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Effects of Graded Methionized Yeast on the Growth, Nutrient Utilization, and Hematobiochemistry of Hybrid *C. gariepinus* x *H. longifilis* (Hetroclarias)

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Abstract

Sacharomyces cerevisiae contains about 45% protein but lacks some essential amino acids like methionine. Methionine promotes the growth of yeast and inhibits autophagy. We methionized yeast for 48hrs at 28°C and thereafter used it in the formulation of novel diets for hybrid African catfish "heteroclarias". The diets varied in the inclusion of methionine: *Sacharomyces cerevisiae* as follows; F1, 0:250; F2, 200:50; F3,150:100; F4, 100:150; F5, 50:200, and F6, 250:0. Hybrid catfish were stocked at 20 fish per aquaria, subjected to 12D:12L photoperiods and were fed *ad libitum* for 86 days. Hybrids that received feed F5 (200gyeast:50g Meth), had the highest SGR, 2.86±0.05 % day^{-1}, lowest FCR F2, 1.04±0.08, highest weight gain 264.96±0.73g, highest PER 5.92±0.07 and highest DFI of 2.95±01g day^{-1}. Conversely the hybrid fed diets F2 (200gMeth:50g yeast), had the lowest SGR of 0.97±0.01% day^{-1}, highest FCR of 2.53±0.01, lowest weight of 35.03±7.52g, lowest PER 0.80±0.02 and lowest DFI of 0.47±0.02g day^{-1}. All analyzed hematobiochemical indices were optimal for catfish fed F5 (200gyeast:50g Meth) and poorest for those fed F2(200gMeth:50g yeast). Results suggest that 50g kg^{-1} methionine inclusion and methionization of yeast is optimal and yields best growth, nutrient utilization and hematological parameters in diets of hybrid African catfish.

Introduction

Aquaculture remains the fastest growing agriculture sector (FAO 2020) and produces needed cheap proteins (Little et al. 2016). It has been noted that since the year 2000, the growth of sub-Saharan aquaculture production has been at 11% annually. Meanwhile, some African countries had a growth of 12-23% per year (FAO, 2020, Rocha et al. 2022). Africa is a major producer of freshwater aquaculture species and brackish water also (Rocha et al. 2022, Ragasa et al.2022). The growth of aquaculture in sub-Saharan Africa faced several obstacles like poor feed management, paucity of funds, and high feed cost of foreign and local feeds (Kaleem and Sabi 2021; Ragasa et al. 2022). Disease outbreaks have caused much loss in the sub-Saharan aquaculture industry like Ghana, infectious spleen and kidney necrosis, Ramírez-Paredes et al. (2021), and viral; diseases in Tanzania and Uganda (Ragasa et al. 2022). There is a need for diets that would increase fish immunity to combat diseases. Fermented yeast products have been used to enhance the productivity, immunity, and health performances of Nile tilapia (*Oreochromis niloticus*) Abu-Elala et al. (2021).

There is a need to find local raw materials because the cost of imported feed raw materials is exorbitant, making locally produced feed also expensive (Enyidi 2012, Verdegem et al. 2021). There is also the need to reduce the inclusion levels of fishmeal in aquafeed for the sake of cost and the environment (Enyidi et al. 2017). Methionine has been identified as prime essential amino acid in substitution of fishmeal with plant proteins (Locally available plant protein meals can be processed with probiotics and used as alternatives (Enyidi and Ekeh 2018). Yeast *S. cerevisiae* is cheap and has been employed in replacing fishmeal or plant proteins without negative effects on growth and nutritional performances in the diets of Arctic charr (Vidakovic et al. 2016), rainbow trout Vidakovic et al. (2020), and Nile tilapia (Abass et al. 2018). Moreso, significant mortality has been recorded in fish culture due to poor rearing environments, pathogenic microbial proliferation, and poor nutrition (Kautsky et al. 2000; Enyidi and Okoli 2019). It has been noted that the microbial communities of the fish picture that of the aquatic ecosystem (Enyidi and Okoli 2019, Nolorbe-Payahua 2020, Bi et al. 2021). Diseases outbreaks have wreaked havoc in many sub-Saharan aquaculture sectors,

