the study. The values of pH, $N0_2^-$, NH_3-N and TSS increased significantly ($p<0.05$) in all treatment diets over time. In both phases, clay treated diets had lower values for measured parameters ($p<0.05$) compared to those of control. In clay diets, values increased ($p<0.05$) with quantity of AB and high inclusion level. Fish feed intake reduced as water quality deteriorated, and uneaten feeds contributed to increase in poor water quality. Thus, high water stability of clay treated diets attributed to maintenance of good water quality as compared to the control.

The results showed that dietary clay enhanced feed binding, which affected digesta viscosity. This reduced the rate of feed evacuation in the gut, and, thus, enhanced proper breakdown and absorption of nutrients. Higher inclusion levels of bentonite clay and increased quantity of AB are not suitable for improved growth and feed utilisation, as increased in digesta viscosity led to fermentation in the gut. An optimum level of performance was recorded at 1500 mg/kg inclusion with natural bentonite (B1500) with a significantly ($p<0.05$) better performance as compared to control and acid activated bentonite. A clay combination blend up to 50% (A50B50) at low inclusion level (500 mg/kg) of acid activated bentonite is considered optimum.

UITTRELSEL

Akwakultuur bly die vinnigste groeiende diereproduksiesektor en is geleidelik besig om visserye in te haal as 'n bron van vis vir voedsel. Die uitdaginge gekoppel aan swak kwaliteit voere, voerbenutting en waterkwaliteit is herhalende kwessies wat die groei van die akwakultuurbedryf verhinder.

Hierdie studie het die effek van natuurlike bentoniet (NB) en sy suurgeaktiveerde vorm (SB) ondersoek as voertoevoeging op akwavoere met verwysing na die fisiese kwaliteit, groeiprestasie, spysverteringsontruimingstempo en waterkwaliteit met die Afrika baber (*Clarias gariepinus*) as die eksperimentele spesies. Bentonietklei het aanwending as 'n voertoevoeging in veekunde en akwakultuur gevind. Dit word gebruik as 'n mikotoksienbinder om voerbenutting te verbeter. Die aanwending van 'n dieetgeaktiveerde bentonietvorm is nog nie ten volle toegelig na die beste van ons kennis nie.

Akwavoere se fisiese eienskappe, insluitend waterstabiliteit, voerduursaamheid, voermassadigtheid en die loging van voedingstowwe is ondersoek. Dieetbentonietklei is tot kommersiële akwavoere bygevoeg en elke behandeling was vier keer herhaal. Waterstabiliteit, voerduursaamheid, voermassadigtheid en voedingstofloging is almal beduidend beïnvloed ($p < 0.05$) deur behandeling. Gemete parameters (waterstabiliteit, voerdigtheid en voerduursaamheid) het toegeneem ($p < 0.05$) met die hoeveelheid SB in die kleimengsel en teen 'n hoë insluitingvlak in vergelyking met die kontrole. Waardes het toegeneem met 'n verhoogde insluitingsvlakke van klei in voer, terwyl voedingstofloging se waarde gedaal het met hoeveelhede SB en hoë insluiting. SB het hoër waardes in vergelyking met NB en die kontrole. Hierdie resultate bevestig die potensiële benutting van NB en SB as voerbinders. Hul vermoë om voerkorrels se fisiese eienskappe te verbeter, sal na verwagting voerbenutting verbeter, en tesame goeie waterkwaliteit handhaaf met verhoging in visgroei.

Akwavoere met ‘n bentonietkleimengsel, en elk teen verskillende insluitingsvlakke het die groeiprestasie, voeromsettingsverhouding (VOV), spesifieke groeitempo (SGR), rooibloedselle (RBS), en haematokrit (HKT) verbeter ($p < 0.05$). Parameters het verbeter met 'n toename in die hoeveelheid van natuurlike bentoniet en lae insluitingsvlakke. Groeiprestasie het toegeneem ($p < 0.05$) in vergelyking met die kontrole tot en met ‘n bentoniet insluiting van 1500 mg/kg en ‘n afname het plaasgevind by 3000 mg/kg. Die inhoud van ru-proteïen (RP), vet, vog, as en

abdominale vet in die hele liggaamsamestelling is beduidend beïnvloed ($p < 0.05$) deur die behandelingsdiëte. Abdominale vet en weefselvetinhoud het toegeneem met bentoniet insluiting.

Om die effek van NB en SB op spysverteringontruimingstempo van *Clarias gariepinus* te evalueer, is vis ewekansig toegewys aan behandelingsdiëte en *ad libitum* gevoer. Vier (4) vis is ewekansig gekies per behandeling om post-voeding maaginhoud (MI) en spysverteringsvullingsinhoud (SV) by (5, 30, 60 en 120 minute) te meet. Die kontrole dieet het hoër waardes vir MI by 5 min getoon wat beduidend verskil het ($p < 0.05$) in vergelyking met kleidiëte. MI het afgeneem ($p < 0.05$) met verloop van tyd in alle diëte en kleidiëte het hoër waardes ($p < 0.05$) tussen (30-120 min) getoon in vergelyking met die kontrole. In die kleidiëte, het MI vinniger afgeneem met verloop van tyd met 'n verminderde hoeveelheid SB en lae insluitingsvlak. Die SV van die kontrole dieet het hoër waardes ($p < 0.05$) met verloop van tyd getoon in vergelyking met kleidiëte.

Die waterkwaliteitsparameters (pH, $N0_2$-, NH_3-N en TSS) is geëvalueer in 'n statiese belugte tenk. Akwavoere is gevoer vir die volwasse visse teen 3% liggaamsmassa-verhouding, twee keer per dag. Elke behandelingsdieet is vier (4) keer herhaal, en 10 visse is ewekansig toegewys aan elke tenk. Die watertemperatuur was 25 ± 2°C en opgeloste suurstof (OS) was 3-9 mg/L. Watermonsters is daagliks versamel uit elke tenk. In in fase I, is waterkwaliteit geëvalueer oor sewe (7) dae en in fase II oor vyf (5) dae. Geen mortalitiete is aangeteken tydens die studie. Die waardes van pH, $N0_2$-, NH_3-N en TSS het aansienlik toegeneem ($p < 0.05$) in alle behandelingsdiëte met verloop van tyd. In beide fases, het kleibehandelde diëte laer waardes vir gemete parameters ($p < 0.05$) in vergelyking met die kontrole getoon. In kleidiëte het waardes toegeneem ($p < 0.05$) met die hoeveelheid van SB en hoë insluitingsvlak. Visvoerinname het verminder soos die waterkwaliteit verswak het, en ongevrete voere het verhoog in hierdie swak waterkwaliteitsomgewing. Dus, 'n hoë waterstabiliteit van kleibehandelde diëte kan bydrae tot die handhawing van goeie waterkwaliteit in vergelyking met die kontrole.

Die resultate het getoon dat dieetkleie verbeter voerbindinge, wat die verteringsmengsel se viskositeit beïnvloed. Dit het die tempo van voerontruiming in die spysvertering verbeter. Hoër insluitingsvlakke van bentonietklei en die verhoogde hoeveelheid van SB is nie geskik vir 'n beter groei en voerbenutting nie, want ‘n toename in verteringsmengsel se viskositeit het gelei tot fermentasie in die spysvertering. 'n Optimale vlak van prestasie is aangeteken by 1500 mg/kg

insluiting met natuurlike bentoniet (B1500), met 'n beduidende ($p < 0.05$) beter prestasie in vergelyking met die kontrole en suurgeaktiveerde bentoniet. 'n Klei kombinasie mengsel tot en met 50% (A50B50) teen 'n lae vlak insluiting (500 mg/kg) van suurgeaktiveer bentoniet, word as optimaal beskryf vir aanwending.

ACKNOWLEDGEMENTS

First and foremost to Him who brought me out from mere clay and set my feet upon the rock; forever you will be my God, redeemer and pillar that holds my life...thank you Jesus.

To Dr Khalid Salie and Mr. Lourens de Wet, who provided mentorship and extra ordinary supervision, I am indeed grateful for your contributions.

My heartfelt gratitude goes to the Dean of the Faculty of AgriSciences, Prof Danie Brink, and the Head of the Department Animal Sciences, Prof Kennedy Dzama, as well as all laboratory technologists who assisted me all through the degree programme.

I appreciate the efforts of staff at the Welgevallen Experimental Farm, including Henk Stander, Desmare van Zyl, Anvor Adams, Amy Landsdell, Nanje Olivier and all the student interns.

I appreciate the financial support provided by Stellenbosch University through the Department of Animal Sciences.

Furthermore, to Prof Mathews Ojo, Prof John Akande, Dr A. O Ogunleye, Dr Foluke Aderemi, Dr Tunde Lawal, Dr Femi Alabi and all staffs at Bowen University, Nigeria. Space cannot allow me to mention you all individually; a deep thank you for your encouragements and platform to achieve this goal.

Dn and Mrs Jacob Olatunji Ayoola, whose selfless sacrifices and fervent prayers as parents brought me this far…You are the best. My siblings, John, Ruth and Tobi, thank you for all your encouragement and assistance.

To my wife Deborah Ayoola and my two children; Johnpaul and Michelle, who came to this world during my study period; thank you all for your sacrifices, understanding and love.

Finally to all those who contributed towards this height in my life, whose name are not mentioned…I am grateful, the Lord reward you all according to your deeds.

TABLE OF CONTENT

LIST OF FIGURES

LIST OF TABLES

LIST OF PLATES

CHAPTER 1

RATIONAL FOR APPLICATION OF DIETARY CLAY MINERAL AS FEED ADDITIVE IN AQUAFEEDS'

1.1 Introduction

Aquaculture is referred to as the farming of aquatic organisms and includes fish, crustaceans, aquatic plants and molluscs. These organisms can be farmed in fresh, brackish and sea water environments. Aquaculture is the fastest growing food production system in the world and African aquaculture production is expanding steadily (Brummett *et al.*, 2008; Bostock *et al.*, 2010), with freshwater fish production contributing the largest proportion. In 2012, aquaculture set new increases in production and now provides almost half of all fish for human food. This share is projected to rise to 62% by 2030 (FAO 2014).

According to FAO, (2012), China is currently the largest producer of seafood, responsible for most of the increase in world per capita fish consumption, particularly from aquaculture, despite a downward revision of China's production statistics for recent years. China's share in world fish production grew from 7 % in 1961 to 61.5% in 2008. Fish and its products consumed in developed countries consists of imports, and, owing to steady demand and declining domestic fishery production (down 10% in the period 2000–2010), the dependence on imports, in particular from developing countries, is projected to grow in coming years (FAO, 2012; Olsen & Hasan, 2012).

Fish are a valuable source of protein and essential micronutrients for humans, providing about 3.0 billion people with almost 20% of their intake of animal protein. A portion of 150 g of fish can provide about 50–60% of an adult's daily protein requirement. In 2010, fish accounted for 16.7% of the global population's intake of animal protein and 6.5% of all protein consumed. Fish proteins can represent a crucial nutritional component in some densely populated countries where total protein intake levels may be low. It can also provide livelihoods for over 10 million Africans, many of whom are small-scale operators supplying food to local and sub-regional markets (Bostock *et al*., 2010; Munguti *et al*., 2014; FAO 2014). Despite its success, aquaculture is faced with challenges of feed quality, water quality and caused poor growth and production.

1.2 An Overview of Africa's Fisheries and Aquaculture

By continent, the annual aquaculture production growth was fastest in Africa (11.7%) and Latin America and the Caribbean (10%) in the first twelve years of the new millennium. Africa's aquaculture is characterized by rapid development in freshwater fish farming in sub-Saharan Africa, most notably in Nigeria, Uganda, Zambia, Ghana, South Africa and Kenya, while brackish water fish farming is found in Egypt. Major cultured fish species are African catfish (*Clarias gariepinus*), tilapia spp. (mostly *Oreochromis niloticus* and *O. mossambicus*) and trout (*Oncorhynchus mykiss*) (Pouomogne *et al.*, 2010b; Munguti *et al.*, 2014; FAO, 2014). Despite notable achievements, the continent's fisheries and aquaculture industries are faced with challenges, such as poor government policies, inadequate funding for quality research, marketing, and funding to expand production (Brummett, 2008).

Africa's fisheries are dominated by production of protein for human consumption and the contribution of aquaculture to total fisheries production varies sharply among countries (Miller & Atanda, 2011). The contribution of fishery activities to national economies is multifaceted. In addition to supplying food, capture and aquaculture production contributes to gross domestic product (GDP), provides livelihoods for fishers and processors, is a source of hard currency (from exports of fishery products), and boosts government revenues through fisheries agreements and taxes (Brummett 2008; Pouomogne *et al*., 2010; FAO 2014). The value added by the fisheries sector in 2011 was estimated at more than US$24 billion, 1.26% of the GDP of all African countries. Aquaculture is still developing in Africa and is mostly concentrated in a few countries such as Egypt, South Africa, Morocco, Uganda and Nigeria with an estimated value of almost US$3 billion per year (FAO, 2012).

About 27.3% of the people engaged in fisheries and aquaculture are women, with marked distribution as fishers (3.6%), processors (58%), and aquaculture workers 4% (Brummett 2008;

FAO, 2012). Western and southern Africa provides high percentages of processors with large female employment, while in eastern Africa the number of fishers often exceeds that of processors (Munguti *et al.*, 2014; FAO, 2014). According to FAO (2014), at the country level, Nigeria ranks first with almost 2 million people engaged in the fisheries and aquaculture sector, followed by Morocco (almost 1.4 million) and Uganda (almost 1 million). Breaking this down, in terms of the number of fishers, Morocco (870 000) tops Nigeria (790 000), Uganda (470 000) and Mali (350 000). In terms of processors, Nigeria (more than 1 million) has almost double the number of Morocco (slightly more than 500 000), followed by Uganda (420 000) and Ghana (385 000). However, in aquaculture, the picture is very different with Egypt (580 000) having more people employed in the sector than all the other countries of Africa combined, followed by Nigeria (135 000) and Uganda (53 000).

1.3 Culture of African catfish (*Clarias gariepinus*)

The African catfish, *C. gariepinus* (Burchell 1822), is one of the most important species currently being farmed in Sub-Sahara Africa (Manuel *et al.*, 2014). *C. gariepinus* is a native species of tropical and subtropical fresh waters. *C.gariepinus* has been widely farmed in heated waters outside its natural range (Oellermann & Hecht, 2000; Singh *et al.*, 2012). Its rapid growth at high densities, ability to breathe air and to withstand poor water quality, and its tasty flesh makes it an excellent candidate for aquaculture (Manuel *et al.*, 2014). According to Schram *et al.*, 2010, *C. gariepinus* tolerates plasma NH_4^+ concentrations within physiological concentrations over a range of water ammonia concentrations that would be lethal to many other fishes. However, the high ammonia concentrations did affect the fish, as revealed by other parameters: plasma glucose, plasma osmolarity, gill morphology, specific growth rate (SGR), total feed intake (TFI) and feed conversion rate (FCR) were negatively affected, resulting in low production. Hence, management of good water quality is important for optimum production, specifically for key parameters such as nitrogenous compounds.

1.4 Sustainable Aquaculture and its environmental impacts

Aquaculture is the only significant new contributor to world food supplies in centuries, and it should have a bright future (Anticamara *et al.*, 2011). However, aquaculture is coming of age at a time of increased ecological awareness and ecological activism. The aquaculture industry must counter criticisms about the adverse environmental effects and lack of sustainability. Ecological concern and better environmental management are very important to ensure mankind's survival (Claude and Schmittou, 1999; Hlava, 2014). Good environmental conditions are also necessary for culture of aquatic animals and it is in the self-interest of aquaculture to protect the surrounding environment. An adaptable aquaculture production technology system whose ecological and economic viability can persist indefinitely is a sustainable aquaculture system (Bostock *et al.*, 2010)

Adequate implementation of an environmental monitoring program has been adopted by aquaculture industries in developed countries (Bostock *et al.*, 2015). In Africa and other developing countries, policies to ensure sustainable aquaculture practices are less implemented in the industries and amidst farmers alike (Brummett, 2008; Miller & Atanda, 2011). Therefore, Africa aquaculture industry should be proactive and work towards developing and implementing systems of environmental friendly production practices for preventing or mitigating adverse environmental impacts.

Discharge of aquaculture wastewater can lead to eutrophication and disruption of natural ecosystems in receiving water bodies. A controlled waste production strategy is necessary in order to maintain sustainable aquaculture growth. Along with fertilisation, aquafeeds' is the major source of waste matter in aquaculture (Sindilariu, 2007; Mackie *et al.*, 2009). Therefore, aquaculture waste management should be approached through diet formulation and/or feeding strategy. The introduction of highly digestible feed has reduced solid waste excretion, and further reductions can be achieved through careful selection of ingredients and processing to improve nutrient availability (Hlava, 2014).

The focus of our research is on clay minerals as a measure to improve feed utilisation and thus enhance sustainable aquaculture management practices. Bentonite clay mineral application in aquaculture has few published works. Reported studies focused on adsorption and ion exchange agents in treatment of cultured and toxin adsorption from the gut (Williford *et al.*, 1992; Indresh *et al.*, 2013; Carraro *et al.*, 2014). Therefore, the application of dietary clay minerals in aquafeeds' to improve feed quality, maintain good water quality, nutrient utilisation, and enhance fish growth on total production is considered important.

1.5 Motivation of study

Fish produce nitrogenous wastes through catabolism of amino acids. The efficiency of fish nitrogen (N) assimilation has important implications for water quality and profitability of fish production. Protein sources, such as fish meal and soybean meal, are the most expensive components of formulated feeds and improvement in the efficiency of N assimilation and utilisation will thus improve the economics of fish production (Reddy *et al.*, 2009; Schram *et al.*, 2010).The inherent efficiency of nutrient utilisation by fish implies that N loading of aquaculture ponds may be limited by the capacity to assimilate nitrogenous excretion, which may have a deleterious impact on water quality and fish growth.

The majority of freshwater and marine teleost fishes are ammonioteles and excrete most of their nitrogenous wastes as ammonia across the gills to the water (Wilkie, 2002; Wright & Wood, 2009). High concentrations of ammonia in water lead to rapid accumulation of ammonia in plasma and tissues, where it is mainly present as NH_4^+ at physiological pH. High internal NH_4+ causes neurotoxicity (Wilkie, 2002; Wright, 2007) . Fish also excrete faecal solid wastes that settle into the sediment along with senescent phytoplankton and other particulate organic matter. Faecal solids may account for up to 50% by weight of feed (dry weight) applied to the pond. A large fraction of this organic matter is rapidly hydrolysed and mineralized, representing an additional source of ammonia. Therefore, high ammonia concentration caused by high feed loads and high fish densities, is an important limiting factor for intensive aquaculture. Ammonia concentrations in water should therefore be kept below species-specific threshold levels.

Nitrite is another potentially toxic nitrogenous compound that may accumulate in fish ponds. Nitrite is released as an intermediate product during nitrification and denitrification. The toxicity of nitrite is expressed through the competitive binding of nitrite to haemoglobin forming methaemoglobin, which does not have the capacity to carry oxygen in blood (Lefevre *et al.*, 2011). Nitrite and nitrate, which are oxidative products of ammonia, induced a large variety of physiological disturbances, in which the former has more toxic effect upon accumulation in freshwater fish (Jensen, 2003; Li & Meng, 2010; Tomasso, 2012). Therefore, in order to achieve maximum yield in intensive fish culture, maintenance of good water quality is important. Since nitrogenous waste affecting water quality in aquaculture are products of feed metabolism, treatment of feed to ensure maximum utilisation of nitrogen compounds and its absorption by fish could enhance good water quality, adequate feed utilisation and increase in yield.

According to Amany (2009), Li *et al.* (2010), and Slamova *et al.* (2011), Na- montmorillonite from bentonite rock can be used as scrubber applied directly to adsorb nitrogenous waste and as feed additives to bind toxins from the gut. Recommendations were made to further investigate the binding ability of clay minerals with protein. They suggested studies to investigate reduction of digestive enzymes activities and possible solution to poor water turbidity that arises after application of clay mineral as scrubber. It is envisaged that the outcomes of the study will complement previous work and direct strategic decision making in relation to application of clay mineral as a feed additive to improve feed quality, nutrient utilisation, fish growth and maintain good water quality in aquaculture.

1.3 Aim of the study

Assessment of the effect of natural and acid-activated bentonite clay mineral as feed additives on water quality, feed physical quality and growth performance parameters of *Clarias gariepinus.*

1.4 Objective of the study

The objective was to determine the effect of natural and acid-activated bentonite combinations at low and high inclusion levels on:

i. Feed quality, including feed durability, feed density and water stability
ii. Production performance of catfish

iii. Feed viscosity/feed flow rate
iv. Body composition of catfish
v. Water quality parameters in a production system, including pH, excretory N ($N0_2$-N, NH_3-N) and TSS.

1.5 Hypotheses

1. H_0 - Bentonite clay minerals as feed additive in aquafeeds' will not improve feed physical quality

   H_1 - Bentonite clay minerals as feed additive in aquafeeds' will improve feed physical quality quality

2. H_0 - Bentonite clay minerals as feed additive in aquafeeds' will not affect growth and body composition of *C. gariepinus*

   H_1 - Bentonite clay minerals as feed additive in aquafeeds' will affect growth and body composition of *C. gariepinus*.

3. H_0 - Bentonite clay minerals as feed additive in aquafeeds' will not affect gut evacuation rate of *C. gariepinus*.

   H_1 - Bentonite clay minerals as feed additive in aquafeeds' will affect gut evacuation rate of *C. gariepinus*.

4. H_0 - Bentonite clay minerals as feed additive in aquafeeds' will not maintain good water quality, of *C. gariepinus*.

   H_1 - Bentonite clay minerals as feed additive in aquafeeds' maintain good water quality, of *C. gariepinus*.

1.6 Concluding remarks

This study will aim to address application of natural and acid-activated bentonite clay mineral as feed additives on feed quality and growth performance of *C. gariepinus*. Effect of these clay minerals on reduction of excretory nitrogenous waste to maintain good water quality will be elucidated. The research will evaluate feed utilisation with clay minerals as feed additive fed to *C. gariepinus*. The study is supported by previous research on related application of clay minerals in livestock animals (poultry and ruminant). The envisaged output is to provide additional knowledge on application of clay minerals in aquaculture, complementing our understanding and interpretation of the application and development of fish aquaculture.

1.7 References

Amany, B. 2009. Pathological studies on effects of aflatoxin. *Egypt J.comp.Path & Clinic. Path* Vol 22(1), 179 -193.

Anticamara, J.A., Watson, R., Gelchu, A. & Pauly, D. 2011. Global fishing effort (1950–2010): Trends, gaps, and implications. *Fisheries Research*. 107(1-3):131–136.

Bostock, J., McAndrew, B., Richards, R., Jauncey, K., Telfer, T., Lorenzen, K., Little, D., Ross, L.,. 2010. Aquaculture: global status and trends. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*. 365(1554):2897–912.

Bostock, J., Mcandrew, B., Richards, R., Jauncey, K., Telfer, T., Lorenzen, K., Little, D., Ross, L.,. 2015. Aquaculture : global and. Trend. *Phil. Trans. R. Soc*. B (2015) 365, 2897-2912 doi:10.1098/rstb.2010.0170

Brummett, R.E., Lazard, J. & Moehl, J. 2008. African aquaculture: Realizing the potential. *Food Policy*. 33(5):371–385.

Carraro, A., De Giacomo, A., Giannossi, M.L., Medici, L., Muscarella, M., Palazzo, L., Quaranta, V., Summa, V.. 2014. Clay minerals as adsorbents of aflatoxin M1 from contaminated milk and effects on milk quality. *Applied Clay Science*. 88-89:92–99.

Hlava, D. 2014. Effects of supplementary feeding in carp ponds on discharge water quality : a review. *Aquacult Int* (2014) 22:299–320.

Indresh, H., Devegowda, G., Ruban, S. & Shivakumar, M. 2013. Effects of high grade bentonite on performance, organ weights and serum biochemistry during aflatoxicosis in broilers. *Veterinary World*. 6(6):313.

Jensen, F.B. 2003. Nitrite disrupts multiple physiological functions in aquatic animals. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*. 135(1):9–24.

Lefevre, S., Jensen, F.B., Huong, D.T.T., Wang, T., Phuong, N.T. & Bayley, M. 2011. Effects of nitrite exposure on functional haemoglobin levels, bimodal respiration, and swimming performance in the facultative air-breathing fish Pangasianodon hypophthalmus. *Aquatic toxicology (Amsterdam, Netherlands)*. 104(1-2):86–93.

Li, J., Li, Y. & Meng, Q. 2010. Removal of nitrate by zero-valent iron and pillared bentonite. *Journal of hazardous materials*. 174(1-3):188–93.

Mackie, A., Woszczynski, M., Farmer, H., Walsh, M.E. & Gagnon, G.A. 2009. *Water Reclamation and Reuse*. 81(10):1406–1419.

Manuel, R., Boerrigter, J., Roques, J., van der Heul, J., van den Bos, R., Flik, G. & van de Vis, H. 2014. Stress in African catfish (*Clarias gariepinus*) following overland transportation. *Fish physiology and biochemistry*. 40(1):33–44.

Miller, J.W. & Atanda, T. 2011. The rise of peri-urban aquaculture in Nigeria. *International Journal of Agricultural Sustainability*. 9(1):274–281.

Munguti, J.M., Kim, J.-D. & Ogello, E.O. 2014. An Overview of Kenyan Aquaculture: Current Status, Challenges, and Opportunities for Future Development. *Fisheries and aquatic sciences*. 17(1):1–11.

Oellermann, L.K. & Hecht, T. 2000. Comparison of the fillet yield, protein content and amino acid profile of Clarias gariepinus and the Clarias gariepinus 5 Heterobranchus longifilis hybrid. *Aquaculture Research*. 31(7):553–556.

Olsen, R.L. & Hasan, M.R. 2012. A limited supply of fishmeal : Impact on future increases in global aquaculture. *Trends in Food Science & Technology*. 27(2):120–128.

Pouomogne, V., Brummett, R.E. & Gatchouko, M. 2010a. Impacts of Aquaculture Development Projects in Western Cameroon. *Journal of Applied Aquaculture*. 22(2):93–108.

Pouomogne, V., Brummett, R.E. & Gatchouko, M. 2010b. Impacts of Aquaculture Development Projects in Western Cameroon. *Journal of Applied Aquaculture*. 22(2):93–108.

Reddy, P.V.B., Rama Rao, K. V & Norenberg, M.D. 2009. Inhibitors of the mitochondrial permeability transition reduce ammonia-induced cell swelling in cultured astrocytes. *Journal of neuroscience research*. 87(12):2677–85.

Schram, E., Roques, J.A.C., Abbink, W., Spanings, T., de Vries, P., Bierman, S., de Vis, H. van & Flik, G. 2010. The impact of elevated water ammonia concentration on physiology, growth and feed intake of African catfish (Clarias gariepinus). *Aquaculture*. 306(1-4):108–115.

Sindilariu, P. 2007. Reduction in effluent nutrient loads from flow-through facilities for trout production : a review.*Aquaculture Research*, 2007, 38, 1005^1036

Singh, A.K., Srivastava, S.C., Ansari, A., Kumar, D. & Singh, R. 2012. Environmental monitoring and health risk assessment of African catfish Clarias gariepinus (Burchell, 1822) cultured in rural ponds, India. *Bulletin of environmental contamination and toxicology*. 89(6):1142–7.

Slamova, R., Trckova, M., Vondruskova, H., Zraly, Z. & Pavlik, I. 2011. Applied Clay Science Clay minerals in animal nutrition. *Applied Clay Science*. 51(4):395–398.

State, T. & Fisheries, W. FAO; 2014. *The State of World Fisheries and Aquaculture 2014*.

Tomasso, J.R. 2012. Environmental nitrite and aquaculture: a perspective. *Aquaculture International*. 20(6):1107–1116.

Wilkie, M.P. 2002. Ammonia excretion and urea handling by fish gills: present understanding and future research challenges. *The Journal of experimental zoology*. 293(3):284–301.

Williford, C.W., Reynolds, W.R. & Quiros, M. 1992.*Applied Clay Science*, 6 (1992) 277-291

World fisheries and aquacultur*e*. FAO; 2012.

Wright, P.A. 2007. *Primitive Fishes*. (Fish Physiology). *Fish Physiology* Vol 26, 283-318Elsevier.

Wright, P.A. & Wood, C.M. 2009. A new paradigm for ammonia excretion in aquatic animals: role of Rhesus (Rh) glycoproteins. *The Journal of experimental biology*. 212(Pt 15):2303–12.

CHAPTER 2

A REVIEW OF INORGANIC NITROGEN PRODUCTS, BENTONITE AND ITS ACID-ACTIVATED FORM

2.1 Introduction

Nitrogenous compounds are essential life-sustaining components to various aquatic microorganisms, and these include ammonia, nitrites and nitrates, which are the most common dissolved inorganic nitrogen forms in aquatic ecosystems. They originate mostly from the natural decomposition of organic matter and metabolic by-products of protein catabolism yielding ammonia-nitrogen (N) (Randall & Tsui 2002; Camargo *et al*., 2005; Wright & Wood 2012). Ammonia-N and nitrite-N are the most toxic forms to aquatic fish, while nitrate-N is relatively less toxic in aquatic systems. These pollutants can disrupt various physiological mechanisms in similar ways although the underlying causes can be substantially different depending on the nitrogenous waste, species, and impact on survival or growth of the aquatic organism (Tilak *et al*. 2007; Bouwman *et al*. 2011) . These compounds are therefore of a ubiquitous concern and are a major limiting factor in the aquaculture industry, since the trend is a move toward more intensive systems with a reliance on higher feed inputs (Bouwman *et al*. 2011; Lefevre *et al*. 2011, 2012).

2.2 Ammonia production

The major end-product of protein catabolism in animals is ammonium and may be excreted as ammonia, urea or uric acid, depending on the animal (Randall & Tsui 2002; Romano & Zeng 2013). Animals cannot store excess amino acids, unlike carbohydrates and lipids, which can be stored as glycogen and triglycerides, respectively. Thus, excess dietary amino acids in the body after absorption for growth and maintenance of protein are preferentially degraded to carbohydrates and lipids in the liver, approximately 40-60% of the nitrogen intake from food is excreted within 24 hrs (Tacon & Forster 2003; Weihrauch *et al.* 2009;

Schram *et al.* 2014). In fish with high-protein diets, their dietary carbon is extracted from the carbon chain of amino acids after the removal of the α-amino group.

Ammonia is highly toxic to fish and unless sufficient water, aerated cultured media or adsorbent material is present to dilute or reduce the excreted ammonia, sub-lethal or lethal levels may accumulate. Under the high stocking densities found in hatcheries and intensive culture facilities, sub-lethal ammonia levels may reduce growth, damage gills and other organs and be a predisposing factor in bacterial gill disease and cause high mortality (Reddy *et al*., 2009; Görg *et al.*,*Ge* 2010a; Häussinger & Görg 2010a;).

Amino acids can be metabolized in the intestine of fish before reaching the liver. However, (Tsui, Hung, Nawata, Wilson, Wright *et al.*, 2009) concluded that deamination of certain amino acids occurs in the intestine of the rainbow trout after feeding. (Wright & Wood, 2009) reported that postprandial amino acid metabolism occurred in the intestine of juvenile *Oxyeleotris marmorata*. Under adverse environmental conditions where ammonia excretion is reduced, some fish can reduce the rate of ammonia production from amino acid catabolism to slow down the production of ammonia internally. During exercise or hypoxia, ammonia can also be produced through the deamination of AMP in the skeletal muscle (Häussinger & Görg, 2010; Schram *et al.*, 2010).

Much of the ammonia produced in fish comes from the α-amino group of amino acids that are catabolized. Several amino acids, including alanine, are converted to glucose by fish hepatocytes, a process regulated hormonally in the same way as it is in mammals (Wright & Wood, 2009; Schram *et al.*, 2010).The rate of alanine and glutamine degradation by catfish hepatocytes can account for 50 and 85% of the total ammonia excreted by the fish (Görg *et al.*, 2010b; Romano & Zeng 2013;). Ammonia can be produced either directly in the cytosol of hepatocytes by specific deaminases (histidase, asparaginase, serine dehydratase, and threonine dehydratase); or via the combined actions (transdeamination) of cytosolic aminotransferases and mitochondrial glutamate dehydrogenase (GDH). Transdeamination is the primary mechanism for catabolism of amino acids in fish liver (Häussinger & Görg 2010b; Wright & Wood 2012). Since GDH is localized exclusively in the matrix of fish liver mitochondria, it is within this compartment that ammonia is released through the route of transdeamination, which involves the deamination of glutamate. Glutaminase, which releases NH_3 from the amide-function of glutamine, is also present in the mitochondrial matrix of some fish species. Thus, ammonia released in the matrix of liver mitochondria has to permeate the mitochondrial membranes before excretion (Görg *et al.,* 2010a; Bouwman *et al.*, 2011).

In pond culture system with abundant water, it is evident that the breakdown of amino acids in the feed and decomposition of organic waste from faeces and uneaten feed resulted in ammonia production. Therefore, efficient feed utilisation with good nutrient absorption can limit ammonia production in aquaculture systems. This can help maintain good water quality and improve fish growth.

2.2.1 Ammonia toxicity and accumulation in aquatic system

Ammonia in water is either unionized ammonia (NH_3) or in the ammonium ion form (NH_4^+). The relative concentration of the two forms in an aqueous solution is mainly affected by pH. Un-ionized ammonia is the more toxic form and predominates when pH is high, while the ammonium ion is relatively nontoxic and predominates when pH is low. However, this concentration increases dramatically as pH increases (Körner *et al.,* 2001; Wright & Wood 2012).

The effect of ammonia on growth (or production) is the most important measure of its sub-lethal effects. From a physiological basis, it represents the summation of a number of individual effects on various systems, organs and biochemical pathways. From an ecological basis, changes in growth represent changes in the basic flow of energy and may have significant effects on the ability of an organism to survive and reproduce (Schram *et al*., 2010; Romano & Zeng, 2013).

Ammonia is more toxic to fish at elevated pH and temperature, which shifts the ionization equilibrium towards the toxic, unionized form. The risk of elevated pH and unionized ammonia is greater in poorly buffered, low alkalinity ponds at high temperatures (Grommen *et al.*, 2002; Kumlu & Eroldog 2004;). It is generally accepted that metabolic processes of animals increase with increasing temperature. These metabolic processes include food consumption, digestion (enzyme activity), growth, ammonia excretion, and oxygen consumption.

Temperature, through its effect on metabolic rate, has been shown to be directly related to ammonia excretion rates of fish. Compiled normalized data on acute toxicity in various species of fish indicate that the effect of increased temperature on toxicity is minor between 3^0C and 30^0C in freshwater systems (Randall & Tsui 2002; Kumlu & Eroldog 2004; Romano & Zeng 2013). However, as metabolic activities and environmental factors increase ammonia accumulation and toxicity, ammonia toxicity poses a dangerous threat to the growth and survival of cultured fish, and its accumulation needs to be maintained below the toxic level for the cultured fish.

2.3 Nitrite production

Nitrite is an intermediate and important product in bacterial nitrification and denitrification processes in the nitrogen cycle. High concentrations of nitrite can be found in water receiving nitrogenous effluents, in various hypoxic environments or in effluents from industries producing metals, dyes and celluloid (Kroupova, Machova & Svobodova, 2005). High nitrite concentrations cause great problems in the intensive culture of commercial fish species and ornamental fish. In these production systems, where recirculating water systems that remove waste ammonia from water are commonly used, an increase in nitrite toxicity in the system is usually prevalent (Philips *et al.*, 2002; Tomasso, 2012a). This method of ammonia detoxification usually leads to the hazard of possible incomplete oxidation of ammonia accompanied by the accumulation of nitrite in the system. Upon the initiation of the nitrification process in biological filters or during imbalance in the process, concentrations of nitrite can reach (50 mg/L) or more (Grommen *et al.*, 2002; Jensen, 2003) On the other hand, when nitrification in biological filters is established or during imbalance in the process, concentrations of nitrite can increase to toxic levels, which may result in mass fish mortality (Tomasso, 2012a).

In addition to aforementioned abiotic factors, the high density of fish in aquatic ponds is associated with a large production of waste products, ammonia excretion, and nitrite production or their accumulation (Roques *et al.*, 2013).Thus, manipulation of aquafeeds' to enhance nutrient absorption, utilisation and reduce feed wastage for organic decomposition could be a useful tool to control accumulation and toxicity (Wright & Wood, 2012).

2.3.1 Uptake and accumulation of nitrite by freshwater aquatic organisms

Aquatic animals are at higher risk of nitrite intoxication than terrestrial animals, since nitrite in ambient water can be actively taken up across the gill epithelium and can accumulate to very high concentrations in the body fluids. Freshwater fishes and crustaceans are hyperosmotic to their environments and require an active uptake of ions across the gills to compensate for ions lost with the urine and via passive efflux across the gills (Kroupova *et al.*, 2005; Jensen & Hansen, 2011). Boudreaux *et al*. (2007) explained the current view on mechanisms of ion uptake in the gills of freshwater fish that an H-ATPase situated in the apical membrane of epithelial cells extrudes protons and creates the driving force for Na^+ entry via sodium channels. The protons originate from hydration of CO_2 to H^+ and HCO_3^-, catalysed by carbonic anhydrase inside the epithelial cells, and the formed HCO_3^- serves as counter ion for Cl^- uptake via an apical Cl^- / HCO_3^- exchange mechanism. In this way, the H-ATPase is thought to energise Cl^- uptake from diluted fresh water by creating a favourable gradient for the apical exit of HCO_3^- ; thus, freshwater fish are at higher risk of nitrite toxicity as compared to marine fish (Boudreaux *et al.*, 2007; Jensen & Hansen, 2011, Roques *et al.*, 2013).

Nitrite exists in two forms – nitrite (NO_2^-) and nitrous acid (HNO_2). These forms are primarily controlled by pH. Virtually all of the total nitrite present at pH levels of cultured aquatic organisms is in the ionized form (Kroupova *et al.*, 2005). The pH-mediated ionization of nitrite is the opposite of that of ammonia, in which the percentage of unionized ammonia (NH_3) increases with increasing pH, while that of the ionized ammonium (NH_4^+) decreases (Tomasso, 2012b).

Tomasso (2012b) suggested that the increased tolerance to nitrite in seawater was due to the increased level of calcium in seawater, relative to fresh water. The author also felt that calcium could not explain all of the observed differences and other factors were possibly involved. (Jensen, 2003) worked on coho salmon (*Oncorhynchus kisutch*) and indicated that environmental chloride was responsible for the increased tolerance to nitrite, and the investigator speculated that the mechanism might be a competitive inhibition for uptake of nitrite. A shared uptake route for nitrite and chloride is also supported by the fact that fish with high brachial Cl^- uptake rates (e.g. rainbow trout, perch, pike, channel catfish) are more sensitive to nitrite than species with low uptake rates (eel, carp, tench) (Jensen, 2003; Jensen & Hansen, 2011). Furthermore, environmental hypercapnia (elevation of ambient CO_2 tension), which leads to an acid base regulatory decrease in brachial Cl^- influx, also reduces NO_2^- uptake (Philips *et al.*, 2002).

Studies on fish and crustaceans revealed that nitrite induced a large variety of physiological disturbances, many of which arise from toxicity. However, freshwater fish are more sensitive to nitrite toxicity than marine fish; therefore, adequate measures are required to reduce toxicity of nitrite in freshwater fish production (Jensen, 2003; Boudreaux *et al.*, 2007; Tomasso, 2012a).

2.3.2 Abiotic factors influencing nitrite accumulation in aquatic water.

a. pH

Wee *et al.* (2007) found in their experiments that the pH of aquatic water was the decisive parameter in nitrite-oxidizing bacteria activity inhibition. Gu *et al.*, (2011), on the other hand, came to the conclusion from sewage plant monitoring data with lab-scale reactor tests that nitrite accumulation is independent of the pH, but is rather due to low dissolved oxygen, suppressing Nitrobacter. During denitrification, nitrite production occurs significantly at high pH ranges (Jensen, 2003). Also, Wuertz *et al.* (2013) observed that a more alkaline pH (>7.8) resulted in a lower denitrification rate and in an increasing nitrite concentration in the effluent (26 mg N L^{-1}at pH 9). Therefore, maintaining a neutral or relatively slighty acidic pH can ameliorate the accumulation of nitrite (Lefevre *et al.*, 2012).

b. Ammonium (NH_4^+) or free ammonia (NH_3)

The equilibrium of ammonium and ammonia in water is dependent on the pH. Ammonium can cause nitrite accumulation (e.g.,(Philips *et al.*, 2002; Jensen & Hansen, 2011; Tomasso, 2012b) but the effect of free ammonia (FA) seems to be more pronounced (Tomasso, 2012b). FA is a competitive inhibitor of nitrite oxido-reductase activity, which is located on the cell membrane of nitrite oxidative bacteria (NOB) (Camargo *et al.*, 2005). According to Philips *et al.* (2002), the slightly less basic pH optimum of Nitrobacter (7.2–7.6) compared to Nitrosomonas (7.9–8.2) appears to be reflected in the higher sensitivity of Nitrobacter to FA, which is aggravated at a higher pH.

Philips *et al*. (2002) established the threshold of inhibition of NOB between 0.5 and 3 mg NH_3-N mg^{-1} viable NOB biomass. The inhibiting effect of FA on NOB may be the result of a combination of several factors, such as initial NH_4^+ concentration, pH and temperature (Jensen, 2003). It is further said that the inhibition by FA is such that the effect of temperature, alkalinity and ammonium load is masked when the concentration of free ammonia is above certain values (Tomasso, 2012a).

c. Temperature

At temperatures above 25°C, nitrite ions may become a severe problem. Philips *et al.*, (2002) determined that the ammonia and nitrite oxidation rates increase 2.6 and 1.8 times per 10°C in a physiologically relevant temperature range. The growth rates of the nitrifying bacteria executing the two steps of nitrification, the ammonia oxidative bacteria (AOB) and nitrite oxidative bacteria (NOB), change with temperature, as the optimum temperature for nitrition is higher than that for nitratation (Camargo *et al.*, 2005; Alonso & Camargo, 2006).

The maximum specific growth rate of <u>Nitrobacter</u> is significantly higher than that of <u>Nitrosomonas</u> temperatures between 10 – 20 °C. At temperatures higher than 25 °C, however, the maximum specific growth rate of <u>Nitrobacter</u> is approximately in the same range as that of <u>Nitrosomonas</u>, possibly leading to nitrite accumulation (Camargo & Alonso, 2006; van Rijn *et* *al*., 2006). At higher temperatures, the growth rate of the NOB is lower than that of the AOB (Alonso & Camargo, 2006; Gu *et al*., 2011). However, describing the equilibrium of NH_3 / NH_4^+ and of HNO_2 / NO_2^-, temperature plays a role in the respective concentrations in water. An increase in temperature causes an increase in FA concentration, which results in higher activity of nitrifying bacteria and accumulation of nitrite (Philips *et al*., 2002).

d. Dissolve oxygen concentration (DO)

The dissolved oxygen concentration is an important parameter for both AOB and NOB. Low oxygen concentrations induced, for instance, a marked decrease in the rate of NO_2^-production by pure cultures of *Nitrosomonas spp* (Philips *et al*., 2002; van Rijn *et al*., 2006a). However, AOB seem to be more robust towards low DO than NOB. In other words, oxygen deficiency due to low DO more significantly influences the activity of NOB than that of AOB (Jensen, 2003). The ammonia-oxidising population had a greater specific affinity for O_2 than the nitrite oxidising bacteria. Cessation of the oxygen supply resulted in an immediate accumulation of nitrite in the mixed cultures of *Nitrosomonas europaea* and *N. hamburgensis* that had been growing at 80% O_2 saturation (Tomasso, 2012a). Apparently, reduction in concentration of DO in a cultured media resulted in an accumulation of nitrite.

2.4 Nitrate production

Ammonium tends to be oxidized to nitrate by aerobic chemoautotrophic bacteria (Nitrosomonas and Nitrobacter, primarily), even if levels of dissolved oxygen decline to a value as low as 1.0 mg O_2/L (Camargo *et al*., 2005; Li, *et al*., 2010). Concentration of nitrate in freshwater and marine ecosystems usually is higher than those of ammonium and nitrite (Scott & Crunkilton, 2000; Álonso & Camargo, 2013). Nitrate (but also ammonium and nitrite) may, however, be removed from water by aquatic plants, algae and bacteria, which assimilate it as a source of nitrogen (Li *et al*., 2010). However, when concentrations of dissolved oxygen decrease to minimum values, facultative anaerobic bacteria (e.g. Pseudomonas, Micrococcus, Bacillus) can utilize nitrate as a terminal acceptor of electrons, resulting in the ultimate ion form of nitrate (Camargo *et al*., 2005; Adeyemo, *et al*., 2008).

Besides production through natural sources, inorganic nitrogen (NH^{+}_{4}, NO^{-}_{2}, NO^{-}_{3}) can enter aquatic ecosystems via anthropogenic sources such as animal farming, urban and agricultural runoff, industrial wastes, and sewage effluents (Ling *et al.*, 2011; Chen *et al.*, 2012). Human activities, which include the extensive use of nitrogen fertilizer and combustion of fossil fuels, have increased the atmospheric deposition of inorganic nitrogen (mainly in the form of NO^{-}_{3}) (Camargo *et al.*, 2005; Camargo & Alonso, 2006). Thus, concentrations of nitrate in ground and surface waters are increasing globally, causing one of the most prevalent environmental problems responsible for water quality degradation, which pose a threat to aquaculture and require adequate management (Bostock *et al.*, 2010; Bouwman *et al.*, 2011; Zhang *et al.*, 2013).

2.4.1 Nitrate toxicity and accumulation

Nitrate does not normally reach toxic concentrations in natural environments or in recirculating systems with high water exchange and has, therefore, received comparatively less attention as a material water quality hazard (Guillette & Edwards, 2005; Junaidi & Hashida, 2010). The absence of patho-physiological effects in most aquatic species at ecologically-relevant concentrations of nitrate rationalizes the belief that nitrate is relatively non-toxic (Camargo *et al.*, 2005; Junaidi & Hashida, 2010). While nitrate is less toxic than ammonia or nitrite on mg/L basis, nitrate commonly rises to levels far in excess of those of the other compounds in intensive aquaculture environments with limited water exchange (Li *et al.*, 2010; Álonso & Camargo, 2013; Zhang *et al.*, 2013).

The main toxic action of nitrate on aquatic animals is due to the conversion of oxygen-carrying pigments (e.g., haemoglobin, haemocyanin) to forms that are incapable of carrying oxygen (e.g., met-haemoglobin) (Scott & Crunkilton 2000; Adeyemo *et al*., 2008; Schram *et al*., 2014). Nevertheless, owing to the low brachial permeability to nitrate, the NO_{3}^{-} uptake in aquatic animals seems to be more limited than the uptake of NH_{4}^{-} and NO_{2}^{-}, contributing to the relatively low toxicity of nitrate (Jensen, 2003; Cho *et al.*, 2010). Nitrate toxicity to freshwater and marine fish increases with increasing nitrate concentrations and exposure times (Scott & Crunkilton, 2000). Furthermore, nitrate toxicity can depend upon the cationic composition of the solution; freshwater fish appear to be more sensitive to nitrate toxicity than marine fish.

