Animal Feed Science and Technology 281 (2021) 115098

Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

Effect of feeding frequency on performance, nutrient digestibility, energy and nitrogen balances in juvenile African catfish (*Clarias gariepinus*) fed diets with two levels of crystalline methionine

Folasade E. Elesho [a], Saskia Kröckel [b], Elisa Ciavoni [a], David A.H. Sutter [b], Johan A. J. Verreth [a], Johan W. Schrama [a,*,1]

[a] *Aquaculture and Fisheries Group, Wageningen Institute of Animal Science (WIAS), Wageningen University, P.O. Box 338, 6700, AH, Wageningen, The Netherlands*

[b] *Skretting Aquaculture Research Centre, P.O. Box 48, 4001 Stavanger, Norway*

A R T I C L E I N F O

Keywords:
Utilization efficiency
African catfish
Feeding method
Amino acid utilization
Feeding frequency

A B S T R A C T

In this study, the effect of feeding frequency and its interaction with crystalline methionine supplementation level on performance, digestion, energy and nitrogen balances was assessed. The experiment had a 2 × 4 factorial design, testing two levels of crystalline methionine (Met) and four feeding frequencies. The two diets contained Met either just fulfilling or exceeding the Met requirement of African catfish (*Clarias gariepinus*). African catfish with initial mean weight of 44 g were allocated to one of four feeding frequencies (six, two, one time (s) per day and two times out of three days) in a recirculation aquaculture system. Fish were fed an equal daily ration for 32 days. Performance parameters, nutrient digestibility, body composition, and nutrient balances were evaluated. Except for digestible nitrogen intake and dry matter body content, none of tested parameters was affected by the interaction between dietary Met levels and feeding frequencies. Growth, energy and nitrogen gain were unaffected by feeding frequency as well as dietary Met level. FCR was low, being 0.84 averaged over all treatments. However, feeding frequency affected feed intake and the apparent digestibility coefficient (ADC) of nutrients. Feeding at lowest frequency was accompanied by a lower feed consumption compared to other frequencies ($P < 0.001$). At higher frequency, ADC was higher for macronutrients but lower for phosphorus and magnesium. It was concluded that the asynchronous availability of AA for protein synthesis, which is often suggested to cause a sub-optimal utilization of crystalline AA, was not influenced by feeding frequency. However, feeding at a low frequency hampered daily feed intake of African catfish. Whereas, higher frequency improved nutrient digestibility, though it did not result in improved growth probably due to the higher energy required for maintenance related to physical activities at higher frequency.

Abbreviations: Met, methionine; FF, feeding frequency; CAA, crystalline amino acid; ADC, apparent digestibility coefficient; BW, body weight; DM, dry matter.

* Corresponding author.
E-mail address: johan.schrama@wur.nl (J.W. Schrama).

[1] Full postal address: Aquaculture and Fisheries Group, Wageningen Institute of Animal Science (WIAS), Wageningen University, P.O. Box 338, 6700, AH, Wageningen, The Netherlands.

https://doi.org/10.1016/j.anifeedsci.2021.115098
Received 6 March 2021; Received in revised form 14 July 2021; Accepted 17 September 2021
Available online 22 September 2021

1. Introduction

Plant protein sources are increasingly used as alternative to marine protein sources in fish diets. However, plant ingredients contain antinutritional factors, which may hamper digestion and metabolism (NRC, 1993). In addition, the amino acid (AA) composition of plant ingredients is less favorable for fish (Ambardekar and Reigh, 2007), because they are often deficient in one or more essential AA (e.g., lysine and methionine). To overcome imbalances in AA composition, crystalline amino acid (CAA) are usually supplemented to diets. Dietary addition of crystalline methionine improved the growth of African catfish when fed diets based on plant-protein (Elesho et al., 2021). Nevertheless, species such as channel catfish (*Ictalurus punctatus*), carp (*Cyprinus carpio*), hybrid striped bass (*Morone saxatalis F X Morone chrysops M*) and tilapia (*Oreochromis niloticus*) have been reported to utilize CAA less efficiently compared to protein-bound AA in the diet (Lumbard, 1997; Nose and Lee, 1974; Teshima, 1990; Yamada et al., 1981; Zarate et al., 1999). This sub-optimal utilization has been ascribed to the rapid absorption of the CAA in the gastrointestinal tract compared to protein-bound AA, which are more slowly released (Nwanna et al., 2012; Plakas and Katayama, 1981; Zarate et al., 1999). Basically, it is suggested that this difference in moment of absorption may result in quick catabolization of the absorbed CAA and thus lost rather than used for protein synthesis (Ambardekar and Reigh, 2007; Nwanna et al., 2012).

