METHIONINE REQUIREMENT OF HYBRID TILAPIA (*OREOCHROMIS NILOTICUS* X THAI/RED TILAPIA: *OREOCHROMIS MOSSAMBICUS*) COMPARED WITH NILE TILAPIA IN NIGERIA

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Abstract

A twelve-weeks feeding experiment was conducted at fish nutrition laboratory of Nigerian Institute for Oceanography and Marine Research (NIOMR) in Lagos, Nigeria, to determine the methionine requirement of *Oreochromis niloticus* x *O. mossambicus* compared with the local *O. niloticus* strain. Six graded methionine level (0. 57, 0.67, 0.77, 0.87, 0.97 and 1.17) % were incorporated into a plant based basal diet in a completely randomized 2*6 factorial design with triplicates in a water recirculation system. Methionine levels significantly weight gain (WG), specific growth rate (SGR), thermal growth coefficient (TGC), feed conversion ratio (FCR), haematological indices and whole-body proximate composition ($P<0.05$). Significant strains and methionine levels were observed for WG and SGR, while strain and methionine independently affected feed intake and FCR. Polynomial regression revealed optimum performance at 0.87% methionine inclusion in the hybrid. The tumour necrosis factor alpha (TNF α) and insulin-like growth factor (IGF) increased with methionine levels. The estimated methionine requirements were 37.06 and 33.8g methionine /kg protein in the hybrid and *O. niloticus* respectively. It was concluded that 33.5g methionine/Kg and 37.06g methionine /Kg protein will be necessary for inclusion into the diets of the local tilapia and hybrid strains respectively for optimum growth performance.

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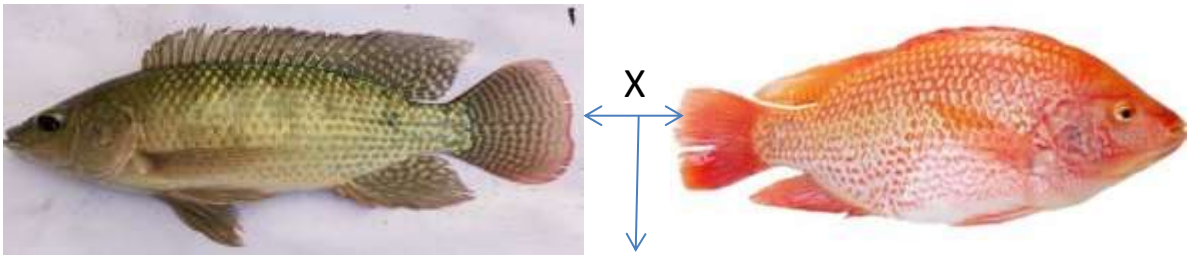
Key Words: Methionine; hybrid tilapia; local strain; growth performance.

1.0 Introduction

Fish farming has emerged to become one of the fastest growing and considered as an important agricultural activity that can address global nutritional deficiency and contribute to poverty alleviation by providing inexpensive protein in developing countries. Nigeria is one of the top aquaculture producers in Africa and the leading producer in Sub-Saharan Africa (SSA), with numerous opportunities for large-scale production (Kaleem and Sabi, 2021). Omeje et al. 2020 reported Tilapia species, *Clarias* and *Heterobrachus* (Catfish), *Cyprinus carpio* (common carp) and *Heterotis niloticus* as the most cultured species of fish in Nigeria.

Tilapias are native to Africa and are one of the most cultured fish in the world. Nigeria's aquaculture focuses mainly on freshwater fish, with catfish species majorly cultured and produced (WorldFish, 2018) where tilapia served as supplemental feed in polyculture. However, in recent times, tilapia is now deliberately cultured for harvest in Nigeria especially with the advent of technology of all-male production. All-male technique has been used to mitigate the problem of early sexual maturity and prolificacy resulting to over population and stunted growth (Yisa et al. 2022). Scientists have also discovered that hybrid red tilapia has greater growth potential and produces fish with slightly higher EPA- eicosapentaenoic acid and DHA-docosahexaenoic acid content (Roshada *et al.*, 2002). Hybrid all-male tilapia using simple cross-breeding between two different strains of *Oreochromis niloticus* to exploit non-additive genetic variance resulting from heterozygosity at many loci are been produced (Yisa et al., 2022) which some farmers obtain for farming tilapia in Nigeria. Presently, four red tilapia strains from Taiwan Province of China, Thailand, Malaysia and the Stirling strain, dominate the red tilapia industry (Somu et al., 2021). The methionine requirement of Nile tilapia as reported by NRC (2011) is 0.75% of dry diet or 2.68% of dietary protein. The local feed formulation for hybrid tilapia being cultured by Nigerian fish farmers depends on nutrient requirement of related species. However,

for optimum performance of any fish, it is necessary to know its specific nutrients requirements to apply into its feed. There is dearth of information regarding the methionine requirement of the hybrid strain of *Oreochromis niloticus* cross with *O. mossambicus*. Therefore, the objective of this study under the development and scaling of sustainable feeds for resilient aquatic food system in Sub-Saharan Africa was designed to determine the methionine requirement of hybrid strain of tilapia compared with that of *Oreochromis niloticus* in Nigeria.



a. *Oreochromis niloticus* **b. *Oreochromis mossambicus***

Plate 1: Parental (a & b) of Hybrid Tilapia

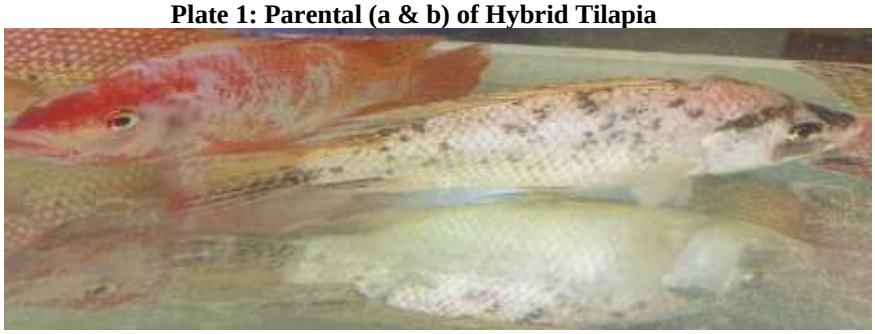


Plate 2: Hybrid Tilapia strain (*O. niloticus* X *O. mossambicus*)

2.0 Materials and Methods

2.1 Ethic Law

The experiments were conducted based on animal ethic law of Nigeria and approval was obtained (project number CMUL/HREC/03/23/1169) from The Health Research Ethics Committee of College of Medicine, University of Lagos, Nigeria.

2.2 Experimental design

Twelve weeks experiment was conducted at fish Nutrition laboratory of Nigerian Institute for Oceanography and Marine Research, Victoria Island, Lagos, Nigeria using a completely randomized 2 x 6 factorial design with three replicates in a water recirculation system. The two factors were the hybrid (improved strain) and the pure Nile tilapia (local strain) whose response depended on the six levels of methionine (0. 57, 0.67, 0.77, 0.87, 0.97 and 1.17%) inclusion into basal diets formulated and fed to triplicate groups of the fish. The re-circulatory system accommodated the 36 number of 80L experimental unit (54 x 44 x 34 cm^3) which formed the replicates.

2.3 Experimental Diets

The 35% crude protein basal diet was prepared using leguminous plants (soybean meal and groundnut cake) as protein sources with other ingredients as premix (to supply vitamins and trace-minerals), lysine, salts, soybean oil and cellulose (Table 1). The treatments were obtained by substituting a quantity of cellulose in the basal diet with supplemented graded level of DL-Methionine at 0.57, 0.67, 0.77, 0.87, 0.97 and 1.17% (Table 1). Supplementation started at 0.57% with increments of 0.1% based on the established methionine requirement for Nile tilapia (Parental strain) of 0.75% of the dry diet (NRC, 2011), while some authors gave a range of 0.5 – 0.7%. Preparation of the feeds involved milling the dry ingredients of each of the experimental diets (Table 1). Warm water (15-20% of the diet mixture) of about 40°C was added to make dough which was pelleted with a 2mm die. The pellets were dried at 60°C for 4-6 hours using an oven (Weswox 220v AC 50HZ Electric laboratory oven, Ambala Haryana, India). A sample of each experimental diet was taken to central laboratory of National Institute for freshwater Fisheries Research (NIFFR), New Bussa, Niger State to analyse for proximate composition (Dry Matter, crude protein, crude fat, ash, crude fibre, nitrogen free extract and energy) and amino acid profile was analysed at Afe-Babalola University, Ado-Ekiti (ABUAD)

Laboratory, Ekiti State. Each experimental feed was fed to satiation to triplicate groups of the fingerlings of improved (hybrid) and pure local strains of Nile tilapia.

Table 1: Composition of 35% crude protein (CP) experimental diets fed to hybrid and local strain of *Oreochromis niloticus* 0 - 12 weeks.

Ingredients	DIETS						
	BASAL	1	2	3	4	5	6
Methionine (%)	0.00	0.57	0.67	0.77	0.87	0.97	1.17
Cellulose	1.20	0.63	0.53	0.43	0.33	0.23	0.03
Soybean meal	44.00	44.00	44.00	44.00	44.00	44.00	44.00
Groundnut cake	32.50	32.50	32.50	32.50	32.50	32.50	32.50
Wheat bran	3.24	3.24	3.24	3.24	3.24	3.24	3.24
Cassava flour	13.80	13.80	13.80	13.80	13.80	13.80	13.80
Soybean oil	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Dicalcium phosphate	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Premix*	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Lysine	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Ascorbic acid	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
DM%	89.82	90.39	90.49	90.59	90.69	90.78	90.98
Ash%	7.74	7.75	7.75	7.75	7.75	7.75	7.75
GE MJ/kg	17.38	17.51	17.53	17.56	17.58	17.60	17.65
DE MJ/kg	12.35	12.47	12.49	12.51	12.53	12.55	12.59
CP%	35.00	35.51	35.60	35.69	35.78	35.87	36.05
Dig CP%	31.09	31.60	31.69	31.78	31.86	31.95	32.13
Lipid%	5.93	5.93	5.93	5.93	5.93	5.93	5.93
Fibre%	6.43	6.43	6.43	6.43	6.43	6.43	6.43

***** Provides per kg: Vitamin A, 1500000 IU, Vitamin D_3 3000000 IU, Vitamin E 200000mg, Vitamin K 2mg; Vitamin B_1 800mg; Vitamin B_2 1600mg; Choline chloride 500mg; Niacin 5970mg; Vitamin B_6 1500mg; Vitamin B_{12} 4mg; Folic acid 300mg; Biotin 40mg; Manganese 20323mg; Iron 1200mg; Zinc 15996mg; Iodine 191mg; pantothenic acid 4000mg; Copper 800mg; Cobalt 0.3 mg; Selenium 60 mg; vitamin C 240g, Choline 87000 mg.