The processes of nitrate-N uptake in freshwater aquatic organism is substantially less complex since active transport does not appear to be involved, while gill permeability to nitrate-N is likely low, thereby reducing passive diffusion. Camargo & Alonso (2006) reported that the channel catfish *Ictalurus punctatus* was able to tolerate a nitrate concentration of 90 mg NO_3-N/L without affecting their growth and feeding activity after an exposure of 164 days and concluded that the acute toxicity of nitrate to *I. punctatus* was independent of water temperature.

Consequently, it is believed that one of the main reasons nitrate-N is less toxic is due to reduced hemolymph accumulation (Jensen, 1996; Adeyemo *et al.*, 2008; Zhang *et al.*, 2013). However, prolonged exposure to elevated levels of nitrate may decrease the immune response, induce haematological and biochemical changes indicative of a pathologic response, and may increase mortality.

2.5 Clay minerals in aquaculture

Clay minerals (aluminosilicates) have found its applications in various disciplines since its discovery in the 17th century. The zeolite and bentonite group of minerals stands out among the rest, and its physical and chemical characteristics contributed to many areas of applications. Their abundance and availability has aroused considerable interest in research from various disciplines (Article, 2004; Safaeikatouli *et al.*, 2010; Tang *et al.*, 2014).

The general applications of the clay minerals (aluminosilicates) in the various processes, which include industries engineering, petroleum discovery, recovery and refining, agriculture and others, are attributed to their structure and composition. Their particle size, surface chemistry, particle shape, surface area, and other physical and chemical properties are related to a particular application. Other characteristics such as viscosity; colour; plasticity; green, dry and fired strength; absorption and adsorption; abrasion; and others are attributed to its applications (Murray, 1991, 2000; Al-anber, 2010).

The aluminosilicates clays are of different types (Figure 2.1) and each is characterized with different properties that enhance its application. The tectosilicates and phylosilicates have gain wide applications over the years in aquaculture, with bentonites and zeolites as the widely used clay types of the aluminosilicates (Slamova *et al*., 2011; Zamparas *et al*., 2012).

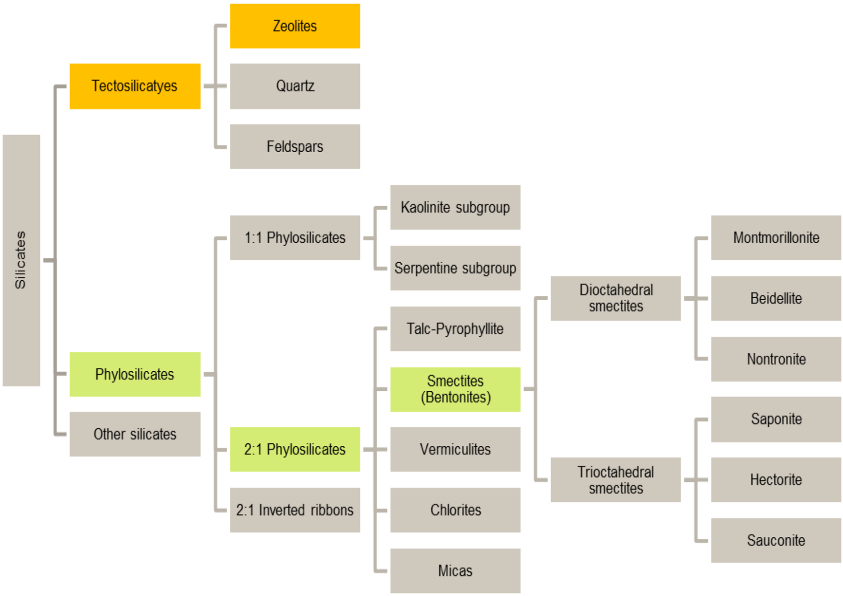

Figure 2. 3 : Classification of aluminosilicates

Clinoptilolite, clay from zeolites, has been found very effective in removing ammonia from water by means of its excellent ion exchange capacity. Many researchers have investigated ammonia removal from water streams by means of ion exchange through direct applications and varied concentrations (Williford *et al*., 1992; Briggs 1996; Ashrafizadeh *et al*., 2008; Álonso 2009; Malekian & Eslamian 2011; Zhou & Boyd 2014b). The particle size and concentration affects the rate of adsorption and absorption in clinoptilolite. The rate of absorption increases with concentration of application and smaller particle size in aqueous solution. The larger surface area in particle size and ability of ions to move freely enhances the ion exchange capacity, therefore increasing the absorption of nitrogenous compounds (Bernal 1993; Barlokov 2008). Furthermore, factors that influences removal of nitrogenous compounds from aquatic system by clay minerals includes pH, temperature, cation exchange capacity (CEC), and presence of other exchangeable ions in solution (Ashrafizadeh *et al.*, 2008; Doktorgrades 2009; Álonso 2009; Jović-jovičić *et al.*, 2010).

Pollution by heavy metals is of great concern due to the increased awareness of the potentially hazardous effects of elevated levels of these materials in the environment. Heavy metals can pose a threat to aquatic life and can enter the food chain, thus, posing a threat to humans (Salem & Akbari 2011; Taylor *et al.*, 2013). Adsorption has been proven to be a successful method for removal of heavy metals from wastewater. Activated carbon is highly effective in adsorbing heavy metals from wastewater but its high cost limits its use. Adsorption using aluminosilicates clays is an inexpensive material and makes the removal of heavy metals from wastewater an economically viable alternative (Kaya & Oren 2005; Al-anber 2010; Mesci 2011; Padilla-Ortega *et al.,* 2013b).

Clay mineral can increase pellet durability when added to complete feed at concentrations of between 1 and 5% (Asgharimoghadam *et al.*, 2012). Since bentonite is authorized for use as anti-caking agents in food (Opinion 2012) and can be reasonably assumed to demonstrate similar properties when applied to feed or feed materials. Dietary clay mineral in feed contaminated by radioactive fallout reduced levels of radiocaesium in animals and their products (Phillips 1999; Indresh *et al.,* 2013; Zychowski *et al.*, 2013). Clay minerals in animal diet act as gut protectants (enterosorbents), which rapidly and preferentially bind aflatoxins from the digestive tract and thus reduce their absorption into the organism (Phillips 1999; Carraro *et al.*, 2014). In that manner, adverse effect of aflatoxins on efficiency and liver function is minimized without marked defects in mineral metabolism of the animals (Ellis 2000; Amany 2009).Using clay minerals, mainly montmorillonite, to alleviate aflatoxin toxicity was started in the late 1970s (Zychowski *et al.*, 2013).

Dietary clay minerals have a role to play in aquaculture. Their accessibility and low cost as compared to other absorbents in feed manufacturing created an edge. Dietary zeolites have received wide research in the past, while research on bentonite application as a feed grade is inadequate.

2.6 Origin of Bentonite clay

Bentonites are clays whose properties, such as crystal structure and size, cation exchange capacity (CEC), hydration and swelling, thixotropy, binding capacity, impermeability, plasticity and tendency to react with organic compounds make them advantageous for a variety of applications (Ladhari *et al.,* 2010; Li *et al.*, 2010; Theng, 2012). The rocks in which these smectite minerals are dominant are bentonite, which is used to describe the industrial mineral. It is a clay material altered from a glassy igneous material, usually a tuff or volcanic ash (Slamova, *et al.*, 2011). Smectites are 2:1 type of aluminosilicates having crystal lattice that consists of two-dimensional layers where a central octahedral sheet of alumina is fused to two external silicate layers (Al-anber, 2010). Isomorphic substitution within the layers generates negative charges that are counterbalanced by easily replaceable alkali or alkaline earth cations. These cations are defined as exchangeable cations (Lünsdorf *et al*., 2000; Hua *et al.*, 2013).

The smectite group of clay minerals consists of several clay minerals, but the two most important are sodium montmorillonite and calcium montmorillonite (Eya *et al.,* 2008; Carraro *et al.*, 2014). Both the octahedral and tetrahedral sheets in smectites can have substitutions, which creates a charge imbalance in the 2:1 layer. Alumina substitutes for silica in the tetrahedral sheet and iron and magnesium substitute for aluminium in the octahedral sheet (Lira *et al.*, 2014a; Safaei *et al.*, 2014). This net positive charge deficiency is balanced by exchangeable cations absorbed between the unit layers and on the edges. If the dominant exchangeable cation is sodium, then the mineral in the smectite group is sodium montmorillonite, and if it is predominantly calcium, it is calcium montmorillonite (Murray, 1991, 2000; Jović-jovičić *et al.*, 2010).

2.6.*1* Structure and properties of smectites (montmorillonite)

Smectite is a 2:1 layer clay mineral and has two silica tetrahedral (T) sheets bonded to a central alumina octahedral (O) sheet

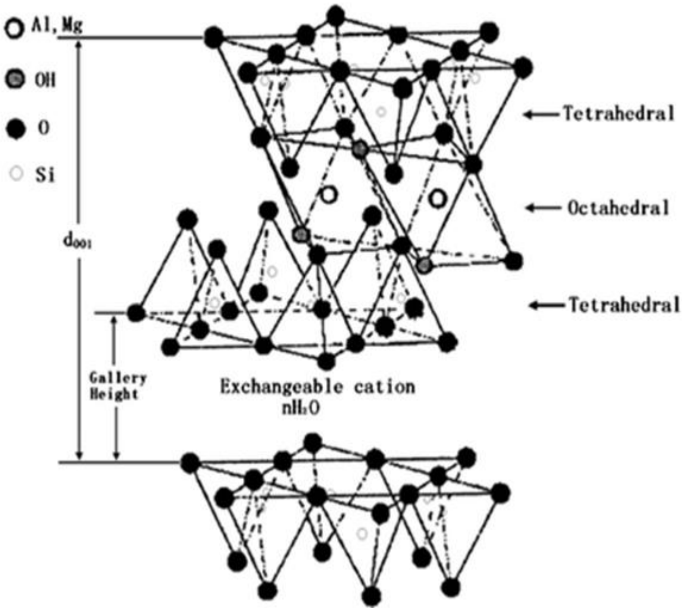

Figure 2. 4 : Bentonite folder - physiochemical (Murray, 1991)

The net negative charge of 2:1 (TOT) layers arising from the isomorphic substitution in the octahedral sheets of Fe^{2+} and Mg^{2+} for Al^{3+} and in tetrahedral sheets of Al^{3+} for Si^{4+} is balanced by exchangeable cations such as Na^{+} andCa^{2+} located between the layers and around the edges (Mesubi *et al.*, 2008; Slamova *et al.*, 2011). The mineral is called Na-smectite (NaS) or Ca-smectite (CaS) corresponding to the exchangeable cation, which is Na^{+} or Ca^{+}. Industrial bentonites predominantly contain either Na-montmorillonite (NaM) or Ca-montmorillonite (CaM), and to much lesser extent other smectites. Bentonites are called Na-bentonite (NaB), Ca-bentonite (CaB) and NaCa-bentonite (NaCaB) corresponding to the abundance of the exchangeable cations. The equivalent amount of exchangeable cations in one kilogram smectite as well as other clay minerals and clays is defined as cation exchange capacity (CEC) (Murray, 2000; Al-anber, 2010).

The high charge on the smectite layer is satisfied by water layers containing cations such as sodium, calcium, magnesium, iron and lithium. Smectites have a high base exchange capacity that generally is of the order of 60-100 mEq/100 g of clay (Murray, 1991). Smectites are normally very thin flakes of extremely small particle size, which gives the material a high surface area (Zamparas *et al.*, 2012). These characteristics give smectites a high degree of absorbency for many materials and when mixed with water, the high charge and fine particle size give the fluid a high viscosity (Luckham & Rossi, 1999). The colour of smectites can vary from white to tan to brown or brownish green or blue-green in colour, which also controls the use in some instances (Slamova *et al.*, 2011).

The formula for montmorillonite is

$(Si_{7.8}\,Al_{0.2})(Al_{3.4}Mg_{0.6})\,O_{20}\,(OH)_4$

The theoretical composition without the interlayer material is

SiO_2, 66.7%, Al_2O_3, 28.3%, H_2O, 5%

The above formula indicates that there is substitution of Si_4^+ by Al_3^+ in the tetrahedral layer and of Al_3^+ by Mg_2^+ in the octahedral layer. Thus, the net layer charge of montmorillonite is:

$[7.8\,(+4)]+[0.2(+3)]+[3.4\,(+3)]+[0.6\,(+2)]+[20\,(-2)]+[4\,(-1)]=-0.8$ charge/unit cell.

The resulting negative net charge is balanced by exchangeable cations adsorbed between the unit layers and around their edges (Murray, 1991; Al-anber, 2010).

Montmorillonite is a clay mineral with substantial isomorphic substitution. Exchangeable cations in the 2:1 layers balance the negative charges generated by isomorphic substitution. The uptake kinetics of cation exchange is fast and the cations such as Na^+ and Ca^{2+} form outer-sphere surface complexes, which are easily changed to solute ion by varying the cation-ionic solution of composition (Luckham & Rossi, 1999; Attramadal *et al.*, 2012). In addition, to cation exchange there is a pH-dependent uptake of metals on montmorillonite. In this adsorption process, adsorbate ions are bound to the clay surface by sharing several ligands (generally oxygen) with adsorbent cations as isolated complexes. With increasing pH or adsorbate cation concentration, metal precipitation can occur (Shu-li *et al.*, 2009; Slamova *et al.*, 2011).

In the montmorillonite structure, interlayer swelling occurs when it is exposed to water. The swelling procedure depends on valences and atomic radii of the exchangeable cations (Jović-jovičić *et al.*, 2010). Al and Si atoms exposed to the crystallite edges are partially hydrolysed to silanol (SiOH) and aluminol (AlOH) groups. These unsaturated edge sites are much more reactive than the saturated basal sites (Murray, 2000; Al-anber, 2010). Montmorillonite of the bentonite clay possesses a high adsorption capacity over other natural clay minerals. This characteristic enhances its wide range of applications since its earliest discovery and in recent times (Slamova *et al.*, 2011; Zamparas *et al.*, 2012).

2.7 Acid-activated Bentonite.

Acid activation is treatment of clay minerals with inorganic acid and usually at high temperature. Acid treatment of clay minerals is an important control over mineral weathering and genesis (Jovanovi & Janakovi 1991; Foletto *et al*., 2011; Faghihian & Mohammadi 2013). Acid activation of clay minerals is a process for selectively leaching out part of the starting material by utilizing solubility differences towards the leaching solution. Acid-leached products of clay minerals consist mainly of amorphous silica (Faghihian & Mohammadi, 2013). However, the chemical composition of the reaction products from the same type of clay minerals can depend on the impurities present. Acid activation of bentonite refers to the controlled, partial dissolution of the raw materials by mineral acids (Noyan *et a*l., 2007; Önal 2007). Upon acid treatment, cations are leached from the octahedral and tetrahedral sheets, impurities such as calcite are dissolved, and exchangeable cations are replaced by hydrogen ions (Önal & Sarıkaya, 2007; Faghihian & Mohammadi, 2013). Simultaneously, the edges of platelets open. All these changes result in an increase in the specific surface area and pore volume of the clays (Önal & Sarıkaya, 2007; Makhoukhi *et al.*, 2009; Liang *et al.*, 2013).

The treatment of clay minerals with concentrated inorganic acids usually at high temperature replaces exchangeable cations with H^+ ions with simultaneous partial elimination of Al^{3+} and other cations from both tetrahedral and octahedral sites, but leaving the SiO_4 groups largely intact (Noyan *et al.*, 2007). It was reported that acid activation followed by thermal treatment increases the adsorbent capacity (Jeenpadiphat & Tungasmita, 2013). This process generally increases the surface area and acidity of the clay minerals, along with the elimination of several mineral impurities and partial dissolution of the external layers (Motlagh *et al.*, 2011). The change of surface area and porous structure of the clays due to acid treatment depends on the particular clay mineral, other clay minerals and non-clay minerals. The overall chemical composition, the type of cations between the layers, acid treatment, process temperature, process period, and other environmental factors determine the properties of the acid-activated bentonite (Noyan *et al.*, 2007; Sar, 2007; Tomić *et al.*, 2011; Al-Khatib *et al.*, 2012).

2.8 Application of bentonite in animal production

Bentonites (E558) are currently authorised for use as food additives, used as binders, anti-caking agents and coagulants under the category technological additives to a maximum of 20 g/kg feeding stuffs (Opinion 2012, EFSA, 2012). Bentonites can increase pellet durability when added to a complete feed at concentrations of between 1% and 5%. Bentonites are authorised for use as anti-caking agents in food without restriction and can be reasonably assumed to demonstrate similar properties when applied to feed or feed materials. Bentonites added to feed contaminated by radioactive fallout or made available to grazing animals reduced levels of radio caesium in animals and their products (Phillips, 1999; Indresh *et al.*, 2013; Zychowski *et al.*, 2013).

Bentonites in animal diets act as gut protectants (enterosorbents), which rapidly and preferentially bind aflatoxins from the digestive tract and thus reduce their absorption into the organism (Phillips, 1999; Carraro *et al.*, 2014b). In that manner, an adverse effect of aflatoxins on efficiency and liver function is minimized without marked defects in mineral metabolism of the animals (Ellis, 2000; Amany, 2009). Using clay minerals, mainly montmorillonite, to alleviate aflatoxin toxicity was started in the late 1970s (Zychowski *et al.*, 2013). Bentonites were tested in numerous animal feed trials, which included chickens, turkey poults, ducklings, pigs, lambs, mink, trout, tilapia, dairy cows, and goats (Murray, 1991, 2000; Safaeikatouli *et al.*, 2010; Indresh *et al.*, 2013). Bentonites are authorised for use without restriction as anti-caking agents in food (EFSA, 2012). It showed similar properties when applied to feed or feed materials. The high cation exchange capacity and adsorption properties further demonstrated its potential as feed additive in aquafeeds' to improve growth and bind nitrogenous waste product in fish production.

2.9 Effect of bentonite on adsorption of protein and nucleic acid.

Clay minerals, i.e. bentonite (montmorillonite), can adsorb various biomolecules, including proteins, DNAs and RNAs in natural environment, and this ability has found applications in the earth's biochemical evolution and origin of life, the drug delivery systems, enzyme immobilization, protein fractionation and even gene-engineering (Yu *et al.*, 2013). Binding forces, such as cation exchange, electrostatic interactions, hydrophobic affinity, hydrogen bonding and van der Waals forces, are responsible for protein adsorption on clay minerals (Yu *et al*., 2013; Carraro *et al.*, 2014;).

The permanent negative and variable charge exhibited by clay minerals due to exchangeable cations, enhance the adsorbtion of protein molecules through electrostatic interactions, which depend on the negatively and positively charged states of protein molecules, pH of the environment and iso-electric point (Yu *et al*., 2013; Lira *et al*., 2014). Types of clay minerals play a critical role in binding amounts of proteins and adsorption mechanisms. Clay properties such as surface area, cation exchange capacity, charge density and degree of swelling are generally related to the maximum adsorption amount. Generally, montmorillonite exhibits higher adsorption capacity over some other types of clay minerals (Safaeikatouli *et al.*, 2012; Yu *et al.*, 2013).

According to Eya *et al.*, (2008), during dietary application of bentonite on juvenile trout, decreased fat content and increased protein content were observed. The higher moisture content of fish fed diet with 10% bentonite corresponded with lower fat content. The significant increase in whole body crude protein content with diets containing bentonite correlated with improved feed efficiency suggests that bentonite contributed towards a more efficient conversion of feedstuff nitrogen to animal protein. Also, Carraro *et al.*, (2014) noticed the subtraction of protein from the milk linearly increased with the amount of bentonite, up to approximately 40% of the initial protein content, during removal of aflatoxin from contaminated milk. Benetoli *et al.*, (2007), Safaeikatouli *et al.*, (2012), Carraro *et al.*, (2014) and Lira *et al.*, (2014b) all agreed that the sequestration of proteins is facilitated at low pH, when proteins are protonated and easily adsorbed.

2.10 Concluding remark

The uses of bentonite powders depend on quality and quantity of their smectites and other minerals, with the type of valence and the amount of exchangeable cations (Murray, 2000; Slamova *et al.*, 2011). Bentonite may be used both naturally and after some physicochemical treatments such as acid activation, ion exchange, and heating according to the application areas (Kaya & Oren, 2005; Makhoukhi *et al.*, 2009; Foletto *et al.*, 2011; Faghihian & Mohammadi, 2013). The physical (binding), chemical and adsorption properties of natural bentonite depend on the crystal structure of their constituent clay minerals. Bentonites are rich in smectite, regardless of their mode of origin (Gu *et al.*, 2011). In addition to smectite, bentonites also contain quartz, mica/illite, feldspar, calcite, carbonates, plagioclase, etc. (Kaya & Oren 2005; Jović-jovičić *et al.* ,2010; Gunn *et al.*, 2013).Natural bentonites usually do not exhibit sufficient adsorption and binding properties and, therefore, they may be treated in order to enhance these properties. Acid-activated bentonite powders have been used in diverse applications, such as adsorbent of heavy metals from aqueous solution, catalyst and bleaching earth, and also in the preparations of carbonless copy paper, electrode, pillared clay, organoclay, and nano composites (Vuković *et al.*, 2005; Yener & Bic, 2012; Yener *et al.*, 2012).

Water quality is an important tool for successful aquaculture practices, in which nitrogenous waste products form an integral part of water pollution in fish culture. Thus, management of water quality through the use of bentonite and acid activated form as feed additives will be tested in this research on *Clarias gariepinus*. Fish growth, nutrient utilisation and other parameters will be considered to validate application of dietary inclusion of clay minerals in aquafeeds'.

2.11 References

Adeyemo, O.K., Ajani, F., Adedeji, O.B. & Ajiboye, O.O. 2008. Acute toxicity and blood profile of adult clarias gariepinus exposed to lead nitrate. *Internet Journal of Hematology*. 4(2).

Al-anber, M.A. 2010. Removal of high-level Fe $^{3+}$ from aqueous solution using natural inorganic materials : Bentonite (NB) and quartz (NQ). *Desalination*. 250(3):885–891.

Al-Khatib, L., Fraige, F., Al-Hwaiti, M. & Al-Khashman, O. 2012. Adsorption from aqueous solution onto natural and acid activated bentonite. *American Journal of Environmental Sciences*. 8(5):510–522.

Álonso, A., 2009. International Science conference 4. , IV(June), pp.6–11.

Alonso, A. & Camargo, J.A. 2006. Short Communication Toxicity of Nitrite to Three Species of Freshwater Invertebrates. *Environmental Toxicology DOI 10.1002/tox*90–94.

Álonso, A. & Camargo, J.A. 2013. Nitrate causes deleterious effects on the behaviour and reproduction of the aquatic snail Potamopyrgus antipodarum (Hydrobiidae, Mollusca). *Environmental science and pollution research international*. 20(8):5388–96.

Amany, B. 2009. Pathological studies on effects of aflatoxin . *Egypt J.comp.Path & Clinic. Path* Vol 22(1), 179 -193.

Article, R. 2004. Kaolin , bentonite , and zeolites as feed supplements for animals : health advantages and risks. *Vet. Med. – Czech*, 49, 2004 (10): 389–399

Ashrafizadeh, S.N., Khorasani, Z. & Gorjiara, M., 2008. Ammonia Removal from Aqueous Solutions by Iranian Natural Zeolite. *Separation Science and Technology*, 43(4), pp.960–978

Attramadal, K.J.K., Tøndel, B., Salvesen, I., Øie, G., Vadstein, O. & Olsen, Y. 2012. Aquacultural Engineering Ceramic clay reduces the load of organic matter and bacteria in marine fish larval culture tanks. *Aquacultural Engineering*. 49:23–34.

Barlokov, D., 2008. Natural zeolites in the water treatment process. , *Slovak Journal of Engineering*, 6: 12, pp.8–12.

Benetoli, L.O.B., de Souza, C.M.D., da Silva, K.L., de Souza, I.G., de Santana, H., Paesano, A., da Costa, A.C.S., Zaia, C.T.B. V. 2007. Amino acid interaction with and adsorption on clays:

FT-IR and Mössbauer spectroscopy and X-ray diffractometry investigations. *Origins of life and evolution of the biosphere : the journal of the International Society for the Study of the Origin of Life*. 37(6):479–93.

Bernal, M.E., 1993. Natural zeoilite and sepiolite as ammonium and ammonia adsorbent materials, *Bioresource Technology* 43 (1993) 27-33

Bostock, J., McAndrew, B., Richards, R., Jauncey, K., Telfer, T., Lorenzen, K., Little, D., Ross, L.,. 2010. Aquaculture: global status and trends. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*. 365(1554):2897–912.

Boudreaux, P.J., Ferrara, A.M. & Fontenot, Q.C. 2007. Chloride inhibition of nitrite uptake for non-teleost Actinopterygiian fishes. *Comparative biochemistry and physiology. Part A, Molecular & integrative physiology*. 147(2):420–3.

Bouwman, a. F., Pawłowski, M., Liu, C., Beusen, a. H.W., Shumway, S.E., Glibert, P.M. & Overbeek, C.C. 2011. Global Hindcasts and Future Projections of Coastal Nitrogen and Phosphorus Loads Due to Shellfish and Seaweed Aquaculture. *Reviews in Fisheries Science*. 19(4):331–357.

Briggs, M.R.P., 1996. The effects of zeolites and other alumino-silicate clays on water quality at various salinities Univ of Maryland. , pp.301–312.

Camargo, J. a & Alonso, A. 2006. Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: A global assessment. *Environment international*. 32(6):831–49.

Camargo, J. a, Alonso, A. & Salamanca, A. 2005. Nitrate toxicity to aquatic animals: a review with new data for freshwater invertebrates. *Chemosphere*. 58(9):1255–67.

Carraro, A., De Giacomo, A., Giannossi, M.L., Medici, L., Muscarella, M., Palazzo, L., Quaranta, V., Summa, V. 2014a. Clay minerals as adsorbents of aflatoxin M1 from contaminated milk and effects on milk quality. *Applied Clay Science*. 88-89:92–99.

Chen, L., Yu, S., Huang, L. & Wang, G. 2012. Impact of environmental conditions on the removal of Ni(II) from aqueous solution to bentonite/iron oxide magnetic composites. *Journal of Radioanalytical and Nuclear Chemistry*. 292(3):1181–1191.

Cho, D.W., Chon, C.-M., Jeon, B.-H., Kim, Y., Khan, M.A. & Song, H. 2010. The role of clay minerals in the reduction of nitrate in groundwater by zero-valent iron. *Chemosphere*. 81(5):611–6.

Doktorgrades, E., 2009. Zeolites in fissures of crystalline basement rocks. Thesis submitted to Albert-Ludwigs-Universität Freiburg im Breisgau.

Ellis, R.W. 2000. Reduction of the bioavailability of 20 m g r kg aflatoxin in trout feed containing clay. *Aquaculture* 183:179–188.

Eya, J.C., Parsons, A., Haile, I., Jagidi, P. & Virginia, W. 2008. Effects of Dietary Zeolites (Bentonite and Mordenite) on the Performance Juvenile Rainbow trout Onchorhynchus myskis.*Australian Journal of Basic and Applied Sciences*, 2(4): 961-967, 2008

Faghihian, H. & Mohammadi, M.H. 2013. Surface properties of pillared acid-activated bentonite as catalyst for selective production of linear alkylbenzene. *Applied Surface Science*. 264:492–499.

Foletto, E.L., Colazzo, G.C., Volzone, C. & Porto, L.M. 2011. Sunflower oil bleaching by adsorption onto acid-activated bentonite. *Brazilian Journal of Chemical Engineering*. 28(1):169–174.

Görg, B., Morwinsky, A., Keitel, V., Qvartskhava, N., Schrör, K. & Häussinger, D. 2010. Ammonia triggers exocytotic release of L-glutamate from cultured rat astrocytes. *Glia*. 58(6):691–705.

Grommen, R., Hauteghem, I. Van & Wambeke, M. Van. 2002. An improved nitrifying enrichment to remove ammonium and nitrite from freshwater aquaria systems. *Aquaculture* 211 (2002) 115–124

Gu, L., Yu, X., Xu, J., Lv, L. & Wang, Q. 2011. Removal of dichloroacetic acid from drinking water by using adsorptive ozonation. *Ecotoxicology* (2011) 20:1160–1166

Guillette, L.J. & Edwards, T.M. 2005. Is nitrate an ecologically relevant endocrine disruptor in vertebrates? In *Integrative and Comparative Biology*. Vol. 45. 19–27.

Gunn, I.D.M., Meis, S., Maberly, S.C. & Spears, B.M. 2013. Assessing the responses of aquatic macrophytes to the application of a lanthanum modified bentonite clay , at Loch Flemington , Scotland , UK.*Hydrobiologia* DOI 10.1007/s10750-013-1765-5

Häussinger, D. & Görg, B. 2010. Interaction of oxidative stress, astrocyte swelling and cerebral ammonia toxicity. *Current opinion in clinical nutrition and metabolic care*. 13(1):87–92.

Hua, W., Li, N., Shen, D., Hui, C., Xiang, C., Lin, C. & Yun, C. 2013. Applied Clay Science Adsorption of proteins and nucleic acids on clay minerals and their interactions : A review. *Applied Clay Science* 80–81 (2013) 443–452

Indresh, H., Devegowda, G., Ruban, S. & Shivakumar, M. 2013. Effects of high grade bentonite on performance, organ weights and serum biochemistry during aflatoxicosis in broilers. *Veterinary World*. 6(6):313.

Jeenpadiphat, S. & Tungasmita, D.N. 2013. Acid-activated pillar bentonite as a novel catalyst for the esterification of high FFA oil. *Powder Technology*. 237:634–640.

Jensen, F.B. 1996. Uptake, elimination and effects of nitrite and nitrate in freshwater crayfish (Astacus astacus). *Aquatic Toxicology*. 34(2):95–104.

Jensen, F.B. 2003. Nitrite disrupts multiple physiological functions in aquatic animals. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*. 135(1):9–24.

Jensen, F.B. & Hansen, M.N. 2011. Differential uptake and metabolism of nitrite in normoxic and hypoxic goldfish. *Aquatic toxicology (Amsterdam, Netherlands)*. 101(2):318–25.

Jovanovi, N. & Janakovi, J. 1991. Pore structure and adsorption properties of an acid-activated bentonite. *Applied Clay Science*, 6 (1991) 59-68

Jović-jovičić, N., Milutinović-nikolić, A., Banković, P. & Dojčinović, B. 2010. Synthesis , Characterization and Adsorptive Properties of Organobentonites. *Eleventh Annual Conference of the Materials Research Society of Serbia* 117(5):849–854.

Junaidi, M.S. & Hashida, N.H. 2010. Impact of catfish density on the waste production, water quality and mortality: Comparison between running and stagnant water system. *Indian Journal of Animal Research*. 44(3):183–187.

Kaya, A. & Oren, A.H. 2005. Adsorption of zinc from aqueous solutions to bentonite. *Journal of Hazardous Materials* B125 (2005) 183–189

Körner, S., Das, S.K., Veenstra, S. & Vermaat, J.E. 2001. The effect of pH variation at the ammonium / ammonia equilibrium in wastewater and its toxicity to Lemna gibba. 71:71–78.

Kroupova, H., Machova, J. & Svobodova, Z. 2005. Nitrite influence on fish : a review.*Vet. Med. – Czech*, 50, 2005 (11): 461–471

Kumlu, M. & Eroldog, O.T. 2004. Effects of temperature on acute toxicity of ammonia to Penaeus semisulcatus juveniles. *Aquaculture* 241 (2004) 479–489

Ladhari, A., Ben Daly, H., Belhadjsalah, H., Cole, K.C. & Denault, J. 2010. Investigation of water absorption in clay-reinforced polypropylene nanocomposites. *Polymer Degradation and Stability*. 95(4):429–439.

Lefevre, S., Jensen, F.B., Huong, D.T.T., Wang, T., Phuong, N.T. & Bayley, M. 2011. Effects of nitrite exposure on functional haemoglobin levels, bimodal respiration, and swimming performance in the facultative air-breathing fish Pangasianodon hypophthalmus. *Aquatic toxicology (Amsterdam, Netherlands)*. 104(1-2):86–93.

Lefevre, S., Jensen, F.B., Huong, D.T.T., Wang, T., Phuong, N.T. & Bayley, M. 2012. Haematological and ion regulatory effects of nitrite in the air-breathing snakehead fish Channa striata. *Aquatic toxicology (Amsterdam, Netherlands)*. 118-119:48–53.

Li, J., Li, Y. & Meng, Q. 2010. Removal of nitrate by zero-valent iron and pillared bentonite. *Journal of hazardous materials*. 174(1-3):188–93.

Liang, H.N., Long, Z. & Yang, S.H. 2013. Effect of acid-activated bentonite on rheological properties of paper coatings. *Zhongguo Zaozhi Xuebao/Transactions of China Pulp and Paper*. 28(4):38–42.

Ling, H., Lu, A., Wang, C., Li, Y., Chen, P., Zhou, J. & Wang, J. 2011. Treatment of municipal landfill leachate with organically modified bentonite. *Clays and Clay Minerals*. 59(5):518–524.

Lira, E., Salazar, F.N., Rodríguez-Bencomo, J.J., Vincenzi, S., Curioni, A. & López, F. 2014a. Effect of using bentonite during fermentation on protein stabilisation and sensory properties of white wine. *International Journal of Food Science & Technology*. 49(4):1070–1078.

Lira, E., Salazar, F.N., Rodríguez-Bencomo, J.J., Vincenzi, S., Curioni, A. & López, F. 2014b. Effect of using bentonite during fermentation on protein stabilisation and sensory properties of white wine. *International Journal of Food Science & Technology*. 49(4):1070–1078.

Luckham, P.F. & Rossi, S. 1999. The colloidal and rheological properties of bentonite suspensions. *Advances in Colloid and Interface Science*82:43–92.

Lünsdorf, H., Erb, R.W., Abraham, W.R. & Timmis, K.N. 2000. “Clay hutches”: a novel interaction between bacteria and clay minerals. *Environmental microbiology*. 2(2):161–8.

Malekian, R. & Eslamian, S.S., 2011. Use of Zeolite and Surfactant Modified Zeolite as Ion Exchangers to Control Nitrate Leaching. , pp.715–719.

Makhoukhi, B., Didi, M.A., Villemin, D. & Azzouz, A. 2009. Acid activation of Bentonite for use as a vegetable oil bleaching agent. *Grasas y Aceites*. 60(4):343–349.

Mesubi, M.A., Adekola, F.A., Odebunmi, E.O., Adekeye, J.I.D., State, O. & Science, M. 2008. Beneficiation and characterisation of a bentonite from north-eastern Nigeria. *Journal of the North Carolina Academy of Science*, 124(4), 2008, pp. 154–158

Motlagh, K., Youzbashi, A.A. & Rigi, Z.A. 2011. Effect of acid activation on structural and bleaching properties of a bentonite. *Iranian Journal of Materials Science and Engineering*. 8(4):50–56.

Murray, H.H. 1991. Overview - clay mineral applications. *Applied Clay Science*, 5 (1991) 379-395.

Murray, H.H. 2000. Traditional and new applications for kaolin , smectite , and palygorskite : a general overview.*Applied Clay Science* 17 2000 207–221

Noyan, H., Onal, M. & Sarikaya, Y. 2007. The effect of sulphuric acid activation on the crystallinity, surface area, porosity, surface acidity, and bleaching power of a bentonite. *Food Chemistry*. 105(1):156–163.

Önal, M. 2007. Swelling and cation exchange capacity relationship for the samples obtained from a bentonite by acid activations and heat treatments. *Applied Clay Science*. 37(1-2):74–80.

Önal, M. & Sarıkaya, Y. 2007. Preparation and characterization of acid-activated bentonite powders. *Powder Technology*. 172(1):14–18.

Opinion, S. 2012. Scientific Opinion on the safety and efficacy of bentonite as a technological feed additive for all species *EFSA Journal* 2010;10(7):2787

Philips, S., Laanbroek, H.J. & Verstraete, W. 2002. Origin , causes and effects of increased nitrite concentrations in aquatic environments. *Environmental Science & Bio/Technology* 1: 115–141.

Phillips, T.D. 1999. Dietary Clay in the Chemoprevention of Aflatoxin-Induced Disease. *Toxicological sciences* 52 (Supplement), 118–126

Randall, D.J. & Tsui, T.K.N. 2002. Ammonia toxicity in fish. *Marine Pollution Bulletin* 45 (2002) 17–23

Reddy, P.V.B., Rama Rao, K. V & Norenberg, M.D. 2009. Inhibitors of the mitochondrial permeability transition reduce ammonia-induced cell swelling in cultured astrocytes. *Journal of neuroscience research*. 87(12):2677–85.

van Rijn, J., Tal, Y. & Schreier, H.J. 2006. Denitrification in recirculating systems: Theory and applications. *Aquacultural Engineering*. 34(3):364–376.

Romano, N. & Zeng, C. 2013. Toxic Effects of Ammonia, Nitrite, and Nitrate to Decapod Crustaceans: A Review on Factors Influencing their Toxicity, Physiological Consequences, and Coping Mechanisms. *Reviews in Fisheries Science*. 21(1):1–21.

Roques, J.A.C., Schram, E., Spanings, T., van Schaik, T., Abbink, W., Boerrigter, J., de Vries, P., van de Vis, H.,(2013). The impact of elevated water nitrite concentration on physiology, growth and feed intake of African catfish Clarias gariepinus (Burchell 1822). *Aquaculture Research*. 2013, 1–12.

Safaei, M., Boldaji, F., Dastar, B., Hassani, S., Mutalib, M.S.A. & Rezaei, R. 2014. Effects of Inclusion Kaolin, Bentonite and Zeolite in Dietary on Chemical Composition of Broiler Chickens Meat. *Asian Journal of Animal and Veterinary Advances*. 9(1):56–63.

Safaeikatouli, M., Jafariahangari, Y. & Baharlouei, A. 2010. Effects of dietary inclusion of sodium bentonite on biochemical characteristics of blood serum in broiler chickens. *International Journal of Agriculture and Biology*. 12(6):877–880.

Safaeikatouli, M., Boldaji, F., Dastar, B. & Hassani, S. 2012. The effect of dietary silicate minerals supplementation on apparent ileal digestibility of energy and protein in broiler chickens. *International Journal of Agriculture and Biology*. 14(2):299–302.

Salem, a. & Akbari Sene, R., 2011. Removal of lead from solution by combination of natural zeolite–kaolin–bentonite as a new low-cost adsorbent. *Chemical Engineering Journal*, 174(2-3), pp.619–628

Sar, Y. 2007. Preparation and characterization of acid-activated bentonite powders. *Powder Technology* 172 (2007) 14–18

Schram, E., Roques, J.A.C., Abbink, W., Spanings, T., de Vries, P., Bierman, S., de Vis, H. van & Flik, G. 2010. The impact of elevated water ammonia concentration on physiology, growth and feed intake of African catfish (Clarias gariepinus). *Aquaculture*. 306(1-4):108–115.

Schram, E., Roques, J.A.C., van Kuijk, T., Abbink, W., van de Heul, J., de Vries, P., Bierman, S., van de Vis, H. 2014. The impact of elevated water ammonia and nitrate concentrations on physiology, growth and feed intake of pikeperch (Sander lucioperca). *Aquaculture*. 420-421:95–104.

Schram, E., Roques, J.A.C., Abbink, W., Yokohama, Y., Spanings, T., de Vries, P., Bierman, S., van de Vis, H. 2014. The impact of elevated water nitrate concentration on physiology, growth and feed intake of African catfish Clarias gariepinus (Burchell 1822). *Aquaculture Research*. 45(9):1499–1511.

Scott, G. & Crunkilton, R.L. 2000. Acute and chronic toxicity of nitrate to fathead minnows (Pimephales promelas), Ceriodaphnia dubia, and Daphnia magna. *Environmental Toxicology and Chemistry*. 19(12):2918–2922.

Shu-li, D., Yu-zhuang, S.U.N., Cui-na, Y. & Bo-hui, X.U. 2009. Removal of copper from aqueous solutions by bentonites and the factors affecting it. *Mining Science and Technology (China)*. 19(4):489–492.

Slamova, R., Trckova, M., Vondruskova, H., Zraly, Z. & Pavlik, I. 2011. Applied Clay Science Clay minerals in animal nutrition. *Applied Clay Science*. 51(4):395–398.

Tacon, A.G.J. & Forster, I.P. 2003. Aquafeeds's' and the environment : policy implications.*Aquaculture* 226 (2003) 181–189.

Tang, Z., Wen, C., Li, P., Wang, T. & Zhou, Y. 2014. Effect of zinc-bearing zeolite clinoptilolite on growth performance, nutrient retention, digestive enzyme activities, and intestinal function of broiler chickens. *Biological Trace Element Research*. 158(1):51–57.

Taylor, P. et al., 2013. Desalination and Water Treatment Zinc removal from aqueous solutions by adsorption onto bentonite. , (August 2014), pp.37–41.

Theng, B.K.G. 2012. *Developments in Clay Science Volume 4*. Vol. 4. *Developments in Clay Science Volume 4*. Vol. 4. 245-318.

Tilak, K.S., Veeraiah, K. & Raju, J.M.P. 2007. Effects of ammonia, nitrite and nitrate on haemoglobin content and oxygen consumption of freshwater fish, Cyprinus carpio (Linnaeus). *Journal of environmental biology / Academy of Environmental Biology, India*. 28(1):45–7.

Tomasso, J.R. 2012a. Environmental nitrite and aquaculture: A perspective. *Aquaculture International*. 20(6):1107–1116.

Tomasso, J.R. 2012b. Environmental nitrite and aquaculture: a perspective. *Aquaculture International*. 20(6):1107–1116.

Tomić, Z.P., Logar, V.P., Babic, B.M., Rogan, J.R. & Makreski, P. 2011. Comparison of structural, textural and thermal characteristics of pure and acid treated bentonites from Aleksinac and Petrovac (Serbia). *Spectrochimica acta. Part A, Molecular and biomolecular spectroscopy*. 82(1):389–95.

Tsui, T.K.N., Hung, C.Y.C., Nawata, C.M., Wilson, J.M., Wright, P.A. & Wood, C.M. 2009. Ammonia transport in cultured gill epithelium of freshwater rainbow trout: the importance of Rhesus glycoproteins and the presence of an apical Na+/NH4+ exchange complex. *The Journal of experimental biology*. 212(Pt 6):878–92.

Vuković, Z., Milutinović-Nikolić, A., Krstić, J., Abu-Rabi, A., Novaković, T. & Jovanović, D. 2005. *Current Research in Advanced Materials and Processes*. Vol. 494. (Materials Science Forum). Stafa: Trans Tech Publications Ltd.

Vuuren, D.P. van, Bouwman, L.F., Smith, S.J. & Dentener, F. 2011. Global projections for anthropogenic reactive nitrogen emissions to the atmosphere: an assessment of scenarios in the scientific literature. *Current Opinion in Environmental Sustainability*. 3(5):359–369.

Wee, N.L.J., Tng, Y.Y.M., Cheng, H.T., Lee, S.M.L., Chew, S.F. & Ip, Y.K. 2007. Ammonia toxicity and tolerance in the brain of the African sharptooth catfish , *Clarias gariepinus*. *Aquaculture* 82:204–213.

Weihrauch, D., Wilkie, M.P. & Walsh, P.J. 2009. Ammonia and urea transporters in gills of fish and aquatic crustaceans. *The Journal of experimental biology*. 212(Pt 11):1716–30.

Williford, C.W., Reynolds, W.R. & Quiros, M., 1992. Clinoptilolite removal of ammonia from simulated and natural catfish pond waters. *Applied Clay Science*, 6(4), pp.277–291

Wright, P. a & Wood, C.M. 2012. Seven things fish know about ammonia and we don't. *Respiratory physiology & neurobiology*. 184(3):231–40.

Wright, P.A. & Wood, C.M. 2009. A new paradigm for ammonia excretion in aquatic animals: role of Rhesus (Rh) glycoproteins. *The Journal of experimental biology*. 212(Pt 15):2303–12.

Wuertz, S., Schulze, S.G.E., Eberhardt, U., Schulz, C. & Schroeder, J.P. 2013. Acute and chronic nitrite toxicity in juvenile pike-perch (Sander lucioperca) and its compensation by chloride. *Comparative biochemistry and physiology. Toxicology & pharmacology : CBP*. 157(4):352–60.

Yener, N. & Bic, C. 2012. Applied Surface Science Simultaneous determination of cation exchange capacity and surface area of acid activated bentonite powders by methylene blue sorption. *Applied Surface Science* 258 (2012) 2534– 2539

Yener, N., Biçer, C., Önal, M. & Sarıkaya, Y. 2012. Simultaneous determination of cation exchange capacity and surface area of acid activated bentonite powders by methylene blue sorption. *Applied Surface Science*. 258(7):2534–2539.

Yu, W.H., Li, N., Tong, D.S., Zhou, C.H., Lin, C.X. (Cynthia) & Xu, C.Y. 2013. Adsorption of proteins and nucleic acids on clay minerals and their interactions: A review. *Applied Clay Science*. 80-81:443–452.

Zamparas, M., Gianni, A., Stathi, P., Deligiannakis, Y. & Zacharias, I. 2012. Applied Clay Science Removal of phosphate from natural waters using innovative modi fi ed bentonites. *Applied Clay Science*. 62-63:101–106.

Zhang, L.S., Wang, Y.Y., Meng, F.S., Zhou, Y.X. & Yu, H. Bin. 2013. Toxicity of nitrate-n to freshwater aquatic life and its water quality criteria. *Huanjing Kexue/Environmental Science*.

34(8):3286–3293.

Zychowski, K.E., Hoffmann, A.R., Ly, H.J., Pohlenz, C., Buentello, A., Romoser, A., Gatlin, D.M. & Phillips, T.D. 2013. The effect of aflatoxin-B1 on red drum (Sciaenops ocellatus) and assessment of dietary supplementation of NovaSil for the prevention of aflatoxicosis. *Toxins*. 5(9):1555–73.