Saccharomyces cerevisiae has been reported to be an important part of fish gut microbiomes (Gatesoupe 2007). The protein content of *S. cevevisiae* is about 45% protein, while lipids are 1% and crude fiber 27% (Raven and Walk, 1980). Yeast has an excellent amino acid profile and high content of lysine, but it is deficient in sulfur-containing amino acids such as methionine and cystine (Huige, 2006). The effects of yeast can be enhanced by the inclusion of methionine which is essential for their multiplication (Thomas and Surdin-Kerjan, 1997). Yeast, however, can directly utilize methionine as a sole sulfur source for its growth (Thomas and Surdin-Kerjan, 1997). Hybrid *Clarias gariepinus* X *H. longifilis* have been reported to utilize diets with 2% levels of dried brewers yeast effectively with the determination of optimal levels beyond 2% inclusion being open for further investigation (Essa, et al., 2011). Oleaginous yeast, *Lipomyces starkeyi* was grown in a media of lignocelluloses and has been used as a substitute for vegetable oil in the diets of Arctic char (*Salvelinus alpinus*) Blomqvist et al. (2018). Methionine is one of the essential amino acids and is an aliphatic, nonpolar α-amino acid that contains sulphur (Li et al., 2009. Methionine is an important supplement in aquafeed (Figueiredo-Silva et al., 2015, Machado et al., 2015; Azeredo et al., 2017, Elesho et al. 2021). Methionine cannot be synthesized denovo by fish and must be supplemented in the diets (Li et al. 2009; Chen et al. 2019). Methioine supplementation has been used severally in previous researches like, for African catfish *C. gariepinus*, Elesho et al., (2021); hybrid tilapia (*Oreochromis niloticus*× *O. reochromis mossambicus*) Figueiredo-Silva et al., (2015), for rainbow trout Gaylord et al., (2009); and for sea bream (Takagi et al., 2001).

This research seeks to find the effects of different combination levels of methionine and yeast, and their effects on the growth and nutrient utilization of hybrid *C. gariepinus* and *H. longifilis* (Heteroclarias).

Materials and Methods

Experimental Feed and Feeding of Fish

The soybean, corn meal, millet and palm oil, and vitamin C were purchased from Ariaria Market Aba Abia State Nigeria. The vitamin premix, *Saccharomyces cerevisiae*, and methionine were purchased from the veterinary and agro market section of New Market Enugu, Enugu State Nigeria. The grains were oven-dried at 40°C for 1 hour. The dried grains were milled into powder with a locally made attrition mill. Known weights of the ingredients according to Table 1 were weighed using an electronic balance. The ingredients were hand-mixed in a bowl and an appropriate homogenous mixture was achieved after 25 minutes of mixing. During the blending, 500mls of water was added to the mixture. The resulting dough was used during the methionization of yeast.

Methionization of Yeast

Known quantities of *S. cerevisiae* and methionine per feed type were measured using an electronic scale according to Table 1. The yeast and methionine were mixed separately in an opaque glass beaker and kept aside in a cool, place (28˚C), for about 48hrs to allow yeast uptake of the methionine. The blended ingredients per feed type as stated above (Table1), were mixed with the methionine –yeast mixture and stored in a locally constructed bioreactor for 48 hours at 28˚C for the growth of yeast and fermentation to take place. Feed F1 was not mixed with methionine and F6 was not equally mixed with *Saccharomyces cerevisiae*. After 48 hours the fermentation was arrested by dough was preconditioned by steam cooking in an airtight pressurized pot for 20 min at 110°C according to Enyidi (2012). The preconditioned dough was hotly pelleted and dried at 40°C in an electric oven equipped with thermostats. The dried pellets were placed in a black nylon bag and stored in a freezer at -20°C till used. Fish were hand-fed at 0900 h and 1700 h. The fish were fed to apparent satiation in the morning. In the evening fish were restricted to eating only 3% of their body weight. The restriction rations of feed were adjusted daily based on the calculated increase in body weight as the trial continued for 28 days. Fish were not fed for 24 hours before weighing.

Experimental Design and the Experimental Fish

The design of the experiment was Completely Randomized Designed (CRD). Fingerlings of hybrid African catfish were obtained by crossing gravid female African catfish *Clarias gariepinus* and male *Heterobranchus longifilis.* The fingerlings were transported to the wet labs of the FISHARM department, Michael Okpara University of Agriculture Umudike. The fish were acclimated for 12 days. During acclimation to the laboratory conditions, the fish were fed a 35% crude protein dry diet prepared in our laboratory.

Groups of 20 hybrid catfish juveniles, average weight 24.82±2.23 were stocked into 15-liter glass aquaria, with three replicates per treatment, 20 tanks in total. The aquaria were supplied aerated borehole water (30.0±1.5°C) and about 80% of the water was changed daily by siphoning. Care was taken to prevent stressing the fish.

