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Effect of Replacing Fishmeal with Water Spinach (*Ipomoea aquatica*) on Growth, Feed Conversion and Carcass Composition for Nile Tilapia Fry (*Oreochromis niloticus*)

Ramzy A. Yousif *[1], Obied J. Abdullah[1], Ahmed M. Ahmed[1], Mohammed I. Adam[1], Fawzi A. Mohamed Ahmed[1] and Omer A. Idam[2]

[1]*Department of Fisheries and Wildlife Science, Sudan University of Science and Technology, Khartoum, Sudan*
[2]*Department of Fish Production and Technology, Faculty of Animal Production*

***Corresponding Author:** Yousif R. A Department of Fisheries and Wildlife Science, Sudan University of Science and Technology, E-mail: ramzy@sustech.edu.sd /ramzy173@gmail.com*

ABSTRACT

The use of alternative feeds aims to maintain productivity and reduce animal production costs. The objective of this study was to determine the nutritional value of Water Spinach (WS) (Ipomoea aquatica), as well as replacement of fishmeal (FM) by water spinach in diets for Nile tilapia fry, Oreochromis niloticus. To determine the growth performance of Nile tilapia fed different levels of water spinach, 150 fish (3.25±0.44g; 3.6±0.2cm) were randomly distributed in flow through system of 15 plastic aquariums each containing about 20 liters, and fed during 7 weeks with isoprotein (30% digestible protein) and isoenergetic (14.35 kJ g-1 digestible energy) diets containing replacement levels of 0, 25, 50, 75 and 100% of FM digestible protein by WS digestible protein. The experimental design was completely randomized with five treatments and three replicates. The protein efficiency ratio, absolute body weight, live weight gain and feed conversion ratio content significantly decreased in fish fed diets containing WS levels above 25%. Therefore, WS can replace up to 25% of FM without impairing juvenile Nile tilapia growth performance, feed efficiency, and body composition.

Keywords: *Growth performance, Replacement, Water Spinach, Orechromis niloticus.*

INTRODUCTION

Tilapia is one of the most popular aquaculture species and is farmed in more than 120 countries and territories. However, global tilapia aquaculture production is highly imbalanced, with the top ten countries in 2015 accounting for over 90 percent of the 5.7 million tonnes of global production (FAO, 2018). Tilapia is second - most important cultured finfish worldwide and farmed globally by many small holders. Nile tilapia (*Oreochromisniloticus*) ranks 6th among the most important cultured species, providing food, jobs, domestic and exporting earnings. Tilapia is an important protein source especially for poor consumer because they are an omnivorous diet, are tolerant to high density in aquaculture and relatively diseases resistant (FAO, 2017).

Fishmeal is recognized by nutritionist as high quality, very digestible feed ingredient that is favored for addition to the diet of most farm animals, especially fish and shrimp. Fishmeal carries quantities of energy per unit weight and excellent source of protein, lipids, minerals and vitamins, carbohydrate very little in fishmeal (Mile & Chapman, 2015).

Water spinach (*Ipomoea aquatic*) is wild plant that grows in water or moist soils and belongs to family *Convolvulaceae* (Salih, 1991). Water spinach offer nutritive values with significant quantities of essential amino acids, nonessential amino acids, macro and micro salts crude fibers, fatty acids, organic acids and polyphenols (Doka *et al.*, 2014), the leaves of this plant is enriched with important vitamins (Igwenyi*et al.*, 2011; &Misra& Misra., 2014). Water spinach leaves contained high amount of essential amino acids (4765 mg/100 g) and non-essential amino acids (11669 mg/100 g) representing a total amino acids content of 16434 mg/100 g. The most abundant components of essential amino acids were leucine (1365 mg/100 g), Tyrosine + phenylalanine (1124 mg/100 g), lysine (682 mg/100 g) and threonine (606 mg/100 g) (Kavishree et al, 2008). The leaves of this plant is enrich with important vitamins, namely thiamine, riboflavin, niacin, pyridoxine, cyanocobalamin, ascorbic acid, a- tocopherol, and phylloquinone (Igwenyi et al 2011 and Misra et al., 2014).Water spinach (Ipomoea aquatic) is an aquatic vegetable distributed in Southern Asia, India, and China. The aerial parts of I. aquatic is a common vegetable eaten by different social groups of South-eastern Asia, India, China, Southwestern Pacific Islands, and African countries, namely Sudan, Nigeria, Tanzania, and Somalia (Doka et al., 2014; Igwenyi et al., 2011). Water spinach (Ipomoea aquatic), locally known in western Sudan as (Arkala), In Sudan the herb is used to treat stomach and intestinal troubles. Until recently, little attention has been given to the use of wild plants as food in Sudan. By learning more about the protein, fat and mineral content of each plant, one can better assess their importance in the nutritional well-being of the communities (Doka et al, 2014).