CHAPTER 3

EFFECT OF DIETARY NATURAL AND ACTIVATED BENTONITE ON PHYSICAL QUALITY OF AQUAFEEDS’

Abstract

The study was undertaken to evaluate the effects of natural bentonite (NB) and its acid activated (AB) form as feed binders on the physical quality of aquafeeds’. Bentonite (a clay mineral) is a binder used in feed technology to improve physical pellet quality. Physical quality of feed varies with ingredient composition and processing condition and may interfere with feed intake, nutrient digestibility, waste dispersal and, therefore, growth performance of the fish. Aquafeeds’ physical quality parameters, including bulk density, durability, and water stability (disintegration and leaching), were studied in two phases. In phase I, NB and AB were, respectively, added as additives into a commercial catfish feed as a clay blend at combination levels (0:100, 25:75, 50:50) and at two inclusion levels for each ratio low and high (500 mg/kg and 1500 mg/kg) clay/feed with a control diet. In phase II, NB and AB were added separately as additives at different inclusion levels (0, 500, 1000, 1500, and 3000 mg/kg). Feeds were evaluated for water stability over eight time intervals (30 mins, 1, 2, 3, 4, 5, 6, and 7 hrs) and feeds were analysed for nutrient leaching (% ash and crude protein) at (30 mins, 1 hr, 3 hr and 7 hours). Each test was replicated four (4) times. Water stability, feed durability, feed bulk density and nutrient leaching were all significantly affected ($p<0.05$) by treatments. In both phases, measured parameters, excluding nutrient leaching, increased with quantity of AB in the clay blend and at high inclusion level. Clay treated feeds had significantly ($p<0.05$) higher values for all parameters measured as compared to those of control feed. Values increased with increase in level of inclusion. AB had higher values as compared to NB and control. These results validate utilisation of NB and AB as a feed binder; their ability to improve pellet physical qualities may enhance feed utilisation, water quality and fish production.

3.1 Introduction

Animal species require different physical properties for their respective feeds. This means that different quality standards are used. For aquafeeds', characteristics such as flowability, sinking velocity, durability, hardness, water absorption and water solubility are important (Behnke, 2001). Fish feeds used in intensive aquaculture production should be resistant to mechanical stress during transport, handling and in pneumatic feeding devices (Fagbenro & Jauncey, 1995a), but should still have a texture and size that can facilitate high feed intake as well as efficient digestion by the fish (Fahrenholz, 2012). Water stability and bulk density are also important pellet quality characteristics that vary according to the salinity and temperature of the water, which control sinking velocity and buoyancy (Fagbenro & Jauncey, 1995a; Fahrenholz, 2012).

The water stability of aquatic feed pellets depends upon the nature and quantity of the binding material. If the pellet is too hard, it is difficult for the animals to ingest; and if it is loosely blended, the pellet would disintegrate faster, leading to wastage of the feed and spoilage of the water (Ruscoe *et al.*, 2005; Argüello-Guevara & Molina-Poveda, 2013a). An efficient binder should be accessible and economical in relation to rate of inclusion and price (Fahrenholz, 2012). It is essential to consider these facts while selecting a binding agent. The use of binders is important in the manufacture of moist feeds and experimental diets in aquaculture.

Binders improve water stability, aid pre-hension, thus increasing feed efficiency, increased faeces cohesion, enabling more reliable faeces collection for digestibility studies. They also reduce feed wastage and improve water quality (Fagbenro & Jauncey, 1995a; Gatlin *et al.*, 2007).Feed processing influences the rate of degradation and the rate of passage of feed components of animals (Behnke, 2001; Cho & Bureau, 2001). Pelleted diets can affect animal performance in a variety of ways. These include: decreased feed wastage, reduced selective feeding, decreased ingredient segregation, less time and energy expended for pre-hension, destruction of pathogenic organisms, thermal modification of starch and protein, etc. (Goelema *et al*. 1999; Thomas & van der Poel, 1996a; Cho & Bureau, 2001; Zang *et al.*, 2009).

In aquaculture, physical integrity of feed, with minimal disintegration and nutrients leaching in water is an important management tool that ensures production success (Argüello-Guevara & Molina-Poveda, 2013a). The true nutritional value of feeds depends not only on the amount of nutrients, but also on their availability and quality at the time of ingestion and digestion by cultured species (Cho & Bureau, 2001). It has been reported that dietary origin accounted for at least 70% of reduction in water quality due to waste outputs and uneaten feed during production (Partridge & Southgate, 1999). Leaching of water-soluble nutrients, such as free vitamins and minerals and amino acids and pellets break into smaller particles reducing its nutritional value after 1 hr of immersion in water was reported by Rosas *et al.* (2008a). This leaching may cause the eutrophication of water, leading to a toxic build of nitrogenous compounds, poor animal growth, inefficient feed conversion and mortality of fish (Ruscoe *et al.*, 2005; Rosas *et al.*, 2008b; Orire *et al.*, 2010). However, binders have been studied to improve feed physical integrity and enhance utilisation by aquatic animals (Rosas *et al.*, 2008b).

Fish pellet with good physical quality is important for preventing attrition and fragmentation of the pellet. Pellet breakage during transportation enhances feed wastage, which will negatively affect the economic result in fish production (Hansen & Storebakken, 2007a). Durability is a physical quality parameter of feed pellets. Durability measures the amount of fines returned from a batch of feed pellets under standard conditions. In feed manufacturing, pelleted feeds are subject to shearing and abrasion during transportation and handling. This induces fines in the feed. For the purpose of feeding ease, economic of production and water quality, pellets need to have a certain resistance against the stresses exerted on them during transportation and distribution to the fish (Thomas & van der Poel, 1996b).

Pellets are sensitive to shearing actions at the places where they are cut off after leaving the die. These cuts impact new surfaces which are sensitive to further deterioration through fines. Pellets that are not properly cooled can have a reduced durability due to stresses in the pellet between the (cooled) outer layer and the (still) warmer inner layer. The improper cooling resulted in a brittle outer layer, with physical properties differing from those of the inner kernel (Thomas & van der Poel 1996b, 1997). These differences in physical properties create stresses in the pellet which cause the outer layer to crack under less optimal conditions. These cracks will allow for an easier formation of fines.

However, binders can have detrimental effects on various digestive processes. For example, alginates and guar gum were effectively used in dry moist and wet feeds for rainbow trout but they accelerated gastrointestinal transit time and depressed the apparent digestibility of protein and fat (Brinker, 2007a). Rosas *et al.*, (2008a) reported that octopuses fed alginate-bound crab meat lost weight and died, revealing that this type of binder limits nutrient absorption from the diet. In contrast, when gelatin was used as binder, energetic balance was similar to that obtained in animals fed natural crab, indicating that octopuses can digest gelatin. Apparently, gelatin promotes absorption of nutrients similar to that observed when octopuses were fed pieces of fresh crab. An experiment was conducted to evaluate the suitability of yam starch as a local alternative binding agent in aquatic feed, which is effective and nutritive. The binding property of yam starch in feed pellet increased significantly with the levels of inclusion in fish feed production. Five percent (5%) inclusion level was found to be appropriate in producing desirable water stable pellets that are also firm to handling during transportation and storage (Orire *et al.*, 2010)

Bentonite is clay altered from glassy igneous material such as volcanic ash or turf (Murray, 2000). Bentonite properties and structure are affected by the acid activation, ion exchange, heating, hydrothermal treatments and some physicochemical processes. The properties of bentonite clay that can be affected include: strength, swelling capacity, plasticity, cohesion, particle size, cation-exchange capacity, pore structure, surface area, catalytic activity, etc. (Luckham & Rossi 1999; Mesubi *et al.* 2008). This clay mineral is currently authorized for use as feed and food additives (Opinion, 2012). Bentonite, because of its superior dry strength, binding capacity, natural occurrence and relatively low cost, has been used in animal production to improve the feed quality and animal growth with few emphasis on aquaculture with varying success (Article, 2004; Carraro *et al.*, 2014; Tang *et al.*, 2014). However, the application of acid-activated bentonite is new in animal and aquaculture production to the best of our knowledge. Therefore, this study aimed to investigate the effects of natural and acid-activated bentonite on water stability, feed durability, feed density and nutrient leaching of pelletized African catfish (*C. gariepinus*) feed.

3.2 Materials and method

3.2.1 Experimental Facilities

The experiment was carried out at the aquaculture facilities at Welgevallen Experimental Farm of Stellenbosch University (SU). The location on a geographical position system (GPS) is as follows: coordinates 33^{o}56' 33.95" S and 18^{o}51'56.15" E, Stellenbosch, Western Cape area, South Africa.

3.2.2 Dietary treatments

Table 3. 6 : Inclusion of bentonite blend in *C. gariepinus* feed

S/N0	Treatments	Designation	Inclusion level of clay (mg/kg of basal diet BD)
I.	Basal diet only * BD	T_1	No inclusion
II.	Basal diet only * BD	T_2	No inclusion
III.	BD + NB (low level)	T_3	500 mg NB (low level)
IV.	BD + NB (high level)	T4	1500 mg NB (high level)
V.	BD + 75 % NB + 25 % AB	T_5	375 mg NB : 125 mg AB (low level)
VI.	BD + 75 % NB + 25 % AB	T_6	1,125 mg NB : 375 mg AB (high level)
VII.	BD + 50 % NB + 50 % AB	T_7	250 mg NB : 250 mg AB (low level)
VIII.	BD + 50 % NB + 50 % AB	T_8	750 mg NB : 750 mg AB (high level)
IX.	BD + 25 % NB + 75 % AB	T_9	125 mg NB : 375 mg AB (low level)
X.	BD + 25 % NB + 75 % AB	T_{10}	375 mg NB : 1,125 mg AB (high level)
XI.	BD + AB (low level)	T_{11}	500 mg AB (low level)
XII.	BD + AB (high level)	T_{12}	1500 mg AB (high level)

- BD : Commercial Montego catfish grower diets

  Natural bentonite – NB Acid-activated bentonite - AB

Table 3.7 : Inclusion level of natural and activated bentonite in *C. gariepinus* feed

S/N0	Treatments	Designation	Inclusion level of clay (mg / kg of basal diet)
I.	Basal diet only *BD	Control	No inclusion
II.	BD + NB 500	NB 500	500 mg NB
III.	BD + AB 500	AB 500	500 mg AB
IV.	BD + NB 1000	NB 1000	1000 mg NB
V.	BD + AB 1000	AB 1000	1000 mg AB
VI.	BD + NB 1500	NB 1500	1500 mg NB
VII.	BD + AB 1500	AB 1500	1500 mg AB
VIII.	BD + NB 3000	NB 3000	3000 mg NB
IX.	BD + AB 3000	AB 3000	3000 mg NB

- BD : Commercial Montego catfish grower diets

Natural bentonite – NB
Acid activated bentonite - AB

3.2.3 Feed preparation

Experiment IA was comprised of 12 test diets of natural, acid-activated bentonite or combinations, while experiment IB had 9 test diets of natural and acid-activated bentonite inclusion levels, prepared by adding the clay mineral as feed additive to a commercial catfish feed. The inclusion and combination rates are described in table 3.1 and 3.2, respectively. In preparing the diets, dry commercial catfish feed was obtained from Montego feed company, Cape Town, South Africa. Feed was prepared by grinding hot extruded commercial pellet, and the re-forming it with a cold extruder (70-80^0 C). Extruder machine with dice hole of size 3 mm was used. A corresponding quantity of clay form was added to 5 kg of feed mixed for 5 min. in a Hobart A-200 mixer. 1.8 litres of water was added gradually and mixed for another15mins until a homogenous paste was obtained and became dough like, which was then extruded through a

Hobart A-200 pellet mill with 3-mm diameter die into strands. The strands were broken to 3 mm long pellets, packed in plastic bags and stored air tight immediately.

3.2.4 Measurement of Physical feed quality parameters

3.2.4.1 Water stability (leaching and disintegration)

The experimental unit was a chambered grid designed to create turbulent water conditions in each chamber, consisting of 98 chambers with a volume of 400 dm^3 each. The system was adapted as described by Viljoen and De Wet (2008). The unit consists of an outer shell into which two sub-units fit as shown in Plate 3.1. The first sub-unit is a rectangular cuboid structure supporting a right circle cylinder matrix with an air stone fitted underneath the open bottom of each cylinder. The second sub-unit fits on top of the first sub-unit and consists of plastic rectangles welded together to form a grid of chambers, over each cylinder matrix. All chambers share the same water, but each chamber will only receive air from its own air stone, thus creating a whole set of different chambers, yet with similar turbulence conditions. The system was built inside an in vitro lab at the feed technology unit, Division of Aquaculture at Welgevallen Experimental Farm

<u>Water stability test (disintegration)</u>

The water stability test was adapted from De Wet *et al.* (2002) and Viljoen and De Wet (2008). The test system consisted of 100 stainless-steel test containers with wire mesh tops and bottoms (see plate 3.3) placed in the chamber grid filled with water and air stone to create turbulence (see plate 3.1 and 3.2). The water temperature was maintained at 24^0 C. The chamber grid was positioned to sustain the test containers in top water (see plate 3.4) and ensure good water flow over the feed. Feed samples were placed and weighed in the test containers (approximately 20 g feed sample per container, see plate 3.5). Containers with feed samples were placed in an oven for 12 hours at 60^0 C to further reduce the moisture content prior to placement into the chamber grid and weighed (initial weight). After placement in the chamber grid, each treatment feed was evaluated for water stability over time intervals of 30mins and 1, 2, 3, 4, 5, 6, and 7 hours. Each test was replicated 4 times. Following the water stability test, dietary treatments were dried at 60^0 C for 12 hours in the test containers after which final weight was recorded.

Water stability (%) was calculated as:

$$= \frac{(\text{Initial weight (Container+feed) })-(\text{Final weight (Container+feed)})}{(\text{Initial weight (Container+feed)}-(\text{Weight (Container)})} X\ 100\%]$$

3.2.4.2 *Experiment II (A & B): Nutrient leaching analysis*

Feed samples from each replicate of all treatment diets were analysed for proximate analysis in duplicate, prior and after the water stability test from time periods (0, 1, 3 and 7 hrs). Proximate analyses included ash, and crude protein and were conducted according to AOAC (2002); thus, moisture was determined after oven-drying at 105°C to constant weight and ash by incinerating the dried residue for 24 h at 550°C in a muffle furnace. Total nitrogen (N) was determined by Dumas method with a LECO FP 428. Crude protein was estimated as N X 6.25. Crude protein =% Nitrogen x protein conversion factor (6.25)

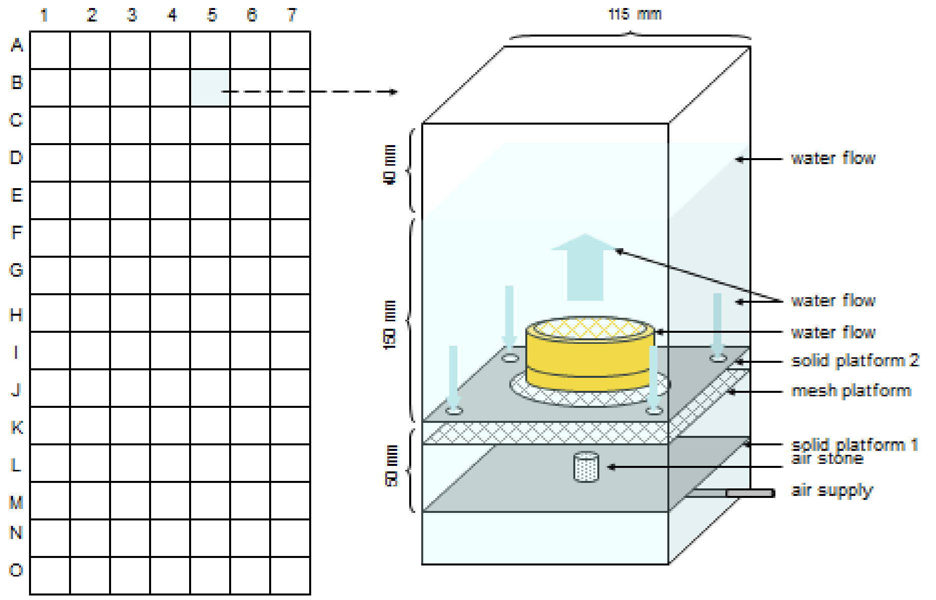

Plate 3. 8 : Water stability chamber

Plate 3. 9 : Installed water stability grid

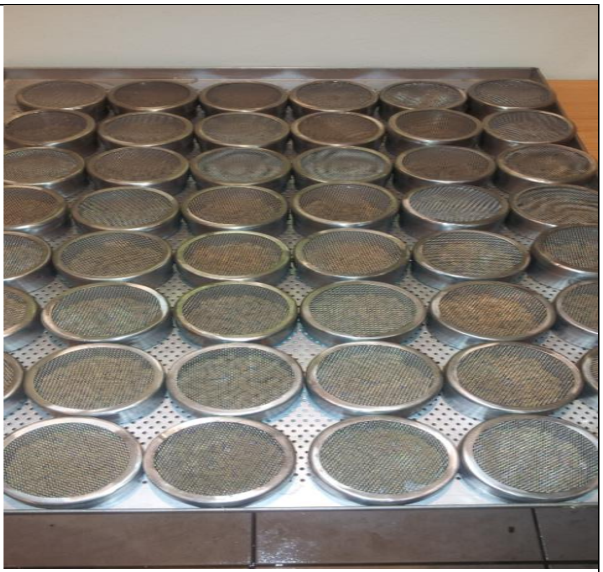

Plate 3. 10 : Stainless steel test containers

Plate 3. 11 : Installed water stability system

Plate 3. 12 : Stainless steel test containers with feed

3.2.4.3 Experiment III (A & B) Pellet Durability test

Pellet durability test

Pellet durability was evaluated using a Pfost durability tester (American Feed Manufacturers Association, 1976). According to the procedure of Pfost (Pfost and Allen, 1962; Pfost, 1963) in (Thomas *et al*., 1997b) durability is determined by inducing fines through an abrasion action of pellets shearing over each other and over the wall of drums (tumbling device: Plate. 3.6). 500 g of sieved pellets are inserted into a drum with dimension (30 cm × 30 cm × 45 cm). After tumbling for 20 min at 50 rpm, the pellets are subsequently sieved using an electric filter (Filter device: Plate 3.7) and the amount of fines passing a sieve with a grid size just smaller than the nominal pellet diameter is determined. In practice, grid-sizes are used of 0.8 X pellet diameter. Durability is then expressed as the ratio of the weight after tumbling over the weight before tumbling, multiplied by 100 (percentage). However, durability is normally expressed as a percentage which gives the amount of fines returned or the amount of pellets recovered.

Pellet durability (%) was calculated as:

$$= [\ \frac{(Weight\ fines)}{(Weight\ of\ pellet)}\ X\ 100\%]$$

Plate 3. 13 : Tumbling device for feed durability

Plate 3. 14 : Filter device for durability test

3.2.4.4 Feed bulk density

The bulk density was measured by weighing the quantity of feed that will fill up a 250-ml beaker, i.e., calculating the relationship between mass of feed and volume of feed.

$$\text{Bulk Density} = \frac{(Weightoffeed)}{(Volumeoffeed)} X\ 100\%]$$

3.2.5 Statistical analysis

All data recordings and calculated values for water stability, feed durability and feed bulk density were computed using Microsoft Excel 2010 and Sharp Model ELW53H Scientific Calculator. Computed data for influence of bentonite clay blend on feed physical qualities was analysed with two-way factorial ANOVA, while influence of natural bentonite and acid-activated at different inclusion levels was analysed with one-way ANOVA. Bonferroni was used to test for the significance of variance ($p<0.05$) for all analysed data among different treatments using SAS 2015 version 12 and XLSTAT version 2015.

3.3 Results

The data for influence of bentonite blend (natural and activated bentonite combinations) on feed water stability are presented in table 3.3, feed durability and feed bulk density in table 3.4, while nutrient leaching was reported in table 3.5 with analysed values.

Table 3.8 : Influence of bentonite blend as feed additive on water stability of *C. gariepinus* feed at time intervals (0.5 – 7hours)

	Treatments						
Period	**A0B0**	**A0B100**	**A25B75**	**A50B50**	**A75B25**	**A100B0**	**SEM**
30min	95.70^{d*}	96.40^{bcd}	95.80^{cd}	97.00^{ab}	96.90^{abc}	97.90^{a}	0.03
1hr	72.60^{e}	75.50^{de}	78.00^{de}	82.20^{c}	86.70^{b}	92.90^{a}	0.08
2hr	62.20^{f}	70.30^{e}	74.20^{d}	77.60^{c}	81.30^{b}	90.80^{a}	0.07
3hr	51.70^{e}	67.40^{d}	69.10^{d}	73.00^{c}	77.90^{b}	86.10^{a}	0.05
4hr	46.10^{e}	58.20^{d}	60.90^{d}	66.60^{c}	75.00^{b}	78.90^{a}	0.07
5hr	35.30^{e}	46.60^{d}	47.90^{c}	58.80^{b}	68.90^{a}	70.10^{a}	0.08
6hr	29.50^{e}	40.50^{d}	47.90^{c}	58.80^{b}	68.90^{a}	70.10^{a}	0.08
7hr	21.80^{f}	37.50^{e}	42.80^{d}	49.60^{c}	65.70^{a}	58.80^{b}	0.06

SEM: Standard error of mean. *Mean values between the rows are verified at $p<0.05$; values on the same row with the same superscript letter are not significantly different using bonferroni post-hoc test. Where: A0B100 = 100% NB, A25B75 = 75% NB: 25% AB, A50B50 = 50% NB: AB, A75B25 = 75% AB: 25% NB, A100B0 = 100% AB, A0B0 = control

As recorded in table 3.3, feed water stability differed significantly ($p<0.05$) among the treatments of bentonite blend at all the time intervals. Acid-activated bentonite at 100% inclusion (A100B0) had the highest value, which was significantly different ($p<0.05$) as compared to other treatments except A50B50 and A75B25 from 30 mins to 6 hr. At 7 hr, 75% activated bentonite (A75B25) had the highest significant value ($p<0.05$) as compared to other treatments (Figure 3.1 and 3.2). The control diet (A0B0) had the lowest values, which were significantly different ($p<0.05$) as compared to dietary clay feed from 30 mins -7hr (Figure 3.1 and 3.2). Feed water stability value increases with quantity of acid-activated bentonite. There was significant interaction ($p<0.05$) between bentonite combinations and inclusion levels (low and high) at all the time intervals. At high inclusion level of bentonite clay (1500 mg/kg), diets have more water stability as compared to low inclusion (500 mg/kg) (Figure 3.3 and 3.4). At 7th hour, the control diet had 72% less water stability as compared to the least clay blend of 82% (Figure 3.5). In all treatment diets, loss in water stability was higher between 30 min – 1hr, as compared to other time intervals (Figure 3.6).

In table 3.4, mean values for feed durability and bulk density are presented. The feed durability (%) and feed bulk density (g/ml) differ significantly ($p<0.05$) among the treatment diets. All dietary clay treated feed had higher values, which differed significantly ($p<0.05$) as compared to those of the control. As recorded for feed durability, activated bentonite at 100% (A100B0) and natural bentonite at 75% (A25B75) had the highest mean values (99.15±0.01), while control diet (A0B0) had the lowest mean value (98.82±0.01). Feed bulk density for bentonite blend combinations are not significantly different ($p>0.05$) but they differ significantly ($p<0.05$) from those of the control.

The nutrient leaching invitro was reported in table 3.5, with data on ash (%) and crude protein (CP) (%) for selected periods (30 mins, 1 hr, 3 hr and 7 hr). The mean percentage ash differed significantly ($p<0.05$) among the treatment diets at all times. The mean values of ash (%) increased with quantity of activated bentonite in the clay blend. Dietary clay treated aquafeeds' increased significantly ($p<0.05$) as compared to that of control. The CP (%) differed significantly ($p<0.05$) among the treatments during all the periods. At 30 mins, the control diet (A0B0) had the highest mean CP value, which differed significantly ($p<0.05$) as compared to those of other treatments except A0B100. The bentonite clay blend (A0B100) had the highest mean CP value at (1, 3 and 7 hr), which differed significantly ($p<0.05$) as compared to that of the control. The mean CP (%) decreased with increase in the quantity of bentonite blend.

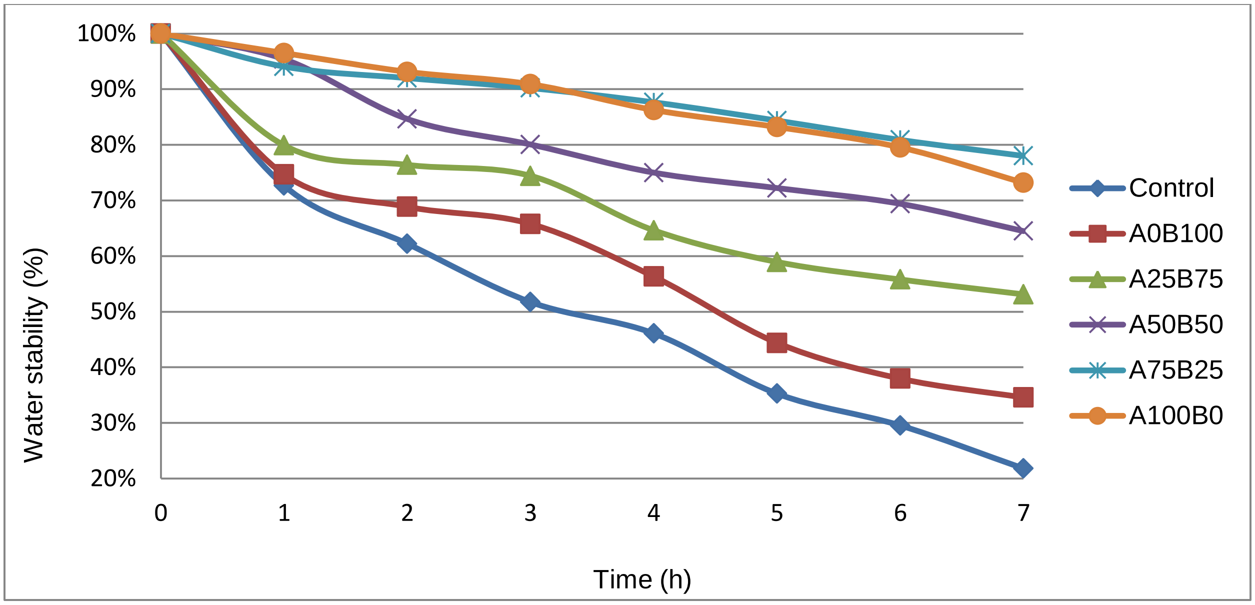

Figure 3.6 : Influence of dietary bentonite blend at low inclusion (500 mg/kg) on water stability. Where: A0B100 = 100% NB, A25B75 = 75% NB, A50B50 = 50% NB: AB, A75B25 = 75% AB: 25% NB, A100B0 = 100% AB

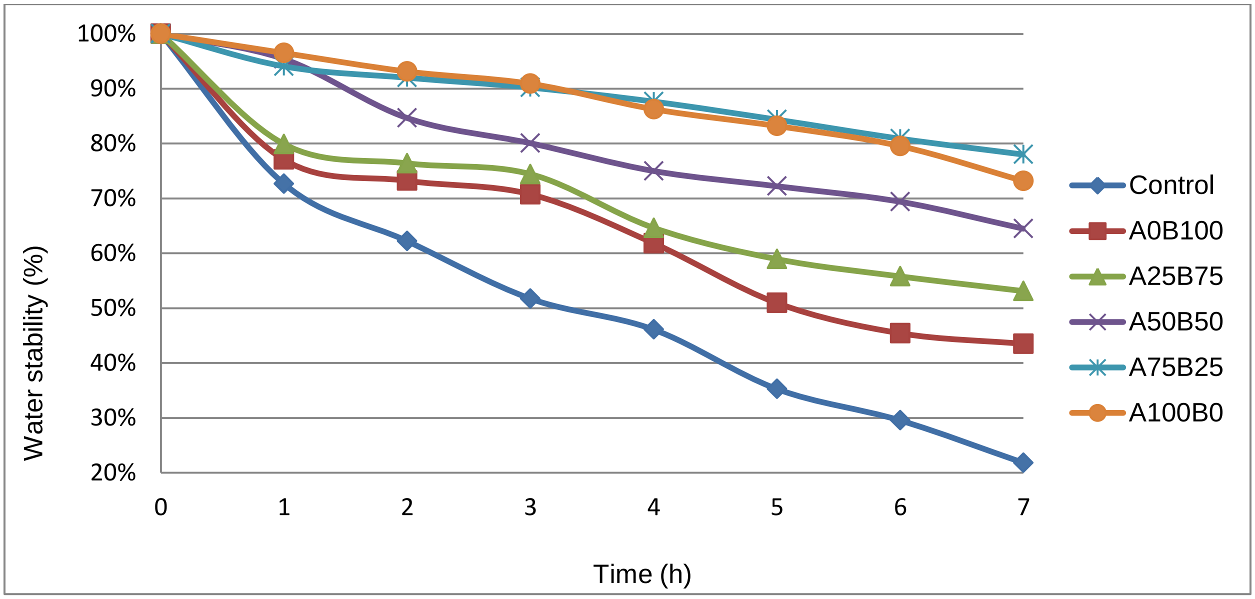

Figure 3.7 : Influence of dietary bentonite blend at high inclusion (1500 mg/kg) on water stability. Where: A0B100 = 100% NB, A25B75 = 75% NB: 25% AB, A50B50 = 50% NB: AB, A75B25 = 75% AB: 25% NB, A100B0 = 100% AB

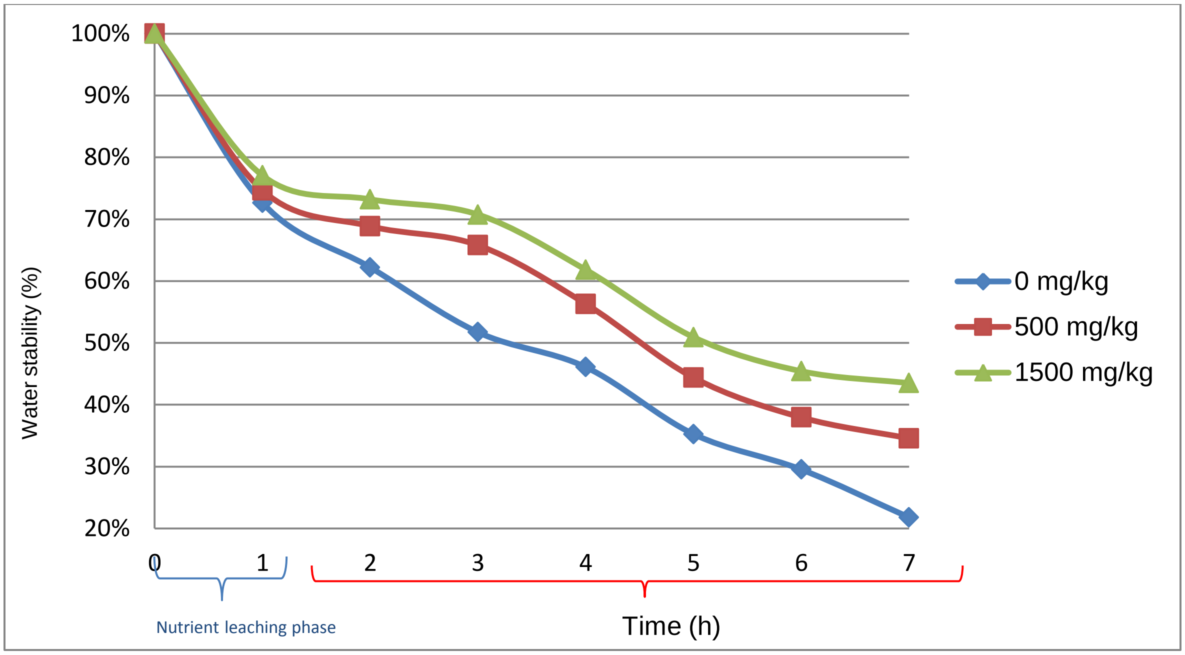

Figure 3. 8 : Influence of low (500 mg/kg) and high inclusion (1500mg/kg) level of natural bentonite on water stability.

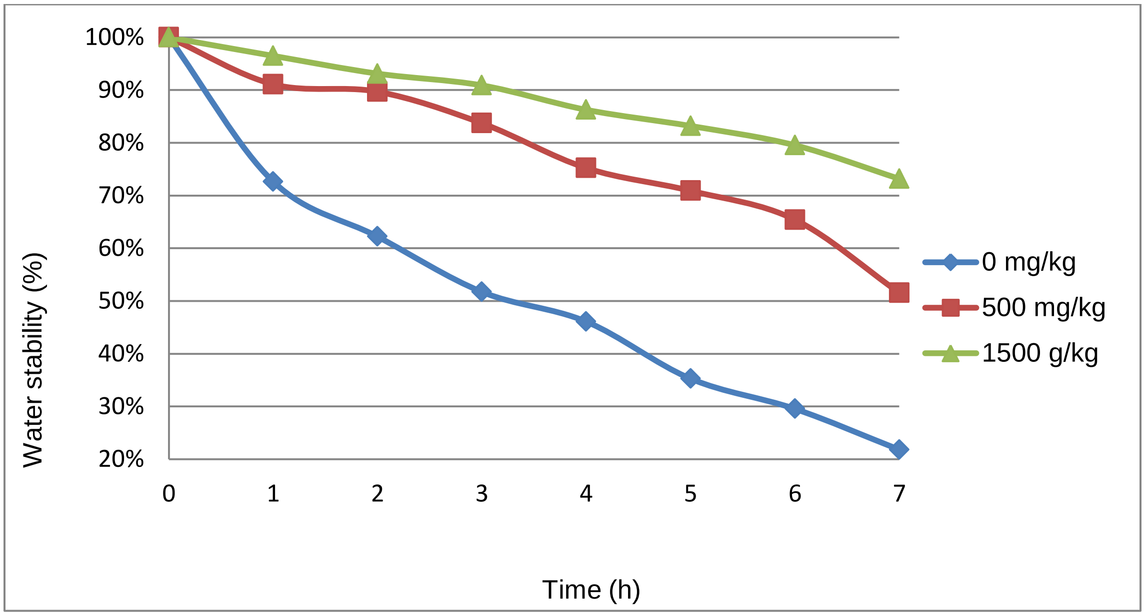

Figure 3. 9 : Influence of low (500mg/kg) and high inclusion (1500mg/kg) level of acid-activated bentonite blend on water stability.

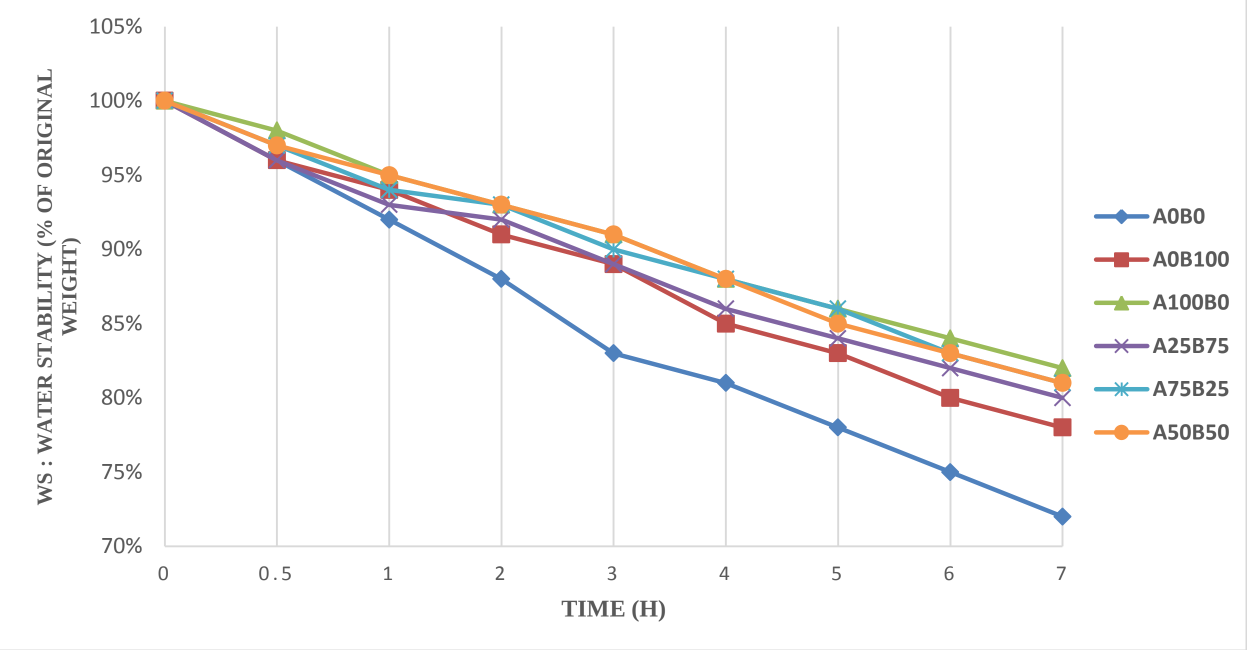

Figure 3.5: Influence of dietary bentonite blend on WS (water stability) based on reduction in percentage of original weight of feed. Where: A0B100 = 100% NB, A25B75 = 75% NB: 25% AB, A50B50 = 50% NB: AB, A75B25 = 75% AB: NB 25%, A100B0 = 100% AB

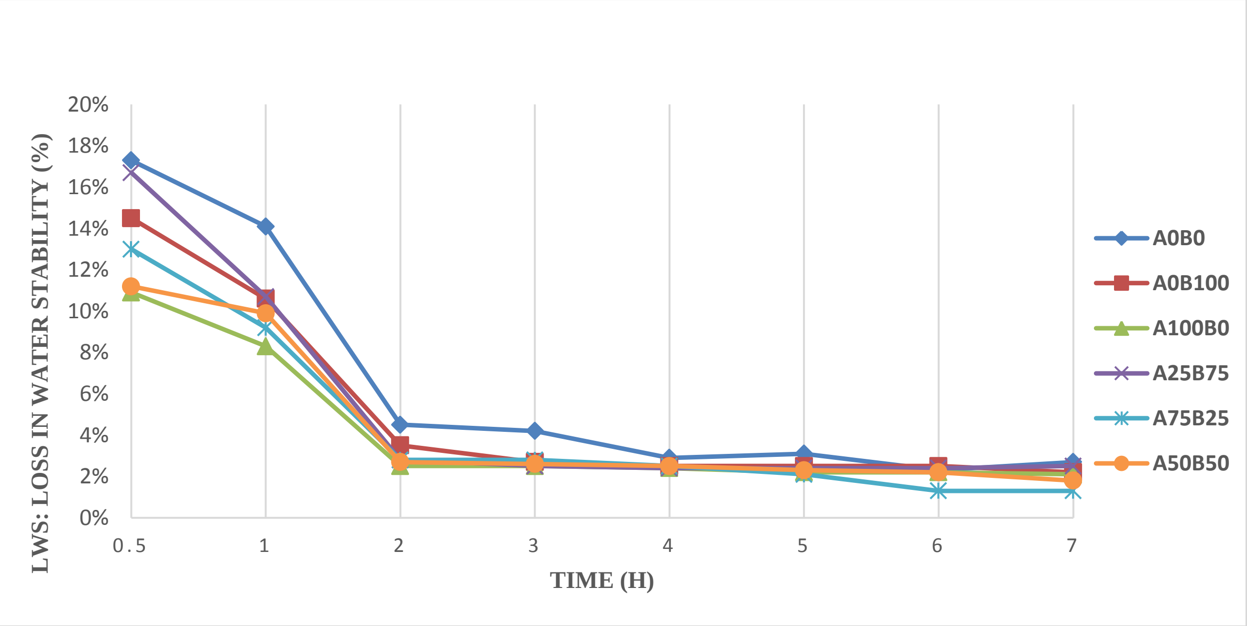

Figure 3.6: Influence of dietary bentonite blend on LWS (% Loss in water stability). Where: A0B100 = 100% NB, A25B75 = 75% NB: 25% AB, A50B50 = 50% NB: AB, A75B25 = 75% AB: NB 25%, A100B0 = 100% AB

Table 3. 9 Influence of bentonite blend as feed additives on feed durability and density of Aquafeeds'

Treatments	**A0B0**	**A0B100**	**A25B75**	**A50B50**	**A75B25**	**A100B0**	**SEM**
Feed durability (%)	98.82^{c*}	99.11^{ab}	99.15^{a}	99.12^{ab}	99.12^{ab}	99.15^{a}	0.01
Feed density (g/ml)	0.98^{b}	1.01^{a}	1.00^{a}	1.01^{a}	1.00^{a}	1.01^{a}	0.003

SEM: Standard error of mean. *Mean values between the rows are verified at p<0.05; values on the same row with the same superscript letter are not significantly different based on bonferroni post-hoc test. Where: A0B100 = 100% NB, A25B75 = 75% NB: 25% AB, A50B50 = 50% NB: AB, A75B25 = 75% AB: 25% NB, A100B0 = 100% AB, A0B0 = control

Table 3. 10 : Influence of bentonite blend as feed additives on nutrient leaching of aquafeeds' (Wet basis)

	Treatments						
Period	**A0B0**	**A0B100**	**A25B75**	**A50B50**	**A75B25**	**A100B0**	**SEM**
Ash (%)							
30min	5.24^{e*}	6.40^{b}	6.60^{b}	6.33^{c}	6.38^{bc}	6.99^{a}	0.01
1hr	4.83^{b}	5.09^{a}	5.05^{a}	5.04^{a}	5.08^{a}	5.11^{a}	0.02
3hr	4.64^{d}	4.82^{c}	4.84^{bc}	4.89^{b}	4.84^{c}	5.02^{a}	0.07
7hr	4.42^{e}	4.61^{d}	4.84^{bc}	4.89^{b}	4.84^{c}	4.94^{a}	0.006
CP (%)							
30min	37.83^{a}	37.46^{a}	36.33^{b}	35.78^{bc}	35.98^{c}	36.39^{b}	0.09
1hr	32.83^{e}	36.67^{a}	35.66^{bc}	35.09^{c}	34.77^{d}	36.22^{ab}	0.05
3hr	28.21^{d}	35.56^{a}	32.60^{b}	31.46^{bc}	30.33^{c}	35.14^{a}	0.07
7hr	24.96^{d}	31.57^{a}	29.26^{c}	30.27^{b}	30.19^{b}	32.38^{a}	0.05

SEM: Standard error of mean. *Mean values between the rows are verified at $p<0.05$; values on the same row with the same superscript letter are not significantly different, based on bonferroni post-hoc test. Where: A0B100 = 100% NB, A25B75 = 75% NB: 25% AB, A50B50 = 50% NB: AB, A75B25 = 75% AB: 25% NB, A100B0 = 100% AB, A0B0 = control

Table 3.6: Influence of bentonite clays (NB & AB) at different inclusion levels on water stability of Aquafeeds' (dry matter basis)

	Bentonite blend and inclusion (mg/kg)									
Period	**0**	**500**		**1000**		**1500**		**3000**		
	A0B0	**AB**	**NB**	**AB**	**NB**	**AB**	**NB**	**AB**	**NB**	**SEM**
30mins	98.06^{a}	95.52^{c}	96.57^{b}	96.19^{b}	94.29^{d}	97.92^{ab}	97.85^{ab}	98.24^{a}	98.20^{a}	0.09
1hr	88.89^{c}	91.45^{d}	90.43^{d}	93.39^{b}	93.25^{b}	92.44^{bc}	92.43^{bc}	96.37^{a}	95.52^{a}	0.21
2hr	64.68^{c}	80.35^{ab}	80.61^{ab}	80.77^{ab}	81.30^{ab}	81.22^{ab}	81.86^{ab}	83.86^{ab}	83.39^{ab}	0.91
3hr	59.34^{f}	73.67^{d}	70.51^{e}	75.74^{c}	74.47^{d}	77.03^{b}	75.67^{c}	79.31^{a}	77.51^{b}	0.23
4hr	52.72^{g}	68.35^{e}	65.39^{f}	73.37^{c}	70.95^{d}	74.43^{bc}	73.29^{c}	76.82^{a}	75.54^{ab}	0.42
5hr	41.78^{g}	59.46^{ef}	57.96^{f}	62.52^{d}	61.52^{d}	66.97^{bc}	65.75^{c}	74.36^{a}	68.50^{b}	0.44
6hr	41.92^{g}	59.43^{d}	47.80^{f}	53.62^{e}	51.81^{e}	67.48^{b}	64.26^{c}	71.89^{a}	65.64^{bc}	0.55
7hr	26.42^{h}	49.62^{f}	44.72^{g}	52.54^{e}	49.72^{f}	62.38^{c}	58.68^{d}	70.68^{a}	64.63^{b}	0.43

SEM: Standard error of mean. *Mean values between the rows are verified at $p<0.05$; values on the same row with the same superscript letter are not significantly different, based on bonferroni post-hoc test. Where : AB : Acid-activated bentonite, NB : Natural bentonite

The result for influence of natural and acid-activated bentonite clay at different inclusion levels on feed water stability are presented in table 3.6. Water stability differed significantly ($p<0.05$) during all periods. At periods 30 mins, control diet (A0B0) had the highest mean water stability, which was significantly different ($p<0.05$) as compared to those of all dietary treated feeds except for bentonite clay inclusion rates of 1500 and 3000 mg/kg At period 2 – 7hr, A3000 had the highest mean water stability, which differed significantly ($p<0.05$) from those of the control and other dietary clay treated feeds. At period 1 – 7hr, control diet had the lowest mean value, which differed significantly as compared to those of other treatments. However, at period 2 - 7hr, mean water stability increased with increase in bentonite clay inclusion. Acid-activated bentonite treated feeds had higher mean values as compared to those of bentonite treated feeds.

Table 3. 7: Influence of bentonite (NB and AB) inclusion levels on feed durability and density of *C. gariepinus* feed

	Bentonite blend and inclusion (mg/kg)									
Parameters	**0**	**500**		**1000**		**1500**		**3000**		
	A0B0	**AB**	**NB**	**AB**	**NB**	**AB**	**NB**	**AB**	**NB**	**SEM**
Feed durability (%)	98.98^{e}	99.02^{de}	98.95^{e}	99.13^{bc}	99.13^{bc}	99.09^{cd}	99.10^{cd}	99.33^{a}	99.20^{b}	0.02
Feed density (g/ml)	0.96^{d}	0.99^{bc}	0.99^{bc}	1.00^{ab}	0.99^{c}	1.01^{ab}	1.01^{ab}	1.01^{a}	1.01^{a}	0.002

SEM: Standard error of mean. *Mean values between the rows are verified at $p<0.05$; values on the same row with the same superscript letter are not significantly different, based on bonferroni post-hoc test. Where; (AB- Acid-activated bentonite, NB- Natural bentonite).

The feed durability and feed bulk density differed significantly ($p<0.05$) between the treatment diets as presented in table 3.7. Both parameters increased with increase in inclusion level of bentonite. Acid-activated bentonite at 3000 mg/kg (A3000) had the highest mean values, which differed significantly ($p<0.05$) as compared to control and other dietary clay treated feeds. The control diet (A0B0) had the lowest mean value, which differed significantly ($p<0.05$) from other treatments except at 500 mg/kg inclusion for durability.