Rearing tanks were aerated, and the water temperature was measured daily and recorded with a Celsius thermometer. The average dissolved oxygen was 6.44±0.2 mg L^{-1}. The oxygen measurement was done with a YSI oxygen meter of model 550A (YSI Inc. Yellow Springs, Ohio, USA). The total gas pressure of the water was analyzed and the value was (100.2±1.0%); as measured with a P4 Tracker total gas pressure meter (Point Four Systems Inc., Richmond BC, Canada). Ammonia (0.28±0.03 mg L-1) was measured fortnightly with an ammonia test kit (Tetra Merke, Melle, Germany). Water pH was 6.9±0.1 and alkalinity 1.03±0.01 mmol L^{-1}.

The tanks were subjected to a photoperiod of L12:D12 and light intensity was c. 8 lux (HD 9221 lux meter, Delta OHM, Padua, Italy). The tanks were cleaned every morning before the feeding of the fish.

The catfish were weighed every two weeks by measuring tank biomass. After the feeding experiment, five catfish were removed from each tank and their length (to 0.1 cm) and weight (to 0.01 g) were recorded individually. The fish were killed by a sharp blow on the head and the liver and visceral fat of the sample fish were removed and weighed (to 0.01 g).

Chemical Analyses

Hemato-biochemical Parameters

The effects of the methionized yeast feed on the fish hematobiochemical parameters of the hybrid catfish were analyzed. A total of 15 fish per treatment feed were used for the analysis. The experiment was conducted in three replicate aquariums per treatment feed. The fish were individually removed from aquarium with a scoop net and quieted by gently hitting it on the head. Caution was taken not to smash the head and cause bleeding, but to give gentle blow that enhances quietness, since African catfish is very active and hardy; immediately the tail was cut for immediate blood collection. A blood sample was collected using sterilized syringes from the caudal vein after gentle cutting. The blood was loaded in a centrifuge operated at 3,800× g for 5 min. The samples of blood serum were separated and stored at −70°C for the analyses of alanine aminotransferase (ALT), aspartate aminotransferase (AST), triglycerides (TG), glucose, total protein (TP), total cholesterol (TC) and The biochemical chemical analyzer (Fuji DRI-CHM 3500i, Fuji Photo Film, Tokyo, Japan) was used in measuring the parameters.

Moisture

The moisture content of the fish muscle was analyzed with fish muscle taken from the dorso ventral side of the fish. The fish was gently killed with a mall blow on the head. Using a surgical blade muscle sample was taken between the dorsal fin and between the pectoral and caudal fins. Care was taken to avoid the

Table 1. Composition of experimental diets varying in the composition of *Saccharomyces cerevisiae* and methionine used in feeding fingerlings African catfish *C. gariepinus* for 86 days

Ingredients	F1	F2	F3	F4	F5	F6
Methionine	0	200	150	100	50	250
Sacchromyces cevevisiae	250	50	100	150	200	0
Soybean	400	400	400	400	400	400
Fish meal	200	200	200	200	200	200
Corn Meal	70	70	70	70	70	70
Millet	50	50	50	50	50	50
Fatty Oil	10	10	10	10	10	10
Vitamin C	10	10	10	10	10	10
Vitamin Premix	10	10	10	10	10	10
Total	1000	1000	1000	1000	1000	1000
Proximate analyses						
Protein	34.32	32.89	35.11	33.78	34.69	34.56
Carbohydrate	15.98	14.69	15.65	15.78	14.98	14.04
Lipid	12.83	11.99	11.02	10.6	11.04	11.05
Fiber	9.08	10	8.78	10.6	9.78	11.02
Moisture	10.06	9.86	9.67	9.12	8.99	10.24
Dry matter	89.94	90.14	90.33	90.88	91.01	89.76

Proximate composition of Vitamin premix per Kg of feed was as follows:Vit.A,4,80000 IU,D ,12000g ;K 0.80g; B1,0.40g;B2,1.20g;B12,8.00mg; Folic acid,0.80g; C,100.00g; Biotin,0.06; Choline chloride,80.0g; manganese,10.0g; Iron,50.0g;Copper,10g;Iodine,0.30g;Cobalt,0.30g;selenium,0.04g skin. Each treatment feed replicates provided 3 fishes for the analysis. The muscles were dried to constant weight at 60°C before being stored.

Crude Protein

Kjeldahl analysis was carried out to evaluate the nitrogen value of the feed and subsequently calculate the protein value. The dried muscle samples were used for the analysis. Tecator kjeltec model 1002 system was used for this experiment. The system analyzed the nitrogen content using block digestion and steam distillation (Tecator kjeltect Hogänäs, Sweden). The resultant crude protein content of the sample was calculated as % N x 6.25.