Culture fish required protein, lipid, energy, vitamins and minerals in their diet for growth, reproduction, and other normal physiological functions. Nutrients for culture may come from various feed source, such as plankton, bacteria, insects and other fish from within the aquaculture ecosystem, and organic matter and processed feeds added to the ecosystem (Hancz, 2011). Natural foods are the best foods for fish and include algae (phytoplankton), zooplankton, detritus, snails, worms, insects and insect larvae, small plants like duckweeds and various other weeds and grasses that are found in a fish pond (WRC, 2010).

With regard to the total sulphur amino acids (namely tyrosine, cystine, methionine and phenylalanine), tyrosine and cystine are best considered semi-essential in that the fish can utilize cystine as a precursor for the biosynthesis of methionine and phenylalanine, thus reducing the dietary requirement for these two essential amino acids. The optimum gross dietary lipid requirements for Nile tilapia ranges between 10 and 15 percent (White *et al.*, 2018).

Materials and Methods

Preparation of Experimental Diets

In this study, firstly proximate composition and fatty acid profile of oilseed meals used in fish feeds were analyzed (Table 1) and then feasibility of replacing fishmeal with water spinach protein for Nile tilapia *Oreochromis niloticus* fry were find out. In this experiment five isonitrogenous 0 % (T0), 25% (T1), 50% (T2), 75% (T3), 100% (T4) fishmeal protein by water spinach protein were formulated (Table 2). All diets were isonitrogenous (30% crude protein); out of which 10% protein was contributed by fish meal.

Crude protein content in the diet was fixed at 30% on the basis of earlier available information (Abdelghany, 2000). All the ingredients were weighed and blended in a Hobart electric mixer thoroughly. These were then steam cooked at 80°C in a volume of hot water. Oil, mineral and vitamin premixes were added to the lukewarm bowl one by one with constant mixing at 60°C. The final diet with bread dough consistency, and then pellets were produced by manual meat grinder with 0.6 mm diameter and later were dried for 24 hrs and subsequently broken into crumbled form and each diet was packed in a plastic bag and stored until used.The amino acid profiles of the experimental diets used in experiment were also analyzed and are given in Table7.

Table1. *Proximate composition profile of ingredients*

Ingredients	FM	WS	GNK	CSM	WM	WB
Protein %	45	21	43,7	38	17	13,7
Fat %	7.5	13.21	16.81	14.87	4.0	7.72
Moisture %	7.0	9.36	6.25	13.69	11	4.12
Ash %	21.3	2.53	10	10.40	4.5	4.37
Fibre %	0.8	6.83	18.38	12.21	7.5	10.47
energy kj/g	14.25	14.35	14.79	14.09	14.85	14.01

Fish Meal (FM), Water Spinach (WS), Ground nut Cake (GNK), Cottonseed Meal (CSM), Wheat middlings (WM) and Wheat bran (WB)

Table2. *Diet composition of the experiment*

Ingredients(g/ 100 g dry diet)	T0	T1	T2	T3	T4
Fish meal[1]	40.00	30.00	20.00	10.00	0.00
Water Spinach[2]	0.00	10.00	20.00	30.00	40.00
Groundnut Cake[3]	20.00	20.00	20.00	20.00	20.00
Cottonseed Meal[4]	3.00	3.00	3.00	3.00	3.00
Wheat middling[5]	20.00	20.00	20.00	20.00	20.00
Wheat bran[6]	11.00	11.00	11.00	11.00	11.00
Oil	3.00	3.00	3.00	3.00	3.00
Mineral premix[7]	1.50	1.50	1.50	1.50	1.50
Vitamin premix[8]	1.50	1.50	1.50	1.50	1.50
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Protein (%)	33.0±0.3	30.6±0.0	28.2±0.2	25.8±0.05	23.4±0.01
Calculated gross energy (kJ g^{-1}, dry diet)	14.22±0.1	14.83±0.3	14.44±0.5	14.05.10	14.20±0.2