2.4 Experimental Fish and Feeding

A batch of 800 fingerlings (400 numbers of hybrid and 400 numbers of pure Nile tilapia) with average initial body weight of 2.01±0.01g was acquired from the Fish Biotechnology and Genetics programme of National Institute for Freshwater Fisheries Research (NIFFR), New Bussa, Niger State, Nigeria. The fingerlings were transported to Lagos in oxygenated polyethylene bags. On arrival to Lagos, equal number of fish were distributed to four (4) holding plastic tanks and acclimatised for 2 weeks. During the 2-week acclimation period, the fish were fed a commercial feed (0.8mm Coppens). The day before the start of the experiment, fish were not fed to enable them to empty their digestive system for actual weight of the fish to be obtained and to reduce stress of handling at stocking. The fish were pooled to a tank before been randomly distributed to the experimental tanks. Twenty (20) fish were stocked in each 80 L tank, and approximately same starting average weight was kept in all the tanks to commence the experiment. Dead fish in the first three days after stocking were replaced seeing it as mortality due to stress of stocking, but any death observed later than the first three days was recorded as mortality due to experimentation. During the experiment, fish mortality was recorded daily. The fish were fed twice daily, morning (8.00 - 9.00 am) and evening (6.00 – 7.00 pm) based on body weight. Uneaten feed was siphoned out 20 - 30 minutes after feeding and dried to be subtracted from quantity supplied to the fish. This was obtained to determine the actual feed consumed per replicate group per week. Water quality parameters were monitored throughout the experimental period. On daily basis and at each sampling, physical observations were made on the fish in each tank to ascertain visual health status.

2.5 Sampling of fish

The fish were sampled biweekly to monitor changes in weight. Weight gain, total and standard length, specific growth rate (SGR), feed conversion ratio, protein efficiency and condition factor were derived from the measurements taken at samplings. At each weighing a sedative containing eugenol (Clove oil at the rate of 40-80mg/Litre of water) was added to the fish in water to avoid stress due to sampling (Fernadex *et al*., 2017). During the experiment, bulk weights and fish counts were recorded throughout the samplings at weeks 2, 4, 6, 8 and 10. At the end of the growth study (week 12 = 84 days), all fish from each tank were weighed individually and the standard and total lengths recorded for the calculation of the final weights, SGR and condition factors. Six of each of the groups of experimental fish were randomly taken for collection of blood samples into heparinised and non- heparinised bottles for haematological and biochemical studies, dissected, their liver and intestine weighed for the calculation of the hepato-somatic index. Following measurements (weight, length and liver), the dissected fish from each tank were pooled and frozen for whole body proximate composition.

2.6 Calculations

Weight gain (g d^{-1}) was calculated as the differences between average initial (Wi) and final (Wf) body weight of fish. Specific growth rate (SGR; % BW Day^{-1}) was calculated as lnWf − lnWi × 100)/t, where lnWf is the natural logarithm of the final weight, InWi is the natural logarithm of the initial weight and t is the duration of the experiment in days (d) (Hardy and Barrows 2005). Total feed intake was taken as the summation of the feed consumed per day throughout the experimental period. Feed conversion ratio (FCR; g g^{-1}) = Feed Intake/weight gain. Protein efficiency ratio (PER) = Weight gain (g)/Protein intake (g). Protein intake = feed supplied X crude protein (%). Protein efficiency ratio (PER) is the increase in weight of fish per crude protein of feed (dry weight). The liver and intestine obtained from each of the fish dissected and weighed were used for the calculation of the hepato-intestine somatic indices, weight of each divided by weight of the fish multiplied by 100. Condition factors (K) were calculated as 100 x W/L^3 (Weight-length relationships).

Thermal Growth Coefficient (TGC) = [(3√W1 -3√W0)/(T x t)] x 1000. Where W1 is Final Weight, W0 is Initial Weight, T is Temperature in ºC, and t is time in days (Alanara *et al*., (2001). Fish survival (%) calculated as number of fish at the end of the experiment divided by the number of fish at the beginning of the experiment x 100.

2.7 Chemical and statistical Analysis

Prior to the analysis of the proximate composition, samples were placed in a Petri-dish and dried using oven (Weswox 220v AC 50HZ Electric laboratory oven, Ambala Haryana, India) at 50 - 60°C until the samples were dried to constant weight. Sample of the initial fish was analysed for proximate composition and inherent amino acid profile. Sample of the diets was also taken for microbial load. At the end of the studies, blood samples collected from random fish samples from each replicate was taken for haematological studies. The fish samples were used for somatic indices studies. Nutrigenomics analysis of the fish was carried out to study the gene expression and protein profiles of fish tissue in response to the dietary plant - based protein used.

The methods for proximate analysis were the standard procedures of AOAC (2015) moisture content was determined by oven drying until a constant weight obtained. The Ash content was determined by incinerating a 2g of each sample in a Muffle Furnace (Gallencamp – England) at 600ºC for 3 hours, the protein content was determined by micro Kjeldah method (Digestion, distillation and titration) the nitrogen was converted by (N x 6.25). Crude lipid was extracted with Diethyl ether (34.6ºC) in a Soxhlet extractor for 6 hours. Crude fibre was by Acid/base digestion using 1.25% H_2SO_4 (v/v) and 1.25% NaOH (w/v) solution. Available Nitrogen Free Extract was calculated by difference. [100 – (A + B + C + D + E)]. A – Moisture, B – Ash, C-Fibre, D – Protein and E – Fat. All proximate components were analysed in triplicate and reported on % dry weight bases. Gross energy was determined with a Bomb calorimeter (Kcal/Kg).

Analysis of Amino Acid was carried out using all solvents and reagents with analytical grades. The GC–MS analysis was performed using a Varian 3800/4000 gas chromatograph / mass spectrometer. The samples were injected into the gas chromatograph on Agilent capillary column, 30m x 0.25mm, 0.25µm film thickness using a temperature program from 70ºC, 2min, 5ºC/min to 110ºC, 10ºC/min to 290ºC, and 16ºC/min to 300ºC. The flow rate of nitrogen, the carrier gas, was 1mL/min. The following conditions were followed: transfer line temperature 250ºC, injector temperature 200ºC; ion source temperature 250ºC, splitter: 10:1. The electron energy was 70eV and the emission current, 100mA. The 15N-methionine was used as the internal standard for quantitative determination. Helium was used as the carrier gas at a flow rate of 1 mL/min. In total, 1 µL of each sample was injected in a split mode utilizing a TriPlus autosampler. The MS instrument was operated in electron ionization (EI) mode with an emission current of 100 µA and a mass

range of 40–800 amu at 70 eV; this was employed to identify the samples. The transfer line, injector, and ion source were all maintained at 250 °C.
The amino acids eluted were alanine (Ala), glycine (Gly), threonine (Thr), serine (Ser), valine (Val), leucine (Leu), isoleucine (Ile), proline (Pro), methionine (Met), phenylalanine (Phe), lysine (Lys), glutamate (Glu), and tyrosine (Tyr).

RNA Extraction from fish tissue
Fish samples' tissue was collected from each replication at the end of the experiment. 1g of specimens was collected from the ventral region of the fish (Cordero et al., 2017) into a well labelled 2ml Eppendorf tube. 1ml of Renal later buffer was used to maintain RNA stability and stored in – 20ºC refrigerators. 300ul of Beta Mercapto Ethanol (BME) was added to 30ml of lysis buffer. 8ml of absolute ethanol was added to the primary wash buffer and 32 ml of ethanol was added. RNA was isolated according to manufacturer's protocol using JENA mini kit.