Crude Lipid

The total lipids of fish muscles were measured by chloroform-methanol extraction at a ratio of 2:1. Total lipid was calculated as the weight difference in non-extracted and extracted muscle samples (Parrish 1999; Kainz et al. 2004). Ash content was calculated by burning a known amount of freeze-dried catfish muscle in a muffle furnace for 24 hours at 550°C.

Calculations and Statistical Analyses

The catfish-specific growth rate (SGR, % day^{-1}) was calculated as 100 * (Ln W2 – Ln W1) * t^{-1}, where W1 and W2 were average weights in g at the start and the end of the experiment, and t was the length of the experiment in days. Food conversion ratio (FCR) was calculated as feed fed (g) * gain (g)$^{-1}$. The economic conversion ratio (ECR) was calculated as the price of the variable part in the diet (USD) * FCR. The protein efficiency ratio (PER) was calculated as FCR * % feed protein * % protein in catfish-1. Daily feed intake (DFI) was calculated as total intake (g)* ((Initial number (N1) - Final number (N2)-1 * (Final weight W2-Initial weight W1-1) *2-1* time*100, where N1 and N2 are initial and final number of fish W1 and W2 are the initial and final weight of fish.

One-way ANOVA was used for testing possible differences in the growth and nutritional parameters and hematobiochemical parameters.

Results

Hematobiochemical Parameters

The hematobiochemical parameters of the hybrid showed that the value of aspartate aminotransferase (AST) was lowest for hybrids fed feed F5, (Yeast 200g:50gMet) 37.5±0.02 µ/L (P<0.05). The AST of hybrids fed feed F5 was significantly lower than all other treatments (P<0.05). The hybrid fed feed F2 (Yeast 50g: Met.200g) had a significantly higher value of AST (87.78±0.04 µ/L) than all other treatment feed (P<0.05). The AST of hybrids fed feed F1, (Yeast 250g: Met.0g) 47.57±0.07 µ/L was significantly (P<0.05) lower than that of hybrid fed feed F6 (Yeast 0g: Met.250g) 79.21±0.03 µ/L. Similarly, the AST values of hybrid fed feed F4, (Yeast 100:Met.150) 49.34±0.12 µ/L, were significantly lower than those fed feed F3 58.64±0.02 µ/L, (P<0.05) (Table 2). Generally, the alanine aminotransferase (ALT) was least for hybrids fed feed F5, 40.24±0.06 µ/L. The hybrid fed feed F2 had an ALT of 108.34±0.08 µ/L, which was not significantly different from the ALT of hybrids fed feed F6 (P>0.05), n=20. The hybrid ALT was similar for those fed feed F1, 52.34±0.02 µ/L, and F4, 50.35±0.08 µ/L (P>0.05). n=20. Blood glucose level was virtually lowest but similar for hybrid fed feed F5, 100.21±0.05 mg/dL, and feed F1, 108.76±0.01 mg/dL (P>0.05). The blood glucose level of hybrids fed feed F1 and F5 were significantly lowered (P<0.05) than that of all other treatment feeds hybrids (Figure 1). The hybrids fed feed F2 (Met. 200g: Yeast 50g) had the highest blood glucose level 195.43±0.04 mg/dL. There were no significant differences between the blood glucose level of hybrids fed feeds F3, F4, and F56 (P>0.05), n=20, (Table 2). The protein levels of the fish were not affected by the feed. There were no significant differences (P>0.05) n=20, in the blood protein of the fish irrespective of treatment feed. Total cholesterol was lowest for the hybrid fed feed F5, 99.03±0.02 mg/dL and this was significantly lower than the blood cholesterol level of hybrids from all other treatment feeds (P<0.05) (Figure 2). The highest cholesterol level was measured from the fish-fed feed 2, 189.34±.05 mg/dL. There was no significant difference between the cholesterol levels of hybrids fed feed F1, 114.42±0.08 mg/dL. And those fed feed F4, 118.07±0.08

Table 2. Hematobiochemical parameters of hybrid African catfish (*C.gariepinus* x *H.longifilis*) fed diets varying in the compositions of methionine and *Sacharomyces cerevisiae* for 86 days

