PRELIMINARY INVESTIGATION INTO THE IMPLICATION OF A SINGLE CELL ORGANISM IN FISH FEED BUOYANCY AND FLOTATION.

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ABSTRACT:

Two Isocaloric Isoproteic 30% crude protein diets were formulated for Clariid catfish and Tilapia with wheat grain starch (WGS) and cassava tuber starch (CTS) incorporated at 10 percent as binding agents. Saccharomyces cerevisiae was included at 2% as floating addictive. The water stability, nutrients retention and flotation of pelleted feeds were observed for 60 minutes. There were generally decreasing trends in stability and retention at increasing time of immersion in water. The lipid retention was higher ($P>0.05$) than proteins in both diets. WGS diet was better ($P<0.05$) than CTS diet in flotation, which was attributed to the presence of gluten protein in wheat products. It is envisaged that a break through in floating feed development in Nigeria aquaculture would save the Nigeria economy from extruded (floating) feed importation.

Key words: Wheat, Cassava, starch, gluten, *Sacharomyces cerevisiae*, water stability, nutrient retention, flotation, fish feed.

INTRODUCTION

The widely used pelleted fish feed are prone to leaching of nutrients into water due to poor stability and disintegration of feed to the bottom waters at feeding. This has been shown to lead to significant quantified losses in aquaculture input management (Falayi, *et. al.* 2003, Lopez-Alverado *et. al*, 1994, Teshima *et. al*, 1993). Another form of feed is extruded floating type. This floating feed is a management tool, which enables the farmers to observe how much, and how actively their fish eat (Mgbenka and Lovell, 1984). However floating feeds are not necessarily better than pelleted sinking feed. Much of the heat labile nutrients in imported floating are lost to heat during extrusion process, even when over fortification of nutrients are made, it can to some extent be viewed as an economic wastage (Hoftmann-LaRoche, 1991, NRC, 1983 Cheftel, 1986).

Little information are available on binding agents needed for fish feed stability and those available are on synthetic organic hydrocolloids for the production of crustacean feeds because of the slow feeding habit (Langdon and Bolton, 1984, Farman Farmainin *et. al*, 1982, Heinen, 1981). Recently some starches have been identified as local binding agents in fin fish feed (Falayi *et al*, 2004, 2003, Orire *et. al.*, 2001).

Feed addictives have also been successfully utilized for buoyancy purpose in food technology (Davidek, *et. al*, 1990, Ponte and Reed, 1982, Magnus, 1982). There is therefore death of information on fish feed buoyancy and flotation.

The objective of this study is to develop improved diets that are buoyant with floating capabilities, as well as having value when it finally sinks, through utilization of biochemical characteristics of feed stuffs and addictives in fish feed production.

MATERIALS AND METHODS:

Two starch components i.e. wheat grain starch (WGS) and cassava tuber starch (CTS) earlier identified by Falayi *et al* (2004, 2003) and Orire *et al.* (2001) were fixed at 10% dry weight, as binding agents and carbohydrate source. *Saccharomyces cerevisiae* was fixed at 2% as floating addictive. Sundried clupeid *Pellonula afzeluis* fish meal was fixed at 15% as only animal protein. Non protein nutrients; minerals and vitamins premix, bone meal and common salt (NaCl) were fixed.

Soybean cake (SBC), groundnut cake (GNC), and Maize middling were formulated by equation method of Halver (1989) as plant protein and carbohydrate sources respectively. All ingredients were singularly ground to fine particulates, weighed according to formulation (Table 1) and mixed in isonitrogenous 30% crude protein diets for dual use of *Tilapia O. niloticus* fingerlings and Clariid catfish juveniles (NRC, 1987 Jauncey and Ross, 1982).

All ingredients were first thoroughly mixed dried by hand in a plastic bowl, before the addition of the floating addictive and the binding agent. The mixture was further kneaded to obtain homogenous hard-paste texture (dough) which was placed inside a hand-pelletizer and rolled out through 2mm die holes into a flat tray in wet form. The wet pellets were cut fresh with knife into about 2cm length each on the tray and immediately covered with another tray and kept in a fairly warm room to allow fermentation for 3 hours.

At the end of the 3 hours, the tray with the pellets was put in electric oven set at 105°C for 2 hours. Replicate samples (100g each) were sent in sealed sampling bottles for proximate analysis following the methods of AOAC (1990).

Water Stability:

Three replicates samples (5g each) of each pelleted feed were placed in nylon sieve sack, tied with string and slowly immersed in a 400ml beaker filled to ¾ of holding capacity with pond water analysed for water qualities (APHA, 1990). Aeration was done through ceiling fans located at the top of ceiling in the laboratory. The pellets were allowed in the medium for the period of tests for 60 minutes at 10 minutes interval. At the end of each test time, the sacks were removed with the aid of the strings, the crumbles allowed to drain for one minute and the content put in a petridish and oven dried at 105°C for 2 hours, cooled in a desiccator and weighed. The new weight represents the left over from the original 5g. The dry matter is also obtained at that period.