Gene expression and qRT-PCR aRNA analysis
Sequences for immune response-related gene Tumour necrosis factor α (TNF α) and Insulin-like Growth Factor (IGF) were profiled in *Oreochromis niloticus* (Singha *et al.*, 2020; Li *et al*., 2021). This was to consider the methionine requirement determination of the hybrid catfish under molecular biology as TNF α has a wide range of biological roles, including the control of cell differentiation, tissue renewal and remodeling, regulation of pro-inflammatory responses as well as the stimulation of other cytokines (Sebastián *et al*., 2012).
For each of the immune response-related genes, house-keeping gene β-actin was profiled for normalization. The qRT-PCR was performed using Bio-Rad CFX96 C1000 in a one-step reaction that combines reverse transcriptase with DNA amplification. A 20 µl reaction volume containing 2 µl RNA, 1 µl Luna Reverse Transcriptase Enzyme Mix (20X), 0.8 µl each of forward and reverse primer, 10 µl Luna Universal One Step Reaction Mix (2X) and 5.4 µl PCR grade water. PCR amplifications were performed in three technical replicates. For growth hormone *Oreochromis niloticus* thermal cycling was initiated with the incubation at 55°C for 10 minutes for reverse transcription of RNA to cDNA, followed by one cycle of initial denaturation at 95ºC for 1 minute, 40 cycles of denaturation at 95ºC for 10 seconds and annealing at 59.1ºC for 30 seconds. For other reactions, the condition was same, except for annealing temperature 57.6ºC for IGF in *Oreochromis niloticus*, 53.5ºC for TNF α in *Oreochromis niloticus* and 54.6ºC for β-actin in *Oreochromis niloticus*. For negative control, qRT-PCR reaction without RNA was considered. The primer sequences for the immune response-related gene (TNF α, IGF and GH) and house-keeping gene β-actin are shown in Table 2
A sample which has an amplification curve with distinct exponential, linear and plateau phases was scored successful. All successful reactions exhibited fluorescence growth curve that crosses the repeat threshold line. The negative control samples should not exhibit any fluorescence growth curves that cross the threshold. The quantified value of RNA was expressed in terms of CT (threshold cycle) value. The relative expressions of TNF α and GH RNA were calculated by following $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001) where β-actin was used as reference gene.
ΔCT = target gene CT value - reference gene CT value
ΔCT being the crossing point deviation of an unknown sample versus a control
ΔΔCT value = ΔCT value of treatment group- ΔCT value of the lowest inclusion group
To calculate CT values according to PCR efficiency correction
The formular CTE = CT*(log(E+1)/ (log (2))
Where E is PCR efficiency of the primers determined by standard curve using serial dilution of RNA. For this purpose, the group with lowest inclusion level was used. The total RNA sample was quantified using a Nano Drop ND1000 spectrophotometer (Nano Drop Technologies, Inc., Wilmington, DE, USA at an absorbance of A260/280. For *Oreochromis niloticus*, the resulting quantity (28ng/µl) was diluted serially 14 ng/µl, 7 ng/µl, 3.5 ng/µl, 1.75 /µl, 0.875 ng/µl. The expression levels between different groups were expressed in relative fold changes.
Data obtained were subjected to two-way ANOVA in SPSS version 25.0 for windows and where significant deference was observed means were separated with Turkey's honest significance difference. Polynomial Regression analysis was used to determine requirement of methionine.

Table 2- Primers used for the quantification of TNF α (Tumor necrosis factor α) and Insulin-like Growth Factor (IGF) in *Oreochromis niloticus*

Primer	Sequence 5'- 3'	Reference
IGFFP	GGACGAGTGCTGCTTCCAAAGC	Singha et al., 2020
IGFRP	TGCTCTTGGCATGTCTGTGTGC	
β-actinFP	AATCCTGCGGAATCCACGAAAC	Singha et al., 2020
β-actinRP	CTCCTTCTGCATCCTGTCAGCG	
TNF-αF	CGTCGTCGTGGCTCTTTGTT	Li et al., 2021
TNF-αR	TGGGGCTCTGTTTTGTCGC	
β-actinF	CGGAATCCACGAAACCACCTA	Li et al., 2021
β-actinR	CCAGACGGAGTATTTACGCTCA	

3.0 Results

The results of the study are presented in tables and figures below.

Table 3: Proximate and Amino acids Composition of Experimental Diets with graded levels of Methionine fed to Hybrid and local strains of *Oreochromis niloticus*.

Parameters	DIETS						
	BASAL	**1**	**2**	**3**	**4**	**5**	**6**
Methionine levels (g/kg protein)	11.72	29.21	31.34	33.80	37.06	40.05	46.59
Moisture Content	7.94	7.94	7.84	8.04	7.94	7.84	8.04
Crude Protein (%)	34.97	33.54	34.45	34.91	34.54	34.45	33.91
Crude Fat (%)	6.70	6.73	6.70	6.68	6.73	7.70	7.73
Crude fibre (%)	6.20	6.20	6.20	6.20	6.20	6.20	6.20
Ash (%)	7.79	7.77	7.80	7.81	7.77	7.80	8.81
Nitrogen free Extract (%)	36.40	37.82	37.01	36.36	37.35	36.02	35.36
Dry Matter (%)	92.06	92.06	92.16	91.96	90.2	90.57	90.55
Gross Energy (Kcal/Kg)	2974.63	2977	2964.55	2974.20	2969.63	2974.83	2994
Amino Acids Composition							
EAA (g/kg CP)							
Phenylalanine	17.57	17.57	17.57	17.57	17.57	17.57	17.57
Histidine	3.36	3.36	3.36	3.36	3.36	3.36	3.36
Isoleucine	6.46	6.46	6.46	6.46	6.46	6.46	6.46
Lysine*	40.37	40.37	40.37	40.37	40.37	40.37	40.37
Leucine	33.87	33.87	33.87	33.87	33.87	33.87	33.87
Methionine	19.0	19.0	19.0	19.0	19.0	19.0	19.0
Valine*	30.3	30.3	30.3	30.3	30.3	30.3	30.3
Arginine	31.63	31.63	31.63	31.63	31.63	31.63	31.63
Threonine	1.96	1.96	1.96	1.96	1.96	1.96	1.96
NEAA							
Glutamic Acid*	59.83	59.83	59.83	59.83	59.83	59.83	59.83
Alanine*	26.67	26.67	26.67	26.67	26.67	26.67	26.67
Aspartic Acid*	36.1	36.1	36.1	36.1	36.1	36.1	36.1
Serine*	17.57	13.2	13.2	13.2	13.2	13.2	13.2
Glycine*	39.13	39.13	39.13	39.13	39.13	39.13	39.13
Proline*	28.87	28.87	28.87	28.87	28.87	28.87	28.87
Tyrosine*	16.23	16.23	16.23	16.23	16.23	16.23	16.23

3.1 Performance of Hybrid and Local Tilapia fed Graded Levels of Methionine

Table 4a and 4b showed the growth performance of hybrid and local tilapia. There were significant differences ($P<0.05$) in the weight gain of the fish in response to the graded levels of methionine. The weight gain (WG) of hybrid and the local significantly increased ($P< 0.05$) with respect to the graded levels of methionine inclusion. There was no significant difference between 0.57%, (29.21g/kg protein) and 0.67% (31.34g/kg protein) groups in hybrid tilapia with the highest value recorded for 0.87% (37.06g/Kg protein) inclusion level. Also, the weight gain in the local tilapia increased with the methionine inclusion levels (Table 4a) with highest value of 3.71g weight gained in 0.77% (33.80g/kg protein). There was also significant difference in the methionine levels on specific growth rate (SGR) for the hybrid compared to local strain. The highest significant SGR ($P<0.05$) was obtained in the group of hybrids that were fed with 0.87% (37.06g/kg protein) level of methionine while there was no significant difference between the groups that fed on diets with 0.57% and 0.67% levels of methionine ($P>0.05$) inclusion. For profitability in aqua feed industries, it will be very necessary to consider inclusion levels of 0.87% and 0.77% for hybrid and local strain respectively. There was significant interaction between strains and methionine levels in terms of WG and SGR. There were also significant main effects of strain and methionine levels in feed intake and feed conversion ratio ($P<0.05$) but no significant interaction. The hybrid responded significantly better compared to the local strain with respect to the parameters under consideration. Survival of the hybrid and local strain was not significantly affected by the methionine levels ($P>0.05$). There was generally high survival across the treatments.

The thermal growth coefficient (TGC) values ranged from 0.25 to 0.30 with the highest value of 0.30 recorded in fish fed with 0.87% methionine level for hybrid tilapia while the TGC values ranged from 0.17 to 0.22 in local tilapia with 0.22 as highest values recorded in 0.67% and 0.77% methionine inclusion level. There were significant main effects of strain and methionine levels on TGC with also significant interactions ($P<0.05$) (Table 4b). The condition factor as an indicator to show the fish 's wellbeing showed no significant difference with respect to the methionine levels and the strains ($P>0.05$).

To estimate the optimal requirement, methionine levels of inclusion expressed as unit per Kg of protein was used as independent factor and the measured growth indices as dependent variables such as WG, SGR and TCG. Polynomial regression was carried out with WG, SGR and TGC on methionine levels resulting in R^2 of 0.31, 0.32 and 0.30 respectively for the hybrid and 0.56, 0.62 and 0.61 for the local tilapia respectively. The R^2 values for both strains indicated an association of methionine with growth of the fish. Association was exhibited in both strains with that of the local higher than that of hybrid. In the case of the hybrid the polynomial regression showed that WG, SGR and TGC increased as methionine levels increases and peaked at 0.87% inclusion and there after reduced at subsequent levels indicating a methionine requirement level with optimum performance attainable at 37.06g/Kg protein, and 37.06kg/protein respectively (Table 4a) (Figures 1, 3, 6 and 5). The regression in the local strain indicated a requirement for optimum performance attainable at 33.5g methionine/kg protein.

Haematological parameters are represented in Table 5a, 5b and 5c. There were significant main effects of strain and methionine levels on the haematological parameters such as white blood cell counts (WBC), red blood cell counts (RBC), Haemoglobin content (HGC), platelets (PTL) ($P<0.05$). There were significant differences ($P<0.05$) in the effect of methionine levels on the blood parameters, although an inconsistent trend was observed. The WBC and PLT were higher in the lower levels of methionine inclusion than the higher levels. Methionine levels significantly ($P<0.05$) affected the serum biochemistry of the fish (Table 6), which correlated with changes in growth patterns, particularly weight gain. There were significant differences in the parameters considered ($P<0.05$). The highest serum protein of 30.62±0.94g/L was observed in the group of hybrids that fed on diet with 37.06g methionine /Kg protein (0.87%) while the highest serum protein of 31.59±0.95g/L was obtainable in local tilapia group that fed on 33.80g of Methionine /Kg protein (0.77%).

The results of the proximate composition of the fish at the end of the experiments revealed a significant increase in crude protein with increase in methionine levels highlighting its influence on growth parameters (Tables 7 & 8). The crude protein increased at higher levels of inclusion and was higher than at lower levels (Table 7).

Figures 8 and 9 present the summarized gene expression and qRT-PCR analysis results, illustrating the levels of TNF α and IGF in both the hybrid and *Oreochromis niloticus* at the end of the experimental period. The TNF α increased with the graded levels of methionine with highest values observed in the groups that were fed with the diet containing 37.06 and 33.8g/kg protein in the hybrid and *O. niloticus* respectively.