Parameters	F1	F2	F3	F4	F5	F6
AST (µ/L)	47.57±0.07^{b}	87.78±0.04^{d}	58.64±0.02$^{c`}$	49.34±0.12^{b}	37.5±0.02^{a}	79.21±0.03^{d}
ALT (µ/L)	52.34±0.02^{b}	108.34±0.08^{d}	70.01±0.07^{c}	50.35±0.08^{b}	40.24±0.06^{a}	106.78±0.06^{d}
Glucose mg/dl	108.76±0.01^{d}	195.43±0.04^{a}	154.56±0.09^{c}	150.37±0.01^{c}	100.21±0.05^{e}	169.56±0.04^{b}
Total protein mg/dL	6.23±0.01^{ns}	6.33±0.09^{ns}	6.10±0.10^{ns}	6.22±0.06^{ns}	6.78±0.07^{ns}	6.9±0.03^{ns}
Total cholesterol mg/dL	114.42±0.08^{b}	189.34±.05^{e}	127.09±0.03^{c}	118.07±0.08^{b}	99.03±0.02^{a}	143.89±0.08^{d}
Triglycerides mg/dL	96.02±0.06^{c}	109.87±0.07^{d}	82..37±0.08^{b}	78.09±0.04^{b}	67.08±0.04^{a}	80.09±0.01^{b}

Where (AST) aspartate aminotransferase and (ALT) alanine aminotransferase mg/dL (P>0.05) Table 2. The cholesterol level of the hybrid fed feed F3, 127.09±0.03 mg/dL, was significantly higher than that of hybrids fed reciprocal diet feed F4, 118.07±0.08 mg/dL. (Table 2). The value of triglycerides was lowest for hybrids fed feed F5, 67.08±0.04 mg/dL but highest for those fed feed F2. 109.87±0.07 mg/dL. Triglycerides were similar for hybrids fed feed F3, 82.37±0.08 mg/dL and feed F6, 80.09±0.01 mg/dL (P>0.05), Table 2.

Growth and Nutritional Parameters

The hybrids accepted the experimental diets and those fed diet F5, (Yeast 200g: Met.50g), had the highest specific growth rate (SGR) of 2.86±0.05 % day^{-1}. The SGR of hybrids fed F5 was significantly higher than all other treatment feed (P<0.05). The catfish fed feed F2 (Yeast 50g: Met.200g), had the lowest SGR of 0.97±0.01% day^{-1} (Table 3). The SGR of hybrids fed feed F$_{-}$1 (Yeast 250g: Met 0g) 2.29±0.09% day^{-1} was next to that of those fed feed 1 but better than that of hybrids fed other treatment diets (P<0.05) n=20.The SGR of the hybrids are stated in (Table 3).

The food conversion ratio (FCR) of the catfish was significantly lowest for those fed diets F5, (Yeast 200g: Met.50g), (FCR=1.04±0.08). The FCR of hybrids fed diets F5 was significantly better than all hybrids fed other treatment diets (P<0.05). The poorest FCR was achieved by the hybrids fed diet F2, (Yeast 0g: Met. 250g). Hybrid FCR detailed in Table 3. The weight gain followed a similar pattern as the FCR. The catfish fed feed F5 had the highest weight gain of 264.96±0.73g. The weight

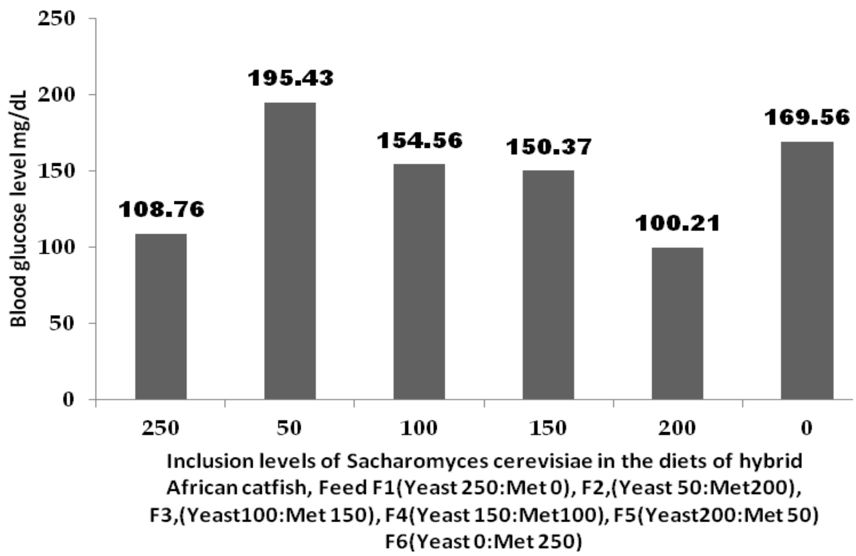

Figure 1. Showing the effects of inclusion levels of methionized yeast (Feed F1 to F6) on the blood glucose levels of hybrid African catfish Heteroclarias.