Table 3. 8: Influence of bentonite (NB and AB) on nutrient leaching of *C. gariepinus* feed

	Bentonite blend and inclusion (mg/kg)									
Time Interval	**0**	**500**		**1000**		**1500**		**3000**		
	A0B0	**AB**	**NB**	**AB**	**NB**	**AB**	**NB**	**AB**	**NB**	**SEM**
Ash (%)										
30mins	6.25^{d}	6.28^{d}	6.29^{d}	6.42^{abc}	6.26^{d}	6.40^{c}	6.41^{bc}	6.47^{a}	6.46^{ab}	0.01
1hr	5.15^{f}	5.31^{e}	5.25^{e}	5.63^{d}	5.65^{d}	5.78^{c}	5.66^{d}	6.29^{a}	5.98^{b}	0.21
3hr	4.44^{g}	4.98^{f}	5.07^{e}	5.57^{d}	5.59^{d}	5.70^{c}	5.54^{d}	5.91^{a}	5.81^{b}	0.02
7hr	4.23^{d}	4.88^{c}	4.88^{c}	5.06^{ab}	4.89^{c}	5.01^{b}	4.89^{c}	5.13^{a}	5.08^{ab}	0.23
CP (%)										
30mins	37.47	37.88	37.39	37.55	37.16	37.25	37.56	37.55	37.48	0.08
1hr	30.23^{e}	32.38^{d}	31.67^{de}	35.00^{b}	34.69^{c}	35.56^{ab}	35.66^{a}	36.02^{a}	36.72^{a}	0.07
3hr	25.75^{e}	28.08^{d}	27.84^{d}	30.08^{c}	29.06^{cd}	30.04^{c}	31.34^{b}	32.09^{b}	33.85^{a}	0.09
7hr	21.45^{e}	24.66^{c}	23.55^{cd}	25.97^{c}	24.94^{c}	27.09^{b}	27.78^{b}	28.09^{ab}	29.07^{ab}	0.06

SEM: Standard error of mean. *Mean values between the rows are verified at $p<0.05$; values on the same row with the same superscript letter are not significantly different, based on bonferroni post-hoc test. Where; (AB- Acid-activated bentonite, NB- Natural bentonite).

The result for nutrient leaching of feed is presented in table 3.8. The ash (%) content increased significantly ($p<0.05$) with increase in bentonite inclusion level. Acid-activated bentonite inclusions had the higher mean values for ash and CP (%) as compared to natural bentonite. The mean ash (%) content decreased over a period of time in all treatments. The CP (%) differed significantly ($p<0.05$) in all treatment diets. CP (%) increased with bentonite clay inclusion level.

Activated bentonite had higher values at 500 mg/kg and 1000 mg/kg inclusion, while natural bentonite had higher values at 1500 mg/kg and 3000 mg/kg inclusion. The values of CP (%) decreased over time in all treatments.

3.5 Discussion

Nutrition is one of the most studied areas within aquaculture; efforts have been focused on the evaluation and preparation of diets with nutritional levels required by the organism, low cost and characteristics such as: palatability, texture, water stability and optimum digestibility that maximize growth and reproductive functions (Argüello-Guevara & Molina-Poveda, 2013b). To achieve retention of the physical integrity of feed, with minimal disintegration and nutrients leaching into water is not easy, not only because of the complicated manufacturing processes, storage and transport, but because the feed remain compact for a sufficient time to be consumed by the species in culture (Charest *et al.*, 2001; Abdollahi *et al.*, 2013). The true nutritional value of feed depends not only on the amount of nutrients, but also on its availability and quality (Argüello-Guevara & Molina-Poveda, 2013a).

Researchers have reported that feed immersed for more than an hour before consumption leads to pellets breaking into smaller particles. Its water soluble nutrients, such as vitamins, minerals, and amino acids, leached, thus, reducing its nutritional value (Brinker, 2007b). This leaching may cause the eutrophication of water, leading to a poor animal growth, inefficient feed conversion and low survival (Fagbenro & Jauncey, 1995b; Ighwela *et al.*, 2013). Binding materials are used as additives in feed manufacturing to improve feed integrity and enhance feed utilisation. The nature and quantity of binder directly influence the physical quality of feed and nutrient absorption by organism (Cheng *et al.*, 2002). Bentonite clay with its acid activation was used as a blend and each at different inclusion levels. Their influence as a blend and whole on pellet water stability, durability, bulk density, and nutrient leaching are discussed.

3.5.1 Water stability (%)

The result of this study validates the importance of adding bentonite clay as binder and corrobates the report of Cuzon *et al.* (1994), which explained that if stability of feed in water is below 70% after 3 hrs, the inclusion of feed binder is necessary to reduce dry matter loss. Bentonite clay has both binding and adsorption properties (Murray, 2000; Jović-jovičić *et al.*, 2010; Gu *et al.,* 2011). These properties bind the feed particles and reduce feed disintegration upon water absorption. It influences the increase in water stability of bentonite diets as compared to control. Thus, the water stability increases with quantity of bentonite clay in the feed. Also, acid-activated bentonite has higher binding and adsorption properties as compared to natural bentonite (Önal, 2007b; Faghihian & Mohammadi, 2013). This explained the increase in water stability seen with acid activated bentonite inclusion. Improved aquaculture production encompasses the administration of quality feed and its utilisation. Most aquaculture wastes are from dietary origin, thus, reduction of waste outputs through the use of highly water stable feed will enhance aquaculture production.

3.5.2 Feed durability (%) and Bulk density (g/l)

All feeds used in intensive aquaculture should be resistant to mechanical stress during transport, handling and in pneumatic feeding devices. At the same time, the feed should have a texture and size that facilitate high feed intake and efficient digestion by the fish (Sørensen, 2012).

The increase in feed durability (%) with inclusion of bentonite can be attributed to its binding, and adsorption properties (Murray, 2000; Jović-jovičić *et al.*, 2010; Slamova *et al.,* 2011). This result agreeds with Hansen & Storebakken (2007b), Kaliyan & Vance Morey (2009), and Kraugerud *et al.* (2011) that feed binders are capable of increasing pellet durability.

Feedstock variables (starch, protein, fibre, fat, lignin and extractives, moisture content, and particle size and its distribution), pre-conditioning processes (steam conditioning/pre- heating and addition of binders), and densification equipment variables (forming pressure, pellet mill and roll press variables) affect the strength and durability of densified products (Kaliyan & Vance Morey, 2009). The factors that increase the strength and durability would also increase the density and specific energy consumption. However, high density or high specific energy input does not necessarily indicate high strength or durability of the densified products (Thomas & van der Poel, 1996c; Kaliyan & Vance Morey, 2009). According to Temmerman *et al*. (2006), there is no relationship between the density and the durability of the biomass pellets and briquettes. According to results of this study, feed bulk density increased with inclusion and quantity of bentonite clay. These results may be useful to predict or explain the rate of diet consumption;

feed with less bulk density are said to require more mass as compared to feed with high bulk density of the same quantity (Thomas, van Vliet & van der Poel, 1998). The swelling characteristics of bentonite clay can be attributed to an increase in bulk density of diets with clay inclusion (Önal, 2007b; Slamova *et al.*, 2011; Faghihian & Mohammadi, 2013).

3.5.3 Nutrient Leaching (%)

The provision of essential nutrients is the primary purpose of any animal ration; the loss of nutrients due to leaching is an important consideration in aquaculture feeds (Fagbenro & Jauncey, 1995a).

The decrease in CP (%) over time for the bentonite treated diets was can be attributed to good water stability to reduce nutrient leaching due to high adsorption and binding properties of bentonite clay (Deng, *et al.,* 2010; Jović-jovičić *et al.*, 2010; Slamova *et al.*, 2011). The increase in combination of natural and acid-activated bentonite reduces nutrient leaching more significantly ($p<0.05$) than the control diet. An increase in binding, cation exchange and adsorption properties due to ionic bonding that exists with the combination may be responsible for improved nutrient leaching in clay treated diet as compared to control (Jović-jovičić *et al.*, 2010; Salem & Karimi, 2010; Yener & Bic, 2012). Leaching of water-soluble nutrients (e.g. amino acids) from formulated food particles is considered to be disadvantageous to fish culture systems, as it increases the incidence of water pollution and can lead to eutrophication (Schram, *et al.*, 2010; Kolarevic *et al.*, 2012; Chezhian et al., 2012).

3.6 Conclusion

In this study, bentonite and its acid-activated form were used as a blend product at high and low levels and separately at different inclusion levels. Blend clay forms improved pellet physical quality (i.e. water stability, durability, bulk density) and reduced nutrient leaching. Bentonite clay blend increased pellet water stability significantly at 7 hrs with ≥ 30% as compared to control diet, while CP (%) increased significantly with 2% in bentonite blend diets over the control diets. Pellet physical characteristics improved significantly with increase in quantity of acid-activated bentonite in the clay blend, and high level inclusion performed better than low level inclusion. Inclusion of either natural or activated bentonite improved the measured pellet physical qualities.

The physical qualities increased significantly with increase in inclusion level, while acid-activated bentonite at all inclusion levels performed significantly better over control and natural bentonite.

3.7 References

Abdollahi, M.R., Ravindran, V. & Svihus, B. 2013. Pelleting of broiler diets: An overview with emphasis on pellet quality and nutritional value. *Animal Feed Science and Technology*. 179(1-4):1–23.

Argüello-Guevara, W. & Molina-Poveda, C. 2013a. Effect of binder type and concentration on prepared feed stability, feed ingestion and digestibility of Litopenaeus vannamei broodstock diets. *Aquaculture Nutrition*. 19:515–522.

Argüello-Guevara, W. & Molina-Poveda, C. 2013b. Effect of binder type and concentration on prepared feed stability, feed ingestion and digestibility of Litopenaeus vannamei broodstock diets. *Aquaculture Nutrition*. 19(4):515–522.

Article, R. 2004. Kaolin , bentonite , and zeolites as feed supplements for animals : health advantages and risks. *Vet. Med. – Czech*, 49, 2004 (10): 389–399

Ayoola, M. O, De wet lourens, Khalid Salie 2015. Evaluating natural and acid activated bentonite as feed additives for improving water stability of feed for African Catfish. *Proc.Aquaculture Ass. of Southern Africa*, Sept 27-Oct 3, pp:53

Behnke, K. 2001. Factors influencing pellet quality. *Feed Tech*. 5(Table 1):19–22.

Behnke, K.C. 1996. Feed manufacturing technology: current issues and challenges. *Animal Feed Science and Technology*. 62(1):49–57.

Brinker, A. 2007a. Guar gum in rainbow trout (Oncorhynchus mykiss) feed: The influence of quality and dose on stabilisation of faecal solids. *Aquaculture*. 267(1-4):315–327.

Brinker, A. 2007b. Guar gum in rainbow trout (Oncorhynchus mykiss) feed: The influence of quality and dose on stabilisation of faecal solids. *Aquaculture*. 267:315–327.

Carraro, A., De Giacomo, A., Giannossi, M.L., Medici, L., Muscarella, M., Palazzo, L., Quaranta, V., Summa, V.,. 2014. Clay minerals as adsorbents of aflatoxin M1 from contaminated milk and effects on milk quality. *Applied Clay Science*. 88-89:92–99.

Charest, D.J., Balaban, M.O., Marshall, M.R. & Cornell, J. a. 2001. Astaxanthin Extraction from Crawfish Shells by Super critical CO2 with Ethanol as Cosolvent. *Journal of Aquatic Food Product Technology*. 10(February 2015):81–96.

Chen, Y.S., Beveridge, M.C.M. & Telfer, T.C. 1999. Physical characteristics of commercial pelleted Atlantic salmon feeds and consideration of implications for modeling of waste dispersion through sedimentation. *Aquaculture International*. 7:89–100.

Cheng, Z.J., Behnke, K.C. & Dominy, W.G. 2002. Effect of Moisture Content, Processing Water Temperature, and Immersion Time on Water Stability of Pelleted Shrimp Diets. *Journal of Applied Aquaculture*. 12(2):79–89.

Chezhian, A., Senthamils, D. & Kabilan, N. 2012. Histological Changes Induced by Ammonia and pH on the Gills of Fresh Water Fish Cyprinus carpio var. communis (Linnaeus). *Asian Journal of Animal and Veterinary Advances*. 7(7):588–596.

Cho, C.Y. & Bureau, D.P. 2001. A review of diet formulation strategies and feeding systems to reduce excretory and feed wastes in aquaculture. *Aquaculture Research*. 32:349–360.

Deng, Y., Luisa, A., Velázquez, B., Billes, F. & Dixon, J.B. 2010. Applied Clay Science Bonding mechanisms between aflatoxin B 1 and smectite. 50:92–98.

Dominy, W.G., Cody, J.J., Chai, M.K., Takamori, T.I., Larsen, B. & Forster, I.P. 2004. A Comparative Study of the Physical and Biological Properties of Commercially-Available Binders for Shrimp Feeds. *Journal of Applied Aquaculture*. 14(3-4):81–99.

Fagbenro, O. & Jauncey, K. 1995a. Water stability, nutrient leaching and nutritional properties of moist fermented fish silage diets. *Aquacultural Engineering*. 14(2):143–153.

Fagbenro, O. & Jauncey, K. 1995b. Water stability, nutrient leaching and nutritional properties of moist fermented fish silage diets. *Aquacultural Engineering*. 14:143–153.

Faghihian, H. & Mohammadi, M.H. 2013. Surface properties of pillared acid-activated bentonite as catalyst for selective production of linear alkylbenzene. *Applied Surface Science*. 264:492–499.

Fahrenholz, A. 2012. Evaluating factors affecting pellet durability and energy consumption in a pilot feed mill and comparing methods for evaluating pellet durability. i–92.

Gatlin, D.M., Barrows, F.T., Brown, P., Dabrowski, K., Gaylord, T.G., Hardy, R.W., Herman, E., Hu, G.. 2007. Expanding the utilisation of sustainable plant products in aquafeeds's': a review. *Aquaculture Research*. 38(6):551–579.

Goelema, J.O., Smits, a., Vaessen, L.M. & Wemmers, a. 1999. Effects of pressure toasting, expander treatment and pelleting on in vitro and in situ parameters of protein and starch in a mixture of broken peas, lupins and faba beans. *Animal Feed Science and Technology*. 78:109–126.

Gu, L., Yu, X., Xu, J., Lv, L. & Wang, Q. 2011. Removal of dichloroacetic acid from drinking water by using adsorptive ozonation. *Ecotoxicology* (2011) 20:1160–1166

Hansen, J.Ø. & Storebakken, T. 2007a. Effects of dietary cellulose level on pellet quality and nutrient digestibilities in rainbow trout (Oncorhynchus mykiss). *Aquaculture*. 272(1-4):458–465.

Hansen, J.Ø. & Storebakken, T. 2007b. Effects of dietary cellulose level on pellet quality and nutrient digestibilities in rainbow trout (Oncorhynchus mykiss). *Aquaculture*. 272(1-4):458–465.

Ighwela, K.A., Bin Ahmad, A. & Abol-Munafi, A.B. 2013. Water stability and nutrient leaching of different levels of maltose formulated fish pellets. *Global Veterinaria*. 10(6):638–642.

Jović-jovičić, N., Milutinović-nikolić, A., Banković, P. & Dojčinović, B. 2010. Synthesis , Characterization and Adsorptive Properties of Organobentonites. 117(5):849–854.

Kaliyan, N. & Vance Morey, R. 2009. Factors affecting strength and durability of densified biomass products. *Biomass and Bioenergy*. 33(3):337–359.

Kolarevic, J., Selset, R., Felip, O., Good, C., Snekvik, K., Takle, H., Ytteborg, E., Baeverfjord, G., 2012. Influence of long term ammonia exposure on Atlantic salmon (Salmo salar L.) parr growth and welfare. *Aquaculture Research*. 44, 1649–1664

Kraugerud, O.F., Jørgensen, H.Y. & Svihus, B. 2011. Physical properties of extruded fish feed with inclusion of different plant (legumes, oilseeds, or cereals) meals. *Animal Feed Science and Technology*. 163(2-4):244–254.

Luckham, P.F. & Rossi, S. 1999. The colloidal and rheological properties of bentonite suspensions. *Advances in Colloid and Interface Science*82:43–92.

Mesubi, M.A., Adekola, F.A., Odebunmi, E.O., Adekeye, J.I.D., State, O. & Science, M. 2008. Beneficiation and characterisation of a bentonite from north-eastern Nigeria. *Journal of the North Carolina Academy of Science*, 124(4), 2008, pp. 154–158

Murray, H.H. 2000. Traditional and new applications for kaolin , smectite , and palygorskite : a general overview.*Applied Clay Science* 17 2000 207–221

Noyan, H., Onal, M. & Sarikaya, Y. 2007. The effect of sulphuric acid activation on the crystallinity, surface area, porosity, surface acidity, and bleaching power of a bentonite. *Food Chemistry*. 105(1):156–163.

Önal, M. 2007. Swelling and cation exchange capacity relationship for the samples obtained from a bentonite by acid activations and heat treatments. *Applied Clay Science*. 37(1-2):74–80.

Opinion, S. 2012. Scientific Opinion on the safety and efficacy of bentonite as a technological feed additive for all species *EFSA Journal* 2010;10(7):2787

Orire, A.M., Sadiku, S.O.E. & Tiamiyu, L.O. 2010. Evaluation of yam starch (Discorea rotundata) as aquatic feed binder. *Pakistan Journal of Nutrition*. 9(7):668–671.

Partridge, G.J. & Southgate, P.C. 1999. The effect of binder composition on ingestion and assimilation of microbound diets (MBD) by barramundi Lates calcarifer Bloch larvae. *Aquaculture Research*. 30:879–886.

Rosas, C., Tut, J., Baeza, J., Sánchez, A., Sosa, V., Pascual, C., Arena, L., Domingues, P., et al. 2008a. Effect of type of binder on growth, digestibility, and energetic balance of Octopus maya. *Aquaculture*. 275:291–297.

Rosas, C., Tut, J., Baeza, J., Sánchez, A., Sosa, V., Pascual, C., Arena, L., Domingues, P., et al. 2008b. Effect of type of binder on growth, digestibility, and energetic balance of Octopus maya. *Aquaculture*. 275(1-4):291–297.

Ruscoe, I.M., Jones, C.M., Jones, P.L. & Caley, P. 2005. The effects of various binders and moisture content on pellet stability of research diets for freshwater crayfish. *Aquaculture Nutrition*. 11(Holdich 2002):87–93.

Salem, A. & Karimi, L. 2010. Physico-chemical variation in bentonite by sulfuric acid activation. *Korean Journal of Chemical Engineering*. 26(4):980–984.

Schram, E., Roques, J.A.C., Abbink, W., Spanings, T., de Vries, P., Bierman, S., de Vis, H. van & Flik, G. 2010. The impact of elevated water ammonia concentration on physiology, growth and feed intake of African catfish (Clarias gariepinus). *Aquaculture*. 306(1-4):108–115.

Schram, E., Roques, J.A.C., van Kuijk, T., Abbink, W., van de Heul, J., de Vries, P., Bierman, S., van de Vis, H.. 2014. The impact of elevated water ammonia and nitrate concentrations on physiology, growth and feed intake of pikeperch (Sander lucioperca). *Aquaculture*. 420-421:95–104.

Slamova, R., Trckova, M., Vondruskova, H., Zraly, Z. & Pavlik, I. 2011. Applied Clay Science Clay minerals in animal nutrition. *Applied Clay Science*. 51(4):395–398.

Sørensen, M. 2012. A review of the effects of ingredient composition and processing conditions on the physical qualities of extruded high-energy fish feed as measured by prevailing methods. *Aquaculture Nutrition*. 18(3):233–248.

Sørensen, M., Ljøkjel, K. & Storebakken, T. 2002. Apparent digestibility of protein , amino acids and energy in rainbow trout (Oncorhynchus mykiss) fed a fish meal based diet extruded at different temperatures. 211:215–225.

Steudel, A., Batenburg, L.F., Fischer, H.R., Weidler, P.G. & Emmerich, K. 2009. Alteration of swelling clay minerals by acid activation. *Applied Clay Science*. 44(1-2):105–115.

Tang, Z., Wen, C., Li, P., Wang, T. & Zhou, Y. 2014. Effect of zinc-bearing zeolite clinoptilolite on growth performance, nutrient retention, digestive enzyme activities, and intestinal function of broiler chickens. *Biological Trace Element Research*. 158(1):51–57.

Thomas, M. & van der Poel, a. F.B. 1996a. Physical quality of pelleted animal feed 1. Criteria for pellet quality. *Animal Feed Science and Technology*. 61(96):89–112.

Thomas, M. & van der Poel, a. F.B. 1996b. Physical quality of pelleted animal feed 1. Criteria for pellet quality. *Animal Feed Science and Technology*. 61(1-4):89–112.

Thomas, M. & van der Poel, A.F.B. 1996c. Physical quality of pelleted animal feed 1. Criteria for pellet quality. *Animal Feed Science and Technology*. 61(1-4):89–112.

Thomas, M., van Zuilichem, D.J. & van der Poel, a. F.B. 1997a. Physical quality of pelleted animal feed. 2. contribution of processes and its conditions. *Animal Feed Science and Technology*. 64(2-4):173–192.

Thomas, M., van Zuilichem, D.J. & van der Poel, A.F.B. 1997b. Physical quality of pelleted animal feed. 2. contribution of processes and its conditions. *Animal Feed Science and Technology*. 64(2-4):173–192.

Thomas, M., van Vliet, T. & van der Poel, a. F.B. 1998a. Physical quality of pelleted animal feed 3. Contribution of feedstuff components. *Animal Feed Science and Technology*. 70(1-2):59–78.

Thomas, M., van Vliet, T. & van der Poel, A.F.B. 1998b. Physical quality of pelleted animal feed 3. Contribution of feedstuff components. *Animal Feed Science and Technology*. 70(1-2):59–78.

Tomić, Z.P., Logar, V.P., Babic, B.M., Rogan, J.R. & Makreski, P. 2011. Comparison of structural, textural and thermal characteristics of pure and acid treated bentonites from Aleksinac and Petrovac (Serbia). *Spectrochimica acta. Part A, Molecular and biomolecular spectroscopy*. 82(1):389–95.

Wright, P. a & Wood, C.M. 2012. Seven things fish know about ammonia and we don’t. *Respiratory physiology & neurobiology*. 184(3):231–40.

Yener, N. & Bic, C. 2012. Applied Surface Science Simultaneous determination of cation exchange capacity and surface area of acid activated bentonite powders by methylene blue sorption. 258:2534–2539.

Zang, J.J., Piao, X.S., Huang, D.S.., Ma, X. & Ma, Y.X. 2009. Feed particle size: Implications on the digestion and performance of poultry. *Asian-Aust. J. Anim. Sci*. 22(September):107–112.

CHAPTER 4

EFFECT OF DIETARY NATURAL AND ACID-ACTIVATED BENTONITE BLENDS ON PRODUCTION PERFORMANCE AND HAEMATOLOGICAL PARAMETERS OF AFRICAN CATFISH (*Clarias gariepinus*)

Abstract

In aquaculture efficient feed utilisation is an important factor affecting the growth of fish and water quality, thus, determining the success of any aquaculture production. Feed binders are additives used to improve pellet quality and feed utilisation. Bentonites are natural or acid activated aluminosilicates with ion exchanging, adsorption, swelling and binding properties. Bentonite supplied as dietary additives to livestock feeds, such as that for swine, ruminants and poultry, improve weight gain, increase feed conversion ratios and promote animal health. The present study is aimed at investigating the natural and acid-activated bentonite clay combination on growth performance of African catfish, *Clarias gariepinus.* Clay combinations were included at 0, 25%, 50%, 75% and 100% with two inclusion levels (low – 500 and High – 1500 mg/kg) in feeds fed to a *Clarias gariepinus.* The experimental diets were fed *ad libitum* to juvenile *Clarias gariepinus* weighing (10 – 15 g) over an 84-days trial period to evaluate the growth performance and haematological parameters. Mean final total length, final weight, daily length gain, daily weight gain, hepatosomatic index (HSI), haematocrit (HCT) value, RBC and survival (%) were significantly ($p<0.05$) affected by treatment diets. Mean growth performance, feed conversion ratio and specific growth rate improved with increase in quantity of natural bentonite and low inclusion level. This study is a pioneer report of natural and acid-activated bentonite combinations as feed additives. Up to 50% at the low inclusion level of the bentonite clay blend is considered optimum.

4.1 Introduction

Over the last decade, spectacular growth has taken place in aquaculture. In the past, most production in developing countries is realized from pond-based or open-water extensive, improved extensive and semi-intensive practices using polyculture farming technologies (Bostock *et al.*, 2010; Miller & Atanda, 2011; Munguti *et al.*, 2014). Recently, the bulk of high value freshwater and marine carnivorous fish in developing countries is produced by intensive farming systems using high-cost nutrient inputs in the form of nutritionally-complete formulated diets (Brummett, 2008; Diana, 2009). It is agreed that global aquaculture production will continue to increase and much of the increased production in developing countries of Asia and Africa is likely to be achieved through the expansion of semi-intensive, small-scale pond aquaculture (Brummett, 2008; Bostock *et al.*, 2010). Nutrition and feeding will play an essential role in the sustained development of aquaculture (Ashley, 2007).

Feed is the biggest source of nutrient loading in fish aquaculture production; clear understanding of its impact is essential for sustainable development and maintaining good water quality in production systems (Nyina-Wamwiza *et al.*, 2012; Pohlenz & Gatlin, 2014). This will help reduce negative impacts and improve predictability of environmental effects (Oliva-Teles, 2012). However, it is generally acknowledged that these impacts can be reduced by feeding fish with more environmentally-friendly diets, developing better feeding strategies and by a sound farm management (Ng & Romano, 2013; Hixson, 2014).

Tacon & De Silva (1997), Oliva-Teles (2012), and Pohlenz & Gatlin (2014) explained the interrelationships among various factors and strategies in dealing with environmental pollution and aquafeeds'. The authors advocated a holistic approach and noted that fish nutritionists can no longer be formulators of nutritionally wholesome diets, but need to consider fresh strategies in diet development and feed cost reduction. Intensive farming of carnivorous fish species is primarily dependent on a supply of nutritionally-complete, formulated diets. However, mitigation of negative impacts of aquafeeds' through development of more environmentally-friendly diets is considered to be a major challenge. Potential pollutants from aquafeeds' are metabolic wastes, which include phosphorus and nitrogen, as well as organic matter, and these affect water quality, leading to reduction in production or loss of fish (Wright & Wood, 2009; Schram *et al.*, 2010; Tomasso, 2012)

Researchers have focused on precise requirements for protein, amino acid and energy for each species and stage of development, as well as strain, in an attempt to formulate environmental-friendly aquafeeds' that will enhance fish production (Bureau 1999; Giri *et al.,* 2000; Belal, 2005; Musa *et al.,* 2012). However, it is acknowledged that nutrient requirements change as the intensity of culture changes (Sørensen *et al.,* 2002). The digestibility of nutrients is not precisely defined in many commercial feeds, which has led to a little setback in completely maintaining environmentally-friendly aquafeeds' through precise nutrient requirements. Consequently, current research shows that feed performances and digestibility can be increased with the use of enzymes that enhance plant protein use and by use of extrusion technology (Wang, 2007; Odedeyi & Fagbenro, 2010; Bowyer *et al.,* 2012). Therefore, continued research on processing techniques and additives for improving feed performances and digestibility are required.

Aquafeed binder type and strength may influence the attractiveness and digestibility of aquafeeds'. Digestibility of feed will ultimately be influenced by the digestibility of the binder. A high concentration of binder may cause a reduction in diet digestibility, while too little binder may not be adequately water stable, resulting in deterioration of water quality and loss of valuable dietary nutrients (Partridge & Southgate, 1999). In formulating aquafeeds' using binders, it is important to balance the digestibility of the binder, effectiveness, and inclusion rate with the cost of the overall production process.

Bentonites are clays whose properties, such as crystal structure and size, cation exchange capacity (CEC), hydration and swelling, thixotropy, binding capacity, impermeability, plasticity and tendency to react with organic compounds, make them advantageous for a variety of applications (Shu-li *et al.*, 2009; Jović-jovičić *et al.*, 2010). Both the sodium and calcium forms of bentonite are used for the production of animal feed pellets. Bentonite is not only an effective binding agent but also an adsorbent of pathogenic micro-organisms, enzymes and toxins and thus improves the growth and health of the animals (Murray, 2000). Bentonites in their natural state have limited adsorption capacity, which is largely due to presence of impurities. This ability is greatly enhanced by treatment with strong inorganic acids. This process alters the structure, chemical composition, and physical properties of the clay while increasing its adsorption capacity and binding properties (Foletto *et al.,* 2013). To our understanding, with available published articles, no study has elucidated the application of acid-activated bentonite in animal husbandry or aquaculture.

C. gariepinus is a highly valued food fish in many African countries. The high cost of formulated commercial fish feeds is a major constraint to the expansion and growth of the aquaculture sector (Oellermann & Hecht, 2000b). This has led to several attempts by nutritionists to ensure adequate utilisation of feed nutrients and reduction in feed wastage. Normally catfish are bottom feeders, but their feeding habits are adaptable and they do filter feed in groups at the water surface, especially if the water quality is good. Hence, feed pellets with good physical quality are important for *C. gariepinus* production. The stomach is muscular and the intestine is thin walled and relatively short, implying a dependence on high-protein foods.

The research into application of dietary bentonite on production performance and physiological parameters in the freshwater *C. gariepinus* is scanty and to the best of our knowledge dietary acid-activated bentonite application is yet to be elucidated. These studies investigated the effect of natural and acid-activated bentonite combinations on production performance and haematological parameters of African catfish (*C. gariepinus*).

4.2 Material and Methods

4.2.1 Experimental Facility.

The experiment was carried out at Welgevallen farm, Division of Aquaculture's (DA) facilities of Stellenbosch University (SU), located on a geographical position system (GPS), coordinates 33^{o}56' 33.95" S and 18^{o}51'56.15 "E, Stellenbosch, Western Cape area, South Africa.

The experimental facilities consisted of a recirculating warm-water (indoor temperature controlled) aquaria system (RAS) that housed a total of 88 plastic tanks, dimensions (70 cm x 40 cm x 38 cm) and volume of 90 L each. The RAS consisted of eight (8) rows; each row had 11 tanks with controlled water recirculating system, a water filtration system, an aeration system, a water pump as well as a central heating system. The filtration system consisted of a mechanical filter and a pump.

Each tank had a separate aeration system providing a constant flow of water (13.6 ± 1.9 L/sec) and air (7.74 mg/L/sec) to each tank. The outflow passed through a mechanical filtration system. Air supply to the tanks was provided by a 1.1 kW blower (Model SCL V4, Incorezzo, Milano, Italy) distributing air via a flexible 5-mm tube with an air stone at the end (Plate 4.1).

Basic water quality parameters, such as temperature (^{o} C), dissolved oxygen (mg/L), and pH, were monitored daily using a HACH HQ 40-d multi-parameter instrument, with HACH LDO probe. Each plastic tank was cleaned at 2-weeks intervals throughout the duration of the study.

Plate 4. 4 : Recirculating aquatic system used during the experiment, incorporating 88 rectangular plastic tanks

4.2.2 Experimental layout and design

The experiment was conducted between October 2014 - January 2015 over 84-days duration. Clay mineral (natural and acid-activated bentonite) was included in commercial aquafeed diets at 10 different inclusion levels with a control diet, resulting in 12 treatments. All treatments were replicated seven (7) times. The experimental diets were fed *ad libitum*, two (2) times daily, and strict precaution was taken to avoid uneaten feed by fish. Tanks of fish were allocated to each treatment diet randomly.

Table 4. 4 : Inclusion level of clay mineral in *C. gariepinus* feed

S/N0	Treatments	Designation	Inclusion level of clay (mg/kg of basal diet BD)
I.	Basal diet only * BD	T_1	No inclusion
II.	Basal diet only * BD	T_2	No inclusion
III.	BD + NB (low level)	T_3	500 mg NB (low level)
IV.	BD + NB (high level)	T_4	1500 mg NB (high level)
V.	BD + 75 % NB + 25 % AB	T_5	375 mg NB : 125 mg AB (low level)
VI.	BD + 75 % NB + 25 % AB	T_6	1,125 mg NB : 375 mg AB (high level)
VII.	BD + 50 % NB + 50 % AB	T_7	250 mg NB : 250 mg AB (low level)
VIII.	BD + 50 % NB + 50 % AB	T_8	750 mg NB : 750 mg AB (high level)
IX.	BD + 25 % NB + 75 % AB	T_9	125 mg NB : 375 mg AB (low level)
X.	BD + 25 % NB + 75 % AB	T_{10}	375 mg NB : 1,125 mg AB (high level)
XI.	BD + AB (low level)	T_{11}	500 mg AB (low level)
XII.	BD + AB (high level)	T_{12}	1500 mg AB (high level)

- BD : Commercial Montego catfish grower diets

  Natural bentonite – NB   Acid activated bentonite - AB

4.2.3 Experimental Animals and Compounds

A total of 1155 mixed sex African catfish (*Clarias gariepinus*) weighing between 10 and 15 g were obtained from a commercial farm Aquaculture Innovations, Grahamstown, South Africa. Fish were bathed in the antibiotic Chloramine-T at 10 mg/L for five (5) minutes. Treatment was repeated for three (3) consecutive days. All fish were left in the quarantine tanks for seven (7) days**.** The fish were randomly allocated to 77 tanks at a stocking density of 15 fish per tank. The fish were allowed to acclimatize for seven (7) days and, after this period, the treatment diets were fed for a period of 84 days. The fish were fed until visual observation of satiation (Baker, 1984) at two periods, i.e. 8.00 hrs and 16:00 hrs.

Clay minerals (natural and acid-activated bentonite) were obtained from Nutrex NV Lille, Belgium). See plate (4:2) for visual appearance of clay minerals. A commercial basal diet for catfish produced by Montego feed mill, Cape Town, South Africa was procured. The clay powders were included as an additive into feed at various treatment ratios (Table 4.1).

Plate 4. 5 : Clay mineral used for the study (From L-R: Natural bentonite, acid-activated bentonite clay)

4.2.4 Feed preparation

Feed was prepared by grinding the hot-extruded commercial pellet and then re-forming it with a cold extruder (70-80 D). All treatment diets were thoroughly mixed and 1.8 L of water was added to 5 kg of feed to form a dough using Mac electronic mixer (See plate 4.3). An extruder machine with dice hole of size 3 mm (Plate 4.4) was used. Pellets were allowed to air dry, using a dry air blower at 60^0C for 12 hrs (Plate 4.5). After drying, a crusher was used to crush and separate the pellets into regular sizes (Plate 4.6), before packaging into airtight containers. Treatment diets were produced weekly and the control diet was without clay mineral inclusion.

Mac electronic mixer

Laboratory extruder

An air dryer chamber

A feed crusher and separator

Plate 4. 6 : Feed processing machines.

4.2.5 Data collection

At the beginning of the experiment, total length (TL, i.e. from tip of snout to the tail end) and body weight of each fish were taken using a calibrated measuring board (measured to the nearest 0.01 mm) and electronic balance, UWE, HGS-300, Capacity: 300 X 0.01 g (measured to the nearest 0.01 g), respectively (Skelton, 2001). All fish were fasted 12 hrs prior to sampling period. Sampling of fish was taken at 21-days intervals and each fish was measured per tank for the aforementioned parameters. Clove oil was used as anaesthesia during all sampling. Feed consumption was recorded and mortality checked daily in each tank.

After the 84-day trial period, one fish was randomly selected from each tank for collection of blood samples for haematological study and the liver was removed and weighed using the electronic weighing balance. Hepatosomatic index (HSI) for all the sampled fish per tank were calculated.

Blood collection and haematological study

Three (3) fishes from each tank was bled via the caudal vein using non-heparinized needles with syringes. At least 2 ml of blood was collected from each fish into sample bottles containing an anticoagulant, ethylene diamine tetra acetate (EDTA) for haematological parameters. Bottles were immediately stored at 4^0C.

Two haematocrit capillary tubes were ¾-filled simultaneously with blood containing EDTA. The vacant end of the tube was sealed with Critoseal. Each tube was placed into the special centrifuge with the sealed end facing outward. Paired samples were placed opposite each other. The cover plate was "finger-tightened" and the lid put into the Down and Lock position. The centrifuge was set to run for 5 minutes. The haematocrit value, following centrifugation, was determined using the Graphic Reader.

The Red blood cell, White blood cell, and Haemoglobin (Hb) were determined using the automatic blood analyser Cell Dyn 3700 haematology analyser at the Department of Physiology, Stellenbosch University. All other constituents of haematological indices were calculated from the whole blood results.

Calculated values

i. Specific growth rate, feed intake and feed conversion rate

Specific growth rate, feed intake and feed conversion rate were calculated over the trial period (84 days); the fish in each tank were counted and individually weighed (Mettler PM 34 Delta range) to the nearest 1 g, to calculate the specific growth rate (SGR) as follows:

$$\mathrm{SGR} = \mathrm{W_t} - \mathrm{W_1} \times \frac{100}{t}$$

Where SGR=specific growth rate (%/d), Wt=mean weight at day 84 (g), W_1= mean weight at day 1 (g) and t = number of days.

Feed was administered twice daily at 0800 h and 1600 h until visually observed satiation. Feed fed per tank was recorded. Uneaten pellets were collected from each tank one hour after the two daily feeding sessions. Feed loss per tank was calculated as the total number of uneaten feed pellets multiplied by 0.0966 g/pellet, determined by weighing 100 feed pellets. Daily feed intake per tank was recorded, defined as the difference between daily feed rate and feed loss. Total feed intake per tank resulted from the sum of the daily feed intake. Total feed intake and biomass per tank were used to calculate feed conversion rate (FCR) as follows:

$$\mathrm{FCR} = \frac{\mathrm{TFI}}{nt \times Wt - n1 \times W1}$$

Where FCR=feed conversion rate (g/g), TFI=total feed intake (g), Wt=mean weight at day 84 (g), W1=mean weight at day 1 (g), n_t =number of fish at day 84 and n1=number of fish at day 1.

$$\mathrm{ADWG} = \frac{Fnwt - Inwt}{nt}$$

Where ADWG = average daily weight gain (g), F_{nwt} = mean final wt (g), I_{nwt} = mean initial weight (g), nt = number of rearing days (84)

$$\mathrm{ADLG} = \frac{Fnlt - Inlt}{nt}$$

Where ADLG = average daily length gain (gmm), F_{nlt} = mean final length (mm), I_{nlt} = mean initial length (mm), nt = number of rearing days (84)

$$\mathrm{RFI} = \left.\frac{TFI}{Fnwt - Inwt}\middle/ nt\right. \times 100$$

Where RFI = relative feed intake (%), F_{nwt} = mean final weight (g), I_{nwt} = mean initial weight (g), nt = number of rearing days (84)

$$\text{Survival (\%)} = \frac{Nft}{Nfl} \times 100$$

Where Nft = number of fish at day 84, Nfl = number of fish at day 1

$$\text{HSI (\%)} = \frac{Liver\ (wt)}{Fish\ (wt)} \times 100$$

Where HSI = hepatosomatic index (%)

4.2.6 Statistical analysis

All data recordings and calculated values, which included total length, body weight, survival rate, average daily length gain, average daily weight gain, feed conversion rate, specific growth rate, hepatosomatic index and haematological indices, were computed using Microsoft Excel 2010 and Sharp Model ELW53H Scientific Calculator. A regression analysis with two-way analysis of variance (ANOVA) was performed. Bonferroni was used to test for the significance of variance ($p<0.05$) for all recorded and calculated data among different treatments using SAS 2015 version 12 and XLSTAT version 2015.

4.3 Results

The production performance parameter results of *C. gariepinus* fed natural and acid-activated bentonite clay combinations in feed during 84-days trial period are presented in Table 4.2, which presents mean initial total length, initial weight, final total length, final weight, average daily length gain (ADLG), average daily weight gain (ADWG), feed conversion rate (FCR), specific growth rate (SGR), hepatosomatic index (HSI) and percentage survival rate (% survival). Table 4.3 records values for low and high inclusion levels of mean initial total length, initial weight, final total length, final weight, average daily length gain (ADLG), average daily weight gain (ADWG), feed conversion rate (FCR), specific growth rate (SGR), hepatosomatic index (HSI) and percentage survival rate (%), respectively. Haematological parameters are shown in Table 4.4, which includes mean haemoglobin (Hb), haematocrit (HCT), red blood cells (RBC), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and white blood cells (WBC).

Table 4. 5 : Production performance of C. gariepinus fed dietary bentonite clay blend

	Treatments						
Parameters	**A0B0**	**A0B100**	**A25B75**	**A50B50**	**A75B25**	**A100B0**	**SEM**
Initial TL(mm)	106.65	104.51	103.69	102.92	104.21	103.08	1.80
Final TL(mm)	328.79^{ab}	342.25^{a}	340.10^{a}	339.86^{a}	328.83^{ab}	321.35^{b}	4.19
Initial Wt. (g)	8.72	8.03	8.03	7.85	8.17	7.80	0.36
Final Wt. (g)	303.76^{ab}	336.97^{a}	331.27^{ab}	321.95^{ab}	299.29^{ab}	287.18^{b}	11.90
ADLG (g)	2.66^{b}	2.87^{a}	2.84^{a}	2.86^{a}	2.69^{ab}	2.61^{b}	0.04
ADWG (g)	3.65^{ab}	4.12^{a}	4.04^{a}	3.96^{ab}	3.65^{ab}	3.45^{b}	0.14
RFI (g)	3.18^{a}	2.85^{bc}	2.73^{c}	2.82^{bc}	2.88^{b}	2.79^{bc}	0.05
FCR	1.39	1.28	1.36	1.43	1.59	1.34	0.12
SGR (%)	3.51	3.92	3.86	3.74	3.45	3.33	0.19
HSI (%)	1.62^{d}	2.21^{c}	2.93^{b}	3.04^{b}	3.04^{b}	3.88^{a}	0.06
Survival (%)	78.10^{a}	73.81^{b}	71.43^{bc}	67.62^{c}	62.86^{d}	59.52^{d}	0.99

SEM: Standard error of mean. *Mean values between the rows are verified at $p<0.05$; values on the same row with the same superscript letter are not significantly different based on bonferroni post-hoc test. Where: A0B100 = 100% NB, A25B75 = 25% AB: 75% NB, A50B50 = 50% AB, 50% NB, A75B25 = 75% AB: 25% NB, A100B0 = 100% AB, A0B0 = control

Table 4.2 showed the growth performance of *C. gariepinus* fed combination of dietary natural and acid-activated bentonite over 84 days. The mean final TL and final weight values were significantly different ($p<0.05$) among the treatments. A100B0 had the lowest mean final TL (321 ± 4.19 mm) and weight (287.18 ±11.90 g) respectively, which differed significantly ($p<0.05$) from those of other treatment diets. The mean values for final TL decreased with increase in the quantity of acid-activated bentonite in the clay blend and mean final weight followed the same trend. Mean ADLG and ADWG of A100B0 differed significantly ($p<0.05$) as compared to A0B100 and A25B75 treatments. A100B0 had the lowest mean ADLG (2.61 ± 0.04), which differed significantly ($p<0.05$) from those of other dietary clay diets but not control. Mean ADLG and ADWG decreased with increase in quantity of acid-activated bentonite in the clay blend. However, low inclusion level had higher values for the parameters as compared to high inclusion level. The inclusion level (low and high) with bentonite blend combination had no significant interaction for mean final TL, final Weight, ADLG and ADWG.

Table 4. 6 : Mean production performance of *C .gariepinus* fed dietary bentonite clay blend at low inclusion and high level (500 & 1500 mg/kg)

	Treatments						
Low Inclusion Level							
Parameters	**A0B0**	**A0B100**	**A25B75**	**A50B50**	**A75B25**	**A100B0**	**SEM**
Final TL(g)	328.79^{ab}	344.44^{ab}	349.42^{a}	343.96^{ab}	337.76^{ab}	322.61^{ab}	5.93
Final Wt. (g)	303.76^{ab}	340.70^{ab}	356.51^{a}	332.03^{ab}	323.93^{ab}	290.33^{ab}	16.83
ADLG (mm)	2.66^{b}	2.91^{abc}	2.96^{a}	2.93^{ab}	2.78^{abcd}	2.62^{d}	0.06
ADWG (g)	3.65^{ab}	4.23^{ab}	4.38^{a}	4.08^{ab}	3.91^{ab}	3.49^{ab}	0.19
RFI (g)	3.18^{a}	2.79^{bc}	2.70^{c}	2.77^{ab}	2.83^{abc}	2.74^{bc}	0.07
FCR	1.39	1.26	1.28	1.42	1.45	1.31	0.18
SGR (%)	3.51	3.96	4.17	3.86	3.76	3.36	0.26
Survival (%)	78.10^{a}	77.14^{ab}	74.29^{abc}	70.48^{bcd}	63.81^{de}	62.86^{ef}	1.40
High Inclusion Level							
Parameters	**A0B0**	**A0B100**	**A25B75**	**A50B50**	**A75B25**	**A100B0**	**SEM**
Final TL(g)	328.79^{ab}	340.06^{ab}	330.79^{ab}	335.75^{ab}	319.91^{b}	320.10^{ab}	5.93
Final Wt. (g)	303.76^{ab}	333.23^{ab}	306.03^{ab}	311.88^{ab}	274.65^{b}	284.03^{ab}	16.83
ADLG (mm)	2.66^{b}	2.83^{abcd}	2.72^{abcd}	2.80^{abcd}	2.60^{d}	2.60^{cd}	0.06
ADWG (g)	3.65^{ab}	4.01^{ab}	3.71^{ab}	3.84^{ab}	3.39^{b}	3.41^{b}	0.19
RFI (g)	3.18^{a}	2.90^{ab}	2.77^{bc}	2.86^{bc}	2.92^{ab}	2.79^{bc}	0.07
FCR	1.39	1.29	1.43	1.45	1.74	1.36	0.18
SGR (%)	3.51	3.87	3.55	3.62	3.17	3.29	0.26
Survival (%)	78.10^{a}	70.48^{ab}	68.57^{cde}	64.76^{de}	61.91^{ef}	56.19^{f}	1.40

SEM: Standard error of mean. *Mean values between the rows are verified at $p<0.05$; values on the same row with the same superscript letter are not significantly different based on bonferroni post-hoc test. Where: A0B100 = 100% NB, A25B75 = 75% NB: 25% AB, A50B50 = 50% NB: AB, A75B25 = 75% AB : 25% NB, A100B0 = 100% AB, A0B0 = control

The relative feed intake (RFI) differed significantly ($p<0.05$) between the treatments. The control diet A0B0 had the highest mean value (3.18 ± 0.07), which differed significantly ($p<0.05$) from those of the dietary bentonite blend diets.

The inclusion level and bentonite blend had no significant interaction for RFI. However, low inclusion level had lower values consistently for all bentonite clay forms as compared to high inclusion.

Mean feed conversion ratio (FCR) and specific growth rate (SGR) values were not significantly different ($p>0.05$) between the treatments diets of bentonites. Mean FCR values linearly improved at bentonite A0B100 (1.28±0.12) and A0B0 (1.39±0.12). The value increased with the quantity of acid activated bentonite. A0B100 had the highest value (3.92±0.19) and lowest value A100B0 (3.33±0.19). There is significant interaction ($p<0.05$) between the inclusion level and bentonite clay form for FCR.