Table 4a: Growth Performance and nutrient utilization hybrid and local strains of *Oreochromis niloticus*.

DL-Meth. Levels	Strains	Initial weight (g/fish)	Initial T Length cm	Wt. Gain g/Fish	Final Total Length Cm/Fish	Final Std Length cm/Fish	SGR g/fish/day	Feed Intake	FCR
29.21	Hybrid	1.98±0.02^{a}	5.76±0.02^{a}	4.68±0.26^{c}	7.13±0.02^{a}	5.63±0.12^{b}	1.09±0.06^{b}	21.63±1.63^{b}	4.62±.17^{a}
31.34	Hybrid	1.98±0.02^{a}	5.66±0.22^{a}	4.61±0.13^{c}	7.57±0.11^{a}	5.90±0.12^{a}	1.09±0.06^{b}	22.31±1.17^{b}	4.83±.07^{a}
33.80	Hybrid	1.98±0.03^{a}	5.70±0.12^{a}	5.29±0.10^{b}	7.23±0.02^{a}	5.76±0.01^{a}	1.17±0.02^{a}	22.79±1.54^{b}	4.31±.12^{b}
37.06	Hybrid	1.98±0.01^{a}	5.43±0.05^{a}	6.39±0.29^{a}	7.23±0.03^{a}	5.70±0.11^{a}	1.08±0.05^{c}	21.87±.43^{c}	3.42±.03^{c}
40.05	Hybrid	2.03±0.11^{a}	5.60±0.02^{a}	4.85±0.21^{b}	7.40±0.12^{c}	5.63±0.12^{b}	1.07±0.04^{bc}	22.82±.63^{b}	4.70±.16^{a}
46.59	Hybrid	2.10±0.12^{a}	5.90±0.11^{a}	4.81±0.32^{b}	7.00±0.06^{b}	5.86±0.12^{a}	1.05±0.08^{a}	23.37±3.65^{a}	4.86±.09^{a}
29.21	Local	1.97±0.08^{a}	3.56±0.02^{a}	3.03±0.9^{b}	6.40±0.01^{a}	2.93±0.01^{a}	0.80±0.28^{a}	8.29±5.72^{c}	2.73±.87^{a}
31.34	Local	1.98±0.08^{a}	3.68±0.02^{a}	3.62±0.04^{a}	6.43±0.12^{a}	3.00±0.02^{a}	0.92±0.01^{a}	7.96±0.15^{c}	2.19±1.65^{c}
33.80	Local	2.00±0.04^{a}	4.05±0.01^{a}	3.71±0.5^{a}	6.30±0.11^{a}	3.00±0.00^{a}	0.92±0.20^{a}	6.77±7.97^{f}	1.82±1.04^{d}
37.06	Local	2.02±0.06^{a}	3.90±0.02^{a}	3.00±0.39^{b}	6.70±0.02^{a}	2.97±0.04^{a}	0..77±0.14^{a}	7.5±2.46^{d}	2.5±.52^{b}
40.05	Local	2.00±0.05^{a}	3.6±0.03^{a}	3.11±0.40^{ab}	6.46±0.02^{a}	2.93±0.02^{a}	0.80±0.33^{a}	7.21±6.99^{f}	2.32±1.68^{b}
46.59	Local	2.00±0.05^{a}	3.87±0.07^{a}	2.81±1.11^{b}	6.66±0.02^{a}	3.03±0.01^{a}	0.68±0.28^{b}	8.1±2.78^{d}	2.88±.79^{a}
DLMeth									
29.21		1.98±.02	3.85±.14	3.85±7.54^{c}	6.77±3.14	4.28±2.63	0.95±40c	14. 96 ±24.97	3.68±.64
31.34		1.98±.11	4.67±.50	4.12±8.51^{b}	7.00±3.20	4.45±2.64	1.01±.38^{a}	15.63±20.65	3.51±1.10
33.80		1.99±.20	4.88±.63	4.50±10.9^{a}	6.76±3.96	4.38±3.41	1.05±.48^{a}	14.78±27.36	3.06±.78
37.06		2.02±.12	4.57±.43	4.69±13.7^{a}	6.96±3.61	4.34±3.22	0.92±.61^{ab}	14.68±22.36	2.96±.80
40.05		2.02±.24	4.93±.66	3.98±.7^{a}	6.93±3.42	4.28±2.89	0.94±.66^{ab}	14.79±27.78	3.51±1.09
46.59		2.05±.21	4.89±.38	3.81±.12	6.83±4.17	4.45±3.52	0.87±.69^{b}	15.75±26.42	3.87±1.35
Strains									
Hybrid		2.00±.20	10.81±.60	28.13±4.54^{a}	7.26±.98	2.98±.20	1.09±.4	22.46±3.7	4.45±.41
Clarias		2.01±.15	9.46±.26	6.69±1.68^{b}	6.49±.22	5.75±0.6	0.82±.13	6.79±9.56	2.41±1.4
Two Way ANOVA									
D L Methionine		NS	NS	**	NS	NS	**	**	NS
Strains		NS	NS	**	**	**	**	**	NS
Interactions		NS	NS	**	NS	NS	**	**	**

Table 4b: Nutrient utilization and somatic index of hybrid and local strains of *Oreochromis niloticus*.

DL-Meth. Levels g/Kg Protein	Strains	Protein Intake (g/fish)	PER	Survival %	Condition Factor	Thermal Growth Coefficient	Hepatho Somatic Index	Intestinosomatic Index
29.21	Hybrid	7.57±.34^{b}	1.61±.06^{b}	76.00±3.85	0.69±.09	0.26±.01	1.58±.14	3.45±.03^{b}
31.34	Hybrid	7.81±.08^{b}	1.69±.03^{a}	80.00±3.85	0.84±.097	0.26±.02	1.44±.10	3.42±.18^{b}
33.80	Hybrid	7.98±.02^{b}	1.51±.05^{c}	80.01±3.85	0.85±.12	0.28±.03	1.44±.12	3.39±.48^{ab}
37.06	Hybrid	7.65±.14^{b}	1.20±.02^{c}	84.55±3.85	1.00±.09	0.30±.01	1.78±.26	4.01±.04^{a}
40.05	Hybrid	7.98±.80^{b}	1.64±.04^{b}	80.00±00	0.87±.16	0.25±.21	1.54±.16	3.67±.12^{b}
46.59	Hybrid	8.17±.46^{a}	1.70±.04^{c}	84.08±4.13	0.76±09	0.26±.01	1.34±.03	3.23±.07^{ab}
29.21	Local	2.90±2.29	0.96±.35^{b}	80.33±7.64	0.79±.25	0.19±.01	1.36±.12	3.25±.40^{b}
31.34	Local	2.79±6.36	0.77±.66^{c}	75.00±7.64	0.75±.53	0.22±.01	1.27±.11	3.13±.13^{b}
33.80	Local	2.36±3.19	0.64±.42^{c}	75.00±7.64	1.19±.17	0.22±.01	1.55±.13	3.55±.24^{a}
37.06	Local	2.62±.99	0.87±.11^{b}	80.00±5.77	0.96±.29	0.19±.01	1.38±.12	3.12±.13^{b}
40.05	Local	2.52±2.79	0.81±.27^{b}	75.00±5.00	0.86±.24	0.19±.01	1.48±.20	3.24±.15^{b}
46.59	Local	2.83±1.12	1.00±.22^{a}	85.00±1.23	0.70±.11	0.17±.01	1.44±.13	3.12±.32^{b}
DLMeth								
29.21		5.23±1.21	1.28±.13	68.05±33.0	.97±.52	0.23±.04^{b}	1.47±.21	3.35±.16^{b}
31.34		5.4±.22	1.23±.44	63.61±35.4	1.03±.72	0.24±.11^{b}	1.35±.20	3.27±.32^{b}
33.80		5.17±0.44	1.08±.80	71.11±27.32	1.49±.75	0.25±.15^{b}	1.50±.13	3.47±.54^{b}
37.06		5.13±8.94	1.04±.11	63.61±35.26	1.14±.53	0.33±.21^{a}	1.38±.20	3.57±.53^{a}
40.05		5.25±0.11	1.23±.56	70.00±33.01	1.25±.42	0.25±.12^{a}	1.51±.17	3.46±1.1^{b}
46.59		5.5±0.36	1.35±.97	66.53±35.64	0.95±.31	0.22±.04^{b}	1.39±.25	3.18±.12^{c}
Strains								
Hybrid		7.86±1.3	1.56±.56	97±3.46	0.83±.14^{b}	0.31±.05	1.35±.41	3.52±.21
Clarias		2.67±0.27	0.84±.12	37.22±8.78	1.46±.64^{a}	0.19±.13	1.41±.35	3.23±.40
Two Way ANOVA								
D L Methionine		NS	**	**	NS	**	NS	**
Strains		NS	NS	**	**	**	**	**
Interactions		NS	**	**	NS	**	NS	NS

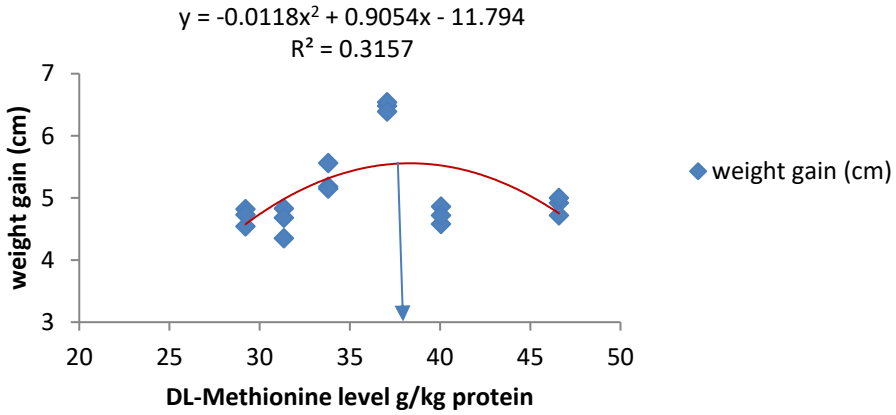


Figure 1: Weight Gain response of hybrid strains to graded levels of DL-Methionine