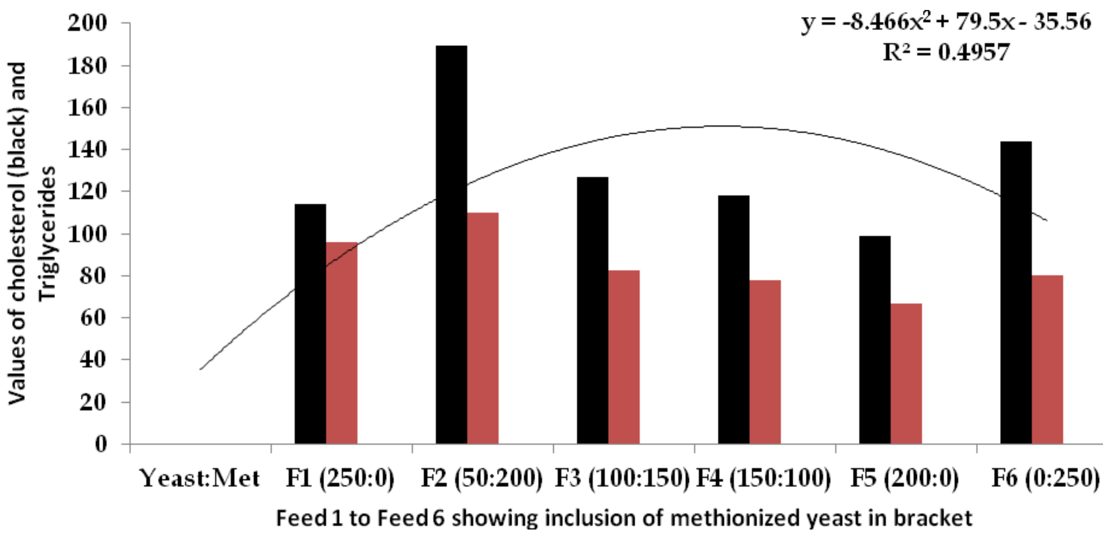

Figure 2. Showing the effects of inclusion of methionized yeast on the cholesterol level (in the black bar) and total triglycerides of hybrid African catfish heteroclarias fed with experimental diets for 86 days.

gain of hybrids fed with F5 was significantly higher than all other feed (P<0.05), n=20. Diet F2 is reciprocal to diet F5 based on the formulation but the weight gain of hybrids fed diet 2 was 35.03±7.52g. The weight gain of hybrids fed feed F2 was significantly the lowest among all treatment feeds (P>0.05), n=20 (Table 3).

Protein efficiency ratio (PER) was best for the hybrid fed feed 5, 5.93±0.07 and this was significantly better than the PER of hybrids fed all other treatment diets (P<0.05). The PER of hybrids fed diet (F2) (0.80±0.02) was the poorest of all treatment diets PER. (Table 3). The hybrid fed feed F5 had the highest daily feed intake (DFI) than those fed other treatment feeds, (P<0.05), n=20, DFI, 2.88±01g day^{-1}. The DFI of the hybrid fed feed F5 reciprocal diet F2, was 1.16±0.02g day^{-1} and this was more than half of F5 DFI. However, feeding the catfish with a diet similar to feeding F5 but without the addition of methionine gave a DFI of 1.16±0.02 g day^{-1} (Table 3). The condition factor (CF) of the catfish didn't follow any clearly defined pattern. Similarly, the lengths of the fish were not significantly different for the hybrids fed diets F1, 9.85±0.42 cm, and diet F5, 9.66±0.16cm (P>0.05) (Table 3).

The costs of producing the feeds were highest for diets F1 and F5, 2.56 USD and 2.55 USD, respectively while the rest were mere above1 US dollars.