[1]Fishmeal 45% CP; [2]Water Spinach21%; [3]Groundnut Cake43.7% CP; [4]Cottonseed Meal 38%; [5]Wheat Middling 17% CP and [6]Wheat bran 13.7%.[7]Mineral mixture (g/100g dry diet) calcium biphosphate 13.57; calcium lactate 32.69; ferric citrate 02.97; magnesium sulphate 13.20; potassium phosphate (dibasic) 23.98; sodium biphosphate 08.72; sodium chloride 04.35; almunium chloride.$6H_2O$ 0.0154; potassium iodide 0.015; cuprous chloride 0.010; mangnous sulphate H_20 0.080; cobalt chloride. $6H_2O$ 0.100; zinc sulphate. $7H_2O$ 0.40 (Halver, 2002). [8]Vitamin mixture (g/100 dry diet) choline chloride 0.500;inositol 0.200; ascorbic acid 0.100; niacin 0.075; calcium pantothenate 0.05; riboflavin 0.02; menadione 0.004; pyridoxine hydrochloride 0.005; thiamin hydrochloride 0.005; folic acid 0.0015; biotin 0.0005; alpha-tocopherol 0.04; vitamin B_{12} 0.00001; LobaChemie, India (Halver, 2002).

Experimental System and Animals

Fry of *Oreochromis niloticus* were procured from Hussien Fadoul Fish Farm, Soba-Khartoum, Sudan. These were transported to hatchery of the Department of fisheries and Wildlife Science, Sudan University of Science & Technology, Khartoum, Sudan, transport of fry in polyethylene sac and stocked in fiber glass for two days in this period fry no feed. After that use of small deep net to caught fries and then weight thesefries and standardized then transfered to medium aquarium and circular pond.

During this period, the fish were fed to apparent satiation by feeding diet consisting of cotton seed meal, wheat bran and wheat middling in the form of dried powder diet twice a day at 8:00 a.m. and 04:30 p.m. For conducting the experiments, *Oreochromis niloticus*fry (3.25 ±0.44g; 3.6±0.2cm) were sorted out from the above acclimated lot and stocked in triplicate groups in 70-L circular polyvinyl tanks (water volume 20 L) fitted with a continuous water flow-through (1-1.5 L min-1) system at the rate of 10 fish per tank for each dietary treatment. Fish were fed test diets in the form of pellets diet to apparent satiation twice daily. No feed was offered to the fish on the day they were weighed. Initial and weekly weights were recorded on a top-loading balance. The feeding trial lasted for 7 weeks. Faecal matter and unconsumed feed, if any, were siphoned off. The unconsumed feed was filtered on a screen soon after active feeding, dried and weighed to measure the amount of feed consumed.

Water Quality Parameters

Water temperature, dissolved oxygen, NO_2, NO_3, pH, and total ammonia during the feeding trial were recorded following standard methods (APHA 1992). The range of water temperature, dissolved oxygen, NO_3, pH, and NH_3 over the 7 weeks feeding trial, based on weekly measurements, were 29.43 ºC, 5.31 mg L^{-1}, 1.39 mg L^{-1}, 7.37 and 0.29 mg L^{-1}, respectively Table (5).

Chemical Analysis

At the begining of experiment, 10 fish were euthanized at stocking and frozen (<-15 ºC) for initial whole-body composition analysis, and at the termination of the seven week feeding trail, all fish were counted and weighted, and 10 fish per trough were ranndomly selected for analysis of whole-body composition. Assessment of proximate composition of ingredients, diets and carcass was made using standard techniques (AOAC 1995). Briefly, crude protein (N x 6.25) was determined (Kejeltec Tecator TM Technology 2300, Sweden), dry matter was determined after drying in a oven at 105 ºC, ash content was determined by incineration in a muffle furnace at 550 ºC for 8 hrs, crude fat (solven extraction with petroleum ether B.P 40-

60ºC for 2-4 h Socs Plus, SCS 4, Pleican Equipments, Chennai, India).