The water stability at that time is calculated as the difference in sample weight after reweighing and expressed as percentage loss of dry matter (%LDM) (Fagbenro and Jauncey, 1995, Renukaradhya and Varghese, 1987).

Nutrient Retention:

Total protein and total lipid in pelleted feed samples before and after immersion were determine by the microkjeldahl and soxhlet extraction of samples methods of AOAC (1990). The values of nutrients (proteins and lipid) retention were also expressed on percentage remaining basis.

Flotation:

Pelleted feeds were tasted for periods of flotation on water in an aquarium measuring 60x60x30cm^3 filled with pond water treated in the same conditions as in the other tests. Three replicates (20 samples each) pelleted feed bound with WGS and CTS were gently placed on the water surface in the tank and watched for period of flotation at 5 minutes interval for 60 minutes. The mean numbers of floating pellets at the end of each time were recorded and expressed as percentage of the initial number.

Statistical Analysis:

All data obtained on the chemical analysis of diets, water stability, nutrient retention, and flotation were subjected to the analysis of variance (ANOVA) and the difference in means were tested for significant using the Duncan multiple range test at 95% confidence level (Duncan 1955).

RESULTS AND DISCUSSION

The proximate analysis of results of the diets is presented on table 1. The similarities in the nutrients composition can be traced to the feed ingredients inclusions, which were only differentiated by the binders involved. There were higher ($P>0.05$) proteins (31.94) higher lipids (11.54) crude fiber (5.80), Nitrogen free extract (33.89) and Ash (10.52) content in WGS than CTS diet which confirmed higher nutrients in wheat product than cassava product (Falalyi *et. al,* 2004, Aduku, 1995 and Oyenuga, 1968).

The water stability of both diet, at initial 10 minutes (98.01 and 97.10), nutrient retention (97.4 and 97.00 (proteins), 99.00 and 99.00 (lipids) were less than 100 percent. This may be due to the inferior binding properties of the local binders (starches) used (Mitchel, 1979). Both diets have soybean cakes (SBC) among the plant nutrients, which may have contributed further to reduction in feed stability as supported by Lim and Doming, (1990) that, the water stability of pellets is inversely related to the level of soybean they contained despite the inclusion of binders. The superiority ($P>0.05$) of WGS over CTS in water stability confirmed the works of Ponte and Reed (1982) and Glucklick and Shelef, (1962) that pregelatinised wheat starch and wheat products are hydrophobic and hence good binding potentials. The successful decrease in crude protein and crude lipid retention at increasing times of immersion (Table 2) represents the loss of free amino acids and free fatty acids present in the diets as a result of disintegration and leaching. Goldbath *et. al.*, (1980) reported that moisture facilitate the breakdown of proteins and loss of amino acids inform of Nitrogen (N). Venugopal and Kashavanath *et. al.*(1984) also observed same at increased moisture content in pelleted feeds. The high physical stability of pellets bound with the binders were averagely effective for protein and lipid nutrients but did not prevent total protein nor total lipid loss through leaching, but served the advantageous purpose of using binder in dry feed (Heinen, 1981) as a method of improving feed consistency, reduction of wastage and loss of feed nutrients to water systems. The insignificant ($P>0.05$) values recorded for lipid retention at every test time (Table 2) could be traced to the unsaturation of oil and insoluble property of fat (except in ether) in the formulated diets as reported by Langdon and Bolton (1984) and Teshima *et. al.* (1993). The higher lipid ($P>0.05$) in WGS diet over CTS diets contributed to its higher stability and nutrient retention as reported by Das *et. al.*. (1994) and Lopez-Alverado *et.al*, (1994) that higher fat level in feed prevent water penetration thus helping it to retain it compactness for long period. This has been the motive behind the lipid walled encapsulation of water-soluble food nutrients for larva fishes by the above authors.