Discussions

The methionization of yeast (*S. cerevisiae*) in the diets of hybrid African catfish had profound effects on their growth and nutrient utilization. Methionine is needed in aquafeed for enhanced growth rate Ebenezer et al. 2020, The high specific growth rate recorded for hybrids in this research is in line with results of previous works done on Nile tilapia, Lara-Flores et al. (2003), Abu-Elala et al. 2021, on African catfish *Clarias gariepinus* Essa et al. (2011) and rainbow trout (Huyben et al. 2017; Vidakovic et al. 2020), who noted improved growth effects as a result of dietary yeast inclusion. There is a recent increasing interest in the usage of yeast protein in feed production (Navarrete and Tovar-Ramírez 2014, Parapouli et al. 2020, Vidakovic et al. 2020, Abu-Elala et al. 2021, Jach et al. 2022). Yeast has been identified as a plausible aquaculture feed ingredient because it breaks down complex substances and improves the nutritional values of plant products (Gause and Trushenki 2011, Enyidi and Ekeh 2018, Madibana and Mlabo 2019, Lape˜na et al. 2020). Yeast has been also used to improve immunity (Torrecillas et al. 2012; Eryalc¸in et al. 2017). However, we noted significant differences in the effects of the diet that has methionine compared to the ones that haven't got methionine. The growth differences between hybrids fed with diets containing methionine and those that contained none or graded levels suggest that methionine is essential in the growth of the fish. Methionine has been noted as a limiting factor in the use of yeast as a feed ingredient (Dilger and Baker, 2007; Candebat et al., 2020; Ruiz et al. 2020, Agboola et al. 2021). Yeast utilizes methionine for growth and bioconverts sodium selenite (Na2SeO3), sodium hydrogen selenite (NaHSeO3), and sodium selenate (Na2SeO4)), both soluble but poorly bioavailable, into useful organic seleno-amino acid, L-selenomethionine (Chasten et al. 2003, Yin et al. 2010, Rai et al. 2019).

A comparison of the lower FCR of catfish fed feed F5 compared to those fed feed F1 suggests that methionine possesses growth and nutrient utilization boosting effects on the hybrids. Besides, the difference in the FCR of hybrid fed feed F1 (Yeast 250g: Met.0) and F5 (Yeast 200g: Met 50g), points to the importance of methionine to yeast performances. Similarly, the performances of hybrids fed diets F1 compared to those fed diet F6 shows the importance of methionine inclusion. This is supported by previous works of Sutters et al. (2013) and Ruiz et al. (2020), who noted that methionine is needed for yeast growth and can be limiting. Yeast utilizes methionine for growth. In a recent study, Agboola et al. (2022) noted that the apparent digestibility coefficient (ADC) of methionine in yeast varies according to the type of yeast. The low FCR of the

Table 3. Growth and nutritional performances of hybrid African catfish (*C.gariepinus* x *H.longifilis*) fed diets varying in their inclusions of methionine and *Sacharomyces cerevisiae* for 86 days

Feeds	F1	F2	F3	F4	F5	F6
Ini no	20	20	20	20	20	20
Final no	20	20	20	20	20	20
Ini Wt	25.76±0.05^{ns}	23.40±0.03^{ns}	25.13±0.02^{ns}	25.03±0.07^{ns}	24.76±0.03^{ns}	40.36±0.08^{ns}
Final Wt	185.10±0.07^{b}	58.43±0.06^{f}	149.40±0.04^{d}	166.56±0.06^{c}	289.73±0.05^{a}	128.01±0.02^{e}
Wt gain	159.33±5.49^{b}	35.03±7.52^{f}	124.26±0.45^{d}	141.53±0.03^{c}	264.96±0.73^{a}	87.63±0.42^{e}
SGR	2.29±0.09^{b}	0.97±0.01^{b}	2.06±0.08^{c}	2.20±0-.03^{b}	2.86±0.05^{a}	1.34±0.05^{d}
FCR	1.26±0.07^{b}	2.53±0.01^{f}	1.81±0.06^{e}	1.64±0.06^{d}	1.04±0.08^{a}	1.40±0.03^{c}
PER	3.59±0.01^{b}	0.80±0.02^{d}	2.75±0.06^{e}	3.23±0.01^{c}	5.92±0.07^{a}	1.96±0.03^{f}
DFI	1.24±0.09^{b}	0.47±0.02^{d}	0.89±0.02^{c}	1.45±0.06^{a}	2.95±01^{a}	0.83±0.05^{c}
Cost $	2.56^{a}	1.90^{c}	2.11^{b}	2.20^{b}	2.55^{a}	1.75^{c}
CF	1.06±0.09^{b}	2.00±0.06^{a}	1.23±0.08^{b}	1.14±0.07^{b}	1.14±0.04^{b}	1.11±0.03^{b}
Length	9.85±0.42^{a}	7.06±0.11^{d}	9.02±0.30^{c}	9.36±0.29^{b}	9.66±0.16^{a}	9.46±0.15^{b}

Means not followed by the same superscript within a row are significantly different (P<0.05)

Where: SGR: Specific Growth Rate % day^{-1}; FCI: Feed Conversion Ratio; PER: Protein; CF: Condition Factor; DFI=Daily Food Intake g; Wt.gain: Weight Gain.