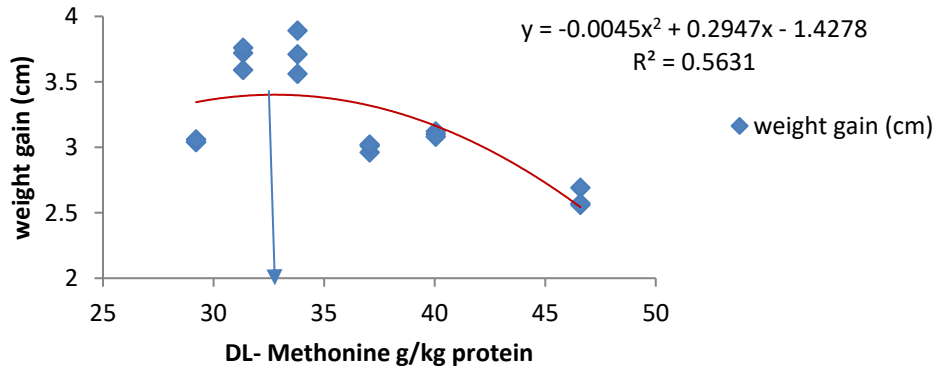


Figure 2: Weight Gain response of local strains to graded levels of DL-Methionine

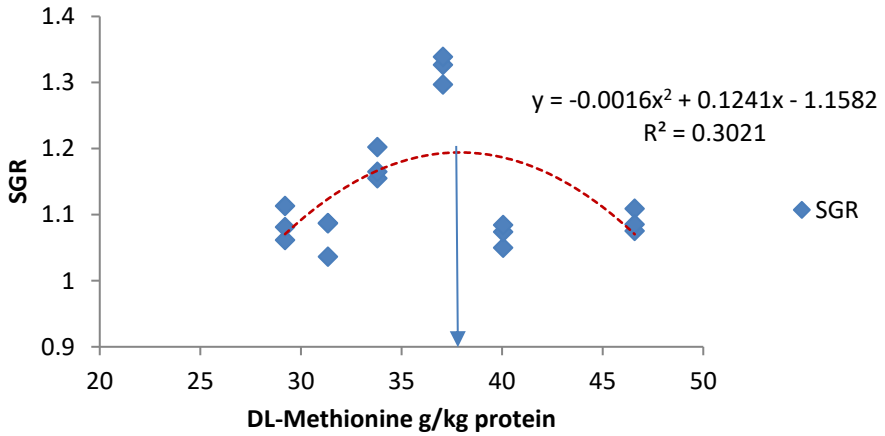


Figure 3: Specific Growth Rate response of Hybrid strains to graded levels of DL-Methionine

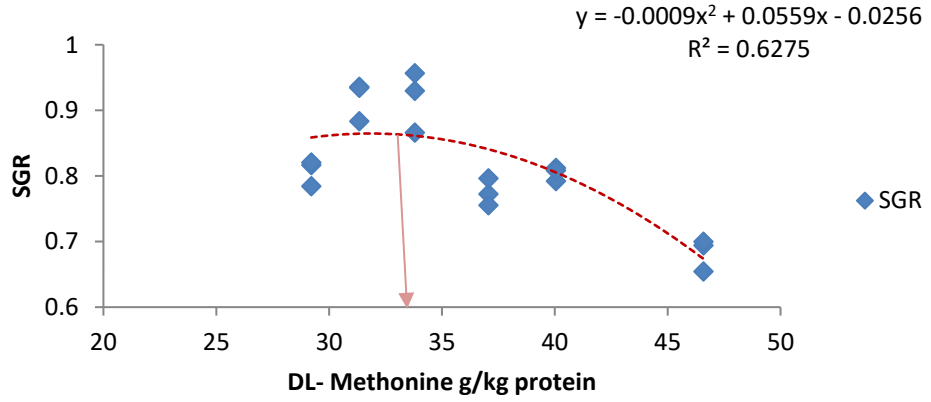


Figure 4: Specific Growth Rate response of local strains to graded levels of DL-Methionine

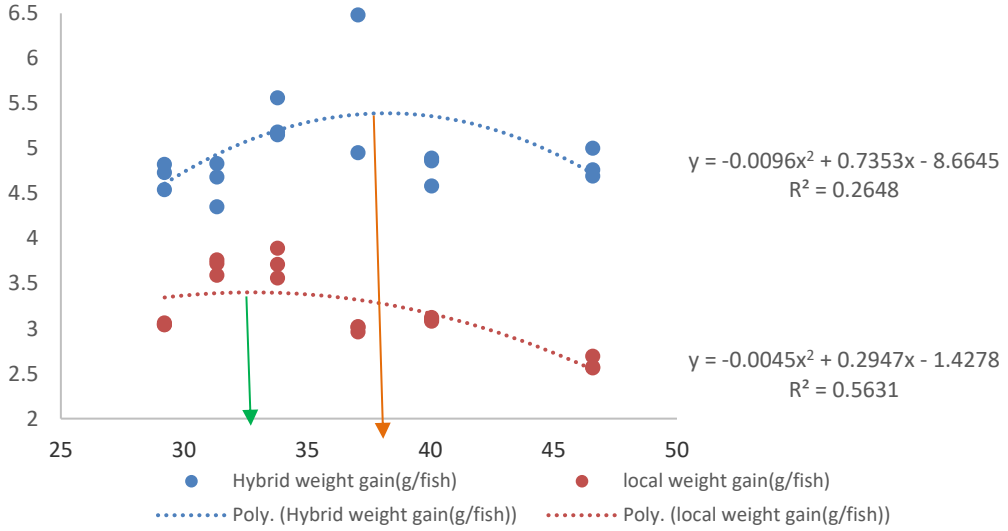


Figure 5: Weight gain of Hybrid and *Oreochromis niloticus* fed dietary levels of Methionine 0-12 weeks.

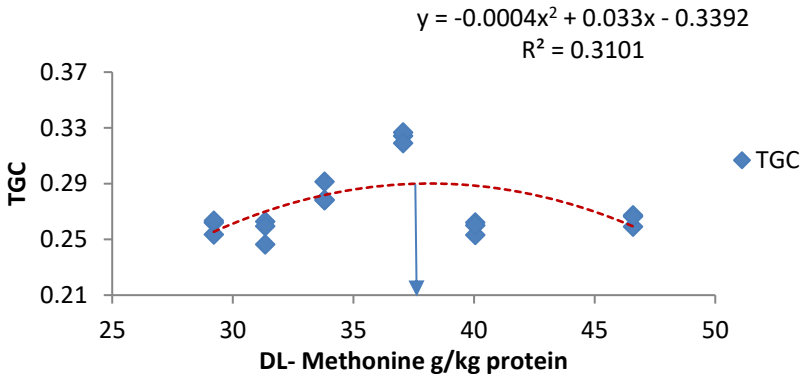


Figure 6: Thermal growth coefficient of Hybrid tilapia fed dietary levels of Methionine 0-12 weeks.

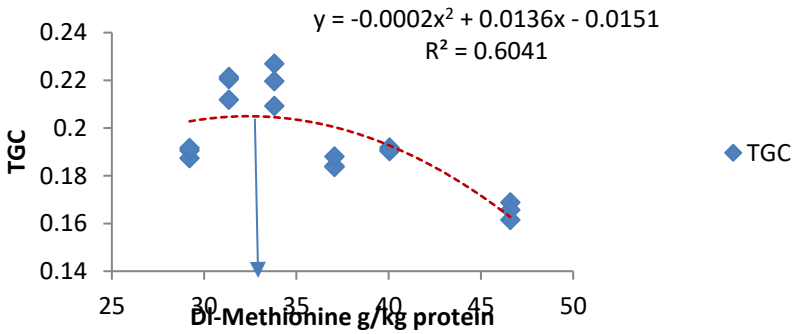


Figure 7: Thermal growth coefficient of local Tilapia fed dietary levels of Methionine 0-12 weeks

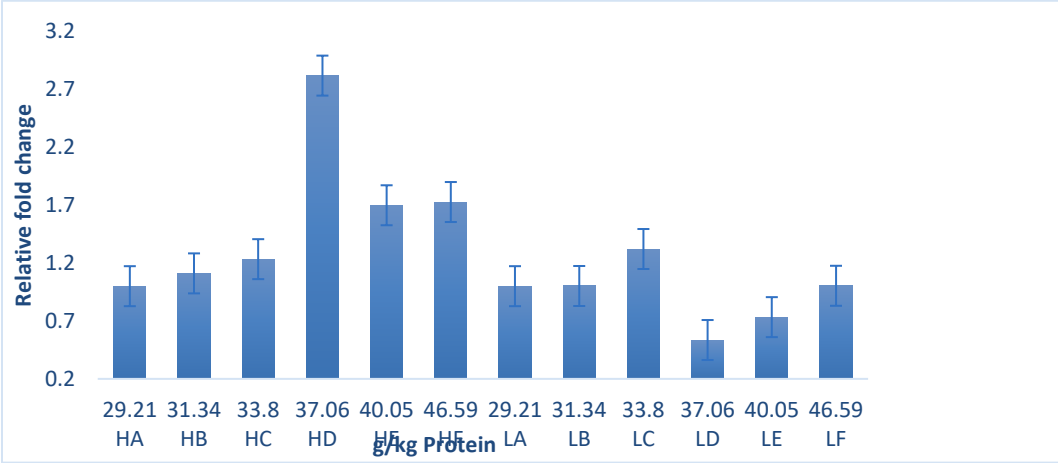


Figure 8: Hybrid and Oreochromis niloticus (Tilapia); Insulin-dependent Growth Factor (IGF)