It is assumed that among other factors, feeding frequency can affect the utilization efficiency of CAA. For instance, feeding at a low frequency may negate the optimum utilization of AA in a diet that contains a combination of CAA and purified ingredients (Van den Borne, 2006; Van den Borne et al., 2006). One way to improve utilization of CAA is feeding multiple times in a day (Ambardekar and Reigh, 2007; Barroso et al., 1999), as this will complement the temporal release of protein-bound AA and at the same time providing more chances for the timely absorption of CAA (Lanna et al., 2016). It was thus hypothesized that increasing feeding frequency will aid the utilization of CAA supplementation in diets deficient in AA, thereby leading to an increased protein deposition in fish. The positive effect of multiple feeding was demonstrated in a study carried out by Zarate et al. (1999) on channel catfish fed diets containing free and protein-bound lysine. A better utilization of free lysine was achieved when the animals were fed five times a day compared to twice a day. Yamada et al. (1981) observed similar results in carp fed free AA from 3 to 18 times daily, as growth increased in proportion to high frequency.

Methionine (Met) is often the first limiting AA in many vegetable proteins, especially legumes (Mai et al., 2006), therefore, its supplementation in fish diets is usually inevitable. In a recent study, we estimated Met requirement of African catfish to range between 18.7 and 21.4 g/kg digestible methionine per unit of digestible protein by feeding fish restrictively twice per day (Elesho et al., 2021). Despite providing nutrients to meet the average daily requirement of animals, the circadian fluctuation in nutrient requirement and availability can be disrupted within a day (Van den Borne, 2006). Feeding at low frequency may diminish the match between nutrient supply and requirement within a day, especially when nutrients with low storage capacity in the body are fed e.g., AA (Van den Borne, 2006). For instance, when plant diets supplemented with CAA are fed, there may be a temporal excess supply of CAA relative to the requirement and thus a sub-optimal utilization for protein deposition may occur.

African catfish is a species of great and still increasing economic importance in Africa and worldwide (Eyo and Ekanem, 2011; Fagbenro et al., 1999). Several authors have suggested that feeding twice or three times per day is sufficient for the optimum growth of African catfish (Aderolu et al., 2010; Eyo and Ekanem, 2011; Marimuthu, 2010). However, none of these studies assessed whether the effect of feeding frequency on growth is dependent on diet composition (crystalline methionine supplementation). Therefore, the objective of the current study was to examine the influence of feeding frequency on digestibility, growth, nitrogen and energy balances of African catfish fed diets supplemented with crystalline methionine. Furthermore, it was assessed if the effect of feeding frequency was dependent on the level of methionine supplementation.

2. Materials and methods

2.1. Ethics statement and research facility

The study (project number 2018.W.0014.002) was in accordance with the Dutch law on the use of animals (Act on Animal Experiments) for scientific purposes and was approved by the Central Animal Experiments Committee (CCD) of The Netherlands. This experiment was conducted in the research facility of CARUS-ARF at Wageningen University, The Netherlands. Fish were kept and handled in agreement with EU-legislation.

2.2. Fish and housing conditions

Mixed sex of African catfish (*Clarias gariepinus*) fingerlings were obtained from a commercial hatchery (Fleuren & Nooijen BV, Nederweert, The Netherlands). At the start of the experiment, 980 fish weighing on average 44 g were randomly assigned (40 fish per tank) into 24 experimental tanks. All tanks were part of a recirculating water system sharing a common reservoir. The system water volume was 5 m^3. Water loss due to evaporation was continuously compensated with the addition of well water. Additional water refreshment was based on NO_3^- removal from the system to keep NO_3^- levels below 500 mg/L. Each tank was equipped with air stones and the water outlet of each tank was connected to a separate swirl separator (AquaOptima AS, column height 44 cm; diameter 24.5 cm) for collection of feces and spilled feed pellets. Water quality parameters were monitored regularly and maintained within optimal levels for African catfish. Average (SD) measured values over the experimental period were as follows: water temperature 27.3 ± 0.15 °C; pH, 7.4 ± 0.19; ammonium, 0.17 ± 0.138 mg/L; nitrite, 0.13 ± 0.019 mg/L; nitrate, 381 ± 33 mg/L; conductivity, 3454 ± 307 mS; and dissolved oxygen concentration, 6.6 ± 0.43 mg/L. Photoperiod was kept at 12 h light: 12 h dark.

