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Gelatinization of Starch in Tilapia Feed.

Bharat Adhikari
Shijan Adhikari

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ABSTRACT

The aim of study was to observe the effect of different levels of starch gelatinization in feed for Nile tilapia. Wheat was extruded with different moisture addition (20%, 25%, 30% and 35%), to tentatively obtain different degrees of starch gelatinization. Five different diets were prepared one with raw wheat and remaining four with gelatinized wheat, using a pasta machine. The degree of starch gelatinization of the diets were assessed by Differential Scanning Calorimetry. All diets with wheat that had been extruded had completely gelatinized starch. The diets (Diets 1-5) were fed to fishes in 10 tanks with 9 to 11 Nile tilapia with a mean initial weight of 120 g for 30 days. Each diet was fed to fish in two tanks. The tanks were supplied with freshwater from a recirculated aquaculture system with a mean water temperature of 27°C. The fish were weighed at the beginning and at the end of the experiment. Daily dietary dry matter intakes were assessed; chemical composition in initial and final fish samples and diets were analyzed. Growth rates, feed conversion ratio, and retention of dietary protein and energy were calculated.

The feed intake of fishes in each tank was on average 91 g per fish, which results in average weight gain of 78 g per fish. Regression analysis showed that the retention of dietary protein (p-value: 0.00821) and energy (p-value: 0.00821) were significantly related to second order polynomial of dietary treatment, however feed conversion ratio and feed intake were not significantly related. It was interesting to observe that at little addition of moisture as 20% in extruding wheat gave complete gelatinization. This shows the advancement in engineering in the field of feed manufacturing technology, which can be beneficial to feed producer and farmers.

Keywords: Extrusion, Gelatinization, Nile tilapia, Moisture, Feed

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Bharat Adhikari (Master in Feed Manufacturing Technology)

Shijan Adhikari (Master in Feed Manufacturing Technology)

Norwegian University of Life Sciences (NMBU)

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List of abbreviations

°C	Degree Celsius
DSC	Differential Scanning Calorimeter
DG	Degree of gelatinization
FAO	Food and Agriculture Organization
FCR	Feed Conversion Ratio
G	Gram
H	Hour
HCL	Hydrochloric acid
L	Liter
Min	Minutes
Mg	Milligram
MM	Millimeter
Mg/dl	Milligram/deciliter
MJ/Kg	Mega joule/kilogram
NMBU	Norges miljø- og biovitenskapelige universitet
RPM	Revolution per minute
Wt.	Weight
%	Percentage

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Introduction

Carbohydrate is an inexpensive source of energy and is extensively being used in animal feed. Carnivorous fishes, in general utilize dietary carbohydrate poorly, varying among species (Shiau & Lei, 1999). However, without starch, other nutrients such as protein are catabolized for energy (Li et al., 2013).Tilapia is an omnivorous fish which can withstand a high level of dietary carbohydrate. Gelatinization will increase susceptibility for starch degradation in digestive tract (Svihus et al., 2005), which consequently can increase the digestibility in Tilapia too. This dissertation aims to seek the degree of gelatinization require to obtain the maximum positive impact on growth and overall body performance.

Carnivorous fishes such as trout and European sea bass have poor utilization of carbohydrates whereas omnivorous fishes such as tilapia can withstand on diet with 41 to 56 percent of carbohydrate (Hemre et al., 2002a). However, the utilization of carbohydrates can be enhanced by wet-heat treatment, which includes pelleting and extrusion. The limited water content and temperature allow a small extent of gelatinization that makes pelleting less efficient than extrusion (Svihus et al., 2005).

Although gelatinization increases digestibility, some study shows that it has negative impact on growth and body performance in some animals such as broiler (Zimonja & Svihus, 2009).

Objective of study

- The first objective was to find out effect of gelatinized starch on Nile tilapia
- The second objective was to reveal the weight gain, FCR, feed intake, protein and energy retention in Nile tilapia fed different level of gelatinized starch and non-gelatinized starch.

With hypothesis of positive impact of gelatinization in tilapia, an experiment was conducted for 28 days. Observations were made on weight gain of the fishes fed with various degree of gelatinized starch. Since, limited study has been carried out to identify the extent of gelatinization for maximum growth this study aims to compare diets with different degree of gelatinization with non-gelatinized starch.

Literature review

Wheat

Wheat (*Triticum* spp.) is a cereal grain cultivated worldwide. In 2010/11, world production of wheat was 653.8 million tons that is estimated to increase to 716.6 million tons in 2013/14(Nation, 2015) The increase in production shows the demand and importance of wheat in human consumption as well as in animal production. Wheat pentosans cause a general inhibition of nutrient digestion affecting starch, fat and protein (Choct & Annison, 1992). Research by Miracle *et el*. (1977) and Stober *et al*. (1979) indicates that the phosphorus in wheat and barley is more available than the phosphorus in corn because these grains contain more total phosphorus than corn (Hayes et al., 1979). Starch yields of durra wheat (*Triticum durum*) were between 24 and 33 % with an average of 29 % (Vansteelandt & Delcour, 1999). The distribution of the two major starch molecules, amylose and amylopectin, that are accumulated within the granules of wheat are formed of concentric zones of alternating amorphous and semi-crystalline regions. Both polymers are made up from chains of α (1→4)-linked glucose residues, but differ in the frequency of α (1 → 6)-branches. The smaller amylose polymers contain few branches and make up 20–30 % of the starch fraction, whereas the larger and heavily branched amylopectin molecules make up the remaining part of starch (Båga et al., 1999). Wheat consists predominantly of starch (about 70-80% dry wt.), with lower amounts of protein (usually about 10-15% dry wt.), lipids (1-2% dry wt.) (Shewry et al., 1997).

Gelatinization of starch

Gelatinization is a process of breaking down the inter-molecular bond present in starch applying heat and water. Starch has semi-crystalline structure with two components – amylose and amylopectin. The bond between semi-crystalline structures irreversibly breaks down as granules swells and burst with the application of heat and water. This leads amylose molecules to leach out of the granules giving enzymes access to glycosidic linkage and consequently increase digestion (Svihus et al., 2005).