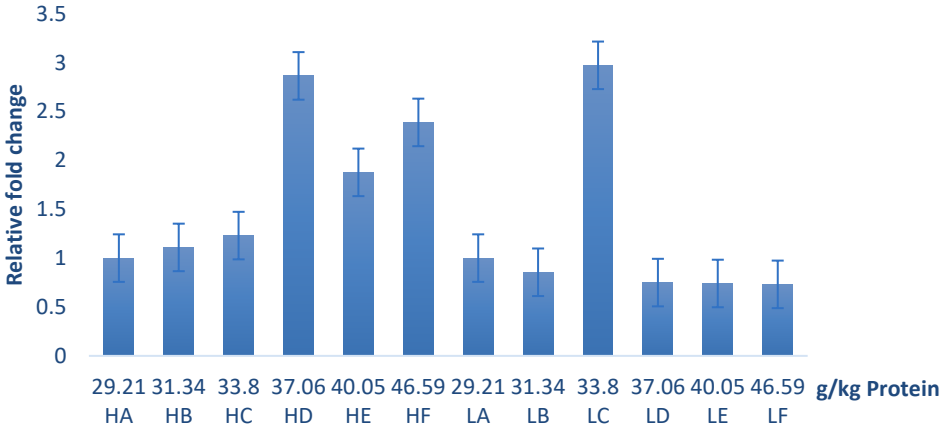


Figure 9: Hybrid and *Oreochromis niloticus* (Tilapia); Tumour Necrotic Factor-α (TNF

Table 5a: Hematological parameters of hybrid Tilapia and local strains of *Oreochromis niloticus* fed graded levels of methionine 0 – 12 weeks.

DL-Meth. Levels	Strains	WBCx109/L	RBCx109/L	HGBg/L	HCTL/L	MCV FL	MCH Pg	MCHC g/L	PLT X 10^9/L
0.57	Hybrid	3.94±0.12^{b}	3.56±0.16^{a}	75.00±8.00^{b}	0.29±0.02^{a}	83.30±3.20^{c}	20.95±1.35^{c}	252.50±25.50^{b}	16.50±0.50^{a}
0.67	Hybrid	4.59±0.08^{a}	3.22±0.07^{a}	93.50±5.50^{a}	0.24±0.02^{a}	72.85±0.35^{d}	27.25±1.35^{b}	374.00±20.00^{a}	15.00±1.00^{a}
0.77	Hybrid	4.23±0.12^{a}	3.13±0.66^{a}	62.00±3.00^{c}	0.22±0.01^{a}	75.15±12.35^{d}	20.43±3.28^{c}	272.00±1.00^{b}	15.00±0.0^{a}
0.87	Hybrid	3.77±0.06^{b}	2.12±0.02^{a}	70.00±6.0^{bc}	0.25±0.01^{a}	120.50±1.46^{a}	32.99±2.52^{a}	273.50±17.50^{b}	13.00±1.0^{a}
0.97	Hybrid	3.34±0.33^{b}	2.51±0.16^{ab}	66.00±1.0^{bc}	0.25±0.00^{a}	102.50±7.80^{b}	26.40±2.10^{bc}	257.00±1.00^{b}	13.50±0.50^{c}
1.17	Hybrid	3.29±0.01^{b}	2.86±0.05^{a}	56.00±2.00^{d}	0.22±0.00^{a}	77.45±0.15^{d}	19.50±0.40^{d}	251.50±4.50^{b}	15.50±0.50^{b}
0.57	Local	4.39±.0.13	4.39±0.13a	89.50±.0.5	0.29±0.0	67.60±1.20^{d}	20.40±0.50^{d}	302.00±2.0^{a}	18.00±1.0
0.67	Local	5.15±0.5	5.15±.0.5	109.0±10.0	0.34±0.02	67.30±2.0^{d}	21.20±0.10^{d}	315.00±8.0^{a}	19.5±1.5
0.77	Local	2.10±.0.09	4.93±.0.07	101.25.00±1.0	0.32±0.01	65.80±1.20^{d}	20.60±0.00^{d}	312.50±5.5^{a}	20.5±.0.5
0.87	Local	2.80±.0.01	5.05 ±.01	105.0±.1.00	0.34±0.00	66.85±0.75^{d}	20.80±0.40^{d}	311.00±2.0^{a}	17.00±00
0.97	Local	2.62±.0.04	4.26±.02	91.0±.2.00	0.29±0.01	69.45±2.85^{d}	21.40±0.60^{d}	308.50±7.5^{a}	15.5±.0.5
1.17	Local	5.71±.0.13	4.74±.01	94.5±4.5	0.30±0.00	63.55±2.31^{d}	19.95±0.05^{d}	314.50±14.5^{a}	18.5±0.5
DLMeth									
0.57		4.24±.0.45	3.97±.0.47	82.25±9.4	0.29±0.0	75.45±8.80^{d}	20.67±0.95^{a}	277.25±31.6^{a}	17.25±1.1
0.67		3.90±.0.76	4.28±.0.99	101.25±11.1	0.29±0.05	70.07±3.35^{a}	24.22±3.42^{c}	344.50±35.1^{a}	17.25±2.7
0.77		3.16±.1.17	4.03±.1.06	81.75±21.7	0.27±0.05	70.47±9.37^{d}	20.51±2.07^{a}	292.25±22.4^{a}	17.75±3.03
0.87		3.28±.0.53	3.58±.1.60	87.50±19.6	0.29±0.04	93.67±29.4^{a}	26.89±6.86^{c}	292.25±23.3^{a}	15.00±2.28
0.97		2.95±.0.13	3.38±.0.97	78.50±13.8	0.27±0.04	85.97±18.7^{d}	23.90±3.06^{c}	282.75±28.6^{a}	14.5±1.18
1.17		4.49±10.32	3.80±.1.03	75.25±21.3	0.26±0.04	70.50±7.82^{a}	19.72±0.35^{c}	283.00±35.8^{a}	17.00±1.7
Strains									
Hybrid		3.86±1.29	2.94±0.57.	70.41±12.9	.25±.03	88.62±18.52	24.58±.5.21	280.08±45.9	14.75±1.34
Local		3.50±.0.98	4.75±.0.4	98.42±8..4	.31±.02	77..69±17.1	20.72±.0.58	310.58±7.9	18.16±1.81
Two Way ANOVA									
D L Methionine		**	**	**	**	**	**	**	NS
Strains		NS	NS	**	**	**	**	**	NS
Interactions		NS	NS	**	NS	NS	**	**	**

Table 5b: Hematological parameters of hybrid Tilapia and local strains of *Oreochromis niloticus* fed graded levels of methionine 0 – 12 weeks.

DL-Meth. Levels	Strains	RDW-SD FL	RDW-CV	PDW FL	MPV FL	P-LCR	PCT	Neut x 10	Lymp x 10
0.57	Hybrid	38.00±.0.9	13.80±0.3	10.20±0.5	9.55±0.05	3.80±0.1	.25±0.05	.76±0.11	2.75±0.02
0.67	Hybrid	40.00±.0.2	13.40±0.1	11.35±0.25	9.65±0.05	3.65±0.15	.26±0.0	1.50±0.28	2.63±0.35
0.77	Hybrid	36.55±0.25	13.50±0.2	12.10±0.4	10.80±0.15	3.80±0.1	.15±0.05	1.20±0.32	2.59±0.56
0.87	Hybrid	36.40±0.7	13.30±0.3	15.00±0.2	10.60±0.2	2.65±0.25	.35±0.05	1.30±0.05	1.70±0.02
0.97	Hybrid	41.60±0.8	14.80±0.7	13.05±0.75	10.95±0.35	3.70±0.1	.33±0.06	1.20±0.30	1.60±0.05
1.17	Hybrid	36.90±0.2	13.80±1.5	15.95±0.15	12.15±1.05	2.35±0.05	.37±0.04	.80±0.01	1.81±0.09
0.57	Local	40.05±3.85	16.55±1.05	5.450±0.15	6.550±0.05	3.80±0.00	.20±0.09	1.5±0.11	2.61±0.69
0.67	Local	34.75±2.45	14.50±0.3	5.850±0.55	6.85±0.35	2.55±0.25	.17±0.01	1.63±0.38	1.32±0.28
0.77	Local	34.30±2.1	14.35±0.45	6.400±0.9	7.50±0.7	4.55±0.05	.15±0.06	.81±0.06	1.07±0.00
0.87	Local	35.15±0.75	14.45±0.05	5.650±0.05	6.55±0.25	3.95±0.35	.17±0.05	1.10±0.02	1.49±0.02
0.97	Local	46.15±2.45	18.75±1.05	7.800±0.4	8.40±0.1	3.40±0.3	.13±0.03	.81±0.06	1.43±0.03
1.17	Local	27.60±1.4	15.10±0.4	5.600±0.00	6.40±0.1	3.60±0.2	.21±0.04	1.88±0.11	3.07±0.00
DLMeth									
0.57		39.02±2.7	15.17±1.65	7.82±0.9	8.05±1.64	3.80±0.05	.22±0.06	1.13±0.41	2.68±0.44
0.67		37.37±3.3	13.95±0.63	8.60±0.9	8.25±1.54	3.10±0.05	.22±0.63	1.57±0.31	1.98±0.77
0.77		35.42±1.81	13.92±0.56	9.25±0.9	9.1±1.88	4.17±0.05	.15±0.41	1.02±0.30	1.83±0.91
0.87		35.77±0.94	13.87±0.65	10.30±0.9	8.57±2.23	3.30±0.05	.26±0.76	1.21±0.13	1.59±0.11
0.97		43.87±2.94	16.77±2.3	10.42±0.9	9.67±3.21	3.55±0.05	.23±0.25	1.04±0.32	1.51±0.10
1.17		32.25±5.17	14.45±1.21	10.77±0.9	9.27±3.21	2.97±0.05	.29±0.6 9	1.34±0.59	2.44±0.70
Strains									
Hybrid		38.24±2.05	13.76±0.78.	12.94±2.09	10.62±.0.98	3.32±0.62	0.28±.0.86	1.15±0.33	2.18±1.34
Local		36.33±6.18	15.62±1.72	6.12±0.92	7.04±0.78	3.64±0.65	0.17±.0.04	1.22±.44	1.83±0.80
Two Way ANOVA									
D L Methionine		**	**	**	**	**	**	**	NS
Strains		NS	NS	**	**	**	**	**	NS
Interactions		NS	NS	**	NS	NS	**	**	**

Table 5c: Hematological parameters of hybrid Tilapia and local strains of *Oreochromis niloticus* fed graded levels of methionine 0 – 12 weeks.