Growth Parameters

The effects of replacing fishmeal with water spinach in diets on growth and conversion efficiency of fingerling *Oreochromis niloticus* during the present experiment was evaluated using following indices:

Live weight gain (LWG; %) = Final individual body weight (FW)-Initial individual body weight (IW)/Initial individual body weight (IW) × 100

Absolute weight gain (g/fish) = Final individual body weight-Initial individual body weight

Feed conversion ratio (FCR) = Dry feed fed/Wet weight gain

Protein efficiency ratio (PER) = Weight gain/ Protein fed

Specific growth rate (SGR; %/day) = ln Final body weight-ln Initial body weight/No. of days × 100

Per cent survival = (Final number of fish/Initial number of fish) × 100

The proximate content analysis of carcass and feed was done. The results from the replicates for each feed samples were used to provide the data for the statistical analysis. All growth data were subjected to analysis of variance (Snedecor and Cochran 1968; Sokal and Rohlf 1981). Differences among treatment means were determined by Duncan's Multiple Range Test at a P<0.05 level of significance (Duncan 1955).

Results

Results in Table 2 and 3. indicated that the overall mean ± SD of the final weight, (WG g/fish), (AWG g/fish), (LWG), (SGR) of Nile tilapia fed on diets with partially substituted by WSM, However, the greatest values of WG (g/fish (36.90±3.50), SGR% (5.44± 0.38) and FCR (1.96±0.18), PER (0.38±0.04) and the greatest values of final body weight (g) (40.4±3.22) were achieved by fish fed on T1.

Table3. *The increment weight of Nile tilapia (g/fish) as affected with WSM incorporation in diets/days*

	T0 (0%)	T1 (25%)	T2 (50%)	T3 (75%)	T4 (100%)
Initial weight	3.60±0.10^{a}	3.50±0.36^{a}	2.53±0.51^{b}	3.50±0.30^{a}	3.13±0.84^{a}
7 days	7.00±0.56^{a}	7.73±0.40^{a}	5.60±0.61^{b}	4.93±0.57^{c}	6.77±1.59^{b}
14 days	9.53±0.40^{b}	11.67±0.55^{a}	8.27±2.25^{b}	7.27±0.25^{c}	9.63±1.48^{b}
21 days	12.77±1.56	16.50±0.56	12.07±3.51	9.53±1.01	13.37±1.72
28 days	17.43±3.40	19.43±1.07	15.83±3.35	13.93±1.45	17.17±0.93
35 days	21.57±4.05	25.13±2.38	19.43±6.41	18.27±1.10	21.50±0.87
42 days	29.40±4.73	35.43±3.66	26.80±7.99	24.87±2.74	25.37±3.91
49 days	40.20±9.43	40.40±3.22	35.63±8.95	32.90±3.38	27.43±6.36

$^{a, b, c}$ *Mean values followed by the same superscript in each column are not significant different (p>0.05)*

Table4. *Growth, survival and feed utilization of Nile tilapia fed experimental diets*

	T0 (0%)	T1 (25%)	T2 (50%)	T3 (75%)	T4 (100%)
IW (g)	3.60±0.10^{a}	3.5±0.36^{a}	2.53±0.51^{b}	3.5±0.30^{a}	3.13±0,84^{a}
FW(g)	40.20±9.43^{a}	40.4±3.22^{a}	35.63±8.95^{b}	32.90±3.38^{b}	27.43±6,36^{c}
AWG (g)	36.60±9.46^{a}	36.90±3.50a	33.10± 8.70^{b}	29.40±3.39^{b}	24.30±6,42^{c}
LWG %	1016.67±29.73^{a}	1054.29±212.08^{a}	1308.3±341.42^{b}	840.0±107.34^{c}	776.36±311.00^{d}
SGR	4.66±1.30^{c}	5.44±0.38^{a}	5.19±1.49^{a}	4.98±0.29^{b}	4.83±0.73^{b}
FCR	2.05±0.5^{a}	1.96± 0.18^{a}	2.28±0.68^{b}	2.47± 0.27^{b}	3.10± 0.78^{b}
PER	0.35± 0.09^{b}	0.38±0.04^{a}	0.36±0.10^{b}	0.36±0.04^{b}	0.33±0,09^{c}
Survival %	98	100	100	98	98