The percentage survival (% survival) was significantly affected by the treatment ($p<0.05$). Treatments A0B0, A0B100 and A25B75 were not significantly different ($p>0.05$) but differed significantly from A100B0 and A75B25. A50B50 is significantly different ($p<0.05$) from A0B0 but not differed significantly ($p>0.05$) from other treatments. A0B0 had the highest mean value (78.10±0.99) and A100B0 with the lowest mean value (59.52±0.99). The inclusion level and bentonite clay form had no significant interaction. Low inclusion level had higher values for survival (%) as compared to those of high inclusion level. The % survival values decreased with increased in quantity of acid-activated bentonite in the clay form.

Hepatosomatic index (%, HSI, Table 4.2) differed significantly ($p<0.05$) among treatments. A0B0, A0B100, A100B0 were significantly different ($p<0.05$) from each other and those of the other treatment diets. But treatments A25B75, A50B50 and A75B25 were not significantly different ($p>0.05$) from each other. A100B0 had the highest mean value (3.88±0.06) and A0B0 the lowest mean value (1.62±0.06).

The haematocrit values (HCT) were significantly different ($p<0.05$) among the treatment diets. A50B50 (34.84±0.53) had the highest mean value, which differed ($p<0.05$) from those of A0B100 and A100B0 (31.97±0.53). Bentonite combinations and inclusion levels showed a significant interaction. Haemoglobin (Hb) mean values were not significantly different ($p>0.05$); A0B0 (12.93±0.24) had the highest mean value and A75B25 (12.6±0.24) the lowest. Mean red blood cells (RBC) values were significantly different ($p<0.05$) among treatments. A0B0 (2.85±0.06) had the highest mean value, which differed from those of A25B75 and A100B0 (2.52±0.06), which were the lowest means. Mean corpuscular haemoglobin concentration (MCHC) values are not significantly different ($p>0.05$) between treatments. A0B100 (39.27±0.86) and A50B50 (36.67±0.86) were the highest and lowest values respectively.

Table 4. 4: Haematological indices of C.gariepinus fed dietary bentonite clay blend

	Treatments						
Parameters	**A0B0**	**A0B100**	**A25B75**	**A50B50**	**A75B25**	**A100B0**	**SEM**
HCT (%)	33.93^{ab}	32.31^{b}	33.16^{ab}	34.84^{a}	33.51^{ab}	31.97^{b}	0.53
Hb (g/dl)	12.93	12.61	12.37	12.74	12.6	12.30	0.24
RBC($10e^{12}$/l)	2.85^{a}	2.65^{ab}	2.56^{b}	2.71^{ab}	2.58^{ab}	2.52^{b}	0.06
MCHC(g/dl)	38.12	39.27	37.48	36.67	37.95	38.55	0.86
MCH(pg)	45.35^{b}	47.72^{ab}	48.48^{a}	47.05^{ab}	49.15^{a}	48.96^{a}	0.69
MCV(fl)	119.10	123.39	131.45	128.83	131.11	127.57	3.54
WBC($10e^{9}$/l)	78.29	77.79	74.07	77.14	76.21	74.07	1.38

Differences between the rows are verified at $p<0.05$, values on the same row with the same letter are not significantly different

Mean corpuscular haemoglobin (MCH) values differed significantly ($p<0.05$). Treatment with highest value A75B25 (49.15±0.69) differed from A0B0 (45.35±0.69) lowest value. There were significant interactions between levels and bentonites but no differences between levels. Mean corpuscular volume (MCV) values were not significantly different ($p>0.05$) for bentonite and levels, with A25B75 (131.45±3.54) having the highest value and A0B0 (119.10±3.54) the lowest value. White blood cells (WBC) values were not significantly different ($p>0.05$) with the highest value A0B0 (78.29±1.38) and lowest values A100B0 and A25B75 (74.07±1.38). However, WBC values were significantly different ($p<0.05$) between levels. High levels had higher values as compared to low levels. No significant interaction between bentonites and levels

4.4 Discussion

4.4.1 Growth performance and feed utilisation

The results obtained in this experiment indicated that dietary bentonite clay blend had a significant ($p<0.05$) effect on production performance of *C.gariepinus* as compared to those of the control. The optimum production performance of *C.gariepinus* was found with combination of acid-activated bentonite of not more than 25% blend at low inclusion level (0.5%).

The improved production performance parameters are in disagreement with the findings made by (Eya *et al.*, 2008) who found that inclusion of bentonite at 5% were the optimum levels for maximum % weight gain, specific growth rate and feed efficiency in rainbow trout. The result are also in contrast with some studies with no effect of dietary clay on trout and tilapia fish species (Dias *et al*., 1998; Kanyılmaz and Tekelioglu, 2009; Yıldırım *et al*., 2009). The inclusion levels of clay used in this study are quite low (0.05 and 0.15%) as compared to previous studies, yet significant improvement in growth parameters measured in this study can be attributed to the quality of clay materials. The binding effect and swelling capacity are characteristics that varied with clay origin and processing (Murray, 1991; Ghahri *et al.*, 2010; Jović-jovičić *et al.*, 2010).

Binders are used to improve feed manufacture and to stabilize diets in water. Lignosulfonates, cellulose, carboxymethylcellulose (CMC), corn starch, wheat starch, arabic gum, polymers, aluminosilicates, among others are used as binders (Rosas *et al.*, 2008a). However, binders are capable of increasing the digesta viscosity through binding of nutrients and other feed constituents (Amirkolaie *et al.,* 2005). Digesta viscosity is an important phenomenon that affects digestibility in fish. An optimum viscosity of ingested feed can enhance digestion, through relatively slow passage of feed through the gut. This encourages proper breakdown of feed materials by digestive enzymes and enhances absorption and utilisation of nutrients, as suspected in this study with the resultant improved growth. This result corrobates Phillips *et al.* (1987) and Eya *et al.* (2008) that attributed improved performance in rainbow trout fed bentonite and mordenite to improved utilisation of nutrients. Dias *et al*. (1998) and Danabas & Altun (2011) further explained that slower passage of pre-digested food through the intestine leads to the improved utilisation of nutrients from the feeding dose of bentonite and zeolite, particularly protein (See chapter 4).

The RFI of control diet (A0B0) had the highest mean value, which was significantly different ($p<0.05$) from dietary clay diets. Although it is expected that the dietary clay treated diets would have higher RFI due to the higher feed density as compared to that of the control (See chapter 3). Therefore, the slow passage of dietary clay treated diets in the gut, which led to reduced stomach emptying rate as compared to control, may be responsible for the observed RFI. The return of appetite in dietary clay treated diets is slower as compared to control diet (See chapter 4). The increased mean FCR with inclusion of acid-activated bentonite in the dietary clay blend can be attributed to high binding effect of activated clay (Ayoola *et al*. 2015). This high binding effect made the aquafeeds' harder as compared to that of the control and increased the time used by digestive enzymes to break down the feed for absorption. High residence time of feed in the gut without digestion enhances fermentation and low feed utilisation cum FCR (Amirkolaie, et al., 2006; Verreth, *et al.,* 2010).

The decrease in growth performance of *C. gariepinus* observed with increase in quantity of acid-activated bentonite and high inclusion level can be attributed to binding characteristics leading to an increase in stomach and intestinal viscosity. The application of acid-activated bentonite in feed for aquafeeds' is very rare and this study may present the first data on this clay as a dietary material. Acid activation of bentonite with inorganic acids increases the cation exchange ability, adsorption, and binding characteristics (Noyan, *et al.*, 2007; Tomić, Logar *et al.*, 2011; Yener *et al.*, 2012). Similar to the observed effect of acid-activated bentonite in this study, Leenhouwers *et al.* (2006) recorded the largest reduction in protein digestibility of *C. gariepinus* at the highest guar gum inclusion level attributed to high viscosity. The binding effect that enhanced these viscous properties seen in acid-activated clay bentonites and high inclusion of bentonite combinations can be related to soluble NSP reducing mixing of digestive enzymes and substrates, enhancing endogenous losses of nutrients and increasing the thickness of the unstirred water layer adjacent to the mucosa, all leading to impaired nutrient digestion and absorption (Leenhouwers *et al.*, 2007; Rosas *et al.*, 2008a; Sinha, *et al.*, 2011).

4.4.2 Survival and Hepatosomatic index

The mean percentage survival value differed significantly ($p<0.05$) and decreased from control treatment diets A0B0 (78.10±0.99) based upon acid-activated bentonite quantity and high inclusion level A100B0 (59.52±0.99). Hepatosomatic index, which is the percentage of wet liver weight to body weight differed significantly ($p<0.05$) among the treatments. It increased from control diet A0B0 (1.62±0.06) with acid-activated bentonite quantity A100B0 (3.88±0.06). In this study, cannibalism was controlled strictly by ensuring minimum variation in fish size at stocking, stocking density and *ad libitum* feeding practices (Martins, *et al.,* 2005; Marimuthu, *et al.,* 2011). An incidence of mortality occurred at days 60 – 67 of the trial period. Dead fish were observed to have distended stomach. Stomachs of carcasses were filled with oil and little feed remnants. At this period, the water temperature was extremely high due to atmospheric conditions (30 – 35^0C). Thus, the high mortality can be attributed to incidence of nutrition syndrome condition known as bloat.

In poultry, the antinutritional effects of soluble NSP are mediated by changes in digesta characteristics, such as increased digesta viscosity and increased fermentation activity (Maisonnier *et al.,* 2001; Abdollahi *et al.*, 2013). High viscosity also increases residence time of the digesta and therefore increases intestinal volatile fatty acids (VFA) production. The resulting drastic changes in the gut ecosystem may decrease nutrient digestion and ultimately have been shown to reduce performance of broiler chickens (Maisonnier *et al.*, 2001). In Nile tilapia, Amirkolaie *et al*. (2006) measured the VFA of digesta fed guar gum incorporated in feed as an indicator for the occurrence of fermentation in the intestine. The total VFA concentration increased as digesta passed through the intestine. This increased microbial fermentation activity as digesta passed through the intestinal tract. VFA are produced when protein is fermented in the intestine (Williams *et al.,* 2001), as fermentation of carbohydrate also produces VFA in Nile tilapia (Amirkolaie *et al.*, 2006). As observed in this study, up to 40% mortality was associated with a high prevalence of bloat in rainbow trout and chinook salmon fed high viscous feed at high water temperature (Staurnes *et al*. 1990; Lumsden *et al*. 2002).

In fish, the amount of acid and pepsin produced is roughly proportional to the amount of stomach distension. Contractions of the stomach wall triturate food and, with the aid of digestive juices, reduce the food to a thin liquid (chyme) which is able to pass through the normally partially open pyloric sphincter and into intestine (Bowyer *et al.*, 2012).The rate of gastric emptying in fish varies with temperature, season, activity, body size, satiety, food type and metabolic rate (Odedeyi & Fagbenro, 2010). The nervous system (enterogastric reflex) and hormonal control regulate the flow of chyme into the proximal small intestine. Receptors in the small intestinal mucosa and intestinal wall are sensitive to excessive stomach distension, acidity, osmolality and high oil levels to regulate the quantity and quality of the chyme (Tacon & De Silva, 1997).

Therefore, there is a negative feedback mechanism operating to protect the intestinal tract from excessive stretching, mucosal damage and nutrient overload. This combined nervous and hormonal feedback mechanism between the intestine and stomach is referred to as enterogastric control (Anderson, 2006). These mechanisms act to reduce the acidity of the chyme. Pellets manufactured with superior binding properties act to slow the disintegration and digestion of feed, as shown to increase the incidence of bloat in rainbow trout and coho salmon (Staurnes *et al.*, 1990). Chyme produced during digestion of pelleted foods with high binding properties in the stomach will contain considerably higher concentrations of nutrients and will be less well digested (Partridge & Southgate, 1999). Limited amount of digestive juice will be available for the breakdown of large volume of feed; chyme produced under these conditions is likely to be a potent activator of enterogastric feedback, leading to increase in acidity of chyme from microbial fermentation (Anderson, 2006).

Hepatosomatic indices (HSI) in this study were significantly ($p<0.05$) affected by the treatment diets. The lowest mean value recorded for the control diet is within the range recorded for fish with normal health status (Sadekarpawar & Parikh 2013; Wang *et al*. 2014; Sink & Lochmann 2014). The values reported for normal fish of same specie used in this study varied with different authors. Therefore, the increased values observed in this study with increased quantity of acid-activated bentonite can be attributed to increased activity of liver during metabolism of diets. The high binding properties increase digesta viscosity and, thus, the rate of metabolism. Consequently, the increase in the mean HSI in this study may be sex related (Boonyaratpalin, *et al.*, 1998; Wilson & Castro, 2010; Sadekarpawar & Parikh, 2013). Fish used in this study were mix sex fish, thus, significant differences ($p<0.5$) among treatments may be due to variation in sex differences among the treatments; further study is suggested to ascertain the significant observation of HSI.

4.4.3 Haematological parameters

Blood parameters are considered patho-physiological indicators and help fish biologists to interpret physiological responses to stress imposed by the environment (Akinrotimi *et al.*, 2007). Haematological indices of most fish have been studied with the aim of establishing normal value ranges and any deviation from them may indicate a disturbance in the physiological process (Zang *et al.*, 2013; Wang *et al.*, 2014)**.** Stress activates non-specific responses in fish, which enables the fish to cope with the disturbance and maintenance of its homeostatic state. But if stress perseveres or persists, the response then becomes mal-adaptive and threatens the fish's health and general wellbeing (Ogunji, *et al.*, 2008). Therefore, in the presence of stressors (contaminants /pollutants), haematological parameters and blood chemistry can be used as standard laboratory tests to determine diseased conditions and metabolic disturbances in fish. Studies have reported varying range of haematological parameters for healthy or normal *Clarias spp.*, with contradicting results. However, values obtained in this experiment were within the normal ranges recommended for *C. gariepinus*, HCT values (35.25±4.14), Hb (12.10±2.12)g/dl, RBC (2.8±0.15)10e^{12}/l, WBC (80±5.45) (Dada & Ikuerowo, 2009; Senthil Kumaran, *et al.*, 2011; Erhunmwunse & Ainerua, 2013).

The haematocrit values (HCT), red blood cells (RBC) and mean corpuscular haemoglobin (MCH) were significantly different (p<0.05) between the treatments diet. These differences may be attributed to sex differences. In the assessment of the blood parameters of goldfish, *Carassius auratus*, Summerfelt (1967) in (Length 2004; Gabriel *et al.*, 2009) observed that males consistently had significantly higher haematocrit values than the females and suggested the need to separate blood component data on the basis of sex to avoid attributing sex differences to other factors.

4.5 Conclusion

The present findings showed that dietary bentonite blend at low and high inclusion had significant effects on the production performance and haematological parameters measured. The positive results from this study were recorded with an increase in quantity of natural bentonite at low inclusion level. Increase in quantity of acid -activated bentonite increased the binding characteristics of the feed and higher digesta viscosity (See chapter 3 and 4). These slow down the digestion with poor absorption of nutrient for growth in fish as reported by Leenhouwers *et al.* (2006), Leenhouwers *et al.* (2007), and Eya *et al.* (2008). An increase in the quantity of acid-activated bentonite is suspected to enhance microbial fermentation in the fish stomach due to high digesta viscosity. It is suspected that fermentation in the gut enhanced production of VFA in the stomach, which led to a syndrome called “bloat”. High water temperature triggered the bloat condition and led to mortality of fish. These observations are related to other studies (Storebakken, 1985; Staurnes *et al.*, 1990; Amirkolaie *et al.*, 2005, 2006; Brinker, 2007; Rosas, *et al.*, 2008b). The haematological profile and hepatosomatic index showed that the inclusion of bentonite clay does not have any toxic or environmental effect on fish. Overall, 100% natural bentonite A0B100 at low inclusion level (0.05 mg/kg) feed had the best performance, while quantity up to 50% acid activated bentonite combination with natural bentonite A50B50 low inclusion level (0.05 mg/kg) feed is optimum. This study showed that natural and acid-activated bentonite combination can improve feed utilisation and growth performance of *C. gariepinus.* High inclusion level and increase in quantity of acid-activated bentonite in high production water temperature can cause mortality. It is suggested that further studies should be carried out to evaluate different inclusion level of dietary clay and access gut fermentation.

4.6 References

Abdollahi, M.R., Ravindran, V. & Svihus, B. 2013. Pelleting of broiler diets: An overview with emphasis on pellet quality and nutritional value. *Animal Feed Science and Technology*. 179(1-4):1–23.

Amany, B. 2009. Pathological studies on effects of aflatoxin on. *Egypt J.comp.Path & Clinic. Path* Vol 22(1), 179 -193.

Amirkolaie, A.K., Leenhouwers, J.I., Verreth, J.A.J. & Schrama, J.W. 2005. Type of dietary fibre (soluble versus insoluble) influences digestion, faeces characteristics and faecal waste production in Nile tilapia (Oreochromis niloticus L.). *Aquaculture Research*. 36(12):1157–1166.

Amirkolaie, A.K., Verreth, J.A.J. & Schrama, J.W. 2006. Effect of gelatinization degree and inclusion level of dietary starch on the characteristics of digesta and faeces in Nile tilapia (Oreochromis niloticus (L .)).*Aquaculture Research* 260:194–205.

Anderson, C.D. 2006. Review A review of causal factors and control measures for bloat in farmed salmonids with a suggested mechanism for the development of the condition. *Journal of Fish Diseases*, 29, 445–453

Ashley, P.J. 2007. Fish welfare: Current issues in aquaculture. *Applied Animal Behaviour Science*. 104(3-4):199–235.

Ayoola, M. O, De wet lourens, Khalid Salie 2015. Evaluating natural and acid activated bentonite as feed additives for improving water stability of feed for African Catfish. *Proc.Aquaculture Ass. of Southern Africa*, Sept 27-Oct 3, pp:53

Belal, I.E.H. 2005. A review of some fish nutrition methodologies. *Bioresource Technology* 96 395–402

Boonyaratpalin, M., Suraneiranat, P. & Tunpibal, T. 1998. Replacement of fish meal with various types of soybean products in diets for the Asian seabass, Lates calcarifer. *Aquaculture*. 161(1-4):67–78.

Bostock, J., McAndrew, B., Richards, R., Jauncey, K., Telfer, T., Lorenzen, K., Little, D., Ross, L.. 2010. Aquaculture: global status and trends. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*. 365(1554):2897–912.

Bowyer, J.N., Qin, J.G., Adams, L.R., Thomson, M.J.S. & Stone, D.A.J. 2012. The response of digestive enzyme activities and gut histology in yellowtail kingfish (Seriola lalandi) to dietary fish oil substitution at different temperatures. *Aquaculture*. 368-369:19–28.

Brinker, A. 2007. Guar gum in rainbow trout (Oncorhynchus mykiss) feed: The influence of quality and dose on stabilisation of faecal solids. *Aquaculture*. 267:315–327.

Brummett, R.E. 2008. African aquaculture : Realizing the potential. *Food Policy*. 33(5):371–385.

Bureau, D.P. 1999. Apparent digestibility of rendered animal protein ingredients for rainbow trout ž Oncorhynchus mykiss /.A*quaculture* 180 1999 345–35

Dada, A.A. & Ikuerowo, M. 2009. Effects of ethanolic extracts of Garcinia kola seeds on growth and haematology of catfish (Clarias gariepinus) broodstock. *African Journal of Agricultural Research* Vol. 4 (4), pp. 344-347

Danabas, D. & Altun, T. 2011. Effects of zeolite (Clinoptilolite) on some water and growth parameters of rainbow trout (Oncorhynchus mykiss Walbaum, 1792). *Digest Journal of Nanomaterials and Biostructures*. 6(3):1111–1116.

Diana, J.S. 2009. Aquaculture Production and Biodiversity Conservation. *BioScience*. 59(1):27–38.

Dias, J., Huelvan, R., Dinis, M. & Metailler, R. 1998. Influence of dietary bulk agents (silica, cellulose and a natural zeolite) on protein digestibility, growth, feed intake and feed transit time in European seabass () juveniles. *Aquatic Living Resources*. 11(4):219–226.

Ellis, R.W. 2000. Reduction of the bioavailability of 20 m g r kg aflatoxin in trout feed containing clay. *Aquaculture* Ž.183 2000 179–188

Erhunmwunse, N.O. & Ainerua, M.O. 2013. Characterization of Some Blood Parameters of African Catfish (Clarias gariepinus). *Aquaculture* 5(3):72–76.

Eya, J.C., Parsons, A., Haile, I., Jagidi, P. & Virginia, W. 2008. Effects of Dietary Zeolites (Bentonite and Mordenite) on the Performance Juvenile Rainbow trout Onchorhynchus myskis. *Australian Journal of Basic and Applied Sciences*, 2(4): 961-967, 2008

Foletto, E.L., Paz, D.S. & Gündel, A. 2013. Acid-activation assisted by microwave of a Brazilian bentonite and its activity in the bleaching of soybean oil. *Applied Clay Science*. 83-84:63–67.

Ghahri, H., Habibian, R. & Fam, M.A. 2010. Effect of sodium bentonite, mannan oligosaccharide and humate on performance and serum biochemical parameters during aflatoxicosis in broiler chickens. *Global Veterinaria*. 5(2):129–134.

Giri, S.S., Sahoo, S.K., Sahu, A.K. & Mukhopadhyay, P.K. 2000. Nutrient digestibility and intestinal enzyme activity of Clarias batrachus (Linn .) juveniles fed on dried ® sh and chicken viscera incorporated diets. *Aquaculture* 71:97–101.

Jović-jovičić, N., Milutinović-nikolić, A., Banković, P. & Dojčinović, B. 2010. Synthesis , Characterization and Adsorptive Properties of Organobentonites. *Eleventh Annual Conference of the Materials Research Society of Serbia* 117(5):849–854.

Kanyılmaz, M., Tekelio˘ glu, N., 2009. Influence of supplementation of various levels of natural zeolite on growth, body composition incommoncarp (Cyprinus carpio L., 1758). C¸ .Ü Fen Bilimleri Enstitüsü Dergisi 19, 116–124 (in Turkish, English abstract)

Kanyılmaz, M., Tekelio, N., Sevgili, H., Uysal, R. & Aksoy, A. 2015. Effects of dietary zeolite (clinoptilolite) levels on growth performance , feed utilisation and waste excretions by gilthead sea bream juveniles (Sparus aurata). *Aquaculture* 200:66–75.

Leenhouwers, J.I., Verreth, J.A.J. & Schrama, J.W. 2006. Digesta viscosity , nutrient digestibility and organ weights in African catfish (Clarias gariepinus) fed diets supplemented with different levels of a soluble non-starch polysaccharide. *Aquaculture Nutrition* 2006 12; 111–116

Leenhouwers, J.I., Veld, M., Verreth, J.A.J. & Schrama, J.W. 2007. Digesta characteristiscs and performance of African catfish (Clarias gariepinus) fed cereal grains that differ in viscosity. *Aquaculture* 264:330–341.

Leenhouwers, J.I., Ortega, R.C., Verreth, J.A.J. & Schrama, J.W. 2007. Digesta characteristics in relation to nutrient digestibility and mineral absorption in Nile tilapia (Oreochromis niloticus L .) fed cereal grains of increasing viscosity. *Aquaculture* 273:556–565.

Leenhouwers, J.I., ter Veld, M., Verreth, J.A.J. & Schrama, J.W. 2007. Digesta characteristiscs and performance of African catfish (Clarias gariepinus) fed cereal grains that differ in viscosity.

Aquaculture. 264(1-4):330–341.

Length, F. 2004. Influence of sex , source , health status and acclimation on the haematology of Clarias gariepinus (Burch , 1822). *African Journal of Biotechnology* Vol. 3 (9), pp. 463-467

M Hixson, S. 2014. Fish Nutrition and Current Issues in Aquaculture: The Balance in Providing Safe and Nutritious Seafood, in an Environmentally Sustainable Manner. *Journal of Aquaculture Research & Development*. 03(03).

Maisonnier, S., Gomez, J. & Carré, B. 2001. Nutrient digestibility and intestinal viscosities in broiler chickens fed on wheat diets, as compared to maize diets with added guar gum. *British poultry science*. 42(1):102–10.

Marimuthu, K., Umah, R., Muralikrishnan, S., Xavier, R. & Kathiresan, S. 2011. Effect of different feed application rate on growth , survival and cannibalism of African catfish , *Clarias gariepinus* fingerlings *Emir. J. Food Agric*. 23 (4): 330-337.

Martins, C.I.M., Aanyu, M., Schrama, J.W. & Verreth, J.A.J. 2005. Size distribution in African catfish (*Clarias gariepinus*) affects feeding behaviour but not growth. *Aquaculture* 250 :300–307.

Miller, J.W. & Atanda, T. 2011. The rise of peri-urban aquaculture in Nigeria. *International Journal of Agricultural Sustainability*. 9(1):274–281.

Munguti, J.M., Kim, J.-D. & Ogello, E.O. 2014. An Overview of Kenyan Aquaculture: Current Status, Challenges, and Opportunities for Future Development. *Fisheries and aquatic sciences*. 17(1):1–11.

Murray, H.H. 1991. Overview - clay mineral applications. *Applied Clay Science*, 5 (1991) 379-395.

Murray, H.H. 2000. Traditional and new applications for kaolin , smectite , and palygorskite : a general overview *Applied Clay Science* 17 207–221.

Musa, S.M., Aura, C.M., Ngugi, C.C. & Kundu, R. 2012. The Effect of Three Different Feed Types on Growth Performance and Survival of African Catfish Fry (Clarias gariepinus) Reared in a Hatchery. 2012.*International Scholarly Research Network Volume 2012*, Article ID 861364, 6 pages

Ng, W.-K. & Romano, N. 2013. A review of the nutrition and feeding management of farmed tilapia throughout the culture cycle. *Reviews in Aquaculture*. 5(4):220–254.

Noyan, H., Onal, M. & Sarikaya, Y. 2007. The effect of sulphuric acid activation on the crystallinity, surface area, porosity, surface acidity, and bleaching power of a bentonite. *Food Chemistry*. 105(1):156–163.

Nyina-Wamwiza, L., Milla, S., Pierrard, M.-A., Rurangwa, E., Mandiki, S.N.M., Van Look, K.J.W. & Kestemont, P. 2012. Partial and total fish meal replacement by agricultural products in the diets improve sperm quality in African catfish (Clarias gariepinus). *Theriogenology*. 77(1):184–94.

Odedeyi, D.O. & Fagbenro, O.A. 2010. Feeding habits and digestive enzymes in the gut of Mormyrus rume (Valenciennes 1846) (Osteichthyes Mormyridae). *Tropical Zoology* 23: 75-89.

Oellermann, L.K. & Hecht, T. 2000. Comparison of the ® llet yield , protein content and amino acid pro ® le of Clarias gariepinus and the Clarias gariepinus 5 Heterobranchus longi ® lis hybrid. *Aquaculture Research*. 31(7) : 553-556

Ogunji, J.O., Uwadiegwu, N., Okogwu, O. & Osuigwe, D.I. 2008. Response of African Catfish, Clarias gariepinus (Burchell, 1822), to Diets of Pigeon Pea, Cajanus cajan, Subjected to Different Processing Methods [electronic resource]. *Journal of the World Aquaculture Society*. 39(2):215–224.

Oliva-Teles, a. 2012. Nutrition and health of aquaculture fish. *Journal of Fish Diseases*. 35(2):83–108.

Partridge, G.J. & Southgate, P.C. 1999. The effect of binder composition on ingestion and assimilation of microbound diets (MBD) by barramundi Lates calcarifer Bloch larvae. *Aquaculture Research*. 30:879–886.

Phillips, T.D., Kubena, L.F., Harvey, R.B., Taylor, D.R. & Heidelbaugh, N.D. 1987. Hydrated Sodium Calcium Aluminosilicate : A High Affinity Sorbent for Aflatoxin1. *Poultry Science* 67:243-247

Pohlenz, C. & Gatlin, D.M. 2014. Interrelationships between fish nutrition and health. *Aquaculture*. 431:111–117.

Robinson, a, Johnson, N.M., Strey, a, Taylor, J.F., Marroquin-Cardona, a, Mitchell, N.J., Afriyie-Gyawu, E., Ankrah, N. 2012. Calcium montmorillonite clay reduces urinary biomarkers of fumonisin B_1 exposure in rats and humans. *Food additives & contaminants. Part A, Chemistry, analysis, control, exposure & risk assessment*. 29(5):809–18.

Rosas, C., Tut, J., Baeza, J., Sánchez, A., Sosa, V., Pascual, C., Arena, L., Domingues, P.. 2008a. Effect of type of binder on growth, digestibility, and energetic balance of Octopus maya. *Aquaculture*. 275:291–297.

Rosas, C., Tut, J., Baeza, J., Sánchez, A., Sosa, V., Pascual, C., Arena, L., Domingues, P. 2008b. Effect of type of binder on growth, digestibility, and energetic balance of Octopus maya. *Aquaculture*. 275(1-4):291–297.

Sadekarpawar, S. & Parikh, P. 2013. Gonadosomatic and Hepatosomatic Indices of Freshwater Fish Oreochromis mossambicus in Response to a Plant Nutrient. *World Journal of Zoology* 8 (1): 110-118.

Safaeikatouli, M., Jafariahangari, Y. & Baharlouei, A. 2010. Effects of dietary inclusion of sodium bentonite on biochemical characteristics of blood serum in broiler chickens. *International Journal of Agriculture and Biology*. 12(6):877–880.

Safaeikatouli, M., Boldaji, F., Dastar, B. & Hassani, S. 2012. The effect of dietary silicate minerals supplementation on apparent ileal digestibility of energy and protein in broiler chickens. *International Journal of Agriculture and Biology*. 14(2):299–302.

Schram, E., Roques, J.A.C., Abbink, W., Spanings, T., de Vries, P., Bierman, S., de Vis, H. van & Flik, G. 2010. The impact of elevated water ammonia concentration on physiology, growth and feed intake of African catfish (Clarias gariepinus). *Aquaculture*. 306(1-4):108–115.

Senthil Kumaran, S., Kavitha, C., Ramesh, M. & Grummt, T. 2011. Toxicity studies of nonylphenol and octylphenol: Hormonal, haematological and biochemical effects in Clarias gariepinus. *Journal of Applied Toxicology*. 31(8):752–761.

Shu-li, D., Yu-zhuang, S.U.N., Cui-na, Y. & Bo-hui, X.U. 2009. Removal of copper from aqueous solutions by bentonites and the factors affecting it. *Mining Science and Technology (China)*. 19(4):489–492.

Sinha, A.K., Kumar, V., Makkar, H.P.S., Boeck, G. De & Becker, K. 2011. Non-starch polysaccharides and their role in fish nutrition – A review. *Food Chemistry*. 127(4):1409–1426.

Sink, T.D. & Lochmann, R.T. 2014. The Effects of Soybean Lecithin Supplementation to a Practical Diet Formulation on Juvenile Channel Catfish , Ictalurus punctatus : Growth , Survival , Hematology , Innate Immune Activity , and Lipid Biochemistry. *Journal of the World Aquaculture Society* Vol.45(2):163–172.

Sørensen, M., Ljøkjel, K. & Storebakken, T. 2002. Apparent digestibility of protein , amino acids and energy in rainbow trout (Oncorhynchus mykiss) fed a fish meal based diet extruded at different temperatures. *Aquaculture* 211:215–225.

Staurnes, M., Andorsdottir, G. & Sundby, A. 1990. Distended, water-filled stomach in sea-farmed rainbow trout. *Aquaculture*. 90(3-4):333–343.

Storebakken, T. 1985. Binders in fish feeds i . Effect of alginate and guar gum on growth , digestibility , feed intake and passage through the gastrointes ~ nal tract of rainbow trout Binders are useful in reducing the wastage from wet and moist fish feeds . Alginate and guar. *Aquaculture* 47:11–26.

Tacon, A.G.J. & De Silva, S.S. 1997. Feed preparation and feed management strategies within semi-intensive fish farming systems in the tropics. *Aquaculture*. 151(1-4):379–404.

Tomasso, J.R. 2012. Environmental nitrite and aquaculture: a perspective. *Aquaculture International*. 20(6):1107–1116.

Tomić, Z.P., Logar, V.P., Babic, B.M., Rogan, J.R. & Makreski, P. 2011. Comparison of structural, textural and thermal characteristics of pure and acid treated bentonites from Aleksinac and Petrovac (Serbia). *Spectrochimica acta. Part A, Molecular and biomolecular spectroscopy*. 82(1):389–95.

Verreth, Æ.J., Vermis, Æ.K., Nelis, H.J., Sorgeloos, Æ.P. & Verstegen, Æ.M. 2010. evacuation in larvae of African catfish *Clarias gariepinus* under different feeding conditions. *Aquaculture International* 18:119–134.

Wang, Y. 2007. Effect of probiotics on growth performance and digestive enzyme activity of the shrimp Penaeus vannamei. *Aquaculture* 269 259–264.

Wang, X., Li, X., Leng, X., Shan, L., Zhao, J. & Wang, Y. 2014a. Effects of dietary cottonseed meal level on the growth , haematological indices , liver and gonad histology of juvenile common carp (Cyprinus carpio). *Aquaculture*. 428-429:79–87.

Wilson, J.M. & Castro, L.F.C. 2010. *Morphological diversity of the gastrointestinal tract in fishes*. First Edit ed. Vol. 30. Fish physiology , pp 1-55. Elsevier Inc.

Wright, P.A. & Wood, C.M. 2009. A new paradigm for ammonia excretion in aquatic animals: role of Rhesus (Rh) glycoproteins. *The Journal of experimental biology*. 212(Pt 15):2303–12.

Yener, N., Biçer, C., Önal, M. & Sarıkaya, Y. 2012. Simultaneous determination of cation exchange capacity and surface area of acid activated bentonite powders by methylene blue sorption. *Applied Surface Science*. 258(7):2534–2539.

Yildirim, Ö., Türker, A. & Şenel, B. 2009. Effects of natural zeolite (clinoptilolite) levels in fish diet on water quality, growth performance and nutrient utilisation of Tilapia (Tilapia zillii) FRY. *Fresenius Environmental Bulletin*. 18(9):1567–1571.

Zang, L., Shimada, Y., Nishimura, Y., Tanaka, T. & Nishimura, N. 2013. A novel, reliable method for repeated blood collection from aquarium fish. *Zebrafish*. 10(3):425–32.

Zychowski, K.E., Hoffmann, A.R., Ly, H.J., Pohlenz, C., Buentello, A., Romoser, A., Gatlin, D.M. & Phillips, T.D. 2013. The effect of aflatoxin-B1 on red drum (Sciaenops ocellatus) and assessment of dietary supplementation of NovaSil for the prevention of aflatoxicosis. *Toxins*. 5(9):1555–73.

CHAPTER 5

THE EFFECT OF DIETARY NATURAL AND ACID-ACTIVATED BENTONITE CLAY ON PRODUCTION PERFORMANCE AND BODY COMPOSITION PARAMETERS OF AFRICAN CATFISH (*Clarias gariepinus*)

Abstract

This study examined the effect of dietary natural bentonite and acid-activated bentonite inclusion levels on growth, feed utilization, and body composition of African catfish (*Clarias gariepinus*) reared in a recirculating aquaculture system (RAS). Efficient feed utilisation is an important factor that determines the success of aquaculture, affecting the growth of fish and water quality. Feed binders are additives used to improve pellet quality and feed utilisation. Natural bentonites have been increasingly used as dietary feed additives to livestock, such as swine, ruminants and poultry, to improve weight gain, increase feed conversion ratio and promote animal health. The acid-activated form of the clay mineral is yet to gain a wide usage as a feed additive. In this study, nine (9) test diets were formulated at four bentonite inclusion levels for each clay type (500, 1000, 1500 and 3000 mg/kg) and a control diet. Mixed sex juvenile catfish (initial weight 10 - 15 g) were fed the test diets *ad libitum* for 70 days. Mean final body weight, average daily weight gain (ADWG), feed conversion ratio (FCR), specific growth rate (SGR), hepatosomatic index (HSI) and viscerosomatic index (VSI) were significantly affected ($p<0.05$) by the dietary treatments. Growth performance increased as compared to control with bentonite inclusion up to 1500 mg/kg and decline at 3000 mg/kg for both bentonite forms. The mean contents of crude protein (CP), fat, moisture, ash and abdominal fat in whole body composition were significantly affected ($p<0.05$) by the treatment diets. Mean abdominal fat and tissue fat content increased with bentonite inclusion. An optimum level of performance was recorded at 1500 mg/kg inclusion with natural bentonite having a significantly ($p<0.05$) better performance over acid-activated bentonite. However, higher inclusion levels (3000 mg/kg) of bentonite clay impaired growth and feed utilisation.

5.1: Introduction

Fish and fishery products represent a very valuable source of protein and essential micronutrients for balanced nutrition and good health. Fisheries and aquaculture are a source not just of health but also of wealth (Brummett 2008; *FAO*, 2012; Munguti *et al.*, 2014). Diet, among other factors, have strong effects on stress tolerance and health, and, therefore, for an adequate growth and resistance to stress and disease problem, fish must be fed adequate quantities of diet that meet all their nutrient requirements (Filbrun *et al.*, 2013). Feeding animals with diet that do not meet nutrient requirements not only affects growth and feed efficiency but also increases susceptibility to disease and induces the appearance of deficiency signs, including altered behaviour and pathological changes. Unbalanced diets may also induce negative interactions or antagonism among nutrients that provoke signs similar to deficiency of nutrients. At very high levels of nutrient, which are unusual in practical diets, toxicity signs may occur (Oliva-Teles, 2012).

In intensive fish production practice, using high protein diet is prevalent. Incidence of hypoxia (dissolved oxygen (DO) <2 mg/L) may occur, which stimulates growth of cyanobacteria, filamentous green algae, and aquatic plants (Filbrun *et al.*, 2013). Hypoxia can stress fish, reduce food consumption, reduce feed utilisation, low immune response to pathogens, and specific growth, which can lead to poor production output (Oliva-Teles, 2012). The increase of ammonia in culture pond enhances poor water quality and may lead to death of fish or poor growth (Trichet, 2010; Pohlenz & Gatlin, 2014). However, the high protein inputs characteristic of most intensive aquaculture systems may lead to a build-up of the intermediate product, nitrite. Nitrite concentrations may also become high in recirculating systems as bio-filters are becoming established, and research has confirmed incomplete oxidation of ammonia to nitrate in recirculating systems (Philips *et al.*, 2002; Lefevre *et al.*, 2014).

The influence of aquafeeds' on discharged water quality will depend upon the composition and physical characteristics of the feed used, the technology used in its production, its digestibility, palatability, quality of the components supplied, and feeding technique. The waste produced can be divided into either solid or gaseous phase. Solid waste, consisting of settle-able and suspended solids, mainly originates from uneaten and/or spilled feed and from excreted faeces. Part of the dissolved waste (i.e. organic substances, ammonia) originates from metabolites excreted by fish through the gills and in urine, the rest originating from disintegration/re-suspension of nutrients from both the settle-able and suspended solid waste fractions (Amirkolaie *et al.*, 2005, 2006). In order to improve this situation, a number of studies have been undertaken that address fish production management and feeding strategy, investigating such issues as new fish feed mixtures with good feed physical characteristics and improved utilisation of the feed supplied (Hlava, 2014).

Bentonite is usually incorporated as an ingredient in animal feed as part of mycotoxin binding strategies to improve feed retention time, increase feed efficiency, promote a healthy digestive tract and enhance performance (Robinson *et al.*, 2012; Carraro *et al.*, 2014). Animal response depends upon the dietary composition, clay treatment (Juan-juan *et al.*, 2010), clay type and purity, incorporation rate and rearing period (Zychowski *et al.*, 2013). Modified forms of bentonite clay (acid-activated, intercalated with inorganic acid) are used in a number of technological applications. Important physical changes in acid-activated bentonite are the increase of specific surface area, binding capacity and average pore volume (Makhoukhi *et al.*, 2009; Motlagh *et al.*, 2011; Tomić *et al.*, 2011).

The African catfish, *C. gariepinus* , is tolerant of a wide range of temperatures, as well as low oxygen levels (Oellermann & Hecht, 2000; Singh *et al.*, 2012). It commands high demand from consumers and is mostly preferred by food fish aquaculturists. This is due to the ideal characteristics of this species (Akinwole & Faturoti, 2007; Jiwyam, 2011; Nyin *et al.*, 2012), which includes high growth rate at high stocking densities, a high food conversion, and good meat quality. The potential of the *C. gariepinus* for aquaculture has been demonstrated for ponds as well as for intensive culture in tanks under controlled indoor conditions (Jiwyam, 2011; Singh *et al.*, 2012; Kumar & Engle, 2014). The formulation of a nutritionally-balanced commercial feeds has contributed to the commercial success of the *C. gariepinus* industry. However, feed costs account for the bulk of operating costs (55% of variable costs and 46% of total costs) in this fish species business (University of Arkansas at Pine Bluff [UAPB], 2011 in (Kumar & Engle, 2014)).

Therefore, good feed quality to enhance feed utilisation and managing water quality is essential for success of fish production. This study evaluated the effects of dietary natural and acid-activated bentonite at different inclusion levels on production performance characteristics and body composition of *C. gariepinus* reared in a recirculating aquatic system (RAS).

5.2: Material and Methods

5.2.1: Experimental Facilities.

The experiment was carried out at Welgevallen farm, Division of Aquaculture's (DA) facilities of Stellenbosch University (SU), located on a geographical position system (GPS), coordinates 33^{o}56' 33.95" S and 18^{o}51'56.15 "E, Stellenbosch, Western Cape area, South Africa.

The experimental facilities consisted of a recirculating warmwater (indoor temperature controlled) aquaria system (RAS) as described in previous chapter.

Basic water quality parameters such as temperature (^{o} C), dissolved oxygen (in mg/L), and pH were monitored daily using a HACH, HQ 40-d Multi instrument, with HACH LDO probe. Each plastic tank was cleaned at two (2)-week intervals throughout the duration of the study.

5.2.2: Experimental layout and design

Table 5. 4 : Inclusion level on bentonite clay (NB and AB) in *C. gariepinus* feed

S/N0	Treatments	Designation	Inclusion level of clay (mg / kg of basal diet)
I.	Basal diet only *BD	Control	No inclusion
II.	BD + NB 500	NB 500	500 mg NB
III.	BD + AB 500	AB 500	500 mg AB
IV.	BD + NB 1000	NB 1000	1000 mg NB
V.	BD + AB 1000	AB 1000	1000 mg AB
VI.	BD + NB 1500	NB 1500	1500 mg NB
VII.	BD + AB 1500	AB 1500	1500 mg AB

VIII.	BD + NB 3000	NB 3000	3000 mg NB
IX.	BD + AB 3000	AB 3000	3000 mg NB

- BD : Commercial Montego catfish grower diets

Natural bentonite – NB	Acid activated bentonite - AB

The experiment was conducted between February (2015) to May (2015) over 70 days. Clay mineral (natural and acid-activated bentonite) was included into commercial aquafeed diets at eight (8) different inclusion levels with a control diet, resulting in nine (9) treatments. All treatments were replicated seven (7) times. The experimental diets were fed *ad libitum*, two (2) times daily, and strict precaution was taken to avoid uneaten feed by fish. Tanks were allocated to treatment diets and fishes were allotted to tanks in a completely randomized design.

5.2.3: Experimental Animals and Compounds

A total of 945 mixed sex African catfish (*C. gariepinus*) weighing between 10 and 15 g, were obtained from a commercial farm Aquaculture Innovations, Grahamtown, South Africa. Fish were quarantined as explained in previous chapter. The fish were fed until visual observation of satiation (Baker, 1984) at two periods, i.e. 8.00hrs and 16:00hrs. Clay minerals (Natural and acid activated bentonite) were obtained from Nutrex NV Lille, Belgium. See plate (5:2) for visual appearance and cation exchange capacity of clay minerals. A commercial basal diet for catfish produced by Montego feed mill, Cape Town, South Africa was procured. The clay powders were included as an additive into feed at four (4) inclusion levels

5.2.4: Water quality maintenance

Water temperature was measured once daily along with total ammonia, nitrite, and pH. Total ammonia-nitrogen and nitrite-nitrogen were measured using a Hach DR/890 colorimeter (Hach Company, Loveland, Colorado). The pH was measured using an Ultrabasic UB-10 pH meter (Denver Instrument, Bohemia, New York). Mean (±SE) daily water temperature was 29.8 ± 0.1°C. Water quality values (mean ± SE) were as follows: pH was 6.9 ± 0.1; total ammonia, 0.3 ± 0.1 mg/L; and nitrite, 0.6 ± 0.2 mg/L.

5.2.5: Feed processing

Feed was prepared as explained in previous chapter.

5.2.6 Data collection

At the beginning of the experiment, total length (TL), i.e. measurement from tip of snout to the tail end, and body weight of each fish were taken using a calibrated measuring board (measured to the nearest 0.01mm) and electronic balance, UWE, HGS-300, Capacity: 300 X 0.01g (measured to the nearest 0.01g), respectively (Skelton, 2001). All fish were fasted 12 hrs prior to sampling period and on sampling day. Sampling of fish was taken at 14-days intervals, and each fish was measured per tank for the aforementioned parameters. Clove oil was used as anaesthesia during all sampling. Feed consumption was recorded and mortality checked in each tank daily.

At 70th day of trial period, two (3) fishes were randomly selected from each tank to collect samples for carcass characteristics, body composition and hepatosomatic index.

5.2.7 Sample collection

The fish were weighed individually and subsequently processed by removing skin, head and viscera; dress-out yield was calculated as weight of dressed fish relative to weight of whole fish. The fish were filleted by removing the muscle from the back and rib bones with a knife, and fillet yield were calculated relative to weight of the whole fish. The fillets were sealed in air-tight plastic bags and frozen until assayed. Each whole-fish sample was ground in a Hobart meat grinder (Hobart Food Equipment, Troy, OH) and analysed in duplicate for proximate composition following the standard methods AOAC (2002). Moisture content was determined by drying samples in an oven at 100^0C for 24 hrs. Samples were then incinerated overnight in a muffle furnace at 600^0C for measurement of ash content (AOAC 2002). Protein was measured by combustion Dumas method with a LECO FP 528 (AOAC 2002). Total fat content of the fish samples were determined by chloroform–methanol extraction (1: 2) (Lee *et al.*, 1996)

5.2.8 Calculated values

ii. Specific growth rate, feed intake and feed conversion rate

Specific growth rate, feed intake and feed conversion rate were calculated over the trial period (70 days), the fish in each tank were counted and individually weighed (Mettler PM 34 Delta range) to the nearest 1 g to calculate the specific growth rate (SGR) as follows:

$$\mathrm{SGR} = \mathrm{W_t} - \mathrm{W_1} \times \frac{100}{t}$$

Where SGR=specific growth rate (%/d), Wt=mean weight at day 84 (g), W_1=mean weight at day 1 (g) and t=number of days.