2.3. Experimental diets

This experiment was designed to assess the effect of feeding frequency and its interaction with crystalline methionine supplementation level on digestion, energy and nitrogen balances. The ingredient composition and proximate analysis of the experimental diets are given in Tables 1 and 2. Two diets were formulated to represent minimal but sufficient supply (required) and oversupply of Met. These experimental diets were formulated to be identical regarding ingredient composition and nutrient concentrations except for the amount of crystalline DL-methionine supplementation and cellulose. The basal diet (Adq-Met diet), was formulated to meet exactly the Met requirement of African catfish (19.2 g/kg crude protein) based on the results of a previous study (Elesho et al., 2021). Since Met can be converted into cystine (Cys), a small amount of hydrolyzed feather meal was included in the Adq-Met diet to avoid low Cys levels. The analyzed Cys level was 6.0 g/kg dry matter (DM). Methionine was then added to create a High-Met diet representing an oversupply of AA. In both diets, protein originated mostly from plant protein ingredients (i.e., fishmeal-free diet). The contrast between the Adq-Met and High-Met diet was created by exchanging 3.0 g/kg cellulose for crystalline DL-methionine. This resulted in an analyzed methionine content of 6.7 g/kg DM (19.3 g/kg crude protein) for the Adq-Met diet and 9.8 g/kg DM (29.1 g/kg crude protein) for the High-Met diet. The contrast between both diets in Met content was equal to the contrast in Met+Cys content. The Met+Cys content was 12.7 and 15.9 g/kg DM at the Adq-Met and High-Met diet respectively. This range in dietary Met content was chosen to test the hypothesis that at low feeding frequencies, the AA utilization is reduced and consequently the amount of Met available for growth at low feeding frequencies is insufficient at the Adq-Met diet but not at the High-Met diet. Yttrium oxide was added as a marker for the determination of the apparent digestibility coefficient (ADC) of nutrients. The experimental diets were extruded as floating pellets (3–3.5 mm diameter) using a twin-screw extruder (Wenger, Sabetha, KS, U.S.A) and were produced by the Skretting ARC Norway. After the extrusion process, pellets were coated with 40 g/kg palm oil in order to prevent leaching of the crystalline Met before uptake. Diets were stored at 4 °C throughout the experimental period.

2.4. Feeding and sampling

At the beginning of the experiment, 20 fish were randomly selected and euthanized by an overdose of phenoxy-ethanol (1.0 mL/L), to determine proximate composition. Before stocking of the tanks, fish were counted while being sedated (0.25 mL/L phenoxy-ethanol) and the total biomass was recorded. The response of African catfish to both Met diets was compared under four feeding frequencies (FF). These were (A) "6/1d-FF", feeding six times per day, at 8:00, 12:00, 16:00, 20:00, 24:00 and 4:00 h; (B) "2/1d-FF", feeding two times per day, at 8:00 and 16:00 h; (C) "1/1d-FF", feeding one time per day at 12:00 h; (D) "2/3d-FF", feeding two times out of three days, at either 8:00 h or 16:00 h with a 36 h interval. These feeding frequencies resulted in an interval between successive feeding of 4, 12, 24 and 36 h. Each combination of diet and feeding frequency was randomly assigned to the experimental tanks in triplicate. During the 32-day experimental period, the daily food allowance was divided into the appropriate number of equal amounts for each feeding frequency. This was done to minimize the variation in response parameters due to variability in feed intake. Fish were hand-fed, except

Table 1
Ingredients composition of the experimental diets[a].

	Adq Met	High Met
Test ingredients (g/kg)		
DL-Methionine	2.8	5.8
Cellulose	3.0	0.0
Basal ingredients (g/kg)		
Soy bean meal	50	
Sunflower meal	25	
Faba bean (dehulled)	150	
Lupine meal	100	
Pea meal	150	
Canola meal	75	
Hydrolyzed feather meal	70.7	
Wheat	100	
Wheat flour	27.7	
Gelatinized wheat starch	100	
Fish oil	45	
Palm oil	40	
Mono calcium phosphate	36	
Calcium carbonate	10	
Yttrium oxide	1.0	
Vitamin and minerals[b]	4.8	
Lysine HCl	5.2	
L-Threonine	2.8	
L-Tryptophan	1.0	

[a] The ingredient composition of the 2 experimental diets were similar except for the content of cellulose and DL-methionine.
[b] Skretting ARC closed formula for vitamin and trace mineral premix to meet requirements specified for fresh water fish, according to NRC (2011) recommendation.