All hydrothermal treatment used in feed processing results in gelatinization of starch, but the extent varies. Pelleting results in range of 10-20% gelatinization and Expander in between 22-35% whereas extrusion may result in complete gelatinization. However, the extent of gelatinization in extrusion depends upon extrusion conditions such as moisture, temperature and processing conditions (Tester & Morrison, 1990). It has been shown that minimum water content of 30 % is required for a gelatinization of wheat (Svihus et al., 2005). Complete starch gelatinization is generally achieved at 20-30% moisture and a temperature of 212ºC (Serrano & Agroturia, 1996).

Gelatinized starch is an effective energy source, since gelatinization increases starch digestibility (Svihus, 2014), as well as protein and energy retention by catabolizing gelatinized starch for growth and energy purpose instead of protein and energy (Peres & Oliva-Teles, 2002). Gelatinized starch improves physical quality of feed by increasing binding between particles in the ingredient mix.

Some of the research carried out regarding utilization of gelatinized carbohydrate has produced contradict results. Positive impact of gelatinized starch on growth and body performance were observed in rainbow trout when starch was 40% gelatinized (Abd El‐Khalek et al., 2009), or in carp with 20% starch gelatinization (Kumar et al., 2008), or in European sea bass at the ratio of 50% (Da Silva & Vázquez, 2014). However, negative impact of gelatinized starch has been observed in broiler (Zimonja & Svihus, 2009) and piglets (Serrano & Agroturia, 1996).

Soybean Meal

Soybean meal is an extracted by-product of soybean oil. In 2010, world production of soybean was 261.9 million tons that increased to 276 million tons in 2013 (FAO, 2015). Apart from being easily available and inexpensive (Hardy, 2006), it has less phosphorous which leads to low effluent waste (Lazzari & Baldisserotto, 2008). On the other hand it has high protein content and good amino acid profile (Gatlin et al., 2007). This has made it promising plant protein source. About 44 percent and 48 percent of crude protein is available on solvent extracted soybean meal with and without hulls respectively (NRC, 1993).

Anti-nutrients such as trypsin inhibitors, lectins, oligosaccharides, antigens, saponins and tannins are present in raw soybean. Although they have adverse effect on digestion and nutrition, through heat process, the effect can be minimized or eliminated (Barros et al., 2011). Further, despite of having good amino acid profile, soybean have limited amount of essential amino acids such as methionine and cysteine that were reported to be deficient in many fish species (Wilson, 2002). High amount of protein content in soybean has produced promising results for its use on diets for species like common carp, tilapia, salmonoid, red drum and marine shrimp (Alam et al., 2012). Different results indicate that it is possible to eliminate fishmeal totally or partially up to 75% with or without methionine supplementation from commercial tilapia diets (Chou et al., 2004; El - Saidy & Gaber, 2002).

Tilapia

Tilapia (*Oreochromis spp*.) is the common name broadly applied to a group of cichlid fishes native to Africa, the Mediterranean, and the Middle East. Tilapias are farmed "extensively" in pond systems or more "intensively" in cages and tanks. Tilapia can be cultured in either fresh or salt water in tropical and subtropical climates. Culture can be constrained in temperate climates where production must be carried out in indoor tanks (Lim and Webster, 2006). Optimal growing temperatures are typically between 22°C and 29° C (Mjoun et al., 2010). Annual production of tilapia is increasing rapidly and production of tilapia between years 2007 - 2012 increases from 1862592 tons to 3197330 tons (Rakocy, 2005). China is the top producer of tilapia in the world (FAO, 2015; Nemati Shizari, 2014).

Table 1 Production of Nile Tilapia (Oreochromis niloticus)

Year	Quantity (ton)	Year	Quantity (ton)
2007	1862592	2010	2537461
2008	2061390	2011	2808741
2009	2240095	2012	3197330

Source: FAO FishStat (Rakocy, 2005)

Importance of tilapia farming

Tilapia is known as \`aquatic chicken\` due to their high growth rate, adaptability to wide range of environmental conditions and ability to grow and reproduce in captivity and feed on low tropic level. It is thus no surprise that these fishes have become an excellent candidate for aquaculture in tropical and subtropical regions. Tilapia has many attributes that make them ideal candidate for aquaculture, especially in developing countries because of their fast growth, tolerance to wide range of environmental conditions (such as temperature salinity, low dissolved oxygen), resistance to stress and diseases (El-Sayed, 2006), availability of genetic diversity, positive response to BioFlocs, omnivorous, variety of production modes and polyculture (Fitzsimmons et al., 2011). The world's total tilapia aquaculture production in 2000 was 1.27 million metric ton and contributed about 3.6% of global total aquaculture production (Gupta & Acosta, 2004).

Materials and methods

Processing of wheat

Prior to making experimental diets the main carbohydrate source wheat was extruded with five different percentages of moisture. Wheat was ground in a hammer mill (E-22115 TF, Muench - Wuppertal, Germany) with 1mm sieve and was passed in extruder machine. All the extruded wheat pellets were dried for one hour, packaged, labeled and kept in cooler (2-5°C).

Table 2 Wheat extrusion parameters

	Diet2	Diet3	Diet4	Diet5
Feeder, Main (Kg/h)	150.0	150.0	150.0	150.0
Extruder. Water (%)	0.0	7.5	15.0	25.5
Conditioner, stem (%)	11.9	12.3	11.9	10.8
Pressure at end plate (bar)	48.9	28.5	19.7	13.4
Drive Power (kW)	20.2	17.4	15.0	14.8
SME (Wh/kg)	134.4	110.5	91.2	84.1
Throughput	150.0	157.5	165.0	175.5
Extruder Section 1	94.2	78.4	56.8	67.9
Extruder Section 2	103.6	104.7	103.6	101.2
Extruder Section 3	131.5	128.6	119.2	123.7
Extruder Section 4	130.6	127.5	115.7	128.7
Extruder Section 5	130.3	120.8	95.4	109.1
Conditioner temperature (°C)	93.1	82.1	81.0	82.0
Screw speed (rpm)	502.0	502.0	501.0	348.0
Torque absolute (Nm)	384.1	331.0	286.7	404.8
Torque real (Nm)	88.3	76.1	66.0	93.1
Temperature at endplate (°C)	127.8	111.2	89.0	91.9
Knife speed (rpm)	547.0	547.0	547.0	547.0

Screw configuration of extruder

								Fortek Mild SME input											Total
Front																			Length
40	20	60	60	80	80	80	100	20	20	120	100	80	60	60	60	60	80	80	1260
R	R	R	R	R	R	R	R	R	L	Polygon	R	R	R	R	R	R	R	R	

Figure 1 Screw configuration of extruder

Experimental diets

Five different complete plant based feeds were prepared for experiment. The ingredients present in feeds were soybean meal, wheat, corn gluten, rapeseed oil, methionine, taurine, phenylalanine, vitamin c 35%, yttrium oxide (Y2O3), mono-calcium phosphate (MCP), sodium alginate and premix (Table 3).