$^{a, b, c}$ *Mean values followed by the same superscript in each column are not significant different (p>0.05)*

Table5. *Mean physical-chemical parameters of the test concentrations water spinach on Water Quality*

	T0 (0%)	T1 (25%)	T2 (50%)	T3 (75%)	T4 (100%)
pH	7.20±0.01	7.53±0.00	7.17±0.07	7.66±0.08	7.28±0.10
NH_3	0.29±0.01	0.22±0.19	0.33±0.10	0.28±0.01	0.32±0.10
NO_2	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
NO_3	1.78±0.01	1.56±0.01	1.22±0.03	1.23±0.05	1.15±0.50
DO	5.55±1.29	5.13±0.33	5.19±1.63	5.32±1.90	5.34±0.24
Temp.	29.60±0.51	29.60±0.01	29.63±0.51	29.10±0.51	29.24±0.51

[a, b, c] *Mean values followed by the same superscript in each column are not significant different (p>0.05).*

Table6. *Whole-body Composition or CarcassComposition of fry Nile tilapiaOreochromis niloticus fed graded concentrations of Water Spinach for 7 weeks*

	Experimental diets				
	T_0	T_1	T_2	T_3	T_4
DM%	25.91 ± 0.20^b	25.10 ± 0.81^b	25.78 ± 0.76^b	26.06 ±1.95^a	23.65 ± 0.32^c
Fat%	5.26 ± 0.50^c	5.36 ± 0.30^c	7.14 ± 0.83^b	11.11 ±0.10^a	10.00 ± 0.01^a
CP%	70.5 ± 0.50^a	68.43 ± 0.40^a	67.05 ± 0.50^a	61.95 ± 0.2^b	60.11 ± 0.7^b
Ash	1.92 ± 0.30^c	6.00 ± 0.26^b	8.11 ±0 .71^b	8.33 ± 0.29^b	15.00 ± 0.25^a

Means in the same row with different superscripts are significantly (P<0.05) different.

The results of whole body content or the carcass composition very important for nutritional value of Nile tilapia. In table 6 dry matter in the experimental ranged from 23.65 to 26.06. The fat in body ranged between 5.26% in T0 to 11.11% in T3. Crude protein in body of fish depended to essential amino acid in the diets. Generally, crude protein decrease with decrease of fish meal, high Crude protein in T0 (70.5%) and lower crude protein in T4 (60.11%). The Ash content between 1.92 in T0 to 15.00 in T4

Table7. *Amino acid composition (% dry matter) of the experimental diets. Calculated based on the plant feedstuff values reported for Nile tilapia (Furuya et al., 2010).*

	T0	T1	T2	T3	T4
Arginine, %	2.43	2.93	2.89	2.57	3.34
Histidine, %	1.22	1.28	1.33	1.39	1.44
Isoleucine %	0.78	1.80	2.08	2.37	2.65
Leucine %	2.52	1.83	3.24	3.61	3.99
Lysine %	2.45	1.14	2.64	2.75	2.53
Methionine %	0.78	0.51	0.84	0.89	0.92
Cystine %	0.38	0.37	0.42	0.90	2.52
Phenylalnine %	1.48	1.30	2.10	2.44	2.76
Tyrosine %	1.05	0.96	1.58	1.87	2.14
Threonine %	1.43	1.63	1.65	2.03	2.23
Tryptophan %	0.37	0.43	0.49	0.55	0.62
Valine %	0.88	2.10	2.31	2.54	2.76

DISCUSSION

Selection of feed ingredients is one of the most important factors for the formulation and commercial production of supplemental quality feed for any aquatic species (Zamal *et al*., 2008; Koumi *et al*., 2009). Although fish meal is the widely used feed ingredients as animal protein source and accepted for its higher protein composition and essential amino acids; it is rather expensive than the available plant protein sources (Vechklang*et al*., 2011). Beside this, the availability of fish meal is decreasing day by day due to its high demand in other than aquaculture industry like livestock, poultry etc. The decreased supply of fish meal in future will dramatically affect the fish production. One approach to reduce fish meal from fish diets is to replace it with alternative less expensive and easily available plant protein, which will allow for continued expansion of aquaculture. In view of this, a number of plant protein source has been evaluated for the replacement of fish meal (Alceste and Jory, 2000; Yue and Zhou, 2008; Francis *et.al* 2001).