Table 2
Analyzed amino acid and nutrients composition of experimental diets (g/kg DM).

	Diets	
	Adq Met	**High Met**
EAA		
Arginine	27.2	27.0
Histidine	7.9	7.7
Isoleucine	14.8	14.5
Leucine	25.2	24.8
Lysine	22.2	21.9
Methionine	6.7	9.8
Phenylalanine	15.4	15.3
Threonine	16.3	15.9
Valine	17.2	16.9
NEAA		
Alanine	14.8	14.1
Aspartic acid	32.5	31.9
Glutamic acid	57.7	56.6
Cystine	6.0	6.1
Glycine	17.5	17.0
Proline	19.7	19.5
Serine	20.5	20.6
Tyrosine	8.7	9.0
SAA	330	329
Nutrients		
Dry matter	914	911
Crude protein	346	336
Crude fat	124	127
Ash	75.0	75.1
Phosphorus	14.7	14.7
Energy (kJ/g)	21.1	20.8

Amino acid composition was determined by the Skretting (ARC) laboratory Norway
DM: dry matter; EAA: Essential amino acids
NEAA: Non-essential amino acids
SAA: Sum of amino acids, which is without tryptophan since tryptophan was not analyzed.

for the feedings of the "6/1d-FF" treatment at 20:00, 24:00 and 4:00 h, where feed was provided using a belt feeder (each feeding period lasted for 30 min). Fish were fed based on their metabolic body weight. Metabolic body weight was calculated as $BW^{0.8}$ with BW expressed in kg. The feeding level was fixed at 14.5 $g/kg^{0.8}/d$ based on the mean initial weight over all diets. Furthermore, to ensure that the feeding levels per fish were equal for all treatments, daily feed rations per tank were increased based on an expected growth using a FCR of 1. In the case of mortality, the daily feeding rations were adjusted for the number of fish in the tank. During the first three days, the feeding level was gradually increased from 20% to 100% of the intended ration. After each meal, the uneaten feed was weighed and the spilled pellets, which were collected by the swirl separators 15 min after feeding was counted per tank. For proximate analysis of the feed, a representative sample from each diet was taken and stored at 4 °C.

Feces were collected for digestibility measurements from week 2 till the end of the trial, 5 days per week (Monday - Friday), using detachable collection bottles (250 mL) connected to settling tanks. The fecal collection bottles were submerged in ice-filled styrofoam boxes to reduce microbial degradation. Feces were collected overnight and stored daily in the morning in aluminum trays at − 20 °C for further analysis. Feces were pooled per tank. Throughout the experiment, fish behavior was monitored and visually inspected for discernible signs of deformities. Mortality was checked 30 min prior to each hand-feeding period. At the end of the feeding trial, fish were sedated and batch weighed after 24 h of food deprivation. Ten fish per tank were randomly selected and euthanized by an overdose of phenoxy-ethanol (1.0 mL/L) for final body composition analysis.

2.5. Chemical analyses on feed, feces and fish body composition

Analyses were performed on the diets, whole fish samples and feces samples. Before chemical analysis, frozen fish samples were sawed into small pieces and homogenized by mincing twice through a 4.5 mm-screen grinder (Gastromaschinen, GmbH model TW-R 70; Feuma). Dry matter (DM), ash and crude protein (CP) were analyzed using a portion of the freshly sampled minced fish. The remainder of the samples were freeze-dried for later determination of crude fat and energy. Fecal samples were freeze-dried, then manually pulverized through a 1 mm screen sieve. Feed pellets were grinded by a grinding machine. Fish, feces, and feed samples were analyzed in triplicate using the same analytical method. DM content was determined by drying the samples to constant weight at 103 °C for at least 4 h (ISO 6496, 1983) and ash content by incineration in a muffle furnace at 550 °C overnight (ISO 5984, 1978). The Kjeldahl method was used for nitrogen analysis (ISO 5983, 1979) and CP contents calculated as N content times 6.25. Crude fat analysis was determined using the Soxhlet method (ISO 6492, 1999). Energy was measured using an adiabatic bomb-calorimeter