Table 3 Ingredients used in feed (g/kg)

Ingredients	Amount (g/kg)
Soybean meal[b]	440
Corn gluten[c]	65
Wheat[d]	410
Rapeseed oil[e]	37
Methionine[f]	5.0
Phenylalanine[g]	0.9
Taurine[h]	1.5
Monocalcium phosphate[i]	10
Premix[k]	10
Vitamin C -35%[l]	0.1
Yttrium oxide (Y2O3)[m]	0.8
Sodium alginate[o]	20

Feed preparation

Five different feeds were prepared in feed lab of NMBU with ten kilo of feed in each batch. Preparation of diets began with grinding of wheat pellet, soybean meal and corn gluten by using 1mm screen (sieve) (Retsch Gmbh Retsch-Allee 1-5, 42781, Haan, Germany). All the ingredients were correctly weighed and mixed uniformly. To make a mixing homogenous the spiral dough mixer (Moretti Forni Grain, Italy) was used for about 20 minutes. During the mixing 3ℓ cold water (30% of total feed weight) and 1ℓ rapeseed oil was added slowly and continuously in feed one and two (0 and 20% moisture added during extrusion). 3.5 l (35% of total feed weight) and one liter of rapeseed oil was added in Diets 3, 4 and 5 (25, 30, and 35% moisture added during extrusion). The different water addition was due to differences in

[b] Soybean meal, Denosoy, Denofa, Fredrikstad, Norway.
[c] Maize gluten, Cargill 13864.
[d] Felleskjøpet, Norway
[e] Food grade, Eldorado, Oslo, Norway.
[f] Adisseo Brasil Nutricao Animal Ltda, Sao Paulo, Brazil.
[g]Adisseo Brasil Nutricao Animal Ltda, Sao Paulo, Brazil
[h] Taurine-JP8, Qianjiang Yongan Pharmaceutical Co., Ltd., Hubei, China.
[i] Taurine-JP8, Qianjiang Yonga Pharmaceutical Co., Ltd., Hubei, China.
[k] Contents per Kg: Vitamin A 2500.0 IU; Vitamin D3 2400.0 IU; Vitamin E 0.2 IU; Vitamin K3 40.0 mg; Thiamine 15.0 mg; Riboflavin 25.0 mg; d-Ca-Pantothenate 40.0 mg; Niacin 150.0 mg; Biotin 3.0 mg; Cyanocobalamine 20.0 g; Folic acid 5.0 mg; Pyridoxine 15.0 mg; Vitamin C: 0.098 g (Stay-C 35, ascorbic acid phosphate, DSM Nutritional Products, Basel, Switzerland); Cu: 12.0 mg; Zn: 90.0 mg; Mn: 35.0 mg; I: 2.0 mg; Se: 0.2 mg; Cd = 3.0 g; Pb = 28.0 g; total Ca:0.915 g; total K 1.38 g; total Na 0.001 g; total Cl 1.252 g;
[l] Stay-C 35, ascorbic acid phosphate, DSM Nutritional Products, Basel, Switzerland.
[l] Trouw Nutrition, LA Putten,Netherland
[m] Metal Rare Earth Limited, Jiaxing, China.

water absorption in the differently extruded wheat preparations. All the feed dough was transferred to a pasta machine (P55DV, Italgy, Carasco, Italy) and was properly conditioned and mixed then cut in pellet at 3.5 mm die size by using the knife at the edge of the craft opening. The prepared feed was kept on drier at 50°C for seven h, cooled to room temperature and stored at 2-5°C.

Fish and rearing unit

The experiment was conducted at Fish Nutrition Laboratory (NMBU) from 21st October to 17th November 2014. The experimental Nile tilapias (generation 12th of selection, donated by Genomar AS, Norway) were hatched at same laboratory and fed on a commercial diet until the individual body weight was approximately 0.1 kg. For the experiment 10 indoor rearing tanks (70 cm× 50 cm × 50 cm) were chosen. The tanks were supplied with freshwater from a recirculation system, with the water level of 50 cm in each tank. Each tank was stocked with 10 fishes. Tanks were kept in 24 hour light system during whole experiment, and the water temperature was 28°C.

First all the tanks and pipes were cleaned and fishes to be used in trial were selected from large population. Fishes were netted with minimum disturbance and anesthetized (Tricanine methanesulfonate-MS-222, 0.1 g/l water, buffered with $NaHCO_3$, 0.1 g/l water, Western Chemical Inc. Washington USA). Fishes were weighed individually. At the same time ten fishes were randomly taken from same population, weighted and kept in freezer at -20°C for initial whole body composition analysis.

At day 28 of feeding trial fishes of each tanks were weighed, liver samples and faeces from last 15 cm of intestine was taken (incision was given from dorsal part of operculum to base of pelvic fin, similarly from dorsal part of operculum to caudal part of anal fin via upper lateral line scale opening abdominal cavity) by firmly squeezing intestine and remaining part of intestinal content was cleaned. After that five fishes from each tank were kept for final whole body composition analysis. At the same time whole-blood glucose was analyzed.

Feeding experiment

The five different feeds were fed to the fishes. Different in sense of percentage of moisture added while extruding wheat. In 10 tanks ten fishes were kept with biomass 1170gram (±10). Feeding one feed for two tanks was done and time of feeding was 8:15 to 9:15 in the morning and 20:15 to 21:15 in the evening every day for 28 days, with electrically driven band feeders. The uneaten feeds were collected from water outlet while feeding and 30 minutes after finishing feeding. Uneaten feed from morning and evening was collected and dried at 105°C overnight. Dietary dry matter was calculated from the difference of daily fed feed and uneaten feed after dried.

Table 4 Proximate compositions of diets (g/kg)

Parameters	Diet1	Diet2	Diet3	Diet4	Diet5
Dry matter	57.9	69.4	80.8	59.1	55.3
Energy (MJ/Kg)	18.5	17.8	17.6	18.4	18.4
Protein	292	280.7	263.1	295.9	291.1
Carbohydrate	280.4	277.5	278	271.4	261.7
Fat	53.2	36.3	32.2	31.7	36.2
Ash	53	51	50	54	54

Ammonium Measurement

Ammonium concentration measurement in tanks water was started one hour after last meal (10:00am) and was continued in every two hours interval till 4:00pm. The measurement was done from inlet and outlet water of tank on day 28. The detail procedure of ammonium calculation is in appendix.