DL-Meth. Levels	**Strains**	**Mono x 10**	Eos X10	Bas x 10	Neut %	Lymph	Mono %	Eos %	Bas %	IG X 10	IG %
0.57	Hybrid	.22±0.01	.00	.21±0.02	22.10±1.35	68.35±1.15	5.70±0.20	.00	3.85±0.35	.01±0.00	.35±0.05
0.67	Hybrid	.15±0.35	.00	.29±0.11	26.10±1.0	66.70±0.5	4.25±0.45	.00	2.95±0.05	.02±0.01	.30±0.00
0.77	Hybrid	.21±0.01	.00	.19±0.11	26.80±0.9	64.60±2.0	5.00±0.40	.00	3.60±0.70	.45±0.44	.25±0.05
0.87	Hybrid	.24±0.02	.00	.48±0.04	23.65±0.45	67.35±0.65	5.45±0.35	.00	3.55±0.75	.76±0.14	.25±0.05
0.97	Hybrid	.25±0.15	.00	.20±0.01	25.90±1.2	62.70±2.8	6.60±0.30	.00	5.80±0.30	.77±0.04	.30±0.00
1.17	Hybrid	.39±0.15	.00	.27±0.08	22.35±1.15	71.00±1.1	4.00±0.20	.00	2.65±0.25	.91±0.30	.30±0.10
0.57	Local	.29±0.10	.00	.14±0.35	38.65±0.95	48.55±1.55	9.65±0.15	.00	3.15±0.75	.04±0.01	1.0±0.00
0.67	Local	.13±0.01	.00	.10±0.02	35.70±3.9	56.50±5.5	4.20±0.50	.00	3.60±1.10	.01±0.01	.04±0.04
0.77	Local	.11±0.05	.00	.10±0.03	40.15±0.15	53.35±0.15	3.15±0.15	.00	3.35±0.15	.01±0.00	.50±0.00
0.87	Local	.09±0.00	.00	.11±0.00	39.25±0.85	53.45±0.65	3.40±0.20	.00	3.90±0.00	.02±0.00	.70±0.00
0.97	Local	.27±0.09	.00	.10±0.02	29.65±1.01	54.60±0.3	12.00±1.5	.00	4.00±1.00	.01±0.01	.60±0.20
1.17	Local	.56±0.02	.00	.19±0.00	35.50±1.4	52.20±0.5	9.05±0.25	.00	3.25±0.05	.02±0.00	.35±0.05
DLMeth											
0.57		.25±0.07	.00	.17±0.04	30.37±.9.12	58.45±.10.9	7.67±2.16	.00	3.50±0.42	.02±0.02	.67±0.352
0.67		.14±0.02	.00	.19±0.12	30.90±.5.84	61.60±.6.5	4.22±0.50	.00	3.27±0.78	.02±0.01	.17±0.14
0.77		.16±0.06	.00	.14±0.09	33.47±.7.33	58.97±.6.29	4.07±1.04	.00	3.47±0.47	.23±0.37	.37±0.14
0.87		.17±0.08	.00	.29±0.20	31.45±.8.56	60.40±.7.6	4.42±1.15	.00	3.72±0.51	.39±0.41	.47±0.24
0.97		.26±0.05	.00	.15±0.05	27.77±.2.28	58.65±.4.7	9.30±3.11	.00	4.90±1.18	.39±0.41	.45±0.20
1.17		.47±0.09	.00	.23±0.07	28.92±.7.29	1.60±.10.32	6.52±2.82	.00	2.95±0.36	.46±0.52	.32±0.07
Strains											
Hybrid		0.25±0.07	.00	0.27±0.12	24.48±.2.1	66.78±3.03	5.17±.0.94	.00	3.73±1.11	0.49±0.41	0.29±0.05
Local		0.24±.0.13	.00	0.12±0..03	36.48±.3.9	53.10±3.19	6.90±3.61	.00	.3.54±0.66	0.02±0.00	0.53±0.31
Two Way ANOVA											
D L Methionine		**	**	**	**	**	**	NV		NS	NS
Strains		NS	NS	**	**	**	**	NV		NS	NS
Interactions		NS	NS	**	NS	NS	**	NV		**	**

Table 6: Serum Bio-Chemistry parameters of hybrid of Hybrid and local tilapia fed graded levels of methionine 0 – 12 weeks.

DL-Meth. Levels	Strains	Protein/g	Aspartate transaminase (AST) U/L	Alanine transaminase (ALT)U/L	Alkaline phosphatase (ALP) U/L	ALBUMIN g/dL	UREA mg/dl	CREATININE g/L
0.57	Hybrid	29.76±0.61^{a}	7.44±.64^{a}	6.30±.87^{a}	32.00±1.41^{d}	3.36±.02^{c}	14.90±.02^{c}	1.71±.02^{c}
0.67	Hybrid	28.57±0.09^{b}	6.41±.09^{b}	5.93±.82^{abc}	41.00±1.41^{c}	3.67±.02^{d}	12.88±.46^{c}	1.63±.08^{a}
0.77	Hybrid	30.53±0.67^{a}	5.58±.11^{bc}	5.06±.91^{bc}	52.00±4.24^{b}	2.82±.03^{c}	14.88±.45^{c}	2.19±.03^{c}
0.87	Hybrid	30.62±0.13^{a}	7.81±.04^{a}	6.61±.07^{ab}	60.00±1.41^{a}	3.51±.05^{d}	16.68±.02	1.64±.04^{d}
0.97	Hybrid	28.71±0.08^{b}	4.93±.08^{c}	4.45±.33^{c}	39.50±2.12^{c}	4.58±.04^{c}	13.73±.06^{c}	1.49±.03^{b}
1.17	Hybrid	28.87±1.34^{b}	5.95±.11^{bc}	5.51.±.43^{bc}	31.00±1.41^{d}	3.69±.04^{a}	15.40±.72^{c}	1.39±.04^{c}
0.57	Clarias	28.92±0.55^{a}	5.08±.34	4.02±.65	32.00±1.41^{b}	2.90±.30	14.80±.74	1.69±.04
0.67	Clarias	30.00±0.53^{b}	6.29±.31	6.04±.1.36	41.00±1.41^{b}	3.12±.13	12.35±.64	1.40±.09
0.77	Clarias	31.59±0.69^{a}	5.48±.09	5.06±.48	52.00±4.24^{c}	2.73±.05	13.80±.11	2.00±.03
0.87	Clarias	28.69±0.06	7.06±.06	6.60±.36	60.00±1.41^{d}	3.99±.10	16.72±.43	1.64±.08
0.97	Clarias	29.91 ±.75	8.12±.29	7.49±0.40	39.50±2.12^{b}	4.13±.03	19.30±.11	1.78±.33
1.17	Clarias	30.58±0.04	6.59±.01	5.85±.63	31.00±1.41^{a}	3.84±.06	20.92±.16	2.13±.09
DLMeth								
0.57		30.34±1.71^{bc}	6.76±.08^{b}	5.66±1.99^{b}	32.75±1.2^{b}	3.13±.35^{b}	14.85±.91^{b}	1.71±.02$^{c\ a}$
0.67		29.31±0.91^{b}	6.35±.25^{b}	5.98±0.92^{ab}	35.00±6.9^{d}	3.39±.24^{c}	12.60±.74^{d}	1.51±.95^{a}
0.77		31.06±0.72^{a}	5.53±.24^{c}	5.06±.59^{ab}	44.50±9.0^{a}	2.77±.78^{e}	14.34±1.1^{b}	2.10±.13^{a}
0.87		29.66±1.11^{ab}	7.44±.74^{a}	6.60±.21^{a}	51.75±9.5^{a}	3.75±.12^{d}	16.70±.28^{c}	1.64±0.07^{a}
0.97		29.31±0.82^{ab}	6.52±.85^{b}	5.97±1.77^{b}	38.75±1.7^{c}	4.36±.12^{d}	16.52±.24^{c}	1.78±0.33^{a}
1.17		29.72±1.25^{ab}	6.27±.76^{b}	5.68±.48^{ab}	29.75±1.7^{b}	4.36±.20^{a}	18.17±1.2^{d}	1.76±.43^{a}
Strains								
Hybrid		28.84±1.34	6.52±1.33	5.81±1.09	34.91±5.5	2.15±.35	14.74±1.29	1.68±.26
Clarias		29.95±1.07	6.43±1.05	5.84±1.26	42.58±10.9	1.8±.41	16.31±3.16	1.82±.28
Two Way ANOVA								
D L Methionine		**	**	**	**	**	**	**
Strains		**	**	NS	**	**	NS	**
Interactions		**	**	**	**	**	**	**

T able 7: Proximate Composition of Hybrid and local Tilapia Fish fed with Graded levels of Methionine 0-12 weeks