(C7000 IKA®, IKA analysentechnik, Weitershem, Germany; ISO 9831, 1998). Yttrium, phosphorus, calcium and magnesium in feed and feces were determined from the ash by using inducted coupled plasma mass spectrometry according to the standard NEN 15510 (ICP-MS, 2007). Amino acids (excluding tryptophan) were analyzed by Skretting ARC, Norway, using an automatic amino acid analyzer (Biochrom 30 +, Biochrom Ltd, Cambridge, UK) and the methods described in the COMMISSION REGULATION (EC) No 152/2009 (Council, 2009).

2.6. Calculations

Calculations of performance parameters (daily weight gain, specific growth rate, feed conversion ratio DM basis and survival) are given in Table 3. The apparent digestibility coefficient (ADC) of macronutrients were calculated according to the following formula described by Cheng and Hardy (2002) using yttrium oxide as inert marker; ADC (%) = 100 × [1 − (yttrium concentration in the feed × concentration nutrient in feces)/(yttrium concentration in the feces × concentration nutrient in feed)]. The concentrations of yttrium and nutrients were expressed on DM basis. Nitrogen (N) and energy balance parameters were calculated per treatment and expressed as; mg/d and kJ/d, respectively (summarized in Table 3). A detailed description of the calculation of balance parameters was previously provided by Saravanan et al. (2012). The utilization efficiency of digested protein was calculated as the amount of nitrogen retained as percentage of the digestible nitrogen intake.

2.7. Statistical analysis

Data were analyzed by two-way ANOVA to test for the effects of feeding frequency and dietary methionine level. Following a significant ANOVA result, means were compared by a multiple comparisons test using Tukey's honest significant difference (HSD). All statistical analyses were performed by using SPSS Statistics, version 23.0 for Windows (IBM Corp., Armonk, NY, USA).

3. Result

This experiment was designed to assess the effect of feeding frequency and its interaction with crystalline methionine supplementation level on digestion, energy and nitrogen balances. Therefore, two diets with different amounts of CAA were fed to African catfish at four different FFs. The experiment was aimed to have equal feed intakes (FI) in all treatments. However, during the first week, some treatment groups were unable to cope with the set feeding level, especially fish fed at 2/3d-FF. As a consequence, we

Table 3
Fish performance and nutrient balances calculations.

Parameters	Formula
Performance parameters	
Growth (g/d)	$Growth = (Wf - Wi)/t$
Specific growth rate (SGR, %/d)	$SGR = 100 \times (\ln(Wf) - \ln(Wi))/t$
Feed conversion ratio on DM basis (FCR)	$FCR = (FI \times DM\,feed)/growth$
Survival (%)	$Survival = NoFishi/NoFishf \times 100$
Nitrogen balance (mg/d)	
Gross nitrogen intake (GNI)	$GNI = FI \times N\,feed$
Digestible nitrogen intake (DNI)	$DNI = (GNI \times ADCcp)/100$
Branchial and urinary loses (BUN)	$BUN = DNI - RN$
Retained nitrogen (RN)	$RN = ((Wf \times CPf)/6.25) - ((Wi \times CPi)/6.25)$
Utilization efficiency of digested protein (EDP; %)	$EDP = RN/DNI \times 100$
Energy balance (kJ/d)	
Gross energy intake (GEI)	$GEI = FI \times E\,feed$
Digestible energy intake (DEI)	$DEI = (GE \times ADCe)/100$
Metabolizable energy intake (MEI)	$\mathrm{MEI} = \mathrm{DE} - \mathrm{BUE}$
Branchial and urinary energy losses (BUE)	$BUE = (BUN \times 24.9) / 1000$
Retained energy (RE)	$RE = W\,f \times E\,f - W\,i \times E\,i$
Heat production (HP)	$HP = RE - ME$
Retained energy as protein (REpro)	$R\,Epro = (RN \times 6.25) \times 23.7$
Retained energy as fat (REfat)	$RE\,fat = RE - RE\,pro$