Chemical composition of diets and fishes

Diets and fish body composition was done by proximate analysis. Sampled fishes were taken from -20 °C freezer and were cut in small piece and were homogenized in a grinder. The ground fish was freeze dried for a week and then samples were again ground with dry ice. The dry matter of fish was determined after the loss of moisture from the sample when heated at 105°C for 20 hours in an oven. Crude protein was analyzed by Kjeltec auto 1035/1038 systems. Energy of both fish and feed was determined by Parr bomb calorimeter whereas HCL hydrolysis followed by diethyl ether extract method was used to determine crude fat. Ash content was analyzed by heating at 500°C in muffle furnace.

Degree of gelatinization

Degree of gelatinization was determined as per the procedure described by Zimonja and Svihus (2009). Feed samples were ground to 0.5 mm. They were assessed for gelatinization by differential scanning calorimetry. One part sample was mixed with two parts of water. The suspension was slowly stirred and for uniform mixing, suspension was run in vortex mixer for 2 min. The suspension was then allowed to stand for 1 h for equilibration purpose. After 1 h, approximately 130 mg sample was heated from 0 to 160°C with an increase of 5°C per min on a Metter DSC 30S (Metter Toledo AG, Schwerzenbach, Switzerland). Silicon oil was used as reference. Determination of enthalpy values was carried out by computer integration of inverse peaks. Degree of starch gelatinization was calculated based on the difference in enthalpy between sample before and after feed processing. The measurements were conducted in duplicate. Since the four samples containing less than 30 percent of moistures were completely gelatinized so, the fifth sample is assumed to get gelatinized and is removed during analysis.

Blood sugar analysis

Blood sugar was analyzed by rapid test kit (ACCU-CHEK® Compact Plus assembled for and distributed in the U.S.A. by Roche Diagnostics, Indianapolis, IN, made in Ireland Roche Diagnostics 9115 Hague Road Indianapolis, IN 46256) by piercing fish anterior to base of caudal fin around lateral line scale. Blood was dropped in black area of test strip and after five seconds blood sugar was displayed in screen in mg/dl.

Fish growth performance, feed and protein utilization

Feed conversion ratio: $(FCR) = \mathrm{Dietarydrymatterintake(g)/weightgain(g)}$ Dietary dry matter intake (g)/weight gain (g).

$$\text{Protein Retention (\%)} = 100 \times \frac{\text{final protein content in fish (g)} - \text{initial protein content in fish (g)}}{\text{Protein intake by fish (g)}}$$

$$\text{Energy Retention (\%)} = 100 \times \frac{\text{final energy content in fish (kj)} - \text{initial energy content in fish (kj)}}{\text{Energy intake by fish (kj)}}$$

Statistical Analysis

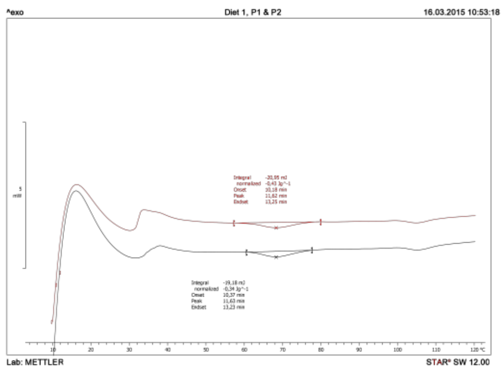
Results were analyzed statistically by 1st and 2nd regressions, and the model giving best fit was chosen. The plots were made in Microsoft Excel, and significance levels were calculated in SAS version 9.4, SAS Institute Inc., Cary, NC, USA. Statistical significance is indicated for $p < 0.05$.

RESULTS

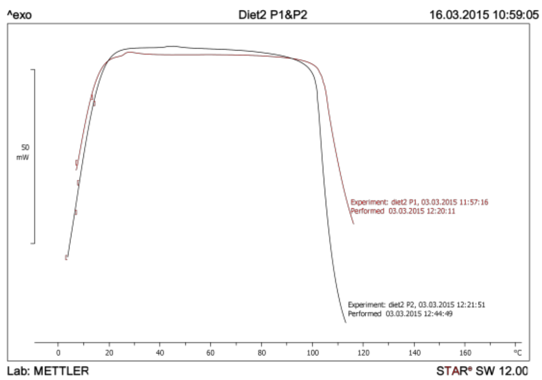
Degree of Gelatinization and Retrogradation

All starch present in extruded wheat were complete gelatinized as seen in DSC curve. Further, there were not any fluctuations in the curve at temperature below 25°C, which signifies that retrogradation phenomenon during storage, was not present in experimental diets fed to fish.

Diet 1



Diet 2



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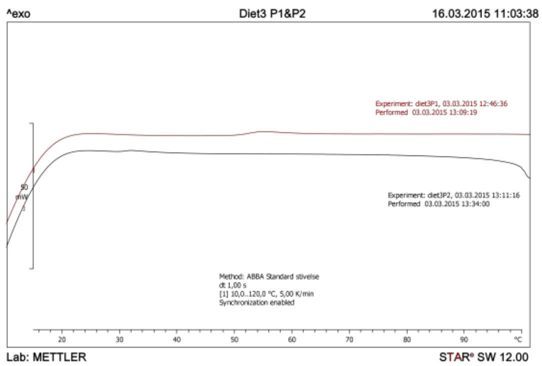

Diet 4

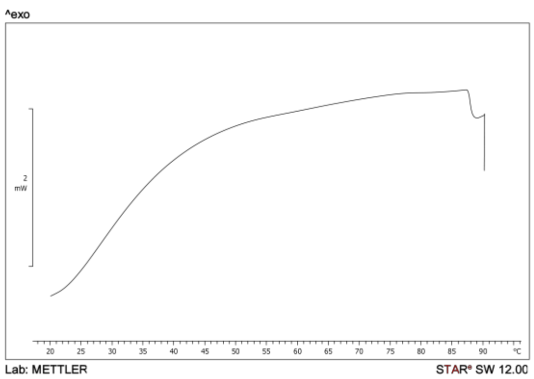

Figure 2 DSC diagrams for Diet 1 (with raw wheat) and Diets 2-4

Incomplete gelatinization of starch was seen for Diet 1 that was not extruded (Figure 1).This is indicated by the uptake of energy seen at approximately 69°C. Visible fluctuations in energy in the temperature range of 50°C - 90°C was not observed for Diets 2-4, signifying complete gelatinization of starch in these diets.

