Effect of Duckweed meal on the rate of mold infestation in stored pelleted fish feed.

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Abstract
The effect of duckweed (*Lemna pauciscostata*) meal on the rate of mould infestation in stored pelleted fish feed was carried out. Freshly harvested duckweed was dried and thoroughly ground into powder using a milling machine. Five dry fish feeds were then prepared using duckweed as a replacement for fishmeal at 0%, 10%, 20% and 30% respectively at 40% crude protein, a diet for catfishes. The resultant pelleted feeds were sun dried for 24hrs and stored in airtight polyethylene bags at room temperature. Quantitative mold count using direct colony counts on pour plate technique with 24hr old culture was carried out bi-weekly until profuse growth were recorded within 24hrs in all experimental feeds. Results showed that mold count from experimental feeds decreased with increasing concentration of duckweed. Ethanolic extracts also showed higher inhibitory properties on radial mycelial growth of all the isolates. Isolates identified were *Fusarium oxysporium, Penicillium digitatum, Aspergillus niger, A.fumigatus, A..flavus, Rhizopus stolonifer* and *R..oryzae.* [Report and Opinion. 2009;1(2):26-31]. (ISSN: 1553-9873).

Introduction.

Feeds are a major cost input into the aquaculture industry and their insufficiency is prominent among the factors responsible for inadequate aquacultural production of fish.

Compounded feeds are prepared with biologically decomposable materials. These materials decompose while in storage due to environmental factors such as temperature and humidity. Change in temperature and humidity affects the moisture content of compounded feed as well as the rate at which chemical changes takes place thereby enhancing invasion and growth of fungi in the feed (Sena and Anderson, 1995; Effiong and Eyo, 1999). Recontamination of feedstuffs by adventitious microorganisms during storage is of primary concern to the feed processor.

Moulds are the principal spoilers of feedstuff in storage (Chow, 1980). Moulds infestation reduces the nutritional value of feeds through loss of dietary lipids and amino acids (Jones, 1987). They also produce mycotoxins, which cause staleness of feed (Chow, 1980). He also stated that there is no effective way of eliminating fungal growth in stored pelleted feed. Their growth can only be controlled.

Research work on the problems of storage of feedstuff \feed has been rather scanty despite the enormous harmful effect it poses on the development of aquaculture in Nigeria (Effiong and Eyo, 2001).

Duckweed meal has been reported to resist attacks by mould for more than 5 years (Skillicorn et al., 1993). Duckweed meal is the compounded form of the group of aquatic macrophytes from the family Lemnaceae.

The dried powdered and directly pelleted forms of this plant have been observed in storage for 13 years without any signs of fungal growth or physical damage, retaining its nutrient content (Mbagwu, 2001).

This study is therefore aimed at determining the effect of duckweed meal on the rate of mould infestation in stored pelleted fish feeds.

Materials and Methods

Freshly harvested duckweed was thoroughly rinsed with clean water and evenly spread on a mosquito net-size mesh outside to sundry and thereafter dried in a forced air oven at 165 ^{0}c for 48 hours and ground to powder with a milling machine according to Mbagwu and Adeniji (1987).

Five dry diets were prepared in which fish meal was replaced with duckweed at 0%, 10%, 20% and 30% levels using the method of Akegbejo Samson (1999) at 40% crude protein, a diet for catfishes.

The various feed ingredients were thoroughly ground into fine meal and mixed together with vitamin premix and salt using hot water. The resultant mixture was pelleted with Moulinese HV6 model pelleting

machine and sun dried for 24 hours. The diets were stored in airtight containers at room temperature for 2 weeks.

1.0g of each feed sample were ground using pestle and mortar, to prepare 10-fold serial dilution. Agar was prepared using sterilized glasswares according to manufacturer's instruction and autoclaved at 121 ^{0}c for 15 minutes. It was allowed to cool to about 37 ^{0}c before 1% streptomycin was added to prevent bacterial contamination (Nwachukwu, 1988).

A 48hour old culture of the isolates were subcultured and incubated at room temperature to produce pure cultures from which stock were prepared and stored. A bi-weekly mould count from each experimental diet was carried out quantitatively using direct colony count on pour plate technique (Miles and Misra, 1938) with 24-hour-old culture. Enumeration continued until profuse growth was recorded within 24 hours in all the experimental diets.

Mould isolates were characterized during sporulation on the basis of cultural and morphological characteristics as well as microscopic examination (Samson and Reenen-Hoekstra, 1988). Sample of duckweed meal was ground using an Automatic Weed Grinder after it was thoroughly washed and air-dried. 5g of this each was measured and blended with 25ml of sterile distilled water (Oyagade, 1994). After thoroughly blending for 7 minutes, the slurry was filtered through a four –layered muslin cloth. The filtrate was passed through a 0.48 millimicron Millipore filter and transferred into sterile bottle.
In order to compare the efficiency of the extraction process, 95 % alcohol was used as the comparative solvent using the same method.

Radial mycelial growth inhibition tests were carried out on the isolates (Van-Etten, 1973; Oloke et al., 1988). The extracts were separately incorporated into molten PDA at 18ml of media to 2ml of extract. Control plates had either sterile water or ethanol without extract.

Agar- extract mixtures were poured into sterile glass petri dishes and allowed to set (Adedayo, 1994). Mycelial plugs of the test organisms of 5.0mm diameter were cut using sterile cork-borer from the advancing margin of the fungal colonies. These were placed at the center of PDA containing concentrations of 5% sterile distilled water or ethanol. All plates were incubated at 25 0c and radial mycelial growth recorded for 72 hours at 24 h0urs interval

Results and Discussion.