DL-Meth. Levels	Strains	MC%	Ash %	Crude Fibre %	Crude Protein %	Crude Fat %	NFE %
0.57	Hybrid	11.19±1.0	16.36±.81^{e}	0.69±.01	44.72±1.45^{d}	21.07±1.3	5.94±1.37
0.67	Hybrid	9.21±.24	14.41±.41^{e}	0.72.±.10	54.19±.46^{c}	19.66±1.16	1.71±1.56
0.77	Hybrid	11.92±.92	15.73±.04^{d}	0.54±.04	47.70±.43^{d}	23.26±1.17	.83±.88
0.87	Hybrid	13.77±1.17	9.99±.47^{d}	0.43±.03	53.83±.51^{a}	18.94±.77	3.03±1.73
0.97	Hybrid	11.44±.23	12.77±.16	0.64±.03	53.57±.40^{a}	20.38±.17	1.17±0.05
1.17	Hybrid	8.83±.53	13.47±1.3^{f}	0.70±.04	57.25±.76^{b}	18.71±.06^{a}	1.02±2.1
0.57	local	4.29±.15	13.81±1.08^{c}	0.12±.12	51.04±.93^{e}	9.78±.66^{b}	20.92±.61
0.67	local	3.92±.19	13.34±.04^{b}	0.21±.01	47.93±.78^{f}	9.31±.25	25.27±.52
0.77	local	5.41±.21	15.98±.90^{b}	0.25±.01	44.37±.09^{c}	5.35±.25^{a}	28.61±.62
0.87	local	4.12±.18	15.33±.59^{a}	0.19±.00	58.75d±.09^{b}	11.61±.98	9.98±.91
0.97	local	3.79±.13	18.11±.30^{a}	0.10±.01	61.21±.83^{c}	6.25±.44	10.45±1.48
1.17	local	4.59±.24	17.33±.41^{a}	0.23±.02	57.66±.44^{f}	6.36±.11^{b}	13.15±1.02
DLMeth							
0.57		7.74±3.83^{bc}	15.08±1.63^{d}	.41±.31^{ab}	47.88±3.6^{d}	15.43±6.25^{a}	13.43±8.26^{a}
0.67		6.56±2.91^{a}	13.87±.69^{c}	.47±.29^{b}	51.06±3.44^{d}	14.48±5.72^{b}	13.49±4.00
0.77		8.66±3.61^{cd}	15.86±.58^{b}	0.39±.15^{ab}	46.03±1.90^{c}	14.30±9.84^{a}	14.72±6.69^{b}
0.87		8.94±5.33^{d}	12.66±2.93^{a}	0.31±.13^{c}	56.29±2.71^{a}	15.27±4.09^{c}	6.50±2.58^{d}
0.97		7.61±4.2^{e}	15.44±2.93^{a}	0.37±.29^{a}	57.39±.42^{b}	13.31±7.74^{bc}	5.81±.5.16^{c}
1.17		6.71±3.65^{b}	15.40±2.28^{b}	0.47±.26^{b}	57.45±.61^{c}	12.53±6.76^{a}	7.08±.58^{b}
Strains							

Hybrid	11.06±1.83	13.81±1.08	.62±.11	51.88±4.44	20.33±1.75	2.28±2.09
Clarias	4.35±.057	15.65±1.86	0.18±.05	53.49±5.44	12.53±6.76	18.06±7.54
Two Way ANOVA						
D L Methionine	**	**	**	**	**	**
Strains	NS	NS	**	**	**	**
Interactions	**	**	**	**	**	**

a-d Means with different superscript significantly different

Table 8: Proximate composition of Hybrid and *Oreochromis niloticus* before subjection to the Experimental diets with graded levels of methionine.

Proximate/EAA	Hybrid Tilapia	Local Tilapia
Moisture Content (%)	70.15	69.50
Ash Content (%)	1.81	1.24
Crude Fat (%)	1.35	1.12
Crude Protein (%)	18.74	18.92
Crude Fibre (%)	1.26	2.16
Carbohydrate (%)	7.95	9.23
Dry matter (%)	29.85	30.50

4.0 DISCUSSION

Methionine inclusion in experimental diets resulted in improved growth and better feed utilization of hybrid strains of *Oreochromis niloticus cross with O. mossambicus* and local breed of *Oreochromis niloticus*. However, it has been reported in various studies that low dietary methionine can limit growth performance and nutrient utilization (Elesho et al., 2021). In this study, growth performance, nutrient utilization indices and other parameters were found to be affected positively with increased levels of methionine. Tilapia feeds were formulated using plant-based ingredients supplemented with synthetic methionine as the limiting amino acid to fill in the knowledge gaps in methionine requirements of hybrid strains. The poor performance of the group that fed on diet with 0.57% methionine inclusion level than other groups indicated that methionine was limiting in the basal diet. The weight gain and specific growth rate of both the hybrid and local tilapia that increased with inclusion levels up to 0.87% methionine inclusion level is an indication of positive effect on the growth parameter which were in consonant with previous studies with juvenile Nile tilapia. Weight gain of *Oreochromis niloticus* was improved when a 35% protein diet formulated from casein was supplemented with either arginine and lysine or tryptophan and methionine (Teshima *et al*., 1986). Growth and feed efficiency of hybrid tilapia, *O. niloticus x Oreochromis aureus*, were not improved when either methionine and lysine or threonine was added to a 24% protein diet containing soybean meal as the main protein source (Liou *et al*., 1986). Tacon *et al*., (1983) reported that growth of Nile tilapia was improved to a level comparable to that obtained from a fish meal-based diet when 0.8% DL-methionine was supplemented to a diet in which 75% of brown fishmeal was replaced by soybean meal. The results of this present study with *Oreochromis mosambicus* cross with *Oreochromis niloticus* (hybrid) compared with local strain of *Oreochromis niloticus* revealed that the fish were able to utilize the dietary methionine included in the plant based basal diet formulated with defatted soybean meal and groundnut cake as the main protein sources with improvement in growth indices up to 0.87% inclusion level (Nguyen and Davis 2009) after which a decline sets in at higher levels of inclusion (Lim and Webster 2006) (Figure 7). When methionine requirement has been met, increasing levels of free methionine supplementation to diets containing groundnut, soybean, fishmeal and crystalline amino acids as protein sources resulted in a significant reduction in specific growth rate of Mozambique tilapia (Jackson and Capper 1982). However, the results of the second experiment showed no adverse effects of excessive levels of methionine on weight gain, survival and FER of juvenile Nile tilapia. The difference in the response of tilapia to excessive levels of methionine may be attributed to the tilapia species used in feeding experiments.

According to Liu *et al*., (2024), methionine is important in the formation of blood proteins, globulins, albumins, antibodies, neurotransmitters, immunoglobulin and hormones. It also assists in the breakdown of fats, preventing their build up in the liver and arteries, which can obstruct blood flow to the brain, heart, and kidneys. Thus, could also be associated with the stability and survival of both the hybrid and local strains of the tilapia throughout the experiment. The physiological function of leukotrienes is orchestrating the recruitment and activation of immune cells therefore facilitating an effective immune response. The leucocytes in the haematological profile analysis ranged between

3.29±0.01 - 4.59±0.08 x 10^9/L in the hybrid and 2.10±0.09 - 5.71±0.13 x 10^9/L in the local strain groups respectively indicating slight variations in immune response between the strains. The relatively stable leucocyte counts suggest that the dietary methionine levels maintained normal immune function and did not induce physiological stress as reported by Wang et al. (2023). In this study, no health challenge was observed in the fish in all the methionine levels which reflected in the haematological parameters accessed (Shannon 2025).

The application of gene expression data along with nutrient utilization for optimization of protein requirement helps in understanding the molecular mechanism of protein on growth of fish. IGF-I expression is one of the most suitable indicators of growth in tilapia (Brown et al. 2012). The growth performance and nutrient utilization study were evaluated along with the gene expression study to get a better and clearer understanding about the molecular mechanism of growth of Hybrid and local tilapia reared in a recirculating system with graded level of methionine. Growth hormones function to promote absorption of amino acids, especially essential amino acids that cannot be synthesized intrinsically in the fish (Farmanfarmaian and Sun 1999; Li *et al*., 2009). The results of gene expression in this study showed that TNF α and IGF increased with the graded levels of methionine with highest observed in the groups that were fed with the diet containing 37.06 and 33.8g/kg protein in the hybrid and *O. niloticus* respectively.

The polynomial regression analysis observed in the hybrid strain compared with the local strain may be attributed to higher capacity for protein deposition and faster growth rate, which may be attributed to genetic improvement and enhanced nutrient utilization and dietary requirements. Genetically improved fish generally exhibit greater rates of protein accumulation and therefore require higher level of essential amino acids to sustain rapid tissue growth. This observation is consistent with the findings of Elesho et al. (2021). In the hybrid strain, weight gain (WG), specific growth rate (SGR), and thermal growth coefficient (TGC) increased progressively with dietary methionine inclusion and reached their maximum values at an estimated requirement of 37.06g methionine /kg protein (0.87% inclusion). Similarly, theoptimum methionine requirement for the local strain was estimated at 33.5g methionine/kg protein. The reduction in growth performance observed beyond these optimum levels suggests that both methionine deficiency and excess can adversely affect fish growth. The reduction in performance with respect to the higher levels of methionine inclusion might be attributed to toxic effect of S-Adenosyl methionine, a product form in methionine metabolism pathway in the liver (Ahmed 2014).

Conclusion

Methionine requirements indicated by this study of both the local tilapia and the hybrid as revealed by the polynomial regression of weight gain, specific growth rate and thermal growth coefficient were 33.5g of methionine/Kg for local tilapia and 37.06g methionine /Kg protein for the hybrid. It will be necessary to encourage feed formulators for the hybrid tilapia to consider this result for optimum performance and maximum output and better income for hybrid tilapia farmers.

Author contribution

C.I. Ayo-Olalusi; conceptualization, investigation, writing-original draft, methodology, validation, visualization, writing -review and editing, data curation. **Ibiyo, L. M. O;** conceptualization, investigation, writing-original draft, methodology, validation, visualization, writing -review and editing, data curation. **Obetta C;** writing -review and editing **Lamien N;** writing -review and editing, **Yossa, R;** conceptualization writing -review and editing. **Ahmad Fatan N;** writing -review and editing. **Owen M;** writing -review and editing.

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Conflicting Interest

The authors have no conflicting interest whatsoever.

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