Survival, Growth and feed Performance

All the fishes survived during experiment. The growth and feed performance are presented in Table 5.

Table 5 Average Growth and Feed performance in Tilapia

Parameters	Diet1	Diet2	Diet3	Diet4	Diet5
Initial weight (g)	117.3	124.0	124.9	112.7	118.5
Final weight (g)	193.3	222.3	188.0	190.3	193.4
Weight gain (g)	76.0	98.3	63.1	77.6	75.0
Feed intake	121.9	101.3	65.8	81.5	84.4
Feed Conversion Ratio[p]	1.63	0.99	0.99	1.03	1.08
Protein retention (%)	32.0	53.7	56.4	53.3	49.6
Energy Retention (%)	19.3	36.9	35.7	39.6	32.6

Feed Intake

Fishes seem to prefer Diet 1, which was not gelatinized (Table 1, Figure 2). It has higher (1218.5 g) level of feed intake than other diets. The model fitted to see the effect of diet on feed intake has suggested that the diets have significant effect on Feed Intake (p-value: 0.021). The model has described around 67 percent of variation present in it.

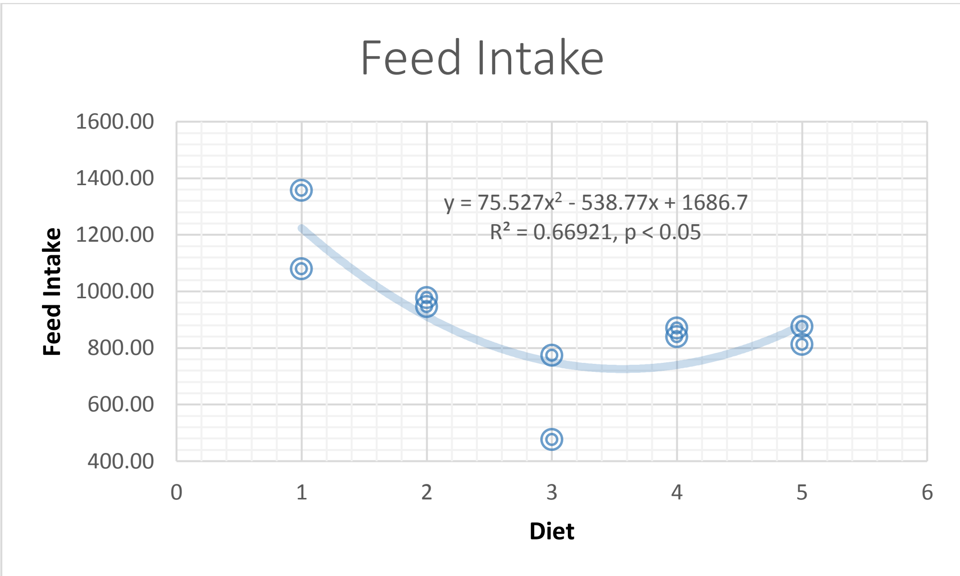

Figure 3 Feed Intake vs. diet with second order trend

[p] DM intake (gm) / Weight Gain (gm)

Weight Gain

Weight gain ranged from 63.1 to 98.3 g per fish with least weight being 63.1 g for Diet 3 and the highest 98.3 g for Diet 2 (Table 1). However, diets did not describe the variation present in weight gain during experiment significantly (p-value: 0.913). A model fitted with weight gain and diet is plotted in Figure 4.

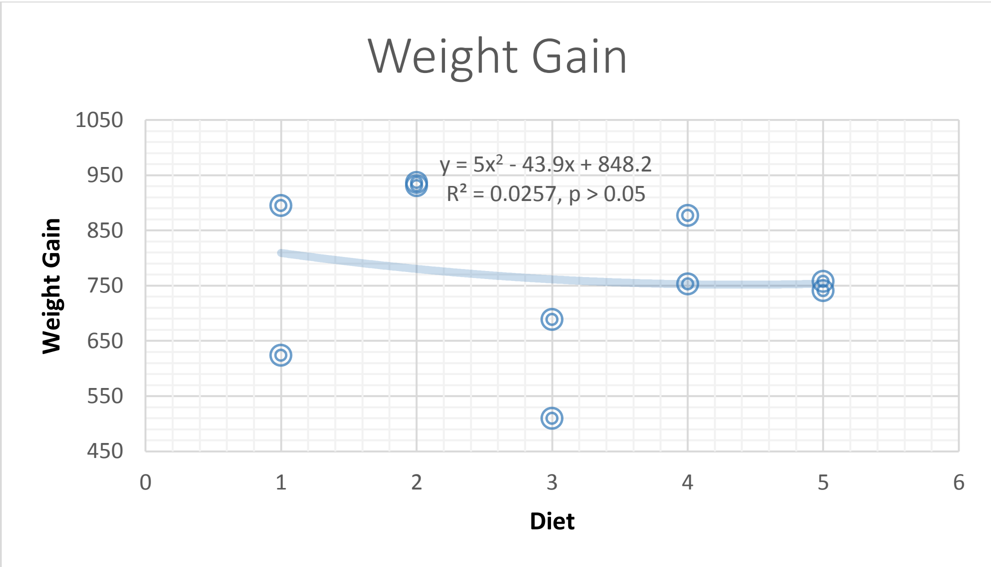

Figure 4 Weight gain vs. Diet plot

Whole body composition of Tilapia fed experimental diets

Average final composition of the whole body of tilapia was modeled with second order polynomial of diets. Although models are not significant the average final protein is found significantly greater than the initial protein (Table 2).