The proximate composition of water spinach leaf meal used in the experiment revealed that the crude protein content were high compared to the result Doka *et al*, 2014. These differences might be due to different environmental conditions such as soil type, local varieties, and processing methods. All the experimental feeds were actively fed upon and accepted by the fish throughout the experimental period which could be as a result of palatability of the feed indicating that the levels of incorporation of water spinach did not affect the palatability of the diets. Growth rates in weight and were calculated from measurements all sample of 10 fish, but also by the survival rate of the fish population. This varied among treatments, and thus in some cases the feed available per fish was influenced by the numbers of fish surviving in the aquarium. For this reason productivity was measured as the growth performance, expressed as weight of fish at the end of the experiment high the weight at the beginning. Using this criterion it was clear that the survival rate had a determining effect on fish productivity compere to the results of Sorphea (2010). Growth rate measured on weight of all fish in the aquarium weekly at 7 week. High weight in final week,in the present study, there were no significant differences in AWG, LWG, SGR, and PER between the 5 treatments, but there was a difference in survival, suggesting that *I. aquatica*cultivation significantly affected survival rather than the growth of *Pelodiscussinensis*in ponds. Although this is the first study to report the effects of *I. aquatica*cultivation on *P. sinensis* growth and survival in pond culture, similar observations on cultured fish species, such as crucian carp *Carassiusauratus* (Chen *et al.*, 2010).

In the present study, the inputs of fishmeal replacement with water spinach meal (0-100%) have been evaluated on Nile tilapia fry. The highest absolute weight gain (36.90g), live weight gain (1.07g) and SGR (5.44) was noticed in T1 (Table 4) as compared to treatments. While comparing the treatments, it was the growth performance of experimental fish had negative impact of increasing water spinach meal in fish diet without T1. Similar results were also reported by Yee Lin *et al*., (2004) and Xu *et al*., (2012) four isonitrogenic and isocaloric diets which contained 100-75% fish meal. After 7 weeks feeding period, no significant (P>0.05) difference was found in live weight gain, feed conversion ratio and protein efficiency ratio among fish fed different experimental diets. (Yee Lin *etal*.,2004). Weight gain, feed conversion ratio and survival rate compared to fish fed of all the treatment (p<0.05) in the present study, the highest absolute weight gain was recorded in T1 (36.90) which was followed by T0 (36.60), T2 (33.10g), T3 (29.40g) whereas, the lowest absolute weight gain (24.30g) was inT4. The recorded absolute weight gain was statistically different between treatments. SGR was not significantly, higher (5.44) in T1 as compared to other treatments. Whereas lowest SGR (4.66) was found in T0. FCR was also no significant higher (3.10) in T4 as compared to other treatment. Whereas, lowest FCR (1.91) was found in T1. The Nile tilapia fed with T0, T1, T2, T3 and T4 diet with no replacement or replacement of fish meal with water spinach meal attended an average net weight of 36.90 respectively. Thus, best growth of Nile tilapia fry was reported when fed with the T1 diet. Nile tilapia provided the diet with 25 per cent replacement of fish meal with water spinach attended an average weight of 36.90±3.50 which was reported to be the highest in fish meal replaced diet. Thus, fish meal replaced by water spinach meal had effect on growth of Nile tilapia compared to the result (Fabusoro *et al.*, 2014).

CONCLUSION

The experiment showed that feeds were actively consumed by the experimental fish; Nile tilapia which brought an increase in weight. Since there was no significant difference (P>0.05) among the means of the treatments, it shows that any of the inclusion level can be used up to 50% inclusion level of water spinach.However, 25% inclusion level of water spinach produced best result in terms of growth. It is therefore recommended that water spinachplant can be incorporated at 25% inclusion without compromising fish growth. There are various alternative protein sources that can be used in aquaculture diets, without affecting growth performance, feed efficiency, and body composition, since the amino acids requirement is considered, water spinach meal (WSM) can be used as plant protein ingredient.

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ABBREVIATIONS

FM Fishmeal, WS Water Spinach, CSM Cotton Seed Meal, WM Wheat Middlings, WB Wheat Bran, GNK Ground nut Cake, DO Dissolved Oxygen

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