Feed was administered twice daily at 0800 h and 1600 h until visually observed satiation. Feed amounts per tank were recorded. For feeding, the known quantity of feed was offered to each tank, and, 45 min after each offering, the remaining feed was siphoned out and dried over night at 105^0C in a laboratory hot air oven. Daily intake of dry matter (DM) was determined by subtracting the residue from the offered feeds (Liu *et al.*, 2011). Daily feed intake per tank was recorded, defined as the difference between daily feed amount and feed loss. Total feed intake per tank resulted from the sum of the daily feed intake. Total feed intake and biomass per tank were used to calculate feed conversion rate (FCR) as follows:

$$\mathrm{FCR} = \frac{\mathrm{TFI}}{nt \times Wt - n1 \times W1}$$

Where FCR=feed conversion rate (g/g), TFI=total feed intake (g), Wt=mean weight at day 90 (g), W1=mean weight at day 1 (g), n_t =number of fish at day 86 and n1=number of fish at day 1.

$$\mathrm{ADWG} = \frac{Fnwt - Inwt}{nt}$$

Where ADWG = average daily weight gain (g), F_{nwt} = mean final wt (g), I_{nwt} = mean initial weight (g), nt = number of rearing days (84)

$$\mathrm{ADLG} = \frac{Fnlt - Inlt}{nt}$$

Where ADLG = average daily length gain (gmm), F_{nlt} = mean final length (mm), I_{nlt} = mean initial length (mm), nt = number of rearing days (84)

$$\text{RFI} = \frac{TFI}{Fnwt - Inwt} \Big/ nt \times 100$$

Where RFI = relative feed intake (%), F_{nwt} = mean final weight (g), I_{nwt} = mean initial weight (g), nt = number of rearing days (84)

$$\text{Survival (\%)} = \frac{Nft}{Nfl} \times 100$$

Where Nft = number of fish at day 84, Nfl = number of fish at day 1

$$\text{HSI (\%)} = \frac{Liver\ (wt)}{Fish\ (wt)} \times 100$$

Where HSI = hepatosomatic index (%), Liver (wt) = liver weight of fish at end of the experiment, Fish (wt) = fish weight at end of the experiment

$$\text{VSI (\%)} = \frac{Viscera\ (wt)}{Fish\ (wt)} \times 100$$

Where VSI = viscerasomatic index (%), Viscera (wt) = viscera weight of fish at end of the experiment, Fish (wt) = fish weight at end of the experiment

5.2.9 Statistical analysis

All data recordings and calculated values, which included total length, body weight, survival rate, average daily length gain (ADLG), average daily weight gain (ADWG), feed conversion rate (FCR), Sspecific growth rate (SGR), hepatosomatic index and survival rate, were computed using Microsoft Excel 2010 and Sharp Model ELW53H Scientific Calculator. A regression analysis was done for data on ADLG, ADWG, FCR and SGR. Slopes or means of all data recorded were compared with one way ANOVA using the GLM. Bonferroni was used to test for the significance of variance ($p<0.05$) for all recorded and calculated data between different treatments using SAS 2015 version 12 and XLSTAT version 2015.

5.3 Results

The production performance of *C. gariepinus* fed natural and acid-activated bentonite clay at different inclusion levels (0, 500, 100, 1500 and 3000 mg/kg) feed during 70days trial period were presented. Table 5.2, presents initial total length, initial weight, final total length, final weight, average daily length gain (ADLG), average daily weight gain (ADWG), feed conversion rate (FCR), specific growth rate (SGR), hepatosomatic index (HSI), viscerosomatic index (VSI), and percentage survival rate (% survival). Abdominal fat and proximate Body composition parameters were recorded in table 5.3.

As shown in table 5.2, final body weight (Final Wt) was significantly different ($p<0.05$) among the treatments. Natural bentonite at 1500 mg/kg feed inclusion (B1500) had the highest value (190.78±4.91) which differed significantly ($p<0.05$) from the control diet (A0B0) and other dietary clay inclusion treatments except natural bentonite at 1000 mg/kg (B1000) and acid-activated bentonite at 1500 mg/kg (A1500). The lowest value for Final Wt (g) was recorded at acid-activated bentonite inclusion treatment at 3000 mg/kg (157.74±4.91). The final total length (Final TL) values were not significantly different among the treatments ($p>0.05$). Highest and lowest values were recorded at B1500 (287.67±4.15) and control treatment (A0B0) (273.47±4.15) respectively.

The average daily length gain (ADLG) are not significantly different among treatments, with highest and lowest values recorded at B1500 (2.26±0.07) and A0B0 (2.07±0.07) respectively. Average daily weight gain (ADWG) are significantly different ($p<0.05$) among treatments. Treatment B1500 had the highest value (2.49±0.05) which differed significantly ($p<0.05$) from control and other treatments except B1000 and A1500. The growth parameters measured increased with dietary clay inclusion till 1500 kg and decreased at 3000 mg/kg. Natural bentonite (B) has higher values as compared to acid-activated bentonite diets (A).

The percentage survival values were not significantly different ($p>0.05$) between the treatments. The highest and lowest value were recorded at B1000 (93.38±3.80) and B3000 (88.10±3.80).

Dietary natural bentonite (B) had higher (%) survival values as compared to acid-activated bentonite.

Table 5. 5 : Production performance of *C.gariepinus* fed bentonite (NB and AB) at different inclusion levels

	Treatments								
Parameters	**A0B0**	**A500**	**B500**	**A1000**	**B1000**	**A1500**	**B1500**	**A3000**	**B3000**	**SEM**
Initial TL(g)	130.21	129.66	130.60	131.45	130.02	131.32	130.11	131.54	130.07	0.83
Final TL(g)	273.47	273.61	280.20	275.84	282.02	282.64	287.67	274.66	278.49	4.15
Initial Wt.(g)	14.37	13.86	14.36	14.55	14.13	14.64	14.44	14.46	14.53	0.28
Final Wt. (g)	159.84^{b}	159.86^{b}	165.00^{b}	163.69^{b}	177.42^{ab}	178.46^{ab}	190.78^{a}	157.74^{b}	168.24^{b}	4.91
ADLG (g)	2.07	2.11	2.25	2.09	2.20	2.17	2.26	2.12	2.12	0.07
ADWG (g)	2.10^{c}	2.11^{c}	2.17^{c}	2.17^{c}	2.31^{abc}	2.40^{ab}	2.49^{a}	2.20^{bc}	2.20^{bc}	0.05
RFI (g)	2.08^{a}	1.85^{bc}	1.83^{c}	1.80^{bc}	1.88^{b}	1.79^{bc}	1.80^{bc}	1.65^{d}	1.70^{d}	0.05
FCR	1.51^{ab}	1.93^{a}	1.78^{ab}	1.98^{a}	1.64^{ab}	1.64^{ab}	1.26^{c}	1.49^{ab}	1.67^{ab}	0.11
SGR (%)	0.51^{b}	0.52^{b}	0.52^{b}	0.52^{b}	0.53^{b}	0.62^{ab}	0.69^{a}	0.50^{b}	0.51^{b}	0.03
HSI (%)	5.02^{a}	4.92^{ab}	4.69^{b}	4.54^{b}	4.48^{b}	4.90^{ab}	4.54^{b}	4.77^{b}	4.50^{b}	0.16
VSI (%)	10.08^{a}	9.00^{b}	8.77^{bc}	7.59^{c}	7.66^{c}	8.03^{bc}	8.38^{bc}	9.33^{b}	8.40^{bc}	0.27
ABD Fat (%)	3.30^{b}	2.05^{c}	1.85^{c}	3.34^{b}	2.22^{c}	3.88^{ab}	3.70^{b}	4.20^{ab}	4.73^{a}	0.20
Survival (%)	90.48	89.52	89.52	93.33	93.38	81.91	87.62	87.62	88.10	3.80

SEM: Standard error of mean * Mean values between the rows are verified at $p<0.05$; values on the same row with the same superscript letter are not significantly different based on bonferroni post-hoc test. Where A0B0 = control, A-acid-activated bentonite, B-natural-bentonite

The relative feed intake (RFI) differed significantly ($p<0.5$) among the treatments. The control diet A0B0 had the higher value (2.08±0.05), which differed significantly from all the dietary clay treated diets. Inclusion level at 3000mg/kg differed significantly ($p<0.05$) from other dietary clay inclusion levels and control. Feed conversion ratio (FCR) values differed significantly ($p<0.05$) among the treatments. B1500 had the best FCR (1.26±0.11) which differed significantly ($p<0.05$) from all other treatment diets. Natural bentonite (B) had higher values for RFI and lower values for FCR as compared to acid-activated bentonite (A) at all inclusion levels.

The specific growth rate (SGR) value is significantly different ($p<0.05$) among the treatments. B1500 had the highest value (0.69±0.03) which differed significantly ($p<0.05$) from other treatments, except A1500. Natural bentonite (B) had higher values as compared to activated bentonite (A) at all inclusion levels.

The percentage hepatosomatic index (% HSI), viscera somatic index (%VSI) and abdominal fat (% ABD Fat) differed significantly ($p<0.05$) among the treatments. Hepatosomatic index (%HSI) had the highest value at control diet A0B0 (5.62±0.16), which differed significantly ($p<0.05$) from other treatments except A500 and A1500. B1000 had the lowest %HSI (4.48±0.16). The control diet A0B0 had the highest value of % VSI (11.38±0.27) which differed significantly ($p<0.05$) from other treatments. A1000 had the lowest value (7.59±0.27).

Abdominal fat which was calculated as percentage of fat to body weight had highest value at B3000 (4.73±0.20), which differed significantly ($p<0.05$) from other treatments except A1500, and A3000. The control diet (A0B0), 1000mg/kg acid-activated bentonite (A1000) and 1500mg/kg natural bentonite (B1500) differed significantly from 500mg/kg bentonite inclusion (A500, B500), and 1000mg/kg natural bentonite (B1000).

Table 5. 6 : Effect of natural and acid activated bentonite inclusion levels on body composition of C.gariepinus

	Treatments									
Parameters	**A0B0**	**A500**	**B500**	**A1000**	**B1000**	**A1500**	**B1500**	**A3000**	**B3000**	**SEM**
Moisture (%)	75.81^{a}	76.63^{a}	77.15^{a}	73.89^{b}	76.98^{a}	75.54^{a}	76.40^{a}	75.61^{a}	76.66^{a}	0.35
Ash (%)	1.08^{c}	1.11^{c}	1.09^{c}	1.03^{cd}	1.62^{ab}	1.53^{b}	1.71^{a}	1.63^{ab}	1.73^{a}	0.05
CP (%)	14.06^{a}	13.08^{bcd}	13.55^{ab}	12.81^{cd}	12.95^{bcd}	12.62^{cd}	13.22^{bc}	12.45^{d}	12.78^{cd}	0.15
Tissue fat (%)	3.61^{c}	3.68^{c}	3.40^{d}	3.97^{b}	3.74^{c}	4.00^{b}	3.91^{b}	4.52^{a}	4.47^{ab}	0.04

SEM: Standard error of mean. *Mean values between the rows are verified at $p<0.05$; values on the same row with the same superscript letter are not significantly different based on bonferroni. Where: A0B0 – control, A- acid-activated bentonite, B- natural bentonite

Table 5.3 showed the values of body composition fed dietary natural bentonite and acid-activated bentonite at different inclusion levels. The values of % Moisture are significantly different ($p<0.05$) among the treatment diets. A1000 had the least value, which is significantly different ($p<0.05$) from all other treatment diets. The % Ash was significantly different ($p<0.05$) among the treatment diets. Ash (%) increased significantly ($p<0.05$) with inclusion level as compared to control.

The % crude protein (CP) was significantly different ($p<0.05$) between the treatment diets. The control diet (A0B0) had the highest value (14.06 ± 0.15), which differed significantly ($p<0.05$) from all inclusion levels of dietary clay treatment diets except for natural bentonite (B500) mg/kg inclusion level. A1000 had the least value (12.45 ±0.15).

The tissue Fat content (%) of sampled fish for all treatments differed significantly ($p<0.05$). A3000 had the highest values (4.52±0.04), which differed significantly ($p<0.05$) from other treatments except B3000. B500 differed significantly ($p<0.05$) from treatment diets, and the control diet had the least values. The fat content increased with increase in inclusion level of dietary bentonite. Activated bentonite (A) had higher values as compared to natural bentonite (B) at all inclusion levels.

5.4 Discussion

The consumption of clay by wild animals in nature and its practice is well documented (Sousa, *et al.*, 2007). This practice, referred to as geophagy, is the deliberate consumption of soil and clay by both animals and humans (Slamova *et al.*, 2011). Several hypotheses have been proposed to explain the geophagic behaviour: detoxification of noxious or unpalatable compounds present in the diet, alleviation of gastrointestinal upsets such as diarrhoea, supplementation of the body with minerals and alleviation of hyperacidity in the digestive tract (Sousa *et al.*, 2007; Wilson & Castro, 2010b). Geophagy may improve food intake through modification of the conditions in the digestive tract, such as pH, buffering capacity, osmotic pressure, and the dilution rate of food (Wilson & Castro, 2010b; Wu *et al.*, 2013).

In livestock and fish husbandry, aluminosilicates clay of alkali and alkaline earth cations are used as additive in livestock and fish feeds due to their detoxifying and nutrient absorption with growth enhancing features (Murray, 2000; Eya *et al.*, 2008). Bentonites, a clay mineral used in animal diet act as gut protectants (enterosorbents), which rapidly and preferentially bind aflatoxins from the digestive tract and thus reduce their absorption into the organism, enhance feed utilisation and improve growth (Robinson *et al.*, 2012; Indresh *et al.*, 2013).

5.4.1 Growth performance and feed utilisation

The improved growth performance found in this study with bentonite inclusion is in agreement with Al-Zubaidy (1992) who reported that the use of bentonite clays as a dietary filler improved growth and increased feed intake in broiler. Bentonite clay improved feed utilisation by slowing the rate of feed flow in the digestive tract, allowing for greater digestion and absorption. In addition, zeolite an aluminosilicates clay type similar to bentonite was demonstrated to have a positive effect on the metabolic utilisation of nutrients in poultry (Eya *et al.*, 2008; Wu *et al.*, 2013; Tang *et al.*, 2014). Tang *et al.* (2014) reported that zeolite clinoptilolite may facilitate blood drainage from villi, and increase the activities of brush border cells and intestinal digestive enzyme, which in turn could improve digestion and absorption of nutrients, and could reduce the rate of food passage in the gut and immobilize enzymes. Thus, the progressive improved growth can be attributed to increase in the passage time of feed in the gut (See chapter 6). This enhances activities of intestinal digestive enzyme through proper breakdown of nutrient and thereby improved nutrient absorption and utilisation.

Bentonites were used in aflatoxin contaminated feeds and diets to reduce bioavailability of the mycotoxin (Robinson *et al.*, 2012; Carraro, De Giacomo, *et al.*, 2014a). Mycotoxins have been linked to poor feed efficiency, reduced growth, disease and death in farm animals (Dschaak, *et al.*, 2010; Slamova *et al.*, 2011). The improved growth in this study may be attributed to ability of bentonite to reduce the mycotoxin effects that may be present in the diets. Although in this study, evaluation of the toxicity in feed was not considered.

However, at bentonite inclusion level 3000 mg/kg (3%), the growth performance declined. This can be attributed to an increase in digesta viscosity, which in turn might enhances fermentation in the gut. The binding properties of bentonite clay could be responsible for high viscosity at high inclusion level. Acid-activated bentonite has a high binding capacity as compared to natural bentonite (Makhoukhi *et al.*, 2009; Motlagh *et al.*, 2011; Yener *et al.*, 2012), this can be responsible for reduced growth performance as compared to natural bentonite. Fermentation in the gut has been reported to reduce feed utilisation, nutrient absorption and growth (Abdollahi *et al.*, 2013; Wu *et al.*, 2013). An alternative explanation that has been widely accepted as explained in broilers fed NSP and sepiolite varied in viscosity. Increase in digesta viscosity could affect intestinal digestion by limiting the diffusion of digestive enzymes and nutrients (Cho & Bureau, 2001) and through primary or secondary activities, increases the microbial activities in the small intestine (Ouhida, *et al.*, 2000).

Bentonite clay has high cation exchange ability. The reduced growth with increase inclusion level in the diets can be attributed to binding and ion exchange with micro and macro minerals present in the feed and fish gut that are important for fish growth. Though this was not measured in the study.

The relative feed intake (RFI) values reduced significantly ($p<0.05$) as compared to control diet. However, with the high density of dietary clay diets as compared to control, RFI is expected to increase in dietary clay diets (See chapter 3). But the binding effect of dietary clay on feed (Ayoola *et al.*, 2015), which slow gut emptying rate and return of appetite can be attributed to this changes (See chapter 6).

5.4.2 Gut morphology and survival rate

As reported in table 5.2, the hepatosomatic index (HSI), viscerosomatic index (VSI) and abdominal fat were significantly ($p<0.05$) affected by treatment diets. The HSI and VSI values are within the values reported for normal fish of this species (Sadekarpawar & Parikh, 2013; Sink & Lochmann, 2014; Wang *et al.*, 2014). The observed differences cannot be fully attributed to diet treatment effects as values do not follow a particular trend. The liver is a major organ in fish, and it has many biological functions. Thus, liver physiology and the liver biochemical index can reflect the nutritional and physiological status of fish (Rodrigues *et al*. 2009; Zhu *et al.* 2012). The significant difference ($p<0.05$) observed in this study for HSI may be sex related as reported by (Boonyaratpalin, 1998; Wilson & Castro, 2010b; Sadekarpawar & Parikh, 2013).

Fish used in this study were mix-sex, further study is suggested to ascertain the significant observation of HSI. On the other hand, this study might have shown stimulating effects of bentonite clay on digestive organ weights (i.e. liver and intestine). The changes noticed in bentonite diets, as compared to control, may suggest changes occurring at the tissue level, such as increased rates of cell proliferation, or increased cell sizes with bentonite inclusion. In *C. gariepinus*, dietary guar gum inclusion was reported to increase digesta viscosity. These high digesta viscosities may explain the observed reduced nutrient digestibility and increases in digestive organ weights (Goelema *et al.*, 1999; Sinha *et al.*, 2011).

The survival rate was not significantly different between the treatments. Though the control diet had the highest value, the decrease in survival rate at bentonite diets inclusion cannot be attributed to bentonite clay treatment.

5.4.3 Whole body composition

In the present study, fat content in whole body was lower in fish fed the diets containing natural bentonite 500 mg/kg, and fat content in whole body increased with the increase in dietary bentonite inclusion as compared to control. This result is in contrast with Eya *et al*., (2008), who found low fat content in fish fed bentonite feed. The high dietary viscosity in bentonite diet, which is associated with binding capacity of bentonite clay, may be responsible for the increased level of abdominal fat and whole body fat content. High viscosity increases residence time of the digesta and therefore increases intestinal Volatile fatty acids (VFA) production (Maisonnier *et al*. 2001; Leenhouwers *et al.* 2007)

The decreased CP (%) with increase bentonite inclusion correspond to reduced growth rate at high inclusion levels. The can be associated with reduction in nutrient utilization at high inclusion level. Also, clay minerals have been reported to bind protein molecules, therefore reduced protein availability or utilization (Johnson *et al.,* 2012, Yu *et al.*, 2013

The values of ash content (%) of fish fed diet with bentonite increased with inclusion level and had a negative correlation with protein. The increased in mineral contents present in clay mineral with high inclusion level is suspected to be responsible. However, further work is suggested to ascertain this results on whole body composition. To the best of our knowledge, very few studies did evaluate the effect of bentonite on whole body composition of fish.

5.5 Conclusion

These findings suggested that incorporation of dietary bentonite into the diets of *C.gariepinus* juvenile to adult level had no adverse effect on performance and whole body composition up to 1500 mg/kg inclusion. Natural bentonite had the better performance over acid-activated bentonite. The inclusion of bentonite up to 1500 mg/kg was the optimum level for measured and calculated parameters. The increased digesta viscosity in the stomach at the high bentonite inclusion diet increased the retention time of digesta and might have stimulated gut fermentation. The cation exchange capacity of the bentonite with protein molecules need further studies to ascertain crude protein utilization for fish growth and body composition.

5.6: References

Abdollahi, M.R., Ravindran, V. & Svihus, B. 2013. Pelleting of broiler diets: An overview with emphasis on pellet quality and nutritional value. *Animal Feed Science and Technology*. 179(1-4):1–23.

Akinwole, A.O. & Faturoti, E.O. 2007. Biological performance of African Catfish (Clarias gariepinus) cultured in recirculating system in Ibadan. *Aquacultural Engineering* 36 (2007) 18–23.

Al-Zubaidy, S.S. 1992. Evaluation of spent bleaching and filtering clay—a bentonite product from palm oil refining as a potential feed ingredient in layer diets. *Animal Feed Science and Technology*. 40(1):13–19.

Amirkolaie, A.K., Leenhouwers, J.I., Verreth, J.A.J. & Schrama, J.W. 2005. Type of dietary fibre (soluble versus insoluble) influences digestion, faeces characteristics and faecal waste production in Nile tilapia (Oreochromis niloticus L.). *Aquaculture Research*. 36(12):1157–1166.

Amirkolaie, A.K., Verreth, J.A.J. & Schrama, J.W. 2006. Effect of gelatinization degree and inclusion level of dietary starch on the characteristics of digesta and faeces in Nile tilapia (

Oreochromis niloticus (L .)). *Aquaculture Research*. 260:194–205.

Ayoola, M. O, De wet lourens, Khalid Salie 2015. Evaluating natural and acid activated bentonite as feed additives for improving water stability of feed for African Catfish. *Proc.Aquaculture Ass. of Southern Africa*, Sept 27-Oct 3, pp:53

Boonyaratpalin, M., Suraneiranat, P. & Tunpibal, T. 1998. Replacement of fish meal with various types of soybean products in diets for the Asian seabass, Lates calcarifer. *Aquaculture*. 161(1-4):67–78.

Brummett, R.E. 2008. African aquaculture : Realizing the potential. *Food Policy* 33 (2008) 371–385.

Carraro, a., De Giacomo, a., Giannossi, M.L., Medici, L., Muscarella, M., Palazzo, L., Quaranta, V., Summa, V., et al. 2014. Clay minerals as adsorbents of aflatoxin M1 from contaminated milk and effects on milk quality. *Applied Clay Science*. 88-89:92–99.

Cho, C.Y. & Bureau, D.P. 2001. A review of diet formulation strategies and feeding systems to reduce excretory and feed wastes in aquaculture. *Aquaculture Research*. 32:349–360.

Dschaak, C.M., Eun, J., Young, A.J., Stott, R.D. & Peterson, S. 2010. E ffects of Supplementation of Natural Zeolite on Intake , Digestion , Ruminal Fermentation , and Lactational Performance of Dairy Cows.*The Professional Animal Scientist* 26 (2010):647–654

Eya, J.C., Parsons, A., Haile, I., Jagidi, P. & Virginia, W. 2008. Effects of Dietary Zeolites (Bentonite and Mordenite) on the Performance Juvenile Rainbow trout Onchorhynchus myskis. *Australian Journal of Basic and Applied Sciences*, 2(4): 961-967,

Filbrun, J.E., Reynolds, C.A. & Culver, D.A. 2013. Effects of Feeding Rate on Habitat Quality in Fish Rearing Ponds. *Journal of the World Aquaculture Society*. 44(2):198–209.

Goelema, J.., Smits, A., Vaessen, L.. & Wemmers, A. 1999. Effects of pressure toasting, expander treatment and pelleting on in vitro and in situ parameters of protein and starch in a mixture of broken peas, lupins and faba beans. *Animal Feed Science and Technology*. 78(1-

2):109–126.

Hlava, D. 2014. Effects of supplementary feeding in carp ponds on discharge water quality : a review. 299–320.

Indresh, H., Devegowda, G., Ruban, S. & Shivakumar, M. 2013. Effects of high grade bentonite on performance, organ weights and serum biochemistry during aflatoxicosis in broilers. *Veterinary World*. 6(6):313.

Jiwyam, W. 2011. The effect of stocking density on yield, growth, and survival of Asian river catfish (Pangasius bocourti Sauvage, 1880) cultured in cages. *Aquaculture International*. 19(5):987–997.

Juan-juan, L.I., De-cheng, S.U.O. & Xiao-ou, S.U. 2010. Binding Capacity for Aflatoxin B 1 by Different Adsorbents. *Agricultural Sciences in China*. 9(3):449–456.

Kumar, G. & Engle, C.R. 2014. Optimizing Catfish Feeding and Stocking Strategies Over a Two-Year Planning Horizon. *Aquaculture Economics & Management*. 18(2):169–188.

Leenhouwers, J.I., Veld, M., Verreth, J.A.J. & Schrama, J.W. 2007. Digesta characteristiscs and performance of African catfish (Clarias gariepinus) fed cereal grains that differ in viscosity. *Aquaculture*. 264:330–341.

Leenhouwers, J.I., ter Veld, M., Verreth, J.A.J. & Schrama, J.W. 2007. Digesta characteristiscs and performance of African catfish (Clarias gariepinus) fed cereal grains that differ in viscosity. *Aquaculture*. 264(1-4):330–341.

Lefevre, S., Wang, T., Jensen, a, Cong, N. V, Huong, D.T.T., Phuong, N.T. & Bayley, M. 2014. Air-breathing fishes in aquaculture. What can we learn from physiology? *Journal of fish biology*. 84(3):705–31.

Liu, X.Y., Wang, Y. & Ji, W.X. 2011. Growth, feed utilisation and body composition of Asian catfish (Pangasius hypophthalmus) fed at different dietary protein and lipid levels. *Aquaculture Nutrition*. 17(5):578–584.

Maisonnier, S., Gomez, J. & Carré, B. 2001. Nutrient digestibility and intestinal viscosities in broiler chickens fed on wheat diets, as compared to maize diets with added guar gum. *British poultry science*. 42(1):102–10.

Makhoukhi, B., Didi, M.A., Villemin, D. & Azzouz, A. 2009. Acid activation of Bentonite for use as a vegetable oil bleaching agent. *Grasas y Aceites*. 60(4):343–349.

Motlagh, K., Youzbashi, A.A. & Rigi, Z.A. 2011. Effect of acid activation on structural and bleaching properties of a bentonite. *Iranian Journal of Materials Science and Engineering*. 8(4):50–56.

Munguti, J.M., Kim, J.-D. & Ogello, E.O. 2014. An Overview of Kenyan Aquaculture: Current Status, Challenges, and Opportunities for Future Development. *Fisheries and aquatic sciences*. 17(1):1–11.

Murray, H.H. 2000. Traditional and new applications for kaolin , smectite , and palygorskite : a general overview.*Applied Clay Science* 17 2000 207–221

Nyina-Wamwiza, L., Milla, S., Pierrard, M.-A., Rurangwa, E., Mandiki, S.N.M., Van Look, K.J.W. & Kestemont, P. 2012. Partial and total fish meal replacement by agricultural products in the diets improve sperm quality in African catfish (Clarias gariepinus). *Theriogenology*. 77(1):184–94.

Oellermann, L.K. & Hecht, T. 2000. Comparison of the ® llet yield , protein content and amino acid pro ® le of Clarias gariepinus and the Clarias gariepinus 5 Heterobranchus longi ® lis hybrid. *Aquaculture Research*. 31(7) : 553-556

Oliva-Teles, a. 2012. Nutrition and health of aquaculture fish. *Journal of Fish Diseases*. 35(2):83–108.

Ouhida, I., Pérez, J.F., Piedrafita, J. & Gasa, J. 2000. The effects of sepiolite in broiler chicken diets of high, medium and low viscosity. Productive performance and nutritive value. *Animal Feed Science and Technology*. 85(3-4):183–194.

Philips, S., Laanbroek, H.J. & Verstraete, W. 2002. Origin , causes and effects of increased nitrite concentrations in aquatic environments. *Environmental Science & Bio/Technology* 1: 115–141, 2002..

Pohlenz, C. & Gatlin, D.M. 2014. Interrelationships between fish nutrition and health. *Aquaculture*. 431:111–117.

Robinson, a, Johnson, N.M., Strey, a, Taylor, J.F., Marroquin-Cardona, a, Mitchell, N.J., Afriyie-Gyawu, E., Ankrah, N. a,. 2012. Calcium montmorillonite clay reduces urinary biomarkers of fumonisin B_1 exposure in rats and humans. *Food additives & contaminants. Part A, Chemistry, analysis, control, exposure & risk assessment*. 29(5):809–18.

Rodrigues, a.p.o., pauletti, p., kindlein, l., cyrino, j.e.p., delgado, e.f. & machado-neto, R. 2009. Intestinal morphology and histology of the striped catfish Pseudoplatystoma fasciatum (Linnaeus, 1766) fed dry diets. *Aquaculture Nutrition*. 15(6):559–563.

Sadekarpawar, S. & Parikh, P. 2013. Gonadosomatic and Hepatosomatic Indices of Freshwater Fish Oreochromis mossambicus in Response to a Plant Nutrient. *World Journal of Zoology* 8 (1): 110-118, 2013

Singh, A.K., Srivastava, S.C., Ansari, A., Kumar, D. & Singh, R. 2012. Environmental monitoring and health risk assessment of African catfish Clarias gariepinus (Burchell, 1822) cultured in rural ponds, India. *Bulletin of environmental contamination and toxicology*. 89(6):1142–7.

Sinha, A.K., Kumar, V., Makkar, H.P.S., De Boeck, G. & Becker, K. 2011. Non-starch polysaccharides and their role in fish nutrition – A review. *Food Chemistry*. 127(4):1409–1426.

Sink, T.D. & Lochmann, R.T. 2014. The Effects of Soybean Lecithin Supplementation to a Practical Diet Formulation on Juvenile Channel Catfish , Ictalurus punctatus : Growth , Survival , Hematology , Innate Immune Activity , and Lipid *Biochemistry*. 45(2):163–172.

Slamova, R., Trckova, M., Vondruskova, H., Zraly, Z. & Pavlik, I. 2011. Applied Clay Science Clay minerals in animal nutrition. *Applied Clay Science*. 51(4):395–398.

Sørensen, M., Ljøkjel, K. & Storebakken, T. 2002. Apparent digestibility of protein , amino acids and energy in rainbow trout (Oncorhynchus mykiss) fed a fish meal based diet extruded at different temperatures. *Aquaculture* 211 (2002) 215–225.

Sousa, C. De, Gomes, F., Baptista, J. & Silva, P. 2007. Minerals and clay minerals in medical geology. *Applied clay science*36:4–21.

Tang, Z., Wen, C., Li, P., Wang, T. & Zhou, Y. 2014. Effect of zinc-bearing zeolite clinoptilolite on growth performance, nutrient retention, digestive enzyme activities, and intestinal function of broiler chickens. *Biological Trace Element Research*. 158(1):51–57.

Tomić, Z.P., Logar, V.P., Babic, B.M., Rogan, J.R. & Makreski, P. 2011. Comparison of structural, textural and thermal characteristics of pure and acid treated bentonites from Aleksinac and Petrovac (Serbia). *Spectrochimica acta. Part A, Molecular and biomolecular spectroscopy*. 82(1):389–95.

Trichet, V.V. 2010. Nutrition and immunity: an update. *Aquaculture Research*. 41(3):356–372.

Verreth, Æ.J., Vermis, Æ.K., Nelis, H.J., Sorgeloos, Æ.P. & Verstegen, Æ.M. 2010. evacuation in larvae of African catfish Clarias gariepinus under different feeding conditions. *Aquaculture International* 18:119–134.

Wang, X., Li, X., Leng, X., Shan, L., Zhao, J. & Wang, Y. 2014. Effects of dietary cottonseed meal level on the growth , haematological indices , liver and gonad histology of juvenile common carp (Cyprinus carpio). *Aquaculture*. 428-429:79–87.

Wilson, J.M. & Castro, L.F.C. 2010b. *Morphological diversity of the gastrointestinal tract in fishes*. First Edit ed. Vol. 30. Fish physiology , pp 1-55 Elsevier Inc.

World Bank and FAO. (2012). The Sunken Billions: The Economic Justification for Fisheries Reform. World Bank, Washington, and Food and Agriculture Organization, Rome. *Available at: https://openknowledge.worldbank.org/bitstream/handle/10986/2596/476060PUB0Sunk101Offic*

Wu, Q.J., Wang, L.C., Zhou, Y.M., Zhang, J.F. & Wang, T. 2013. Effects of clinoptilolite and modified clinoptilolite on the growth performance, intestinal microflora, and gut parameters of broilers. *Poultry science*. 92(3):684–92.

Yener, N., Biçer, C., Önal, M. & Sarıkaya, Y. 2012. Simultaneous determination of cation exchange capacity and surface area of acid activated bentonite powders by methylene blue sorption. *Applied Surface Science*. 258(7):2534–2539.

Zhu, H., Liu, H., Yan, J., Wang, R. & Liu, L. 2012. Effect of yeast polysaccharide on some hematologic parameter and gut morphology in channel catfish (Ictalurus punctatus). *Fish physiology and biochemistry*. 38(5):1441–7.

Zychowski, K.E., Hoffmann, A.R., Ly, H.J., Pohlenz, C., Buentello, A., Romoser, A., Gatlin, D.M. & Phillips, T.D. 2013. The effect of aflatoxin-B1 on red drum (Sciaenops ocellatus) and assessment of dietary supplementation of NovaSil for the prevention of aflatoxicosis. *Toxins*. 5(9):1555–73.

CHAPTER 6

EFFECT OF DIETARY NATURAL AND ACTIVATED BENTONITE BLEND ON RATE OF GUT EVACUATION IN *Clarias gariepinus*

Abstract

Bentonite is a phyllosilicate natural clay material of the montmorillonite group found in silica-rich rocks and altered volcanic ash beds. Bentonite found application as dietary technological and mycotoxin binder in aquafeeds'. Two experiments were designed to evaluate the effect of natural bentonite (NB) and its acid activated form (AB) on gut evacuation rate of *Clarias gariepinus*. Experiment 1; NB and AB were used at combination levels (0: 100, 25:75, 50:50), and two inclusion levels for each ratio low and high (500 mg/kg and 1500 mg/kg). Experiment II; NB and AB were used as feed additives, four inclusion levels for each clay (500, 1000, 1500 and 3000) mg/kg clay/feed respectively. Each experiment comprised of 11 and 9 treatments respectively with four replicates. Fish weighing 240g -250g were randomly allocated to treatment diets and fed *al libitum*. Four fish were selected randomly to measure post feeding stomach content (SC) and intestinal filling (IC) content at (5, 30, 60 and 120mins). The control diet had higher values for SC at 5mins in both experiments which differed significantly ($p<0.05$) as compared to clay diets. SC decreases ($p<0.05$) with time. In clay diets, SC decreases faster over time with reduce in quantity of AB and low inclusion level. The IC of control diets had higher values ($p<0.05$) over time as compared to clay diets in both experiments. The values of IC decreased over time with an increase in inclusion level of each clay and increase in quantity of AB: NB in the diets. The results show that dietary clay reduced the rate of feed evacuation in the gut, which can lead to better feed utilisation and nutrient absorption. Hence high clay inclusion level and quantity of AB in the diet can lead to increase digesta viscosity may be deleterious to fish digestion and nutrient utilization.

6.1 Introduction

The rate at which food is consumed and the efficiency with which it is utilized are important factors to determining growth rate. There is a positive relation between growth and feeding frequency (Verreth *et al.*, 2010). Estimation of food consumption by fish is difficult because of the lack of accurate methods of evaluation of both ingestion and evacuation of food. Methods of quantifying food consumption are useful in nutritional studies with fish, for example they assist in estimation of their quantitative nutrient requirements (Survey *et al.*, 2000; Abdollahi *et al.*, 2013). The factors which affect gut evacuation in fish most strongly include temperature, fish species, fish mass, and feed quality. The research on gastric evacuation of fishes has been with challenges and a definitive pattern has not emerged, largely due to the wide variation in biotic and abiotic factors among experiments (Riche *et al.*, 2004).

Additionally, feeding frequency is strongly correlated with gastric evacuation time (GET). Gastric evacuation rate (GER) is also a function of temperature, fish weight, meal size, dietary composition and energy, and feeding frequency (Survey *et al.*, 2000; Svihus & Zimonja, 2011). Gastric evacuation studies have been used to estimate feed consumption and utilisation in aquaculture. It has been demonstrated that the quantity of available food a fish eats is dependent on stomach fullness, and intervals between meals are a function of the rate of emptying (Nutrition, 2003; Verreth *et al.*, 2010)

Fish culturists can use GER and gastric evacuation time (GET) to develop appropriate feeding strategies for increasing efficiency. Understanding the rate of digestion and its relationship to GER can allow one to predict the return of appetite under a given set of conditions and diets. Demonstrating a consistent relationship between stomach fullness and appetite return will allow an optimal feeding frequency to be predicted (Lam & Flores, 2003). Making food available at an appropriate rate and as soon as appetite has returned can maximize intake and increase feed efficiency (Riche *et al.*, 2004; Verreth *et al.*, 2010).

However, composition of feed ingredients also plays an important role in GET. Digestibility of the ingredients and nutrient composition of the diets are the main factors that affect waste outputs from fish (Survey *et al.*, 2000; Deyoe, 2015). Minimizing waste outputs from aquaculture operations should therefore start at the source, the diet formula and feed manufacturing (Ighwela *et al.*, 2013).

Bentonite is a binder used in feed technology to improve physical pellet quality, as well as binding agent for mycotoxins and endotoxins. Bentonite is clay altered from glassy igneous material such as volcanic ash or tuff. It occurs in two clay forms sodium montmorillonite and calcium montmorillonite (Murray, 2000). Both sodium and calcium bentonites are used in binding animal feed into pellets. The high layer charge, the very fine particle size, the thin flakes, the high cation exchange capacity, and the high surface area result in the physical and chemical properties that determine the many industrial applications. In addition to their binding ability, these minerals act as absorbents for bacteria (Ghahri *et al.*, 2010; Indresh *et al.*, 2013; Carraro *et al.*, 2014) and certain enzymes, which when removed promote the growth and health of the animal (Murray, 2000). Bentonites may also contain other clay- and non-clay minerals as impurities. Thus, bentonites are treated with the inorganic acids such as HNO_3, HCl and H_2SO_4 to remove some of the impurities and thereby obtain more adsorptive materials, binding capacity and high cation exchange (Noyan *et al.*, 2007; Motlagh *et al.*, 2011). They are referred to as acid-activated bentonites.

The sustainable aquaculture requires management practices that enhance feed utilisation and reduced metabolic waste. To the best of our knowledge, no study has reported the effect of bentonite on gut evacuation rate. However, the rate of feed disappearance from the stomach and filling of intestine is thought to be a fair predictor of the return of appetite (Riche *et al.*, 2004). Therefore, this study evaluated the inclusion of natural and acid activated bentonite clay blend on gut evacuation rate in adult *C. gariepinus*

6.2 Materials and Methods

6.2.1 Experimental Facilities.

The experiment was carried out at Welgevallen farm, Division of Aquaculture's (DA) facilities of Stellenbosch University (SU), located on a geographical position system (GPS), coordinates 33o56' 33.95" S and 18o51'56.15 "E, Stellenbosch, Western Cape area, South Africa.

The experimental facilities consisted of a recirculating warm-water (indoor temperature controlled) aquaria system (RAS), see previous chapter for full description. Basic water quality parameters such as temperature (0c), dissolved oxygen (in mg/L), and pH were monitored daily using a HACH, HQ 40-d Multi instrument, with HACH LDO probe. Each plastic tanks was cleaned weekly throughout the duration of the study.

6.2.2 Experimental layout and design

Trial A

The experiment was conducted in the month of April (2015) over 28 days duration. Clay mineral (natural and acid-activated bentonite) was included into a commercial aquafeeds' diet at five different combinations and two inclusion levels (low and high) with a control diet, resulting into 12 treatments. All treatments were arranged to include four (4) time periods (0, 30, 60 and 120 mins).

Trial B

The experiment was conducted in the month of May (2015) over 28 days duration. Clay mineral (natural and acid-activated bentonite) was included into a commercial aquafeeds' diet at four inclusion levels each, and a control diet, resulting in nine (9) treatments. All treatments were arranged to include four (4) time periods (0, 30, 60 and 120 mins).

6.2.3 Experimental Animals and Compounds

A total of 400 unsexed adult African catfish (*Clarias gariepinus*) weighing between 240g and 250g, were randomly allocated to treatment diet. Five fish were stocked into each treatment tank. The fish were fed a commercial catfish feed (grower) for seven (7) days. Treatment diets were offered from 8th – 28th day. The fish were fed until visual observation of satiation (Baker, 1984) at two periods, i.e. 8.00hrs and 16:00hrs. See previous chapters for feed preparation.

Table 6. 5 : Inclusion of bentonite clay combination blend as feed additives in C.gariepinus feed

S/N0	Treatments	Designation	Inclusion level of clay (mg/kg of basal diet BD)
I.	Basal diet only * BD	T_1	No inclusion
II.	Basal diet only * BD	T_2	No inclusion
III.	BD + NB (low level)	T_3	500 mg NB (low level)
IV.	BD + NB (high level)	T_4	1500 mg NB (high level)
V.	BD + 75 % NB + 25 % AB	T_5	375 mg NB : 125 mg AB (low level)

VI.	BD + 75 % NB + 25 % AB	T_6	1,125 mg NB : 375 mg AB (high level)
VII.	BD + 50 % NB + 50 % AB	T_7	250 mg NB : 250 mg AB (low level)
VIII.	BD + 50 % NB + 50 % AB	T_8	750 mg NB : 750 mg AB (high level)
IX.	BD + 25 % NB + 75 % AB	T_9	125 mg NB : 375 mg AB (low level)
X.	BD + 25 % NB + 75 % AB	T_{10}	375 mg NB : 1,125 mg AB (high level)
XI.	BD + AB (low level)	T_{11}	500 mg AB (low level)
XII.	BD + AB (high level)	T_{12}	1500 mg AB (high level)

- BD : Commercial Montego catfish grower diets

Natural bentonite – NB   Acid activated bentonite - AB

Table 6. 6 : Inclusion level of bentonite clay as feed additives in C.gariepinus feed

S/N0	Treatments	Designation	Inclusion level of clay (mg / kg of basal diet)
I.	Basal diet only *BD	Control	No inclusion
II.	BD + NB 500	NB 500	500 mg NB
III.	BD + AB 500	AB 500	500 mg AB
IV.	BD + NB 1000	NB 1000	1000 mg NB
V.	BD + AB 1000	AB 1000	1000 mg AB
VI.	BD + NB 1500	NB 1500	1500 mg NB
VII.	BD + AB 1500	AB 1500	1500 mg AB
VIII.	BD + NB 3000	NB 3000	3000 mg NB
IX.	BD + AB 3000	AB 3000	3000 mg NB

- BD : Commercial Montego catfish grower diets

Natural bentonite – NB   Acid activated bentonite - AB

6.2.4 Data collection

On the 28th day, four fish were selected at random from each tank immediately after feeding *ad libitum*. Fish were killed with a slight hit on the head using a hammer and were dissected to empty the stomach and intestine content. Contents were poured into a separate bag and weight recorded. Random selection of fish and dissection were repeated at (30, 60 and 120mins) for trial A and B respectively

Body weight of each fish was taken using a calibrated electronic balance, UWE, HGS-300, Capacity: 300 X 0.01g (measured to the nearest 0.01g) respectively (Skelton, 2001).

The percentage of stomach and intestine content recovered from each fish was calculated relative to weight of each fish:

i. % Stomach content (SC) = $\frac{Stomach\ content\ (g)}{Weight\ of\ fish\ (g)} \times 100\ \%$

ii. % Intestine content (IC) = $\frac{Intestine\ content(g)}{Weight\ of\ fish\ (g)} \times 100\ \%$

6.2.5 Statistical analysis

All data recordings and calculated values were computed using Microsoft Excel 2010 and Sharp Model ELW53H Scientific Calculator. Computed data were analysed using one way and two way factorial ANOVA where applicable. Data were analysed using linear regression and slopes are compared using GLM. Bonferroni and LSD was used to test for the significance of variance ($p<0.05$) for all recorded and calculated data between different treatments using SAS 2015 version 12 and XLSTAT version 2015.

6.3 Result

Table 6. 7 : The effect of bentonite clay combination blend on feed gut flow rate of *C.gariepinus*

Treatment	5	30	60	120
SC				
A0B0	14.63^{a}	9.79^{c}	4.14^{e}	2.90^{f}
A0B100	11.37^{e}	9.99^{c}	7.86^{d}	4.50^{e}
A25B75	12.05^{d}	10.74^{b}	9.47^{c}	6.82^{d}
A50B50	12.48^{c}	10.76^{b}	9.97^{b}	7.91^{c}
A75B25	12.92^{b}	10.80^{b}	10.05^{b}	9.11^{b}
A100B0	12.63^{c}	12.04^{a}	10.57^{a}	9.64^{a}
SEM	0.06	0.06	0.01	0.05
IC				
A0B0	0	0.41^{a}	0.62^{a}	0.94^{a}
A0B100	0	0.26^{b}	0.40^{b}	0.64^{b}
A25B75	0	0.15^{dc}	0.32^{c}	0.45^{c}
A50B50	0	0.26^{de}	0.38^{b}	0.47^{c}
A75B25	0	0.14^{d}	0.22^{d}	0.39^{d}
A100B0	0	0.07^{e}	0.12^{e}	0.37^{d}
SEM	0	0.005	0.005	0.008

SEM: Standard error of mean. *Mean values between the columns are verified at $p<0.05$; values on the same columns with the same superscript letter are not significantly different based on bonferroni post-hoc test. Where: A0B100 = 100% NB, A25B75 = 25% AB: 75% NB, A50B50 = 50% AB: 50% NB, A75B25 = 75% AB: 25% NB, A100B0 = 100% AB, A0B0 = control

Table 6.3 recorded the stomach content of fish. Treatment diets affected the stomach emptying rate significantly ($p<0.05$) at all the time intervals. At 5 mins post feeding, control diet (A0B0) had the highest value (14.63 %) of body weight, which differed significantly ($p<0.05$) from all treatment diets. Natural bentonite at 100% (A0B100) had the lowest value (11.37%). Over the 120 mins post feeding, stomach content of A0B0 had higher values significantly ($p<0.05$) as compared to all treatment diets and stomach empty rate reduced with inclusion of acid-activated bentonite. At 120 mins, % post feed left in the stomach of fish based on treatment diets fed were;

A0B0 (19.89 %), A0B100 (39.49%), A25B75 (56.59%), A50B50 (63.38%), A75B25 (70.51%) and A100B0 (76.32%). Based on % body weight, at 120mins, the control diet –A0B0 had 3% stomach content (SC), while A100B0 had the highest value for clay treated diet at 10% (Figure 6.1). No significant interaction ($p>0.05$) between the level of inclusion Low (500mg/kg) and High (1500mg/kg) and the clay combinations. Low inclusion level of clay diets had faster stomach emptying rate significantly ($p<0.05$) as compared to high inclusion level clay diets (Figure 6.1 and 6.2).

The intestine contents were all significantly different ($p<0.05$) among the treatment diets at all the time intervals, except at 5 mins when the intestine was empty. A0B0 had the highest values significantly ($p<0.05$) over time. The intestine content reduced significantly ($p<0.05$) with inclusion of acid-activated bentonite. At 120mins, the percentage of feed ingested found in the intestine for each treatment diets were; A0B0 (6.42%), A0B100 (5.63%), A25B75 (3.73%), A50B50 (3.77%), A75B25 (3.02%) and A100B0 (2.93%).