Table 6 Whole body composition of Tilapia

Parameters	Initial	Diet1	Diet2	Diet3	Diet4	Diet5
Dry matter (g/kg)	293.7	287.8	332.7	297.3	306.6	296.0
Energy (Mj/kg)	23.5	22.7	23.5	23.3	23.8	23.3
Protein g/kg	504.5	520.2	518.2	513.6	514.1	525.8
Fat g/kg	285.8	241.0	287.1	297.3	321.2	285.8
Liver lipid g/kg		265.9	335.5	291.3	382.4	269.6
Liver glycogen g/kg		298.9	314.1	314.3	294.9	346.1
Blood sugar mg/dl		4.26	5.42	5.64	4.92	6.04

Feed Conversion Ratio

FCR of tilapia fed experiment Diets 2 and 3 (Table 5, Figure 4) tended to be utilized most efficiently, since these diets have the lowest FCR (0.99). The effect of diet explained around 48 percent of variation in FCR. However, the model is not significant (p-value: 0.1).

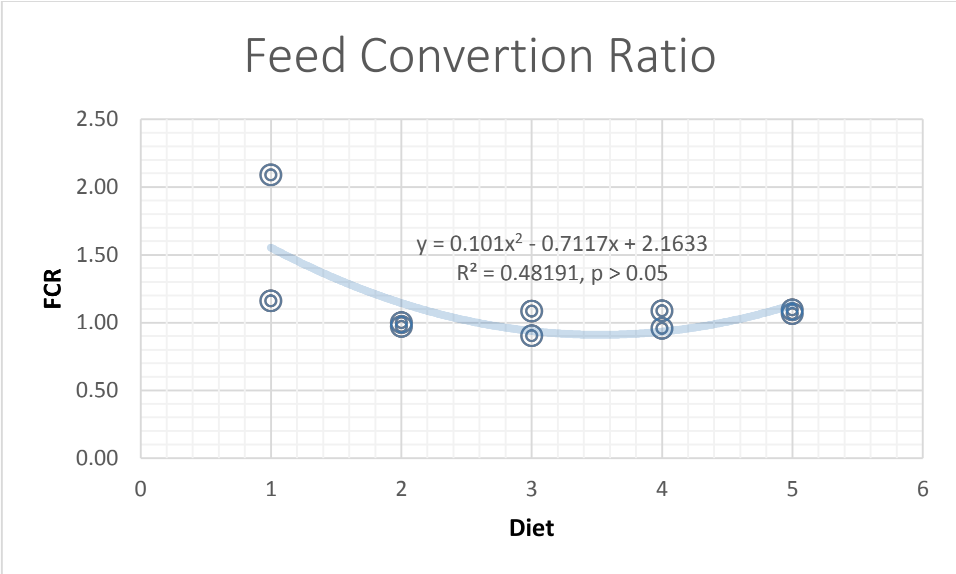

Figure 5 Feed Conversion Ratio vs. Diet with second order fitted trend

Protein Retention

The diets used during the experiments are responsible for more than 74 percent of variation present in protein retention (Figure 5). The fitted model as well as diet and its squared terms are significant (p-value: 0.008). Protein retention ranges from 39.9 to 56.4 with average value of 49.0.

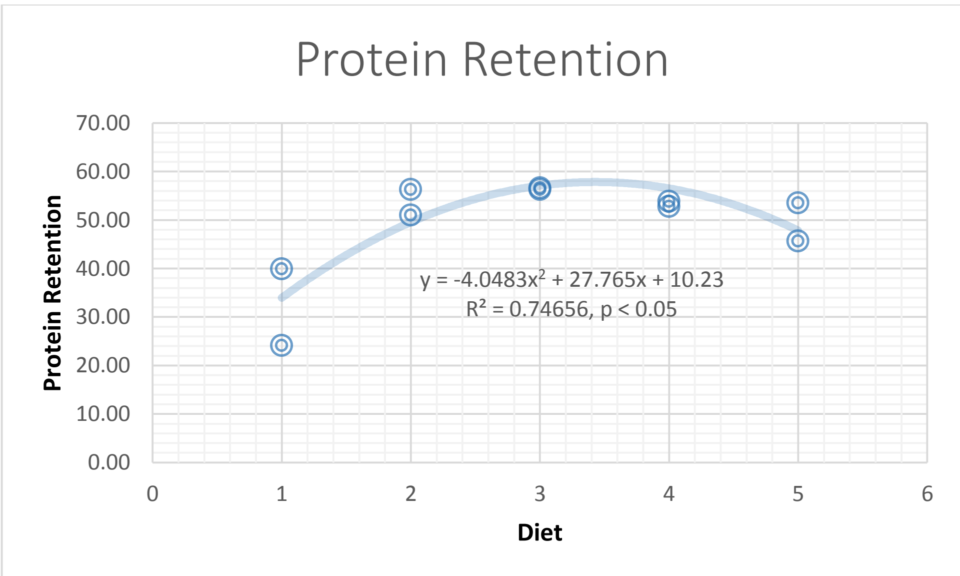

Figure 6 Protein Retention vs. Diet with second order fitted trend

Energy Retention

A similar effect of diet on energy retention is visible as in the case of protein retention (Figure 6). Almost 70 percent of its variation is explained by the second order polynomial of diets used. The model is significant (p-value: 0.016).

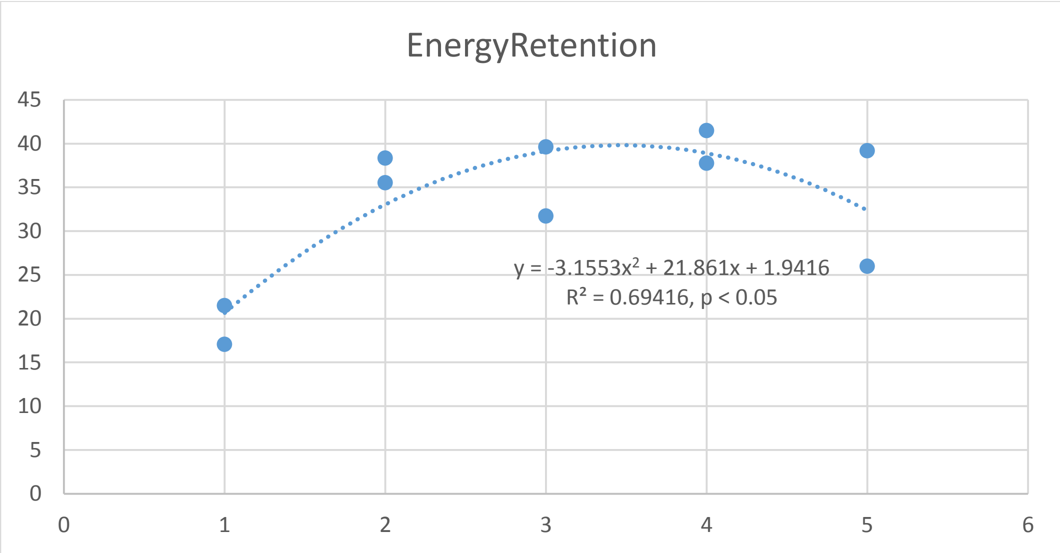

Figure 7 Energy Retention vs. diet

Ammonium measurement

Ammonium concentration in water was found to reach peak after 4 hours of meal and start decreasing thereafter for all diets.