Wi, initial fish weight (g); Wf, final fish weight (g); t, duration of experiment (d); FI, feed intake (g/d); NoFishi and NoFishf, respectively initial and final number of fish per tank; ADCcp, apparent digestibility coefficient of crude protein (%); CPf and CPi, respectively final and initial crude protein content of the fish (g/kg); DMfeed, dry matter content of feed (g/g); Nfeed, nitrogen content of feed (mg/g); Efeed, energy content of feed (kJ/g); Ef and Ei, respectively final and initial energy content of feed (kJ/g); ADCe, apparent digestibility coefficient of the energy (%); 23.7 kJ/g, energy content of protein; 24.9 kJ/g N, energy concentration of NH_3-N as calculated by (Bureau et al., 2003), assuming all N was excreted as NH_3-N.

reduced the rate of increase in feeding level to allow equal ration among treatments. However, only three treatments (1/1d-FF, 2/1d-FF and 6/1d-FF) were able to reach the targeted feeding level after 12 days of the start of the experiment. But the group of fish at 2/3d-FF still lagged behind the other groups and still had substantial feed rejections. These treatments were able to consume only 75% of the targeted feed ration. Consequently, the 2/3d-FF treatment was excluded from the two-way ANOVA and the effect of the diet within the 2/3d-FF was tested by one-way ANOVA. Fig. 1 shows the mean values of FI per FF. Averaged over diets, the group fed 2/3d ate 10.9 g/ $kg^{0.8}$/d of feed and the other FF treatments ate 13.1 g/$kg^{0.8}$/d.

Except for digestible nitrogen intake and dry matter body content, no interaction effect between dietary Met and FF was observed for any of the measured parameters (Tables 4–6). The growth performance of African catfish fed the two diets under varying FF is presented in Table 4. Daily gain and specific growth rate were neither affected by the dietary Met treatment nor the FF treatment. Similarly, FCR on DM basis was unaffected by the applied treatments. Averaged over all treatments, FCR was 0.88. Although, no effect of treatment occurred on survival, fish fed 6 times a day had the highest survival rate.

Means of apparent digestibility coefficient (ADC) of nutrients of all experimental treatments are given in Supplementary Table A. For all of the measured nutrient ADCs, there was no effect of the dietary Met treatment ($P > 0.1$). In contrast, all nutrient ADCs were affected by FF ($P < 0.05$; Supplementary Table A). The main effect of feeding frequency on nutrient ADCs is visualized in Fig. 2. Increasing the feeding frequency from 2/3d to 6/1d increased the digestibility of fat from 90.9% to 94.2% ($P < 0.01$) and of protein from 86.0% to 88.7% ($P < 0.01$). In contrast, phosphorus and magnesium digestibility decreased with increasing feeding frequency ($P < 0.05$).

For the three highest feeding frequencies, there was no effect of dietary Met and FF on fish body composition (Table 5) except for energy content. Feeding frequency had a significant effect on energy content with slightly higher value recorded for fish fed at 6/1d. This was also reflected in a tendency for a higher fat content at 6/1d-FF. At the lowest feeding frequency, 2/3d-FF, dietary Met level affected the ash content ($P < 0.001$), but at the other FF, dietary Met level had no impact on the ash content.

Nitrogen and energy balances of African catfish fed experimental diets under different FFs are shown in Table 6. Digestible nitrogen, energy and metabolizable energy intake were significantly higher in the 6/1d-FF compared to other FFs ($P < 0.001$). This effect was more pronounced in the fish fed the Adq-Met diet. With the increase in dietary Met level, BUN significantly decreased in fish fed High-Met diet at 2/3d-FF ($P < 0.01$). In contrast, both dietary Met and FF did not affect retained nitrogen and energy ($P > 0.05$). The utilization efficiency of digested protein was not influenced by FF. Only a tendency for a reduced utilization efficiency of digested protein was observed in fish fed the Adq-Met diet compared to the High-Met diet in the 2/3d-FF treatment (Table 6).

4. Discussion

This study was conducted to assess the interaction effect of feeding frequency and dietary crystalline methionine level on nutrient digestibility, nitrogen and energy balances in African catfish. Thereby, it investigated the underlying factors causing sub-optimal AA utilization in fish, especially when plant ingredients are used as protein source. Some authors have reported that this problem is often caused by the asynchronous availability of dietary protein-bound AA and CAA supplemented to an AA-deficient diet (Ambardekar and Reigh, 2007; Zarate et al., 1999). Dietary CAA tend to be more quickly released and absorbed by the gastro-intestinal tract than protein-bound AA. This is believed to result in the premature catabolism of CAA (Batterham, 1974), thereby reducing their utilization.