There was no significant interaction ($p<0.05$) between the level of inclusion (Low and High) and the clay combinations. Low inclusion level of clay diets had a faster intestinal filling rate which differed significantly ($p<0.05$) as compared to high inclusion level clay diets (Figure 6.3 and 6.4).

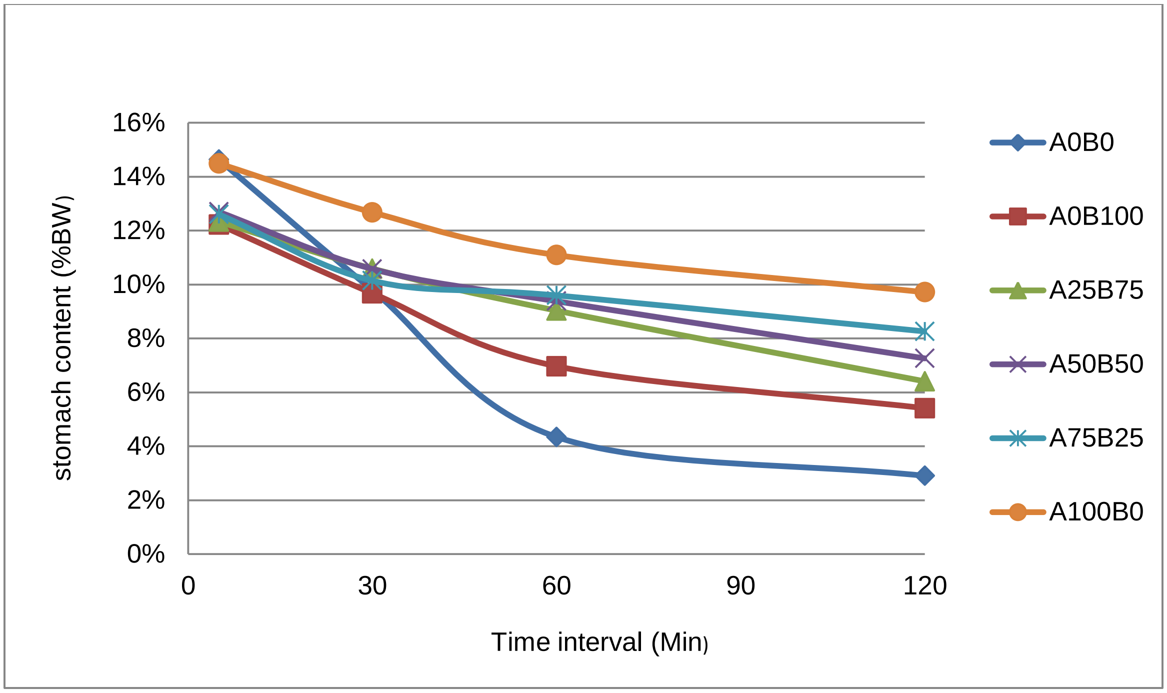

Figure 6. 9 : Effect of bentonite clay combination blend on stomach emptying rate of C.gariepinus at low inclusion level (500mg/kg). Where: A0B100 = 100% NB, A25B75 = 25% AB, 75% NB, A50B50 = 50% AB, 50% NB, A75B25 = 75% AB, 25% NB, A100B0 = 100% AB, A0B0 = control

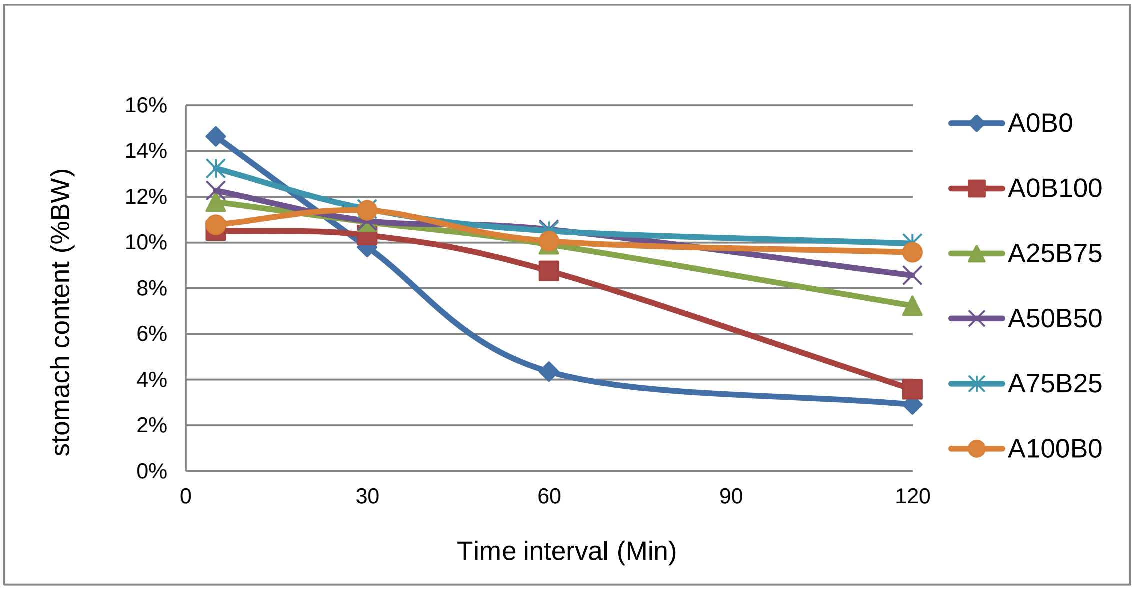

Figure 6. 10 : Effect of bentonite clay combination blend on stomach emptying rate of C.gariepinus at high inclusion level (1500mg/kg). Where: A0B100 = 100% NB, A25B75 = 25% AB, 75% NB, A50B50 = 50% AB, 50% NB, A75B25 = 75% AB, 25% NB, A100B0 = 100% AB, A0B0 = control

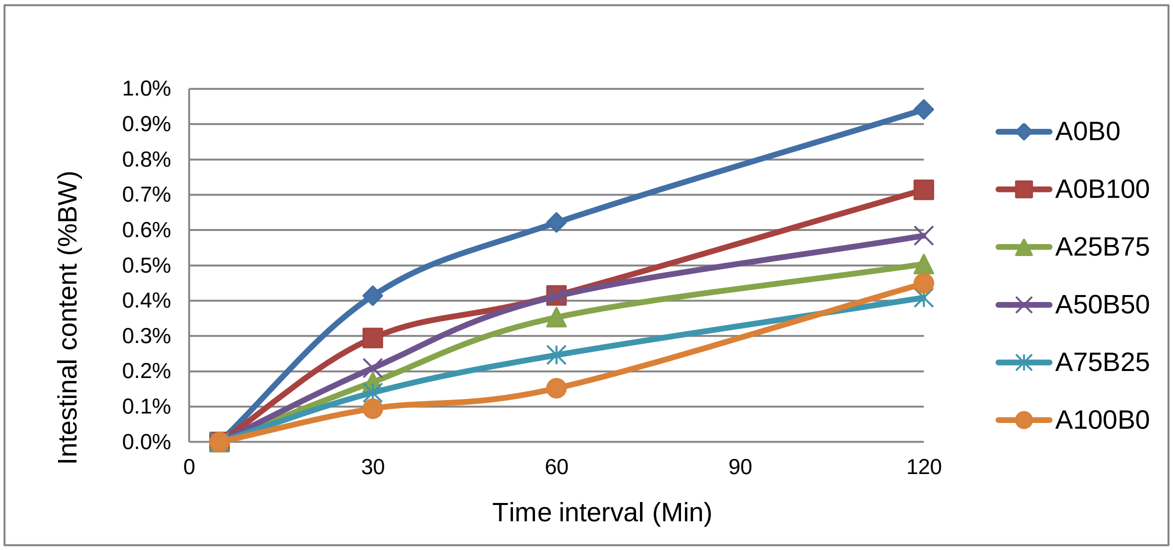

Figure 6. 11 : Effect of bentonite clay combination blend on post-stomach intestinal content of *C.gariepinus* at low inclusion (500mg/kg). Where: A0B100 = 100% NB, A25B75 = 25% AB, 75% NB, A50B50 = 50% AB, 50% NB, A75B25 = 75% AB, 25% NB, A100B0 = 100% AB, A0B0 = control

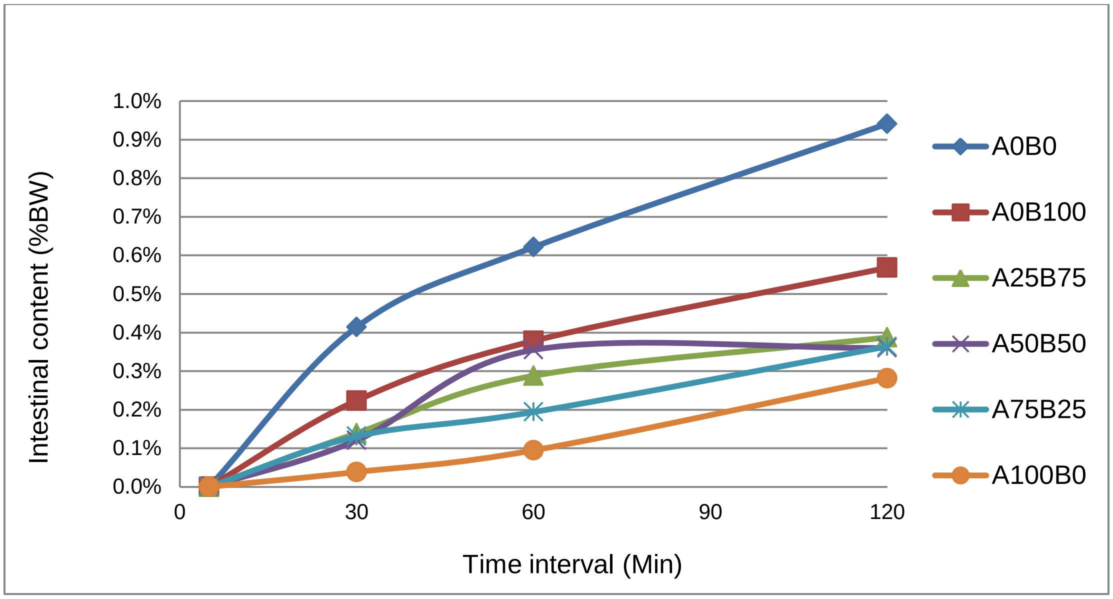

Figure 6. 12 : Effect of bentonite clay combination blend on post-stomach intestinal content of *C.gariepinus* at high inclusion (1500mg/kg). Where: A0B100 = 100% NB, A25B75 = 25% AB, 75% NB, A50B50 = 50% AB, 50% NB, A75B25 = 75% AB, 25% NB, A100B0 = 100% AB, A0B0 = control

Table 6. 8 : The effect of bentonite clay (NB and AB) inclusion levels on gut flow rate of *C.gariepinus*

Treatment	5	30	60	120
SC				
A0B0	12.33^{a}	7.00^{c}	5.84^{b}	3.57^{b}
A500	9.04^{c}	7.86^{ab}	6.02^{ab}	4.20^{ab}
B500	10.17^{bc}	7.60^{ab}	5.97^{ab}	4.03^{ab}
A1000	9.60^{bc}	7.95^{ab}	6.29^{ab}	4.49^{ab}
B1000	9.87^{bc}	7.93^{ab}	5.71^{ab}	4.10^{ab}
A1500	10.08^{b}	7.96^{ab}	6.21^{a}	4.81^{a}
B1500	10.39^{b}	7.94^{ab}	6.19^{ab}	4.42^{ab}
A3000	9.97^{bc}	8.89^{a}	6.26^{a}	4.81^{a}
B3000	10.42^{b}	8.22^{a}	6.23^{a}	4.58^{ab}
SEM	0.27	0.33	0.30	0.23
IC				
A0B0	0	0.50^{a}	0.67^{a}	0.87^{a}
A500	0	0.40^{bc}	0.48^{bc}	0.62^{bc}
B500	0	0.40^{b}	0.53^{b}	0.66^{b}
A1000	0	0.31^{cd}	0.51^{b}	0.55^{cd}
B1000	0	0.35^{bc}	0.51^{b}	0.59^{bc}
A1500	0	0.22^{e}	0.40^{cd}	0.48^{de}
B1500	0	0.26^{de}	0.44^{bcd}	0.53^{cde}
A3000	0	0.21^{e}	0.35^{d}	0.45^{e}
B3000	0	0.25^{de}	0.38^{cd}	0.46^{de}
SEM	0	0.02	0.02	0.02

SEM: Standard error of mean. *Mean values between the columns are verified at $p<0.05$; values on the same columns with the same superscript letter are not significantly different based on bonferroni post-hoc test. Where: A0B0 = control. A= acid-activated bentonite, B= natural bentonite

In table 6.4, the effect of inclusion levels of natural bentonite (B_{clay}) and acid-activated bentonite (A_{clay}) diets on gut evacuation rate was presented. At time interval 5 mins and 30 mins, control diet (A0B0) differed significantly ($p<0.05$) as compared to other treatment diets for the stomach content (SC). The dietary clay B and A showed a trend for stomach flow values. A_{clay} values are higher than B_{clay} at 30 min -120 min. As reported in table 6.4, the intestine content (IC) of control diet (A0B0) had higher values, which differed significantly ($p<0.05$) from all clay treated diets at all the time intervals. Clay inclusion level at 500 and 1000 mg/kg differ significantly ($p<0.05$) as compared to inclusion level at 1500 and 3000 mg/kg. The intestinal content decreased with increase in inclusion level of dietary clay, with the same trend as stomach emptying rate. The control diet had highest value which differed significantly ($p<0.05$) as compared to dietary clay diets. At 120min, SC based on %body weight for B_{clay} diet and control was 4% (figure 6.5), but in figure 6.6, A_{clay} at 3000 mg/kg had the highest value (5%) as compared to control –A0B0 (3.8%). As shown in figure 6.7, at 120min, %body weight of IC of control was 0.88%, and the highest and least value for B_{clay} are B500 – 0.68%, B3000- 0.45%. In figure 6.8, at 120mins % body weight of IC for control diet was 0.88%, A500 – 0.62% had the highest value for A_{clay} treated diet, while A3000- had the least value 0.42%

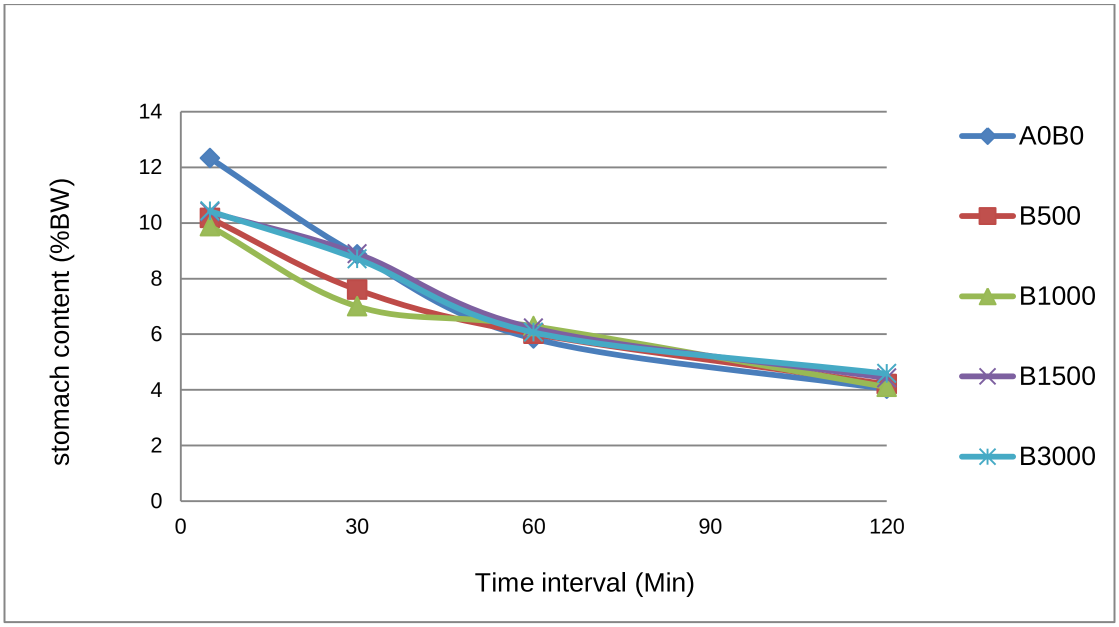

Figure 6. 13 : Effect of natural bentonite clay inclusion levels on stomach emptying rate of *C. gariepinus.* Where: A0B0 – control, B500 – natural bentonite (500mg/kg), B1000 - natural bentonite (1000mg/kg), B1500 - natural bentonite (1500mg/kg), B3000- natural bentonite (3000mg/kg)

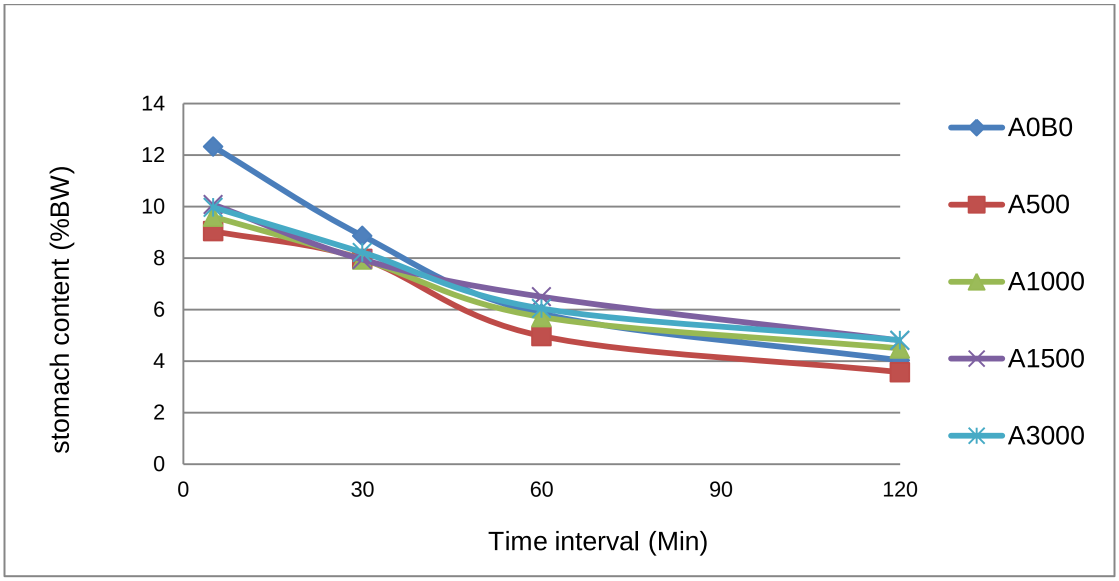

Figure 6. 14 : Effect of acid activated bentonite inclusion levels on stomach emptying rate of *C. gariepinus.* Where: A0B0 – control, A500 – Acid-activated bentonite (500mg/kg), A1000 – Acid-activated bentonite (1000mg/kg), A1500 – Acid-activated bentonite (1500mg/kg), A3000- Acid-activated bentonite (3000mg/kg)

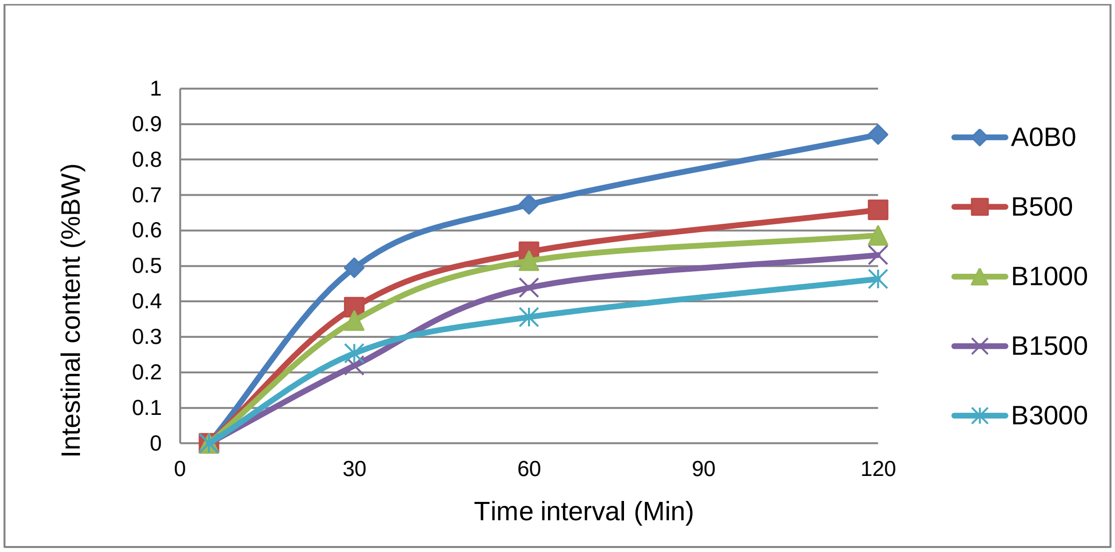

Figure 6. 15 : Effect of natural bentonite clay inclusion levels on post-stomach intestinal content of *C. gariepinus.* Where: A0B0 – control, B500 – natural bentonite (500mg/kg), B1000 - natural bentonite (1000mg/kg), B1500 - natural bentonite (1500mg/kg), B3000- natural bentonite (3000mg/kg)

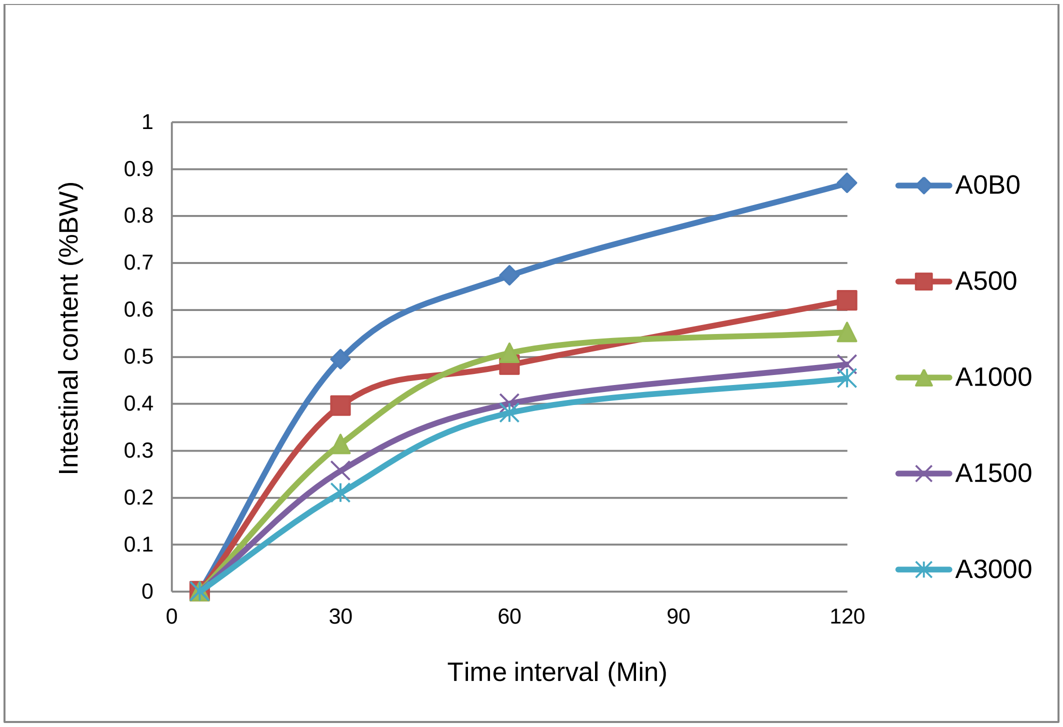

Figure 6. 16 : Effect of acid-activated bentonite clay inclusion levels on post-stomach intestinal content of *C.gariepinus*. Where: A0B0 – control, A500 – Acid-activated bentonite (500mg/kg), A1000 – Acid-activated bentonite (1000mg/kg), A1500 – Acid-activated bentonite (1500mg/kg), A3000- Acid-activated bentonite (3000mg/kg)

6.5 Discussion

Hydrated aluminosilicates clay of alkali and alkaline earth cations, are used in livestock and fish feeds as an additive or filler due to their detoxifying and nutrient utilisation enhancing features (Eya *et al.*, 2008). Bentonite clays have been used in animal feeds as binding and lubricating agents in the pelleting process (Slamova *et al.*, 2011). In addition, bentonite has been used both as a dietary supplement in domestic animal feed to enhance feed utilisation and improve growth (Papaioannou *et al.*, 2004; Ghahri *et al.*, 2010; Safaeikatouli *et al.*, 2010; Indresh *et al.*, 2013). In aquaculture, to the best of our knowledge, no study has considered the utilisation of bentonite and its acid activated form as a whole or its combination on gut evacuation.

6.5.1 Stomach content flow

The effect of a clay combination (NB and AB) blend at two inclusion levels (Low and High) and effect of clay (NB and AB) treatment diets at different inclusion levels were presented in Table 6.3 and 6.4 respectively. The control diet (A0B0) had higher value significantly ($p<0.05$) as compared to clay treatment diets at 5min. This may be attributed to the high density of clay treated diets with swelling capacity (see chapter 3). Bentonites are known to have a high binding and swelling capacity (Mesubi *et al.*, 2008; Shu-li *et al.*, 2009; Slamova *et al.*, 2011). These characteristics of bentonite slow down the passage of feed within the gut as evident with the rate of gut flow/mins. Therefore, the period at which fish appetite returns for feeding increased for dietary clay treatment as compared to control. Thus, this accumulated effect can led to increase in feed intake of control diet as compared to dietary treated diets (See chapter 4 and 5).

The value of stomach content decreases with time significantly ($p<0.05$) among the treatments. At 120mins, % post feed left in the stomach of fish based on treatment diets fed are: A0B0 (19.89 %), A0B100 (39.49%), A25B75 (56.59%), A50B50 (63.38%), A75B25 (70.51%) and A100B0 (76.32%). The control diet (A0B0) had the fastest stomach emptying rate significantly ($p<0.05$) as compared to clay treated diets. The binding characteristic of bentonite clay is responsible for the slow stomach emptying rate as compared to control diet. These binding characteristics led to increase viscosity. Increased viscosity slowed down the passage of ingested feed from the stomach to the intestine as reported in previous studies (Amirkolaie *et al.*,, 2006; Leenhouwers *et al.*, 2007; Sinha *et al.*, 2011).

6.5.2 Intestinal content flow

In table 6.3, the intestinal content differs significantly ($p<0.05$) between the treatment diets at all the time intervals. The control diets had higher values which are significantly different ($p<0.05$) as compared to clay treatment diets. The increased viscosity resulting from the binding effect of clay treatment diets may be responsible for the reduction of intestinal content in clay diets. As reported by previous researchers, non- starch polysaccharides (NSP) were used as binders for aquafeeds'. This resulted in an increase in digesta viscosity in Nile tilapia, fed dietary guar gum supplementation (Slamova *et al.*, 2011). In rainbow trout and common carp, the presence of abnormally loose and sticky material in the stomachs and intestines was observed after feeding with viscous NSP (Knauer *et al.*, 1993).

The binding characteristics and digesta viscosity increases with increase in quantity of acid-activated bentonite (AB). As reported in chapter 3, acid-activated bentonite has a high binding capacity as compared to natural bentonite (Makhoukhi *et a*l., 2009; Motlagh *et al*., 2011; Yener *etal*., 2012, Ayoola *et al.*, 2015).

In both experiments, at 60mins, about 50% of the feed ingested have been emptied from the stomach for all treatment diets, which implies fish could be ready to ingest more feed after 60mins. These results can help relevant stakeholders in aquaculture production to predict the time intervals between feeding for adult *C.gariepinus*. However, further studies are suggested to effectively validate this feeding interval and return of appetite in *C.gariepinus.*

6.6 Conclusion

The result of this study provided information that bentonite clay acts as binder which increases feed resident time in the gut. The binding effect increases digesta viscosity, which slows down the passage of feed in the gut. The process enhances nutrient absorption and thus feed utilisation (See chapter 4 & 5). However, high viscosity reduces nutrient utilisation due to prolonged resident time of digesta in the gut, which results in fermentation (See chapter 4 & 5, Amirkolaie *et al*., 2006; Leenhouwers *et al*., 2007;Sinha *et al*., 2011). Therefore, dietary inclusion of acid-activated bentonite with natural bentonite blend ratio (25:75 = A25B75) at 0.5% and inclusion level of each whole clay at 1.5% are considered optimum. Inclusion or combination blends beyond the recommendation above may possess deleterious effect to the fish or its water culture media.

6.7 References

Abdollahi, M.R., Ravindran, V. & Svihus, B. 2013. Pelleting of broiler diets: An overview with emphasis on pellet quality and nutritional value. *Animal Feed Science and Technology*. 179(1-

4):1–23.

Amirkolaie, A.K., Leenhouwers, J.I., Verreth, J.A.J. & Schrama, J.W. 2005. Type of dietary fibre (soluble versus insoluble) influences digestion, faeces characteristics and faecal waste production in Nile tilapia (Oreochromis niloticus L.). *Aquaculture Research*. 36(12):1157–1166.

Amirkolaie, A.K., Verreth, J.A.J. & Schrama, J.W. 2006. Effect of gelatinization degree and inclusion level of dietary starch on the characteristics of digesta and faeces in Nile tilapia (Oreochromis niloticus (L .). *Aquaculture Research* 260:194–205.

Ayoola, M. O, De wet lourens, Khalid Salie 2015. Evaluating natural and acid activated bentonite as feed additives for improving water stability of feed for African Catfish. *Proc.Aquaculture Ass. of Southern Africa*, Sept 27-Oct 3, pp:53

Becker. K, Richter. H, C.Lu¨ Cksta¨ Dt, and U. Focken. 2003. Evacuation of pelleted feed and the suitability of titanium (IV) oxide as a feed marker for gut kinetics in Nile tilapia. *Journal of fish Biology* 63, 1080–1099.

Brinker, A. 2007a. Guar gum in rainbow trout (*Oncorhynchus mykiss*) feed: The influence of quality and dose on stabilisation of faecal solids. *Aquaculture*. 267:315–327.

Brinker, A. 2007b. Guar gum in rainbow trout (Oncorhynchus mykiss) feed: The influence of quality and dose on stabilisation of faecal solids. *Aquaculture*. 267(1-4):315–327.

Bureau, D.P. 1999. Apparent digestibility of rendered animal protein ingredients for rainbow trout ž Oncorhynchus mykiss /.*Aquaculture* .180 1999 345–358

Carraro, A., De Giacomo, A., Giannossi, M.L., Medici, L., Muscarella, M., Palazzo, L., Quaranta, V., Summa, V.2014. Clay minerals as adsorbents of aflatoxin M1 from contaminated milk and effects on milk quality. *Applied Clay Science*. 88-89:92–99.

Cho, C.Y. & Bureau, D.P. 2001. A review of diet formulation strategies and feeding systems to reduce excretory and feed wastes in aquaculture. *Aquaculture Research*. 32:349–360.

Deyoe, C.W. 2015. Digestion and gut evacuation of channel catfish given pelleted feeds. *Transactions of the Kansas Academy of Science* (1903-),. 76(3):254–260.

Eya, J.C., Parsons, A., Haile, I., Jagidi, P. & Virginia, W. 2008. Effects of Dietary Zeolites (Bentonite and Mordenite) on the Performance Juvenile Rainbow trout Onchorhynchus myskis. *Australian Journal of Basic and Applied Sciences*, 2(4): 961-967, 2008

Ghahri, H., Habibian, R. & Fam, M.A. 2010. Effect of sodium bentonite, mannan oligosaccharide and humate on performance and serum biochemical parameters during aflatoxicosis in broiler chickens. *Global Veterinaria*. 5(2):129–134.

Ighwela, K.A., Bin Ahmad, A. & Abol-Munafi, A.B. 2013. Water stability and nutrient leaching of different levels of maltose formulated fish pellets. *Global Veterinaria*. 10(6):638–642.

Indresh, H., Devegowda, G., Ruban, S. & Shivakumar, M. 2013. Effects of high grade bentonite on performance, organ weights and serum biochemistry during aflatoxicosis in broilers. *Veterinary World*. 6(6):313.

Knauer, J., Britz, P.J. & Hecht, T. 1993. The effect of seven binding agents on 24-hour water stability of an artificial weaning diet for the South African abalone, Haliotis midae (Haliotidae, Gastropoda). *Aquaculture*. 115(3-4):327–334.

Lam, C.D. & Flores, R.A. 2003. Effect of particle size and moisture content on viscosity of fish feed. *Cereal Chemistry*. 80(1):20–24.

Leenhouwers, J.I., Verreth, J.A.J. & Schrama, J.W. 2006. Digesta viscosity , nutrient digestibility and organ weights in African catfish (Clarias gariepinus) fed diets supplemented with different levels of a soluble non-starch polysaccharide. *Aquaculture Nutrition* 2006 12; 111–116

Leenhouwers, J.I., Veld, M., Verreth, J.A.J. & Schrama, J.W. 2007. Digesta characteristiscs and performance of African catfish (Clarias gariepinus) fed cereal grains that differ in viscosity. *Aquaculture*. 264:330–341.

Leenhouwers, J.I., Ortega, R.C., Verreth, J.A.J. & Schrama, J.W. 2007. Digesta characteristics in relation to nutrient digestibility and mineral absorption in Nile tilapia (Oreochromis niloticus L .) fed cereal grains of increasing viscosity. *Aquaculture*. 273:556–565.

Leenhouwers, J.I., ter Veld, M., Verreth, J.A.J. & Schrama, J.W. 2007. Digesta characteristiscs and performance of African catfish (Clarias gariepinus) fed cereal grains that differ in viscosity. *Aquaculture*. 264(1-4):330–341.

Makhoukhi, B., Didi, M.A., Villemin, D. & Azzouz, A. 2009. Acid activation of Bentonite for use as a vegetable oil bleaching agent. *Grasas y Aceites*. 60(4):343–349.

Mesubi, M.A., Adekola, F.A., Odebunmi, E.O., Adekeye, J.I.D., State, O. & Science, M. 2008. Beneficiation and characterisation of a bentonite from north-eastern Nigeria. *Journal of the North Carolina Academy of Science*, 124(4), pp. 154–158

Motlagh, K., Youzbashi, A.A. & Rigi, Z.A. 2011. Effect of acid activation on structural and bleaching properties of a bentonite. *Iranian Journal of Materials Science and Engineering*. 8(4):50–56.

Murray, H.H. 2000. Traditional and new applications for kaolin , smectite , and palygorskite : a general overview. *Applied Clay Science* 17 2000 207–221

Noyan, H., Onal, M. & Sarikaya, Y. 2007. The effect of sulphuric acid activation on the crystallinity, surface area, porosity, surface acidity, and bleaching power of a bentonite. *Food Chemistry*. 105(1):156–163.

Nutrition, A. 2003. Evacuation of pelleted feed and the suitability of titanium (IV) oxide as a feed marker for gut kinetics in Nile tilapia. *Journal of fish Biology* 63, 1080–1099.

Papaioannou, D.S., Kyriakis, C.S., Alexopoulos, C., Tzika, E.D., Polizopoulou, Z.S. & Kyriakis, S.C. 2004. A field study on the effect of the dietary use of a clinoptilolite-rich tuff, alone or in combination with certain antimicrobials, on the health status and performance of weaned, growing and finishing pigs. *Research in Veterinary Science*. 76(1):19–29.

Refstie, S., Landsverk, T., Bakke-McKellep, A.M., Ringø, E., Sundby, A., Shearer, K.D. & Krogdahl, Å. 2006. Digestive capacity, intestinal morphology, and microflora of 1-year and 2-year old Atlantic cod (Gadus morhua) fed standard or bioprocessed soybean meal. *Aquaculture*. 261(1):269–284.

Riche, M., Haley, D.I., Oetker, M., Garbrecht, S. & Garling, D.L. 2004. Effect of feeding frequency on gastric evacuation and the return of appetite in tilapia Oreochromis niloticus (L .). *Aquaculture*. 234:657–673.

Safaeikatouli, M., Jafariahangari, Y. & Baharlouei, A. 2010. Effects of dietary inclusion of sodium bentonite on biochemical characteristics of blood serum in broiler chickens. *International Journal of Agriculture and Biology*. 12(6):877–880.

Salmon, R.E. 1985. Effects of pelleting, added sodium bentonite and fat in a wheat-based diet on performance and carcass characteristics of small white turkeys. *Animal Feed Science and Technology*. 12:223–232.

Shu-li, D., Yu-zhuang, S.U.N., Cui-na, Y. & Bo-hui, X.U. 2009. Removal of copper from aqueous solutions by bentonites and the factors affecting it. *Mining Science and Technology (China)*. 19(4):489–492.

Sinha, A.K., Kumar, V., Makkar, H.P.S., Boeck, G. De & Becker, K. 2011. Non-starch polysaccharides and their role in fish nutrition – A review. *Food Chemistry*. 127(4):1409–1426.

Sinha, A.K., Kumar, V., Makkar, H.P.S., De Boeck, G. & Becker, K. 2011. Non-starch polysaccharides and their role in fish nutrition – A review. *Food Chemistry*. 127(4):1409–1426.

Slamova, R., Trckova, M., Vondruskova, H., Zraly, Z. & Pavlik, I. 2011. Applied Clay Science Clay minerals in animal nutrition. *Applied Clay Science*. 51(4):395–398.

Survey, B.A., Cross, H. & Road, M. 2000. Digestion rate , gut passage time and absorption e ffi ciency in the Antarctic spiny plunderfish. *Journal of Fish Biology* (2000) 57, 908–929

Svihus, B. & Zimonja, O. 2011. Chemical alterations with nutritional consequences due to pelleting animal feeds: a review. *Animal Production Science*. 51(7):590.

Verreth, Æ.J., Vermis, Æ.K., Nelis, H.J., Sorgeloos, Æ.P. & Verstegen, Æ.M. 2010. evacuation in larvae of African catfish Clarias gariepinus under different feeding conditions. *Aquacult Int* (2010) 18:119–134.

Yener, N., Biçer, C., Önal, M. & Sarıkaya, Y. 2012. Simultaneous determination of cation exchange capacity and surface area of acid activated bentonite powders by methylene blue sorption. *Applied Surface Science*. 258(7):2534–2539.

CHAPTER 7

EVALUATION OF WATER QUALITY IN A STATIC AERATED AFRICAN CATFISH (*Clarias gariepinus)* PRODUCTION TANK, FED AQUAFEEDS' CONTAINING DIETARY BENTONITE AND ITS ACID ACTIVATED FORM

Abstract

The potential of *Clarias gariepinus* for aquaculture has been demonstrated for different culture mediums and systems. Production in plastic tanks, with low water availability for exchange was considered. Accumulation of inorganic nitrogen in aquatic systems is mainly attributed to organic waste materials (i.e. faeces and uneaten feed). In this study, bentonite and its acid-activated form are included as feed additives, fed to *C. gariepinus*. The water quality parameters (pH, $N0_2^-N$, NH_3-N and TSS) were evaluated in a static aerated tank. In experiment 1, natural bentonite (NB) and acid-activated bentonite (AB) were respectively added as additives into a commercial *C gariepinus* feed as a clay blend at combination levels (0:100, 25:75, 50:50) and at two inclusion levels for each ratio low and high (500 mg/kg and 1500 mg/kg) clay/feed respectively with a control diet. In experiment II, NB and AB were added separately as additives at different inclusion levels (0, 500, 1000, 1500, and 3000 mg/kg). Restricted feeding at 3% body ratio was administered to adult fish weighing (395 – 405g) and (295 - 305g) for experiment I & II respectively. Each treatment diet was replicated four times, and 10 fish were randomly allotted to each tank. The water temperature was 25±2°C and DO (3 - 9mg/L). Water samples were collected daily from each tank over seven and five days for experiment 1 & II respectively. No mortality was recorded during the study. The values of pH, $N0_2^-N$, NH_3-N and TSS increased significantly ($p<0.05$) in all treatment diets. In experiment 1, A50B50 had lower values ($p<0.05$) as compared to other treatment diets. In experiment II, A500 and B1000 had lower values ($p<0.05$) as compared to the control diet. Uneaten feeds constituted to increase in poor water quality, whereas high water stability of clay treated diets attributed to maintenance of good water quality as compared to control.

7.1 Introduction

Water quality is a main factor in determining success or failure of fish production systems. Fish production capacity can increase by identifying environmental factors and providing an appropriate environment for the fish (Romano & Zeng, 2013). A significant cause of economic loss in aquaculture is mortality of fish through degraded water quality, from nitrogenous metabolic waste of fish and decomposition of uneaten feeds (Cho & Bureau, 2001; Belal, 2005). The most common ionic (reactive) forms of inorganic nitrogen are ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-) in fish culture systems and are potentially toxic to aquatic organisms (Romano & Zeng, 2013). These ions may be present naturally in aquatic ecosystems as a result of atmospheric deposition, surface and groundwater runoff, dissolution of nitrogen-rich geological deposits, N_2 fixation by certain prokaryotes (cyanobacteria, particularly), and biological degradation of organic matter (Wilkie, 1997; Philips *et al.*, 2002; Randall & Tsui, 2002; Zhang *et al.*, 2013).

Ammonia is released across fish gills into the water system and can be responsible for high mortality and reduced growth rates in aquaculture. The two forms of ammonia in water are (un-ionised ammonia NH_3 and ionized ammonium $NH_4^{+)}$; the former is highly toxic to fish (Roques *et al.;* 2012 Tomasso, 2012). Ammonia toxicity is pH dependent; as toxicity increases with higher pH value, other water quality parameters that interact with ammonia toxicity are temperature (a higher temperature resulted in increased un-ionised form, reduction of dissolved oxygen and could increase turbidity (Körner *et al.*, 2001; Kumlu & Eroldog, 2004). In fish, nitrite has resulted in brachial Cl^- uptake inhibition, impairment of the acid-base balance and the electrolyte, reduction of the oxygen carrying capacity of blood by oxidation of haemoglobin to methaemoglobin (Kolarevic *et al.*, 2013; Zhang *et al.*, 2013), changes in gill histopathology, reduction in gross growth efficiency and survival (Stormer *et al.*, 1996; Scott & Crunkilton, 2000; Schram *et al.*, 2014).

The two principal methods of removing inorganic nitrogen in water are: nitrification and ion exchange / adsorption. Nitrification is a two-step oxidation of ammonia to nitrate by autotrophic bacteria, and is an essential part of a recirculating fish culture system (Bower *et al.*, 1981). For nitrification, materials such as oyster shell, rock, sand, etc. are used to prepare a substrate for bacteria (van Rijn *et al.*, 2006a). Ion exchange is a process in which ions of an exchanger (synthetic or natural resin) are exchanged with certain ions in wastewater. Some natural resins, such as clay minerals (e .g zeolites, bentonites, sepiolite etc.) are used to remove heavy metals and inorganic nitrogenous waste from aquatic water (Cho *et al.*, 2010; Li *et al.*, 2010; Zhou & Boyd, 2014).

Bentonites belong to a smectites group of clay minerals formed as a result of weathering of volcanic ashes. The main component of bentonite (over 60%) is montmorillonite, a representative of stratiform silicates containing some amounts of quartz and small admixtures of illite, calcite, mica, chlorites and un-decomposed grains of volcanic glass (Murray, 2000; Shu-li *et al.*, 2009). A promising candidate for reducing levels of ammonia in solution is bentonite. Montmorillonite type clays are hygroscopic and are recognized for their ability to absorb many times their own mass of water, at the same time adsorbing ammonia and other cations (Murray, 2000; Slamova *et al.*, 2011; Taylor, Bellir, Lehocine & Meniai, 2013).

It appears that positively charged materials are held by negatively charged exchange surfaces of the clay (Shu-li *et al.*, 2009). The suggested mechanism is by direct co-ordination of the NH_3 molecule to the surface and/or form ion of the ammonium ion through reaction with water (Williford *et al.*, 1992; Ashrafizadeh, Khorasani & Gorjiara, 2008). Bentonite has been used successfully to reduce ammonia concentrations in aquaria (Williford *et al.*, 1992; Ashrafizadeh *et al.*, 2008); these functions are based on its properties: adsorption, cation exchange and high surface area (Murray, 2000; Jović-jovičić *et al.*, 2010; Zamparas *et al.*, 2012). The major demerit with treatment of clay to reduce nitrogenous compounds in aquaria is the increase in turbidity/total suspended solids accompanying the application of clay minerals (Williford *et al.*, 1992).

The African catfish (*Clarias gariepinus*) is known to be highly tolerant to ammonia toxicity (Ip, *et al.*, 2004; Wee *et al.*, 2007). The fish possess several defence mechanisms to cope with increased internal ammonia. These defence strategies include active excretion of NH_4+, reduced ammonia production by reduction of proteolysis, high ammonia tolerance of tissues and cells. *C. gariepinus* detoxified ammonia to glutamine and could tolerate high levels of glutamine in its brain (Wee *et al.*, 2007). However, despite the tolerance of this fish species to ammonia toxicity, Schram *et al*. (2010) concluded that a water NH_3 concentration of above 24 µM (0.34 mg NH_3-N/L) can cause physiological disturbances, low feed intake, growth and mortality.

Intensive aquaculture production of African catfish implores the use of recirculating aquaria systems, where the major goal is to produce a saleable product as efficiently and cost effectively as possible. This usually implies that the system uses the highest stocking density possible, highest quality feeds and active water quality management as top priority (Ebeling, *et al.*, 2006). In these systems, high levels of ammonia– nitrogen are excreted due to the high protein content of the feed and high production densities. However, the high protein inputs characteristic of most intensive aquaculture systems may lead to a build-up of the intermediate product (Nitrite) due to uneaten feed and excretory nitrogenous products (Schram *et al.*, 2010). Nitrite concentrations may also become high in recirculating systems as bio filters are becoming established, and researches have confirmed incomplete oxidation ammonia to nitrate in recirculating aquaculture systems RAS (Philips *et al.*, 2002; Ebeling *et al.*, 2006).

Therefore, as water quality is an important factor for successful intensive aquaculture production with recirculating systems or intensive pond culture, further investigation is considered to evaluate impact of feed physical qualities in the maintenance of water quality. We hypothesised that dietary inclusion of bentonite and its acid-activated form as feed binder will maintain good water quality in RAS for *C gariepinus* production.

7.2 Materials and Methods

7.2.1 Experimental Facilities

The experiment was carried out at the aquaculture facilities at Welgevallen Experimental Farm, of Stellenbosch University (SU). The location on a geographical position system (GPS) is as follows; coordinates 33°56' 33.95" S and 18°51'56.15 "E, Stellenbosch, Western Cape area, South Africa.

The experimental facilities consisted of a recirculating warm water (indoor temperature controlled) aquaria system (RAS). See previous chapter 4 for detailed information

Experiment 1

7.2.2 Experimental layout and design

The experiment was conducted in February 2015 over 7 day's duration. The bench mark for the experiment duration was set at DO in the tank not less than 3 mg/L. Clay mineral (natural and acid-activated bentonite) were included into a commercial aquafeeds' diet at 5 different combinations and two inclusion levels (low and high) with control diet, resulting in 12 treatments (See Table 7.1). All treatments were replicated 4 times. The experimental diets were feed at a 3% body ratio, 2 times daily. The experimental design was completely randomized.