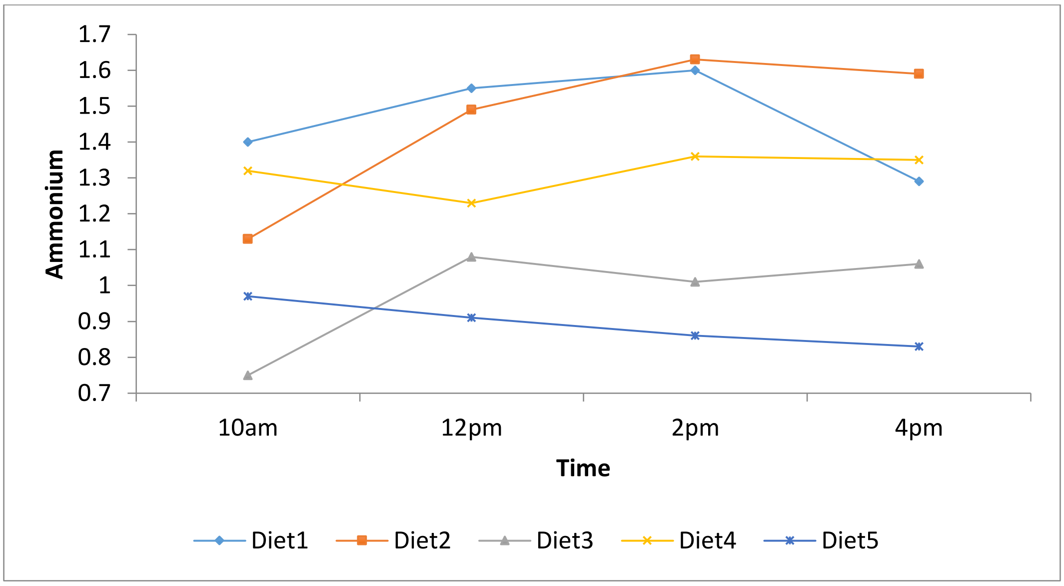

Figure 8 Ammonium measurement at different time interval

Discussion

For optimal utilization of starch by Nile Tilapia present in feed, the main carbohydrate ingredient- wheat was extruded in different moisture level (20%, 25%, 30%, 35%). The purpose was to get different percentage of gelatinization but all were complete gelatinized. We couldn't get the accurate estimate because extruded wheat was not available for DSC. Wheat samples kept in cooler room were thrown away while renovating the room without informing us.

In extruding wheat from feed technology point of view, extruder machine didn't work efficiently while extruding wheat below 20% moisture addition, so the starting was 20%. As the moisture was increased temperature, pressure and shear was decreased and viscosity was increased. At least addition of moisture (7% steam addition) gave complete gelatinization, which is good achievement in regular practice.

Extruded diets were examined in differential scanning calorimeter to determine percentage of gelatinization and found that all the starch were complete gelatinized. The result from Owusu-Ansah et al. (1983) supports the result, who found that at 90 rpm maximum gelatinization occurred at temperature 100°C and feed moisture 23%. Case et al. (1992) reported that wheat flour with 28% moisture was 86% gelatinized whereas corn meal, corn starch and wheat starch with 35% moisture were 86%,85% and 55% gelatinized respectively. The result is contradict with Gomez and Aguilera (1984) who found that, maximum gelatinization was observed at about 28-29% moisture. Below 20% moisture, dextrinization becomes predominant during high- shear cooking-extrusion. The result of Da Silva et al. (1996) shows that, in 35% moisture, percentage of gelatinization is 10.6 ±0.3 which support Gomez & Aguilera (1984).

From previous research we found that gelatinization is limited in feed with low moisture, for complete gelatinization moisture should be high. From the feed technologist point of view it is good achievement that we got complete gelatinization in 20% moisture and the feed is well utilized by fish.

Five feeds were fed to Nile tilapia for 28 days in which weight gain was higher in diet two (20% moisture). The result is supported by Takeuchi et al. (1994) who found, increase in the level of gelatinization of diet increases the growth in tilapia and grass carp as well as improves the nutritive value of diet. There is also another finding that says carnivorous fish show growth promotion when gelatinized starch are included at low level (Hemre et al., 2002b). In contrast, research conducted by Peres and Oliva-Teles (2002) on European Sea bass (*Dicentrarchus Labrax*) found that weight gain, specific growth rate and feed intake was low in gelatinized starch feed than raw starch.

In addition diet two fed fish have low FCR value, the improvement was not seen in other diets. There is no any explanation to this and this should be looked in further research. Diet one was not gelatinized and diet 3,4,5 were complete gelatinized, which support the study of Amirkolaie et al. (2006), who explained that, growth was higher and FCR was lower for fish fed the gelatinized starch compared to those fed the native starch. Increasing the starch content of the diets resulted in an increased growth and improved FCR. Similarly, the study of Booth et al. (2000) says that, FCRs were poorest in fish fed both un-steamed diets and markedly improved for fish fed both the steamed and extruded diets. According to Pfeffer et al. (1991) replacing untreated maize or starch in the diets by the respective extruded maize or starch caused significant reductions of consumption and gain in trout. Feed conversion ratios were improved concurrently, as these reductions were more pronounced in consumption than in gain,

The average weight gain/day of fish fed with diet one, four and five is about 2.7gm while the gain is quite high which is at around 3.52 for the fish fed with diet two. Inverse to the diet two, weight gain of fish fed with diet three is much less (2.28).

Glucose availability in the blood is increased with the increase level of moisture in extrusion except diet4; diet5 shows the highest value as compared to other diets. This shows that starch in the feed was utilized more efficiently as it was gelatinized but there is no obvious explanation to connect the link between increases in moisture while extruding increased availability of glucose and this should be looked in further research. Protein and energy retention was higher in diet2 and lower in diet1 fed two times a day.