It has been reported that fluctuations in nutrient availability may be influenced by the feeding patterns (Van den Borne, 2006). This led to the hypothesis that CAA utilization might not be optimal if the feeding frequency is low. To test this hypothesis, two diets with methionine contents, which is adequate or exceeded the methionine requirement of African catfish (Adq-Met versus High-Met diet), were tested under varying feeding frequencies. Contrary to our expectation, a low feeding frequency did not hamper the utilization

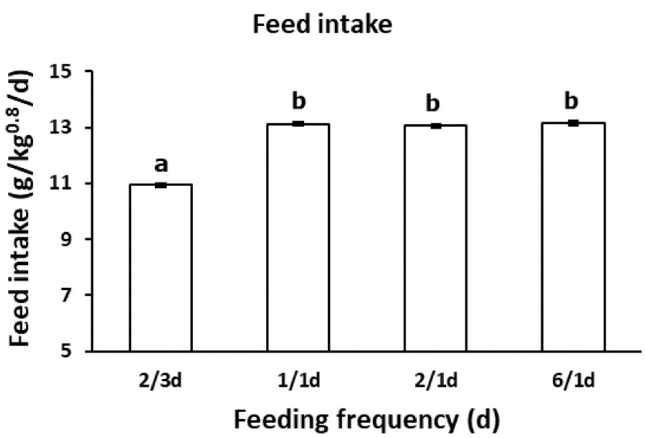

Fig. 1. Effect of feeding frequency (FF) on feed intake in African catfish. Presented values are means over both experimental diets. Means lacking a common letter differ ($P < 0.05$). The applied feeding frequencies were: 2/3d, feeding two times in three days; 1/1d, feeding one time per day; 2/1d, feeding two times per day; 6/1d, feeding six times per day.

Table 4
Growth performance of African catfish during the experimental period.

	Diets										Diets			
	Adq-Met			High-Met							Adq-Met	High-Met		
Feeding frequency	6/1d	2/1d	1/1d	6/1d	2/1d	1/1d			***P*-value**		2/3d	2/3d		***P*-value**
Feeding interval	4 h	12 h	24 h	4 h	12 h	24 h	SEM	D	FF	D x FF	36 h	36 h	SEM	D
Initial BW (g)	43.7	43.6	43.5	43.7	43.7	43.5	0.074	ns	ns	ns	43.4	43.3	0.25	ns
Final BW (g)	103	103	103	101	98	102	1.23	ns	ns	ns	87	85	0.48	0.083
Survival (%)	97.5	94.2	92.5	95.0	95.0	93.3	1.30	ns	ns	ns	92.5	94.2	1.18	ns
Feed Intake DM (g/d)[a]	1.52	1.49	1.49	1.50	1.48	1.52	–	–	–	–	1.19	1.14	–	–
Growth (g/d)	1.85	1.86	1.86	1.78	1.71	1.82	0.039	ns	ns	ns	1.35	1.31	0.013	0.067
SGR (%/d)	2.76	2.77	2.78	2.69	2.61	2.74	0.043	ns	ns	ns	2.23	2.18	0.018	ns
FCR (g/g)	0.82	0.81	0.80	0.84	0.88	0.84	0.022	ns	ns	ns	0.88	0.88	0.009	ns

d, day; D, main effect of diet; FF, main effect of feeding frequency; D x FF, interaction effect between diet and feeding frequency; 6/1d, feeding six times per day; 2/1d, feeding two times per day; 1/1d, feeding one time per day; 2/3d, feeding two times in three days; SGR, specific growth rate; FCR, feed conversion ratio; BW, body weight; DM, dry matter; SEM, standard error of mean; ns, not significant $P > 0.1$.

[a] Feed intake was not statically analyzed because fish were fed restrictively.

Table 5
The effect of dietary methionine and feeding frequency on body composition of African catfish on dry mater basis (DM).