catfish as a result of yeast inclusion is in line with Lara – Flores et al. (2003) and Ran et al. (2015), who noted improved growth rate and FCR of Nile tilapia as a result of dietary yeast inclusion. The lower FCR of the catfish fed feed F5, (Yeast 200g: Met 50g) compared to hybrids fed diets F2, (Yeast 50g: Met 200g), suggests that high inclusion of methionine up to 250g was not expedient in the diets of hybrid African catfish. The performances of catfish fed feed F1, (250g Yeast: 0Meth) and F5 suggests (200g Yeast: 50g Meth) suggests that the inclusion of 50g/kg of methionine and 250g/kg of yeast in the diet, was optimal for the fast growth rate of hybrid African catfish. Recent biochemical researches on the effects of methionione supplementation on the growth of fish noted that, methionine supplementation enhanced faster growth of fish (Elesho et al. 2021, Wang et al. 2021, Ebeneezar et al. 2022). It was noted that methionine enhance faster metabolism and growth through the activation of pathways for nutrient sensing at both the cellular and systemic levels (Wang et al. 2021). Based on our results, the higher inclusion of methionine beyond 50g / kg of feed, did not enhance better growth and nutritional performances. Comparative analyses of growth and nutritional performances of hybrids fed feed F3 and F4 show that the higher the inclusion of methionine the lower the growth performances beyond 50g/kg diet. Supplementation of yeast with methionine has been identified as a plausible means of improving the performance of yeast and preventing autophagy (Sutters et al. 2013), and an increased cellular anabolism and cell proliferation (Walvekar et al., 2018, Wu and Tu 2011, Walvekar and Laxman 2019). The increased growth of yeast cells increased the nutritional values of feed and subsequently the growth and nutritional performances of the hybrid catfish. Methionine is vital in the fish for modifying nutrients metabolism, immunity responses, gene expression, cell signaling, (Li et al., 2007; Wu et al., 2014, Guo et al., 2020;). Methionine supplementation also functions for stimulating elevation of plasma free AA concentrations (Xu et al., 2016; Song et al., 2017). The weight gain and growth rate reduced with increasing methionine. Consequently, the hybrids had enhanced weight gain, protein efficiency ratio, and low FCR. Methionine helps to improve growth, enable proper digestive and assimilation functions of the fish, protection of hepatopancreas and the ileum from lipid peroxidation, and also improve enzymatic antioxidants concerning proteins (Vidakovic et al., 2020). The poor performance of hybrid fed with high inclusion levels of methionine supports Ruiz et al. (2020) who stated that high inclusion of amino acids inhibits the growth of yeast cells. Our results show that yeast should not be included without methionine in the diet of hybrid African catfish. There is a paucity of research articles on the effects of methionized yeast or substitution of yeast with methionine, on hybrid African catfish. Our research is the first research on this concept and provides baseline information for further research.

The high values of the AST and ALT of the hybrid fed feed F2, and F3, suggest that the feed mix had some negative effects on the fish welfare and health. The high methionine content of the diets did not enhance the fast growth rate nor lower FCR. These suggest that high methionine inclusion up to 150g/kg feed and upwards does not support faster growth and may have been toxic to the fish hence the high AST and ALT. Moreover, the high blood sugar and cholesterol suggest that high methionine inclusion could have had some impairment effects on effects on the ability of the fish to utilize carbohydrates and lipids in their diets (Figures 1 and 2). Although we did not do experiments on the hybrid sugar metabolism. More research should be done to know what is responsible for the very poor growth and nutrient utilization of catfish fed high methioninzed yeast diet.

Conclusions

The inclusion of methionized yeast in the diet of the African catfish hybrid is beneficial for proper growth and nutrient utilization. The results showed that methionine is needed in diets that include yeast but is best at 50g /kg feed. Beyond this point, the methionine becomes excess and starts reducing growth rate and weight gain, and protein efficiency ratio. Our results show that yeast protein should be used in hybrid diets but better methioninzed at 50g kg^{-1} feed. Yeast has a good array of amino acids but lacks methionine. Yeast use methionine for growth and proper functioning. Methioninzation of yeast is pivotal for optimum performance of yeast as an aquafeed ingredient. The best inclusion level of methionine is 50g/kg feed. The best inclusion level of yeast is 250g /kg of feed.

Ethical Statement

There were no life threatening or painful experiment carried out with research animal, all procedure were within limit of ARRIVE Guidelines.

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Author Contribution

All authors took part in the design and conceptualization of the work. UDE. contributed the fish meal, yeast, the methionine and methionized the yeast. OB provided the rest of the materials. Both authors carried out running of research. The first draft of the manuscript was written by Enyidi Uche and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Conflict of Interest

The authors have no relevant financial or non-financial interests to disclose.

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