In the experiment water temperature was 28°C and PH7. According to Azaza et al. (2008) experiment conducted in four different temperature (22,26,30,34) and PH range from 6.98 to 7.41 found increase in feed consumption, mean body weight and decreased FCR in fishes kept in 26°C and 30°C than 22°C and 34°C. The experiment is also supported by Coyle et al. (2004) who found 100% survival and high diet acceptance in water temperature 27.4°C and PH8. This shows that temperature and PH were within suitable condition for the growth of Nile tilapia.

The term retro-gradation is used to describe the changes that occur upon cooling and storage of gelatinized starch (Fredriksson et al., 1998). Retrograded starch are resistance to digestion (Haralampu, 2000). From the result we found that our starch were not retrograded. The practical importance of non-retrograded feed is its fresh, pleasant, nutritive and digestible.

Conclusion

Complete gelatinization was achieved by the lowest water supplementation to wheat. No significant effects of water addition in extrusion of wheat were observed for growth or feed conversion. Thus, 20% water addition seems sufficient for extruding wheat for use in tilapia feed. The improved feed intake, retentions of protein and energy observed by comparing the use of non-gelatinized wheat (Diet 1) with that obtained by extruding with 20% water (Diet 2), illustrates the advantage of using gelatinized starch in tilapia feed.

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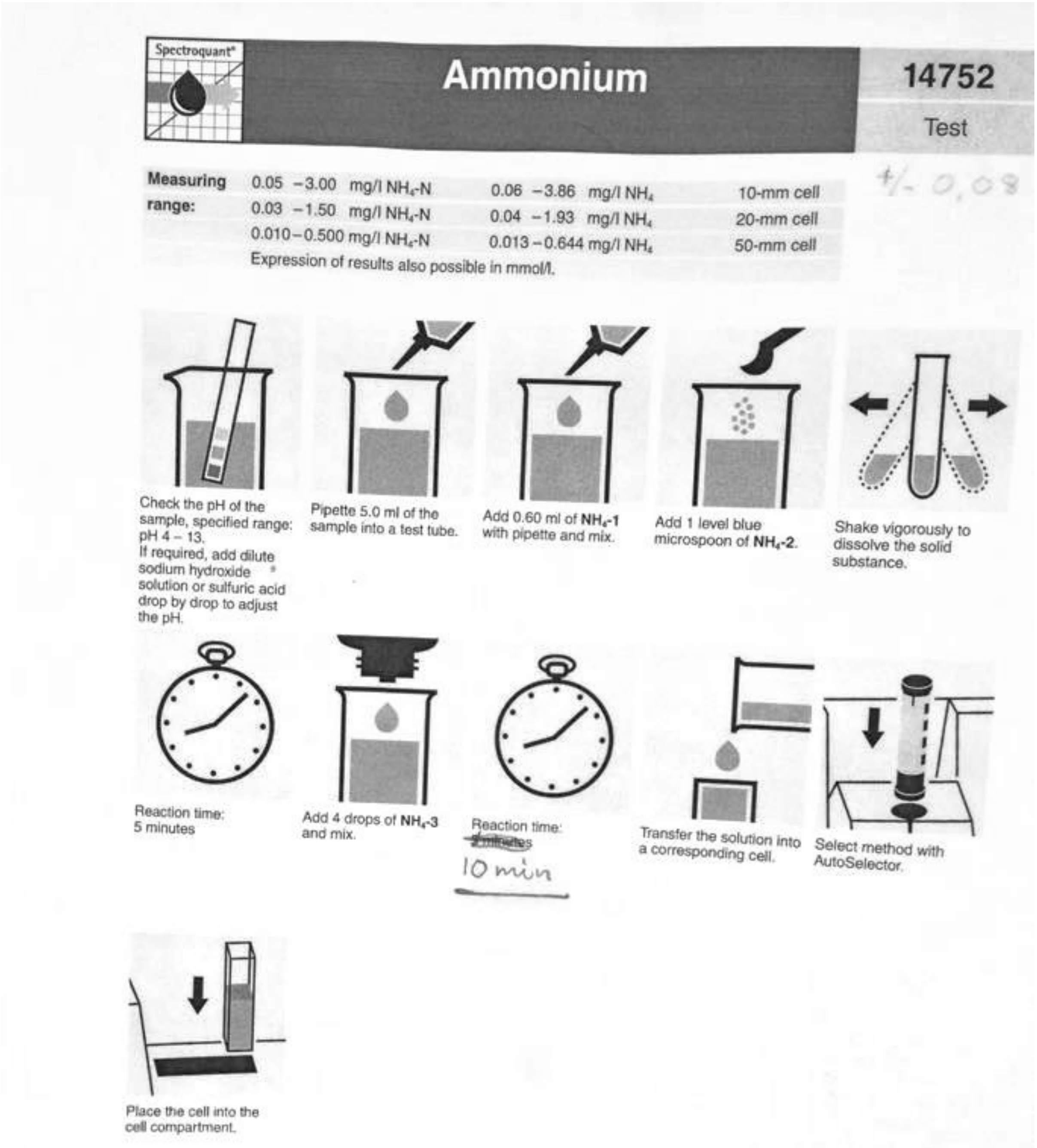
Appendix



Spectroquant®

Ammonium

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+/- 0,08

Measuring range:	0.05 – 3.00 mg/l NH_4-N	0.06 – 3.86 mg/l NH_4	10-mm cell
	0.03 – 1.50 mg/l NH_4-N	0.04 – 1.93 mg/l NH_4	20-mm cell
	0.010 – 0.500 mg/l NH_4-N	0.013 – 0.644 mg/l NH_4	50-mm cell
	Expression of results also possible in mmol/l.		

Check the pH of the sample, specified range: pH 4 – 13. If required, add dilute sodium hydroxide solution or sulfuric acid drop by drop to adjust the pH.

Pipette 5.0 ml of the sample into a test tube.

Add 0.60 ml of **NH_4-1** with pipette and mix.

Add 1 level blue microspoon of **NH_4-2**.

Shake vigorously to dissolve the solid substance.

Reaction time: 5 minutes

Add 4 drops of **NH_4-3** and mix.

Reaction time: ~~5 minutes~~ 10 min

Transfer the solution into a corresponding cell.

Select method with AutoSelector.

Place the cell into the cell compartment.

Norwegian University
of Life Sciences

Postboks 5003
NO-1432 Ås, Norway
+47 67 23 00 00
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