Table 7. 5 : Inclusion level of bentonite clay combination blend in *C.gariepinus* feed

S/N0	Treatments	Designation	Inclusion level of clay (mg/kg of basal diet BD)
I.	Basal diet only * BD	T_1	No inclusion
II.	Basal diet only * BD	T_2	No inclusion
III.	BD + NB (low level)	T_3	500 mg NB (low level)
IV.	BD + NB (high level)	T_4	1500 mg NB (high level)
V.	BD + 75 % NB + 25 % AB	T_5	375 mg NB : 125 mg AB (low level)
VI.	BD + 75 % NB + 25 % AB	T_6	1,125 mg NB : 375 mg AB (high level)
VII.	BD + 50 % NB + 50 % AB	T_7	250 mg NB : 250 mg AB (low level)
VIII.	BD + 50 % NB + 50 % AB	T_8	750 mg NB : 750 mg AB (high level)
IX.	BD + 25 % NB + 75 % AB	T_9	125 mg NB : 375 mg AB (low level)
X.	BD + 25 % NB + 75 % AB	T_{10}	375 mg NB : 1,125 mg AB (high level)
XI.	BD + AB (low level)	T_{11}	500 mg AB (low level)
XII.	BD + AB (high level)	T_{12}	1500 mg AB (high level)

- BD : Commercial Montego catfish grower diets

  Natural bentonite – NB   Acid activated bentonite - AB

A commercial basal diet for catfish produced by Montego Feed Mill, Cape Town, South Africa was procured. All treatment diets were thoroughly mixed, and prepared as reported in chapter 3. Treatment diets were produced weekly and the control diet was without bentonite.

Table 7. 6 : Inclusion level of bentonite clays (NB and AB) in *C.gariepinus* feed

S/N0	Treatments	Designation	Inclusion level of clay (mg / kg of basal diet)
I.	Basal diet only *BD	Control	No inclusion
II.	BD + NB 500	NB 500	500 mg NB
III.	BD + AB 500	AB 500	500 mg AB
IV.	BD + NB 1000	NB 1000	1000 mg NB
V.	BD + AB 1000	AB 1000	1000 mg AB
VI.	BD + NB 1500	NB 1500	1500 mg NB
VII.	BD + AB 1500	AB 1500	1500 mg AB
VIII.	BD + NB 3000	NB 3000	3000 mg NB
IX.	BD + AB 3000	AB 3000	3000 mg NB

- BD : Commercial Montego catfish grower diets

Natural bentonite – NB Acid activated bentonite - AB

Experiment II

7.2.3 Experimental layout and design

The experiment was conducted in February, 2015 over five (5) day’s duration. The bench mark for the experiment duration was set at DO in the tank >/= 7 mg/L. Clay mineral (natural and activated bentonite) were included into a commercial aquafeeds’ diet (see table 7:2) at four (4) inclusion level each, and a control diet, resulting in nine (9) treatments. All treatments were replicated four (4) times. The experimental diets were feed at a 3% body ratio**,** two (2) times daily. The experimental design was completely randomized.

7.2.4 Experimental Animals and Compounds

A total of 800 mix-sex African catfish (*C. gariepinus*) was used for both experiments. 10 adult fish with average total weight of 4 kg were randomly allocated to each tank in experiment 1; the weight of each fish was between 395 – 405g. In experiment II, 10 adult fish with average total weight of 3kg were randomly allocated to each tank. Each fish weighed between 295 – 305g. The same quantity of feed was administered to each tank throughout the experiment. Each tank was cleaned 24 h before data collection.

Table 7. 7 : Summary of parameters measured with method and instrument applied

S/No	Parameters	Unit	Method / Instrument	Reference
1	Temperature	^oC	Oxyguard MK III oxygen meter	OxyGuard International A/S
2	TSS	mg/L	Photometric Method	Hach Company, Loveland, CO, USA
3	Dissolve oxygen (DO)	mg/L	Oxyguard MK III oxygen meter	OxyGuard International A/S
4	pH	NA	Hanna pH 211 microprocessor	Hanna Instruments Woonsocket, RI, US
5	NH_3-N	mg/L	Salicylate Method	Hach Company, Loveland, CO, USA
6	NO_2-N	mg/L	Diazotization Method	Hach Company, Loveland, CO, USA

7.2.5 Data collection

All water taps were closed, with no water inlet or outlet, and only an aerator was provided for each tank throughout the experiment. Water temperature and Dissolve oxygen (DO) were monitored daily. pH, NH_3–N, NO_2-N, and total suspended solids (TSS) were analysed and measured daily from each tank. The methods used for measuring each parameter are:

7.2.6 Statistical analysis

All data recordings and calculated values were computed using Microsoft Excel 2010 and Sharp Model ELW53H Scientific Calculator. Computed data were analysed using one way ANOVA for experiment II and two way factorial ANOVA for experiment I. Bonferroni was used to test for the significance of variance ($p<0.05$) for all recorded and calculated data between different treatments using SAS 2015 version 12 and XLSTAT version 2015

7.4 Results

Table 7. 8 : The effect of dietary bentonite clay combination blend as feed additive, fed to *C.gariepinus* on pH, NH_3-N, NO_2^- N and TSS in a static aerated culture media

Treatment	Time (Days)						
	1	2	3	4	5	6	7
pH							
A0B0	6.87^{a}	7.06^{a}	7.23^{a}	7.36^{a}	7.54^{a}	7.65^{a}	7.73^{a}
A0B100	6.73^{ab}	6.92^{ab}	7.06^{b}	7.16^{c}	7.30^{b}	7.34^{b}	7.45^{b}
A25B75	6.72^{ab}	6.78^{b}	7.04^{bc}	7.11^{cd}	7.21^{cd}	7.27^{c}	7.31^{c}
A50B50	6.72^{ab}	6.84^{ab}	6.99^{bc}	7.08^{d}	7.16^{d}	7.20^{d}	7.26^{c}
A75B25	6.64^{b}	6.77^{b}	6.98^{c}	7.15^{c}	7.21^{c}	7.27^{c}	7.32^{c}
A100B0	6.57^{b}	6.84^{ab}	7.17^{a}	7.24^{b}	7.29^{b}	7.35^{b}	7.39^{b}
SEM	0.04	0.05	0.02	0.01	0.01	0.01	0.01
NH_3-N							
A0B0	13.80^{a}	26.55^{a}	28.00^{a}	40.20^{a}	51.60^{a}	59.40^{a}	67.50^{a}
A0B100	12.80^{ab}	21.98^{b}	19.88^{b}	31.20^{b}	33.20^{b}	42.75^{b}	46.00^{bc}
A25B75	10.95^{bc}	16.43^{cd}	19.00^{b}	20.40^{d}	28.80^{c}	34.43^{d}	36.75^{d}
A50B50	10.30^{c}	14.70^{d}	20.63^{b}	25.65^{c}	29.20^{c}	34.20^{d}	37.50^{d}
A75B25	8.95^{c}	17.85^{c}	21.38^{b}	26.55^{c}	29.80^{c}	36.23^{cd}	39.25^{cd}
A100B0	10.95^{bc}	26.55^{a}	30.88^{a}	37.95^{a}	38.20^{a}	40.28^{bc}	46.75^{b}
SEM	0.52	0.64	0.91	0.87	1.01	1.02	1.54
NO_2^-N							
A0B0	1.91^{a}	3.37^{a}	4.21^{a}	4.29^{a}	6.55^{a}	10.78^{a}	25.60^{a}
A0B100	1.02^{b}	2.25^{b}	3.04^{b}	3.16^{c}	4.09^{d}	9.25^{bc}	18.80^{c}
A25B75	0.46^{c}	1.29^{cd}	2.27^{b}	2.40^{d}	4.52^{c}	8.51^{d}	17.35^{d}
A50B50	0.45^{c}	0.91^{d}	1.26^{c}	1.66^{f}	3.37^{e}	5.50^{e}	11.83^{e}
A75B25	1.18^{b}	1.68^{bc}	2.52^{b}	1.93^{e}	3.65^{e}	8.91^{cd}	18.48^{cd}
A100B0	1.38^{b}	3.51^{a}	4.23^{a}	3.68^{b}	5.35^{b}	9.76^{b}	20.40^{b}
SEM	0.08	0.13	0.21	0.04	0.08	0.17	0.30

TSS							
A0B0	10.25^{bc}	28.00^{a}	41.50^{c}	65.00^{a}	98.00^{a}	115.50^{a}	125.50^{a}
A0B100	9.88^{c}	19.88^{b}	41.75^{c}	52.50^{c}	67.25^{b}	83.25^{b}	88.75^{b}
A25B75	10.03^{bc}	24.50^{d}	41.50^{c}	45.25^{e}	55.50^{de}	65.50^{c}	73.50^{de}
A50B50	11.05^{b}	27.50^{c}	42.25^{c}	46.00^{de}	53.75^{e}	61.25^{c}	69.25^{e}
A75B25	13.13^{a}	32.25^{b}	46.25^{b}	48.50^{d}	60.00^{cd}	68.25^{c}	78.25^{cd}
A100B0	13.75^{a}	37.75^{a}	49.50^{a}	57.50^{b}	62.25^{bc}	79.50^{b}	84.75^{bc}
SEM	0.24	0.57	0.44	0.69	1.35	1.84	1.89

SEM: Standard error of mean. *Mean values between the columns are verified at $p<0.05$; values on the same columns with the same superscript letter are not significantly different based on bonferroni post-hoc test. Where: A0B100 = 100% NB, A25B75 = 25% AB: 75% NB, A50B50 = 50% AB: 50% NB, A75B25 = 75% AB: 25% NB, A100B0 = 100% AB, A0B0 = control

Table 7. 5: The effect of dietary bentonite clay inclusion level as feed additive, fed to *C.gariepinus* on pH, NH_3-N, NO_2^- and TSS in a static aerated culture media

Time (days)	Treatment								
	A0B0	A500	B500	A1000	B1000	A1500	B1500	A3000	B3000	SEM
pH										
1	5.73^{a}	5.47^{bc}	5.49^{bc}	5.54^{bc}	5.44^{c}	5.54^{bc}	5.51^{bc}	5.42^{c}	5.41^{c}	0.03
2	6.77^{a}	6.45^{abc}	6.47^{ab}	6.44^{bc}	6.34^{bc}	6.36^{bc}	6.31^{bc}	6.29^{c}	6.28^{c}	0.04
3	6.83^{a}	6.77^{a}	6.73^{a}	6.67^{a}	6.70^{a}	6.79^{a}	6.69^{a}	6.46^{b}	6.40^{b}	0.04
4	7.12^{ab}	6.92^{bc}	7.02^{ab}	6.98^{b}	6.89^{b}	6.99^{bc}	6.70^{c}	6.70^{cd}	6.68^{cd}	0.04
5	7.27^{a}	7.28^{a}	7.23^{ab}	7.15^{bc}	7.11^{c}	7.17^{bc}	7.15^{bc}	7.09^{c}	7.09^{c}	0.04
NH_3-N										
1	2.98^{ab}	2.88^{abc}	2.50^{c}	2.88^{abc}	2.60^{bc}	2.80^{abc}	3.10^{a}	2.70^{bc}	2.68^{bc}	0.08
2	8.90^{a}	7.60^{abc}	7.00^{c}	7.50^{bc}	7.50^{bc}	7.80^{ab}	7.10^{bc}	7.80^{ab}	7.80^{ab}	0.26
3	14.80^{a}	13.60^{ab}	14.40^{ab}	11.40^{cd}	11.80^{bcd}	10.40^{d}	10.80^{d}	13.06^{bcd}	13.00^{bcd}	0.56
4	25.50^{a}	23.75^{ab}	24.25^{ab}	19.25^{bcd}	14.25^{d}	19.25^{bc}	16.75^{cd}	21.00^{bc}	21.25^{bc}	1.07
5	31.80^{a}	25.90^{ab}	25.20^{ab}	21.30^{c}	21.60^{c}	21.80^{c}	22.80^{c}	22.80^{bc}	22.60^{bc}	1.16
NO_2^-										
1	0.17^{bc}	0.16^{bc}	0.16^{bc}	0.17^{bc}	0.18^{bc}	0.14^{c}	0.17^{bc}	0.20^{ab}	0.23^{a}	0.01
2	0.20^{ab}	0.24^{a}	0.22^{ab}	0.24^{a}	0.22^{ab}	0.14^{b}	0.20^{ab}	0.22^{ab}	0.20^{ab}	0.02
3	0.47^{a}	0.34^{bc}	0.37^{bc}	0.36^{c}	0.33^{c}	0.36^{bc}	0.33^{c}	0.36^{bc}	0.32^{c}	0.03
4	0.89^{a}	0.43^{c}	0.42^{c}	0.46^{c}	0.52^{b}	0.50^{b}	0.62^{bc}	0.70^{b}	0.77^{b}	0.09

5	1.87^{a}	0.69^{d}	0.63^{d}	0.73^{c}	0.75^{c}	0.86^{bc}	0.80^{c}	0.88^{bc}	0.92^{b}	0.17
TSS										
1	2.98	2.88	2.80	2.88	2.82	2.80	2.85	2.85	2.88	0.08
2	14.50^{bc}	12.00^{c}	13.50^{bc}	13.00^{c}	14.00^{bc}	16.00^{abc}	19.00^{ab}	17.50^{abc}	21.50^{a}	1.11
3	24.00^{ab}	19.00^{bcd}	17.00^{c}	15.50^{d}	19.00^{bcd}	19.00^{bcd}	16.00^{d}	26.50^{a}	26.50^{a}	1.23
4	26.50	26.50	24.50	23.00	23.00	27.00	22.00	28.50	27.50	2.21
5	40.50^{a}	30.50^{bc}	29.50^{bc}	29.50^{bc}	28.00^{c}	30.50^{b}	32.00^{b}	34.50^{b}	34.00^{b}	2.13

SEM: Standard error of mean. *Mean values between the rows are verified at $p<0.05$; values on the same columns with the same superscript letter are not significantly different based on bonferroni post-hoc test. Where: A0B0 = control, A= acid-activation, B- natural bentonite

Table 7.4 presented the effect of treatment diets on measured water quality parameters. The pH and NH_3-N values were significantly different ($p<0.05$) among diets at all the time intervals. These values increased ($p<0.05$) with time and the control diet had the highest values. The values increased with the level of acid-activated quantity in the clay blend and quantity of clay in the diet. A50B50 had the lowest pH values from 4th – 7th day. The values of NH_3-N were at the lowest for A25B75, which did not differ ($p>0.05$) with A50B50 and A75B25 over time. There was significant interaction ($p<0.05$) between the level of inclusion and the clay combination blend. The values of NO_2^- and TSS were significantly different ($p<0.05$) with time among the treatment diets. The control diet had the highest values significantly ($p<0.05$) as compared to clay treated diets over all time intervals. The clay treatment diets AB and NB at 100% had higher values as compared to other clay diets blend. The treatment diet A50B50 had the lowest values over time for NO_2^- and TSS. There was significant interaction ($p<0.05$) between the level of inclusion and the clay combination blend.

As reported in table 7.5, NO_2^- and TSS values were significantly affected ($p<0.05$) by treatment diets. The value increased significantly ($p<0.05$) in all treatment diets over time. Dietary clay inclusion at 3000mg/kg had highest values ($p<0.05$) as compared to control and other dietary clay treatment diets at day 1. At day 2, A1500 had least value which was significantly different ($p<0.05$) from other treatments. At day 3 – 5, control diet had highest values significantly ($p<0.05$) as compared to other diets. At day 5, the control diet had the highest value, which differed significantly from other treatment diets. The values of pH and NH_3-N were significantly affected ($p<0.05$) by treatment diets over time. These values increased over time in all treatment diets. The control diet and dietary clay Inclusion levels 500mg/kg had higher values which are significantly different ($p<0.05$) as compared to other treatment diets. Acid-activated bentonite had higher values as compared to natural bentonite.

7.5 Discussion

Intensive aquaculture with RAS applied the use of high quality and quantity of feeds. The systems implied that effluents are discharged to the environment with enhanced nutrient and solid concentrations. Depending on the species and culture technique, up to 85% of phosphorus, 80–88% of carbon and 52–95% of nitrogen input into a fish culture system may be lost to the environment through feed wastage, fish excretion, faecal production and respiration (Zhang *et al.*, 2011; Endut *et al.,* 2012). Although, RAS offer advantages in terms of reduced water utilisation, improved opportunities for waste management, and better hygienic environment with disease management (Van Rijn, 1996; Zhou & Boyd, 2015). To date, the rearing organisms that have been implemented in RASs have expanded to a diverse range of species, from freshwater to seafood products and from hatchery/fingerling to grow-out production (Colt, 2006; Endut *et al.*, 2012). Effective management of inorganic nitrogen accumulation in RAS is still a rising technical problem.

7.5.1 pH.

In table 7.4 and pH values were significantly ($p<0.05$) affected by treatment diets. The values increased significantly over the trial period. In table 7.4, pH at day 1 ranges between (6.64 – 6.87), while at day 7 (7.26 – 7.73). Table 7.5 reported pH values ranges at day 1 (5.43 – 5.73) and day 5 (7.05 – 7.28). Total ammonia in aqueous solution consists of two principal forms, the ammonium ion (NH_4+) and un-ionised ammonia (NH_3), with relative concentrations being pH and temperature dependent. The un-ionised form is most toxic due to the fact that it is uncharged and lipid soluble and thus traverses biological membranes more readily than the charged and hydrated NH_4+ ions (Körner *et al.*, 2001; Spencer *et al.,* 2008; Zhang *et al.,* 2011). Lower value of pH (acidic) reduces concentration of un-ionised ammonia, while higher value of pH increases the toxic effect of ammonia. The results of pH measurement at day 1 for both trials indicated a relatively low concentration of NH_3-N in the production.

The pH value decreased with high clay inclusion level. The results can be attributed to proliferation of algae in the system, resulting from photosynthesis. During daylight, algae and underwater plants remove carbon dioxide from the water as part of the sunlight-driven process of photosynthesis. The relative rates of respiration and photosynthesis within the pond determine whether there is a net addition or removal of carbon dioxide, and therefore whether pH falls or rises. Respiration rates are affected by water temperature and the biomass of plants, animals and microorganisms in the water and bottom sediment (Tucker & Abramo, 2008; Zhang *et al.*, 2011).

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

Adding carbon dioxide “pushes” the chemical reaction toward the right-hand side, forming carbonic acid and hydrogen ions and causing pH to decrease. Removing carbon dioxide “pulls” the reaction to the left, thereby removing hydrogen ions and causing pH to increase (van Rijn *et al.*, 2006a; Tucker & Abramo, 2008).

Furthermore, as the trial progresses, the amount of DO in the culture media reduces due to fish respiration and decomposition of organic waste materials. Reduction of DO gradually reduce feed consumption, thus increasing feed wastage and eutrophication. At low clay inclusion level, the water stability of feed reduces, resulting in high leaching and disintegration, while at high clay inclusion level and high quantity of AB, slow passage of feed in the gut occurred, thus leading to low feed intake (See chapter 6**)**. In this trial, feeding was not based on fish visual observation of satiation, but rather on daily administration of feed regardless of feed un-eaten feed. Thus, increased feed wastage existed with high clay inclusion, leading to eutrophication and proliferation of algae of which photosynthetic action increased pH value.

7.5.2 NH_3-N

The values of NH_3-N were significantly ($p<0.05$) affected by the treatment diets. The values increased significantly ($p<0.05$) over the trial period. In table 7.4, the control diet A0B0, had the highest value 13.80mg/l which differed significantly ($p<0.05$) as compared to other treatments except A0B100, while A75B25 had the lowest value 8.95mg/l. A1500 had the highest value 3.10mg/l which differed significantly ($p<0.05$) as compared to other treatment diets except A1000, B3000 and A3000 with lower values. A500 had the lowest value 2.50mg/l as presented in table 7.5. These values are still lower than (48mg/l) toxic level reported for C gariepinus (Colt & Orwicz, 1991). No explanation can be attributed to the significant difference ($p<0.05$) between the treatment as recorded at day 1, but may be due to fish respiration.

The NH_3-N value increased with high clay inclusion level (>1000mg/kg) and with low inclusion level (less than/equal to 500mg/kg). The results obtained in these trials can be attributed to excretion of ammonia by fish. All animals including fish are ammonogenic. Ammonia is synthesized in the liver, through dietary amino acid catabolism. Digestion of proteins in the intestine releases amino acids, but certain amino acids are metabolized in the intestine before reaching the liver (Randall & Tsui, 2002; Romano & Zeng, 2013). Approximately 40–60% of the nitrogen intake from food is excreted within 24 h, because fish cannot store excess amino acids. Therefore, dietary amino acids in excess of those needed for growth and protein turnover are preferentially degraded over carbohydrates and lipids in the liver (Kolarevic *et al.*, 2013; Görg *et al.*, 2010; Chew & Ip, 2014). The binding properties of clay slow down the passage of feed in the gut **(**See chapter 6**)**, thus increasing nitrogen intake (Leenhouwers *et al.*, 2006; Sinha, *et al.*, 2011). In the control diet and clay treated diets at low inclusion level (</=500mg/kg), NH_3-N increased due to more nitrogen excretion, because these diets has high gut evacuation rate as compared to others (See chapter 6).

However, high clay inclusion level and quantity of acid-activated combination blend reduced feed intake. In this study, feeding was not based on fish visual observation of satiation, but rather on daily administration of feed regardless of feed left. This procedure led to a build-up of organic materials, decrease in DO and increased pH. The build- up of NH_3-N in the system increases with low DO and high pH (Camargo & Alonso, 2006; Ebeling *et al.*, 2006; Chew & Ip, 2014). As reported by some authors, NH_3-N less than/equal to 48mg/l can cause *C gariepinus* mortality (Ghate *et al.* 1993; Ip *et al.* 2004; Schram *et al.* 2010). In this study, no mortality was recorded, although at 7th day fish are found to be weak; a quick water exchange process salvaged the situation.

7.5.3 NO_2^-

The values recorded for $N0_2^-$ were presented in table 7.4 and 7.5. This result suggested that as NH_3-N increases, the synthesis of $N0_2^-$ increases and can be attributed to incomplete oxidation of NH_3-N to $N0_3^-$ (Grommen, *et al.*, 2002; Lefevre *et al.*, 2011). Also, nitrite can be produced from nitrate, through a number of nitrate reductive pathways. For instance, in fast flowing aerobic small streams, ammonia oxidation via nitrification is mainly responsible for elevated nitrite levels, whereas in slow- flowing conditions nitrite concentrations are more attributed to anaerobic nitrate-reducing processes (Philips *et al.*, 2002; Tomasso, 2012b). Therefore, eutrophication that occurs in this study due to feed decomposition at low and high clay inclusion level, control diets, and at increase quantity of AB and NB as explained earlier reduces DO in the system. The DO reduction leads to incomplete NH_3-N oxidation or anaerobic nitrate-reducing process, which increases nitrite level in the system.

7.5.4 TSS

Total suspended solids (TSS) can be of an organic or inorganic nature. Inorganic suspended solids can be derived from sand and clay silting material from erosion and runoff while organic suspended solids derive from animal faeces, uneaten feed and dead fish (Bilotta & Brazier, 2008)**.** In this study, the TSS is mainly organic solids with dead fish excluded. The higher value recorded at control diet can be attributed to the accumulation of uneaten feeds.

The control diet has less water stability as compared to clay treated diets (See chapter 3), thus disintegration of uneaten feeds is faster. The higher value of TSS noticed at high inclusion level of clay (1500 and 3000mg/kg), and high quantity of AB and NB (A100B0, A0B100) as compared to other clay treated diets can be attributed to less feed consumption (See chapter 4). Though these aforementioned clay treated diets have high water stability, as the trial period progresses, feed disintegrated with fish movement occurred. TSS value increased due to uneaten feed quantity, although *C gariepinus* are known to be hardy and capable of living in moulds and withstand relatively low water quality (Akinwole & Faturoti, 2007). The presence of TSS can also act directly on the fish by clogging and being abrasive to their gill structures (Chen *et al.*, 1993; Bilotta & Brazier, 2008), and/or stressing the fish and suppressing their immune system, leading to increased susceptibility to disease and osmotic dysfunction (van Rijn, Tal & Schreier, 2006b).

7.6 Conclusion

In this study, the water quality parameters; pH, NO_2^-, NH_3-N and TSS increased with the trial period. In experiment I, bentonite and its activated form were used as a blend product at (high and low level). Blend clay forms had lower values significantly as compared to control. The optimum performance was found at blend clay form A50B50 as compared to control and other clay blend diets. In experiment II, high and low level inclusions were found appropriate to maintain the water quality parameters as compared to control. B1000 and A500 had optimum performance for each of the clay inclusion levels. Bentonite clay has been described to increase aquafeeds' physical properties. The binding properties increases with acid-activated form and inclusion level (See chapter 3). Uneaten feeds contributed to poor water quality in all treatment diets over time. However, improved physical quality of bentonite clay treated aquafeeds', maintained water quality better as compared to control aquafeeds'. Heavy loadings of culture media with high dietary bentonite clay inclusion and high quantity of acid-activated bentonite aquafeeds' did not maintain good water quality.

7.7 References

Akinwole, A.O. & Faturoti, E.O. 2007. Biological performance of African Catfish (Clarias gariepinus) cultured in recirculating system in Ibadan. *Aquacultural Engineering* 36 (2007) 18–23.

Ashrafizadeh, S.N., Khorasani, Z. & Gorjiara, M. 2008. Ammonia Removal from Aqueous Solutions by Iranian Natural Zeolite. *Separation Science and Technology*. 43(4):960–978.

Belal, I.E.H. 2005. A review of some fish nutrition methodologies. *Bioresource Technology* 96

(2005) 395–402

Bilotta, G.S. & Brazier, R.E. 2008. Understanding the influence of suspended solids on water quality and aquatic biota. *Aquaculture engineering*42:2849–2861.

Camargo, J. a & Alonso, A. 2006. Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: A global assessment. *Environment international*. 32(6):831–49.

Chen, S., Timmonsb, M.B., Aneshansleyb, D.J. & James, J. 1993. Suspended solids characteristics from recirculating aquacultural systems and design implications. *Aquaculture engineering*112:143–155.

Chew, S.F. & Ip, Y.K. 2014. Excretory nitrogen metabolism and defence against ammonia toxicity in air-breathing fishes. *Journal of fish biology*. 84(3):603–38.

Cho, C.Y. & Bureau, D.P. 2001. A review of diet formulation strategies and feeding systems to reduce excretory and feed wastes in aquaculture. *Aquaculture Research*. 32:349–360.

Cho, D.-W., Chon, C.-M., Jeon, B.-H., Kim, Y., Khan, M.A. & Song, H. 2010. The role of clay minerals in the reduction of nitrate in groundwater by zero-valent iron. *Chemosphere*. 81(5):611–6.

Colt, J. 2006. Water quality requirements for reuse systems. *Aquacultural Engineering*. 15(3):169–181.

Colt, J. & Orwicz, K. 1991. Modeling production capacity of aquatic culture systems under freshwater conditions. *Aquacultural Engineering*.102:143–155.

Ebeling, J.M., Timmons, M.B. & Bisogni, J.J. 2006. Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia–nitrogen in aquaculture systems. *Aquaculture*. 257(1-4):346–358.

Endut, A., Jusoh, A., Ali, N., Wan Nik, W.N.S. & Hassan, A. 2012. Effect of flow rate on water quality parameters and plant growth of water spinach (Ipomoea aquatica) in an aquaponic recirculating system. *Desalination and Water Treatment*. 5(1-3):19–28.

Ghate, S.R., Burtle, G.J. & Smith, M.C. 1993. Water quality in catfish ponds subjected to high stocking density selective harvesting production practice. *Aquacultural Engineering*. 12(3):169–181.

Görg, B., Morwinsky, A., Keitel, V., Qvartskhava, N., Schrör, K. & Häussinger, D. 2010. Ammonia triggers exocytotic release of L-glutamate from cultured rat astrocytes. *Glia*. 58(6):691–705.

Grommen, R., Hauteghem, I. Van & Wambeke, M. Van. 2002. An improved nitrifying enrichment to remove ammonium and nitrite from freshwater aquaria systems. *Aquaculture* 211 115–124.

Gutarowska, B., Matusiak, K., Borowski, S., Rajkowska, A. & Brycki, B. 2014. Removal of odorous compounds from poultry manure by microorganisms on perlite--bentonite carrier. *Journal of environmental management*. 141:70–6.

Ip, Y.K., Zubaidah, R.M., Liew, P.C., Loong, A.M., Hiong, K.C., Wong, W.P. & Chew, S.F. 2004. African sharptooth catfish Clarias gariepinus does not detoxify ammonia to urea or amino acids but actively excretes ammonia during exposure to environmental ammonia. *Physiological and biochemical zoology : PBZ*. 77(2):242–54.

Jović-jovičić, N., Milutinović-nikolić, A., Banković, P. & Dojčinović, B. 2010. Synthesis , Characterization and Adsorptive Properties of Organobentonites. *Eleventh Annual Conference of the Materials Research Society of Serbia*, ,117(5):849–854.

Kolarevic, J., Selset, R., Felip, O., Good, C., Snekvik, K., Takle, H., Ytteborg, E., Baeverfjord, G., 2013. Influence of long term ammonia exposure on Atlantic salmon (Salmo salar L.) parr growth and welfare. *Aquaculture Research*, 44, 1649–1664

Körner, S., Das, S.K., Veenstra, S. & Vermaat, J.E. 2001. The effect of pH variation at the ammonium / ammonia equilibrium in wastewater and its toxicity to Lemna gibba. *Aquatic Botany* 71 (2001) 71–78.

Kumlu, M. & Eroldog, O.T. 2004. Effects of temperature on acute toxicity of ammonia to Penaeus semisulcatus juveniles. *Aquaculture* 241 (2004) 479–489

Leenhouwers, J.I., Verreth, J.A.J. & Schrama, J.W. 2006. Digesta viscosity , nutrient digestibility and organ weights in African catfish (*Clarias gariepinus*) fed diets supplemented with different levels of a soluble non-starch polysaccharide.*Aquaculture Nutrition* 2006 12; 111–116.

Lefevre, S., Jensen, F.B., Huong, D.T.T., Wang, T., Phuong, N.T. & Bayley, M. 2011. Effects of nitrite exposure on functional haemoglobin levels, bimodal respiration, and swimming performance in the facultative air-breathing fish Pangasianodon hypophthalmus. *Aquatic toxicology (Amsterdam, Netherlands)*. 104(1-2):86–93.

Li, J., Li, Y. & Meng, Q. 2010. Removal of nitrate by zero-valent iron and pillared bentonite. *Journal of hazardous materials*. 174(1-3):188–93.

Motlagh, K., Youzbashi, A.A. & Rigi, Z.A. 2011. Effect of acid activation on structural and bleaching properties of a bentonite. *Iranian Journal of Materials Science and Engineering*. 8(4):50–56.

Murray, H.H. 2000. Traditional and new applications for kaolin , smectite , and palygorskite : a general overview.*Applied Clay Science* 17 2000 207–221

Philips, S., Laanbroek, H.J. & Verstraete, W. 2002. Origin , causes and effects of increased nitrite concentrations in aquatic environments. *Environmental Science & Bio/Technology* 1: 115–141, 2002.

Randall, D.J. & Tsui, T.K.N. 2002. Ammonia toxicity in fish. *Marine Pollution Bulletin* 45 (2002) 17–23

Van Rijn, J. 1996.The potential for integrated biological treatment systems in recirculating fish culture - A review. *Aquaculture*. 139 (3-4) :181-201

van Rijn, J., Tal, Y. & Schreier, H.J. 2006a. Denitrification in recirculating systems: Theory and applications. *Aquacultural Engineering*. 34(3):364–376.

van Rijn, J., Tal, Y. & Schreier, H.J. 2006b. Denitrification in recirculating systems: Theory and applications. *Aquacultural Engineering*. 34(3):364–376.

Romano, N. & Zeng, C. 2013. Toxic Effects of Ammonia, Nitrite, and Nitrate to Decapod Crustaceans: A Review on Factors Influencing their Toxicity, Physiological Consequences, and Coping Mechanisms. *Reviews in Fisheries Science*. 21(1):1–21.

Roques, J.A.C., Schram, E., Spanings, T., van Schaik, T., Abbink, W., Boerrigter, J., de Vries, P., van de Vis, H., 2012. The impact of elevated water nitrite concentration on physiology, growth and feed intake of African catfish Clarias gariepinus (Burchell 1822). *Aquaculture Research*. (September, 19): 1-12

Safaei, M., Boldaji, F., Dastar, B., Hassani, S., Mutalib, M.S.A. & Rezaei, R. 2014. Effects of Inclusion Kaolin, Bentonite and Zeolite in Dietary on Chemical Composition of Broiler Chickens Meat. *Asian Journal of Animal and Veterinary Advances*. 9(1):56–63.

Safaeikatouli, M., Boldaji, F., Dastar, B. & Hassani, S. 2012. The effect of dietary silicate minerals supplementation on apparent ileal digestibility of energy and protein in broiler chickens. *International Journal of Agriculture and Biology*. 14(2):299–302.

Schram, E., Roques, J.A.C., Abbink, W., Spanings, T., de Vries, P., Bierman, S., de Vis, H. van & Flik, G. 2010. The impact of elevated water ammonia concentration on physiology, growth and feed intake of African catfish (Clarias gariepinus). *Aquaculture*. 306(1-4):108–115.

Schram, E., Roques, J.A.C., Abbink, W., Yokohama, Y., Spanings, T., de Vries, P., Bierman, S., van de Vis, H.. 2014. The impact of elevated water nitrate concentration on physiology, growth and feed intake of African catfish Clarias gariepinus (Burchell 1822). *Aquaculture Research*. 45(9):1499–1511.

Scott, G. & Crunkilton, R.L. 2000. Acute and chronic toxicity of nitrate to fathead minnows (Pimephales promelas), Ceriodaphnia dubia, and Daphnia magna. *Environmental Toxicology and Chemistry*. 19(12):2918–2922.

Shu-li, D., Yu-zhuang, S.U.N., Cui-na, Y. & Bo-hui, X.U. 2009. Removal of copper from aqueous solutions by bentonites and the factors affecting it. *Mining Science and Technology (China)*. 19(4):489–492.

Sinha, A.K., Kumar, V., Makkar, H.P.S., De Boeck, G. & Becker, K. 2011. Non-starch polysaccharides and their role in fish nutrition – A review. *Food Chemistry*. 127(4):1409–1426.

Slamova, R., Trckova, M., Vondruskova, H., Zraly, Z. & Pavlik, I. 2011. Applied Clay Science Clay minerals in animal nutrition. *Applied Clay Science*. 51(4):395–398.

Spencer, P., Pollock, R. & Dubé, M. 2008. Effects of un-ionized ammonia on histological, endocrine, and whole organism endpoints in slimy sculpin (Cottus cognatus). *Aquatic toxicology (Amsterdam, Netherlands)*. 90(4):300–9.

Stormer, J., Jensen, F.B. & Rankin, J.C. 1996. Uptake of nitrite, nitrate, and bromide in rainbow trout, Oncorhynchus mykiss: Effects on ionic balance. *Canadian Journal of Fisheries and Aquatic Sciences*. 53(9):1943–1950.

Taylor, P., Bellir, K., Lehocine, M.B. & Meniai, A. 2013. Desalination and Water Treatment Zinc removal from aqueous solutions by adsorption onto bentonite. *Desalination and Water Treatment* 51 (2013) 5035–5048

Tomasso, J.R. 2012. Environmental nitrite and aquaculture: a perspective. *Aquaculture International*. 20(6):1107–1116.

Tucker, C.S. & Abramo, L.R.D. 2008. Managing High pH in Freshwater Ponds. *Southern Africa regional aquaculture centre* SRAC (4604).

Wee, N.L.J., Tng, Y.Y.M., Cheng, H.T., Lee, S.M.L., Chew, S.F. & Ip, Y.K. 2007. Ammonia toxicity and tolerance in the brain of the African sharptooth catfish , *Clarias gariepinus*. 82:204–213.

Wilkie, M.P. 1997. Mechanisms of Ammonia Excretion Across Fish Gills. *Biochem. Physiol*. Vol. 118A, No. 1, pp. 39–50, 1997.

Williford, C.W., Reynolds, W.R. & Quiros, M. 1992.Clinoptilolite removal of ammonia from simulated and natural catfish pond waters. *Applied Clay Science*, 6 (1992) 277-291

Zamparas, M., Gianni, A., Stathi, P., Deligiannakis, Y. & Zacharias, I. 2012. Applied Clay Science Removal of phosphate from natural waters using innovative modi fi ed bentonites. *Applied Clay Science*. 62-63:101–106.

Zhang, L.S., Wang, Y.Y., Meng, F.S., Zhou, Y.X. & Yu, H. Bin. 2013. Toxicity of nitrate-n to freshwater aquatic life and its water quality criteria. *Huanjing Kexue/Environmental Science*. 34(8):3286–3293.

Zhang, S.Y., Li, G., Wu, H.B., Liu, X.G., Yao, Y.H., Tao, L. & Liu, H. 2011. An integrated recirculating aquaculture system (RAS) for land-based fish farming: The effects on water quality and fish production. *Aquacultural Engineering*. 45(3):93–102.

Zhou, L. & Boyd, C.E. 2014. Total ammonia nitrogen removal from aqueous solutions by the natural zeolite, mordenite: A laboratory test and experimental study. *Aquaculture*. 432:252–257.

Zhou, L. & Boyd, C.E. 2015. An assessment of total ammonia nitrogen concentration in Alabama (USA) ictalurid catfish ponds and the possible risk of ammonia toxicity. *Aquaculture*. 437:263–269.

CHAPTER 8

SUMMARY AND RECOMMENDATIONS

8.1 Summary

Aquaculture is regarded worldwide as one of the fastest growing food-producing sub-sectors, demonstrated by a continuous increase in total production throughout the last decade or more, particularly in a number of developing countries. The African catfish *Clarias gariepinus* is an important species to aquaculture growth and development. It commands high demand from consumers and is mostly preferred as a candidate species by food fish aquaculturists. This is due to the ideal characteristics of this species, which includes high growth rate at high stocking densities, a high food conversion and good meat quality.

Intensive culture of finfish in recirculating aquaculture system (RAS) is a production technique that reuses fish culture water more than once, thereby saving space and water requirement for fish culture. This technology has been successfully adapted worldwide to culture African catfish (*C.gariepinus*). RASs have been developed in response to the increasing environmental regulations in nations with limited access to land and water. RASs offer advantages in terms of reduced water consumption, improved opportunities for waste management and nutrient recycling, better hygienic and disease management and biological pollution control. Despite the advantages posed with the use of RAS, researchers have identified accumulation of inorganic nitrogen products as a concern. Rising nitrite concentrations due to incomplete oxidation by bio filters, has especially been mentioned due to its high toxic to fish health.

Fish produce nitrogenous wastes through catabolism of amino acids. The efficiency of fish nitrogen N assimilation has important implications for water quality and profitability of fish production. Protein sources such as fish meal and soybean meal are the most expensive components of formulated feeds. Thus improvement in the efficiency of N assimilation and utilization is expected to improve the economics of fish production. The inherent efficiency of nutrient utilization by fish implies that N loading of RAS may be limited by the capacity to assimilate nitrogenous excretion, which may have a deleterious impact on water quality and fish growth.

Bentonite (NB) is natural clay of aluminosilicates with properties which include binding, swelling and cation exchange capacity. The clay and its acid-activated form (AB) were used as feed additive to improve aquafeeds' physical properties. Physical integrity of feed, with minimal disintegration and nutrients leaching in water is an important management tool that ensures production success. The true nutritional value of feed depends not only on the amount of nutrients, but also on its availability and quality at the time of ingestion and digestion by cultured species. It has been reported that dietary origin accounted for at least 70% of reduction in water quality due to waste outputs and uneaten feed during production.

The hypothesis set that a diet containing NB and AB as a combination blend or at different inclusion level will enhance feed physical quality which includes water stability, durability, bulk density and nutrient leaching, enhance feed utilization and maintain good water quality during production of *Clarias gariepinus.*

8.1.1 Effect of dietary natural and activated bentonite blend with different inclusion levels on physical quality of aquafeeds'

The study evaluated the effect of natural bentonite (NB) and its acid-activated (AB) form as feed binders on the physical quality of aquafeeds'. Pellet physical qualities include water stability, feed durability, feed bulk density and nutrient leaching. Water stability, feed durability, feed bulk density and nutrient leaching were all significantly affected ($p<0.05$) by treatments. Measured parameters (Water stability, feed density and feed durability) increased ($p<0.05$) with quantity of AB in the clay blend and at high inclusion level. Values increased with an increase in the level of bentonite inclusion. Nutrient leaching decreased ($p<0.05$) with increase in quantity of AB and high inclusion of bentonite as compared to control. AB had higher values ($p<0.05$) as compared to NB and control. These results validate the utilization of NB and AB as a feed binder; their ability to improve pellet physical qualities will enhance feed utilization, water quality and fish production.

8.1.2 The effect of dietary bentonite clay blends on production performance and haematological parameters of African catfish (*Clarias gariepinus)*.

The study investigated the natural and acid-activated bentonite clay combination on growth performance and haematological parameters of African catfish, *Clarias gariepinus.* The experimental diets were fed *ad libitum* to mix-sex juvenile *C.gariepinus* weighing (10 – 15g) over 84 days trial period. Final total length, final weight, average daily length gain, average daily weight gain, relative feed intake (RFI), hepatosomatic index (HSI), haematocrit (HCT) value, red blood cell (RBC) and % survival were significantly ($p<0.05$) affected by treatment diets. Growth performance, FCR and SGR improved with increase in quantity of natural bentonite and low inclusion level. Clay combination up to 50% (AB: NB) at low inclusion (0.5mg/kg,) level of bentonite clay is considered optimum.

8.1.3 The effect of dietary bentonite clay at different inclusion level on production performance and body composition parameters of African catfish (*Clarias gariepinus)*.

This study examined the effect of dietary natural bentonite and acid-activated bentonite inclusion levels on growth, feed utilization and body composition of *Clarias gariepinus*. Mix-sex juvenile fish (initial weight 10 - 15 g) were fed the test diets *ad libitum* for 70 days. Final body weight, average daily weight gain (ADWG), feed conversion ratio (FCR), specific growth rate (SGR), relative feed intake (RFI), hepatosomatic index (HSI) and viscerosomatic index (VSI) were significantly affected ($p<0.05$) by the dietary treatments. Growth performance increased significantly ($p<0.05$) with bentonite inclusion up to 1500 mg/kg and declined at 3000mg/kg as compared to control. The contents of crude protein (CP), tissue fat, moisture, ash and abdominal fat in whole body composition were significantly affected ($p<0.05$) by the treatment diets. Abdominal fat and tissue fat content increased with bentonite inclusion, while fat content and CP were negatively correlated. Results suggested that bentonite inclusion affects the feed retention /

viscosity in the gut. Thus enhancing proper breakdown and absorption of nutrient, a higher inclusion level of bentonite clay is not suitable for improved growth and feed utilization. An optimum level of performance was recorded at 1500 mg/kg inclusion with natural bentonite having a significantly ($p<0.05$) better performance over acid-activated bentonite. However, higher inclusion levels (3000 mg/kg) of bentonite clay impaired growth and feed utilization.

8.1.4 Effect of dietary bentonite blend with different inclusion levels on rate of gut evacuation in African catfish (*Clarias gariepinus).*

The study was designed to evaluate the effect of natural bentonite (NB) and its acid-activated form (AB) on gut evacuation rate of *Clarias gariepinus.* Fish weighing 250g - 300g were randomly allocated to treatment diets and fed *ad libitum*; 4 fish were selected randomly to measure post feeding stomach content (SC) and intestinal filling (IC) content at (5, 30, 60 and 120 min). The control diet had higher values for SC at 5 min which differ significantly ($p<0.05$) as compared to clay diets. At (30 – 120 min), clay treated diets had higher values for SC ($p<0.05$) has compared to control. SC decreased ($p<0.05$) with time. In clay diets, SC decreases faster over time with reduction in quantity of AB and low inclusion level (500mg/kg). The IC of control diets had higher values ($p<0.05$) over time as compared to clay diets. The results show that dietary clay enhanced feed binding, which affected digesta viscosity. This reduced rate of feed passage in the gut. At 60 min, 60% of ingested control diet fed was absorbed from the stomach to the intestine, while 40% absorption was recorded in dietary clay diets. Hence, slow passage of feed in the gut can enhance nutrient absorption, while high digesta viscosity can leads to fermentation in the gut, which may affect feed utilization and water quality.

8.1.5 Evaluation of water quality of a static aerated African catfish (*Clarias gariepinus)* production tank, fed aquafeeds' containing bentonite and its acid activated form as feed additives.

In this study, bentonite and its acid-activated form are included as feed additives, fed to *Clarias gariepinus*; the water quality parameters to include (pH, $N0_2$-, NH_3-N and TSS) were evaluated in a static aerated tank. Feed was fed at 3 % body weight twice daily to adult fish weighing (395

– 405g) and (295 - 305g). Each treatment diet was replicated four times, and 10 fish were randomly allotted to each tank. Water temperature was 25 ± 2^0C, and Dissolve oxygen (DO) range between 3 – 9 mg/L during the study. Water samples were collected daily from each tank over 7 and 5 days for experiment I & II respectively. No mortality was recorded during the study. The values of pH, $N0_2^-$, NH_3-N and TSS increased significantly ($p<0.05$) in all treatment diets. In experiment I, A50B50 had lower values ($p<0.05$) as compared to other treatment diets. In experiment II, A500 and B1000 had lower values ($p<0.05$) as compared to control diet. However, in this study, feed was administered daily to tanks throughout the study period based on the initial body weight of fish. Water quality dropped as the study progress and fish consumption reduced. Thus, uneaten feeds constituted mainly to increase in poor water quality in conjunction with metabolic waste. High water stability of clay treated diets attributed to maintenance of water quality as compared to control.

8.2 Recommendations

The application of dietary bentonite and its acid activated form as feed additives indicated significant effects on feed physical quality, water quality, and production performance of *Clarias gariepinus* as reported in this study. Based on available literatures, this study made use of new clay inclusion levels as compared to existing studies. The combination of natural bentonite and its acid-activated form provides the industry with a range of new products, which require more research for improvement. Therefore the associated research on the performance of these improved aquafeeds' gave more insight into further studies. The following aspects are recommended:

- Evaluation of binding characteristics of bentonite clay forms on micro nutrients (vitamins and minerals) in feed and body of fish species fed with the diet.
- Application of dietary clay mineral forms as feed additives on growth performance of freshwater fish species other than *Clarias gariepinus.*
- Application of dietary clay mineral forms as feed additives on growth performance and water quality of marine fish species, especially since cation exchange properties of clay can hinder its effectiveness as scrubber.