Table 3 represents the numbers and percentages of floating pellets within time, for diets bound with the two agents. All pelleted feed were noticed to have increased in size during fermentation exercise in 3 hours before oven drying took place. The WGS pellets recorded 100 percent flotation in the first 10 minutes and gradually reduced to 80 percent at 20 minutes and 50 percent at the 40^{th} and 45^{th} minutes respectively. The CTS diet was inferior ($P<0.05$) in flotation to WGS from the 5^{th} minutes to end of test time. Magnus, (1982), Ponte and Reed (1982) and Davidek *et al*, (1990) reported that gluten fibers of wheat are proteins and during the dough preparation, gluten becomes stiffened thus contributing to the firms springing consistency as in baked bread. The CTS diet has no gluten protein, which accounts for WGS pellets superiority in flotation. The incorporation of *S. Cerevisiae*, the fermentation process adopted after pelletizing of diets and the oven drying methodologies observed were similar to the technology involved in

bread baking. Part of the starch accompanied the ingredients in the diets were converted to sugar capable of being fermented by the yeast (*S. cerevisiae*) to alcohol with the simultaneous release of carbondoioxide (Co_2) gas. The $C0_2$ gas gave the dough and the pelleted feeds the increase in size earlier observed, with porous structure (Magnus, 1982) and the subsequent air filled spaces in the dried pellets (Ponte and Read, 1982), which made the pellets buoyant and caused them to float on water. The CTS diet only has the air spaces but did not contain gluten protein (Davidek *et. al*, 1990) an added advantage in WGS product, hence the floating time or period was shortened in CTS diet. The effects of both starches were seen in the ability to coat or seal the pellets and prolong the entrapment of the carbondioxide ($C0_2$) gas as well as prevention of water from penetrating the pellets to cause early disintegration (Gluclick and Shelef, 1962).

The higher ($P>0.05$) water stability and higher nutrients retention of WGS and CTS diets confirmed the works of Falayi *et. al.* (2003, 2004) that both are good binders in fish feed. However, the higher ($P<0.05$) flotation capability of the WGS bound diet over the CTS is an added advantage in wheat products in the need for buoyant fish feed development in Nigeria aquaculture in Nigeria.

CONCLUSION

The result confirmed that there are great potentials in wheat and cassava products in fish feed stability, nutrients retention and buoyancy. The results are the preliminary findings in series of Ph. D work considering some biochemical and physical characteristics of feedstuffs and addictives in the development of improved diets for aquaculture.

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Table 1: Diets formulation and proximate composition of pelleted feeds bound with WGS and CTS and *S. cerevisiae* as floating addictive.

Ingredients g^{-100} dry wt.	% INCLUSION IN DIET.	
	WGS	CTS
Maize midlings	40.60	40.60
SBC	15.05	15.05
GNC	15.05	15.05
Fish meal	15.00	15.00
Binder	10.00	10.00
S. cerevisiae	2.00	2.00
Vitamins and Mins. premix	1.00	1.00
Bone meal	1.00	1.00
Salt	0.30	0.30
Proximate analysis:		
Moisture (%)	4.82±0.02	3.98±0.01 NS
Crude protein (%)	31.94±0.02	31.23±0.02 NS
Crude lipid (%)	11.54. ±02	9.80±0.01 NS
Crude fiber (%)	5.80±0.10	5.60±0.02 NS
Ash (%)	10.52±0.02	10.32±0.02 NS
NFE (%)	33.89±0.02	33.77±0.02 NS
Dry matter (%)	95.18±0.02	96.04±0.02 NS

Table 2: Mean percentage water stability and nutrients retention of pelleted feeds bound with WGS and CTS and *S. cerevisiae* as floating addictive.

Time of immersion in water (minutes)	Water stability (%)		Nutrient retention (%)			
	WGS	CTS	Total protein		Total lipid	
			WGS	CTS	WGS	CTS
10	98.01	97.10 NS	97.4	97.02 NS	99.00	99.00 NS
20	95.80	94.20 NS	94.30	94.021 NS	98.45	97.20 NS
30	89.20	88.80 NS	90.51	86.12 NS	98.35	96.80 NS
40	82.50	80.80 NS	86.80	84.56 NS	98.10	96.00 NS
50	72.20	71.8 NS	86.11	82.67 NS	98.0	95.56 NS
60	68.40	66.52 NS	84.36	80.54 NS	97.50	95.10 NS

NS: No significant (P>0.05) difference.

Table 3: Mean % flotation of pelleted feeds bound with WGS and CTS and *S. cerevisiae* as floating agent.

Period of observation (minutes)	Mean No. of pellets tested	Mean No. of flotation in diets		% of flotation in diets	
		WGS	CTS	WGS	CTS
5	20	20	18	100	90 S
>5-10	20	20	16	100	80 S
>10-15	20	18	14	90	70 S
>15-20	20	16	12	80	60 S
>20-25	20	15	10	75	50 S
>25-30	20	13	8	65	40 S
>30-35	20	11	7	55	35 S
>35-40	20	10	6	50	30 S
>40-45	20	10	3	50	15 S
>45-50	20	8	0	40	00 S
>50-60	20	6	0	30	00 S

NS = No significant (P>0.05) difference.
S= There are significant (P<0.05)

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