The bi-weekly fungal count (cfu) from the experimental feed at varying concentrations of duckweed showed decrease in fungal growth with increasing concentration of duckweed (Table 1). This observation could be attributed to the antifungal properties of duckweed acting against the growth of fungal species in the feed. Skillicorn et al., (1993) attributed the long storage characteristic of duckweed meal to the presence of high levels of wax. It could be possible that wax presents physical barriers to the growth of molds, which might impair their utilization of nutrients in the feeds. The molds isolates from the experimental feed samples were ***Fusarium oxysporium, Penicillium digitatum. Aspergillus niger, A. fumigatus, A. flavus, Rhizopus stolonifer*** and ***R. oryzae.***

. Chow (1980) reported that the most common molds involved in the spoilage of feedstuffs belong to the *Aspergillus and Penicillium* species among others. The presence of Aspergillus flavus from the feed indicates the possibility of mycotoxins, compounds produced by this species that are toxic to both humans and fish.

Feedstuffs known to be contaminated by A. flavus include groundnut cake, maize, sorghum, cottonseed cake, copra and cassava (Chow, 1980). The same author however reported that for aflatoxins to be produced, A. flavus must be present alone in a practically pure culture and that the presence of other molds, yeast or even bacteria seems to interfere with aflatoxin production. These findings have also been reported by Abdulhamid (2008).

The effect of duckweed extracts on the radial mycelial growth of fungal isolates from the experimental feeds is shown in Table 2.Differential efficacy on the test organisms was observed between the aqueous and ethanolic extracts of duckweed meal. Ethanol appeared better as an extractant judging from the wider activity spectrum and the resultant effect on the isolates. This observation perhaps suggests the possibility of the occurrence of bioactive substances that are not only soluble in water but also in organic solvent in the plant material. Majekodunmi et al., (1996), and Martinez et al., (1996) reported that a higher activity of extractable natural products were obtained in ethanol compared with aqueous extracts. Odemena and Essien (1995) also reported that the bacterial activity of alcoholic extracts of the roots of fluted pumpkin, Telfaria occidentalis was better than that of aqueous extracts.

Natarajan et al., (2005) reported the antifungal properties of three medicinal plant extracts against Cercospora arachidicola. They reported that fungal growth was gradually suppressed with increasing extract concentration. Similar findings have been reported by Lucia et al., (2002), Silva et al., (2001) and Costa et al., (2000). These reports are similar to the findings of this study.

Olafimihan (2003) working on the antibacterial properties of aqueous and ethanolic extracts of Neem plant reported that the antibacterial activity of the concentrated extract increased with increase in its concentration. This report is similar to the findings from this study with he observation that increasing concentration of duckweed meal in experimental feed resulted in decreasing fungal growth.

The environmental conditions of temperature and relative humidity during the period of the study were high and fell within the ranges that support luxuriant growth of molds in the experimental feed sample. The temperature range varied between 27.2 and 30.6 0c while relative humidity remained constant between 79 and 80%.

According to Chow (1980), growth of fungi is only possible at temperature above 250c and relative humidity values at 65%. There any reduction in fungal growth in the experimental feeds could not be attributed to directly affect the rate of fungal infestation of compounded feed in storage.

Conclusion

The results obtained from this study indicate reduced growth performance in the fungal species isolated from the experimental feed which also signified low infestation rate.

Fungal growth decreased generally with increase in concentration of duckweed meal in feed samples.

The result of this experiment have shown that duckweed has the potential of being a beneficial agent for the control of fungal growth in compounded feed in storage.

Table 1:Percentage composition of experimental feed with different inclusion levels of duckweed meal.

Ingredients (g)	0%	10%	20%	30%
Duckweed	0	2.6	5.2	7.8
Fish meal	26	33.4	20.8	18.2
Yellow maize	48	48	48	48
Soya Bean meal	15	15	15	15
Groundnut cake	6	6	6	6
Vitamin premix	2	2	2	2
Bone meal	2.5	2.5	2.5	2.5
Salt	0.5	0.5	0.5	0.5
Total	100	100	100	100

Table 2: Bi- weekly fungal counts at varying concentrations of duckweed in experimental feed.

Concentration of Duckweed (%).	Fungal count (cfu\ml)(x 10^7)							
	Time (wk)							
	2	4	6	8	10	12	14	16
0	12	20	31	43	72	Profuse	Profuse	Profuse
10	5	9	21	35	52	108	Profuse	Profuse

20	3	11	18	27	48	90	Profuse	Profuse
30	-	-	9	16	21	54	76	Profuse

Table 3: Effect of duckweed extracts on the radial mycelial growth of fungal isolates

Test Organism	Mycelial growth (mm)					
	Aqueous Extract			Ethanolic Extract		
	0%	5%	10%	0%	5%	10%
Fusarium oxysporium	46	21	10	10	-	-
Penicillium digitatum	50	35	24	9	5	-
Aspergillus niger	47	27	18	16	7	2
Aspergillus fumigatus	38	18	12	4	-	-
Aspergillus flavus	50	38	20	16	-	-
Rhizopus oryzae	36	29	16	14	-	-
Rhizopus stolonifer	42	21	13	22	10	4

Table 4:Proximate composition of experimental feed with different inclusion level of duckweed

Feed Sample	% Crude protein	%Ether extract	%Ash content	%Moisture content	%Crude fibre
0%	43.35	14.02	12.30	1.00	6.50
10%	42.56	14.29	12.00	1.00	4.46
20%	41.87	12.83	11.90	2.00	5.13
30%	45.06	11.76	13.29	2.00	4.90

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