		Diets										**Diets**			
		Adq-Met			**High-Met**							**Adq-Met**	**High-Met**		
Feeding frequency	**Initial**	**6/1d**	**2/1d**	**1/1d**	**6/1d**	**2/1d**	**1/1d**			***P*-value**		**2/3d**	**2/3d**		***P*-value**
Feeding interval		**4 h**	**12 h**	**24 h**	**4 h**	**12 h**	**24 h**	**SEM**	**D**	**FF**	**D x FF**	**36 h**	**36 h**	**SEM**	**D**
Dry matter	229	257	262	259	261	260	262	1.24	0.065	ns	0.048	258	253	2.61	ns
Protein (g/kg DM)	647	604	586	597	601	595	597	5.84	ns	ns	ns	594	610	6.09	ns
Fat (g/kg DM)	246	300	302	294	304	300	294	3.32	ns	0.062	ns	292	285	3.59	ns
Ash (g/kg DM)	114	111	110	114	112	116	114	2.19	ns	ns	ns	118	113	0.35	< 0.001
Energy (KJ/g DM)	24.3	25.8	25.7	25.3	25.9	25.6	25.4	0.14	ns	0.02	ns	25.3	25.4	0.16	ns

d, day; D, main effect of diet; FF, main effect of feeding frequency; D x FF, interaction effect between diet and feeding frequency; 6/1d, feeding six times per day; 2/1d, feeding two times per day; 1/1d, feeding one time per day; 2/3d, feeding two times in three days; SEM, standard error of mean; ns, not significant P > 0.1.

Table 6
The relationship between dietary treatments and nitrogen and energy balances in African catfish.

	Diets										Diets			
	Adq-Met			High-Met							Adq-Met	High-Met		
Feeding frequency	**6/1d**	**2/1d**	**1/1d**	**6/1d**	**2/1d**	**1/1d**			***P*-value**		**2/3d**	**2/3d**		***P*-value**
Feeding interval	**4 h**	**12 h**	**24 h**	**4 h**	**12 h**	**24 h**	**SEM**	**D**	**FF**	**D x FF**	**36 h**	**36 h**	**SEM**	**D**
Nitrogen balance (mg/d)														
Gross nitrogen intake[a]	83.7	82.6	82.2	80.6	79.7	81.7	0.21	–	–	–	65.9	61.5	0.07	–
Digestible nitrogen intake	74.5	72.0	72.1	71.3	69.1	71.1	0.23	< 0.001	< 0.001	0.045	56.6	52.9	0.41	0.003
Branchial urinary losses	25.7	23.8	23.4	23.1	23.9	22.5	0.99	ns	ns	ns	21.2	18.2	0.44	0.009
Retained nitrogen	48.7	48.2	48.7	48.2	45.2	48.6	1.01	ns	ns	ns	35.4	34.7	0.68	ns
Utilization efficiency of digested protein (%)	65.4	66.9	67.5	67.6	65.4	68.4	1.44	ns	ns	ns	62.6	65.5	0.90	0.081
Energy balance (kJ/d)														
Energy intake[a]	31.9	31.4	31.3	31.1	30.8	31.6	0.08	–	–	–	25.1	23.8	0.03	–
Digestible energy intake	26.8	25.5	25.6	25.9	24.6	25.6	0.13	0.008	< 0.001	0.085	20.6	19.3	0.20	0.011
Metabolizable energy intake	26.2	24.9	25.0	25.4	24.0	25.1	0.13	0.015	< 0.001	ns	20.0	18.9	0.21	0.015
Heat production	12.3	10.5	11.3	11.4	10.9	11.3	0.26	ns	0.073	ns	9.74	9.22	0.29	ns
Retained energy	13.9	14.4	13.7	13.9	13.1	13.8	0.29	ns	ns	ns	10.31	9.64	0.21	0.09
Retained energy as protein	7.22	7.14	7.22	7.15	6.70	7.20	0.15	ns	ns	ns	5.25	5.13	0.10	ns
Retained energy as fat	6.65	7.25	6.51	6.78	6.44	6.62	0.18	ns	ns	ns	5.06	4.51	0.23	ns
Maintenance energy requirement ($kJ/kg^{0.8}$ /d)	47.1	31.6	38.8	40.4	40.1	38.9	3.53	ns	ns	ns	43.9	40.9	2.41	ns

d, day; D, main effect of diet; FF, main effect of feeding frequency; D x FF, interaction effect between diet and feeding frequency; 6/1d, feeding six times per day; 2/1d, feeding two times per day; 1/1d, feeding one time per day; 2/3d, feeding two times in three days; SEM, standard error of mean; ns, not significant $P > 0.1$.

[a] Nitrogen and energy intake were not statistically analyzed because restricted feed intake was applied.

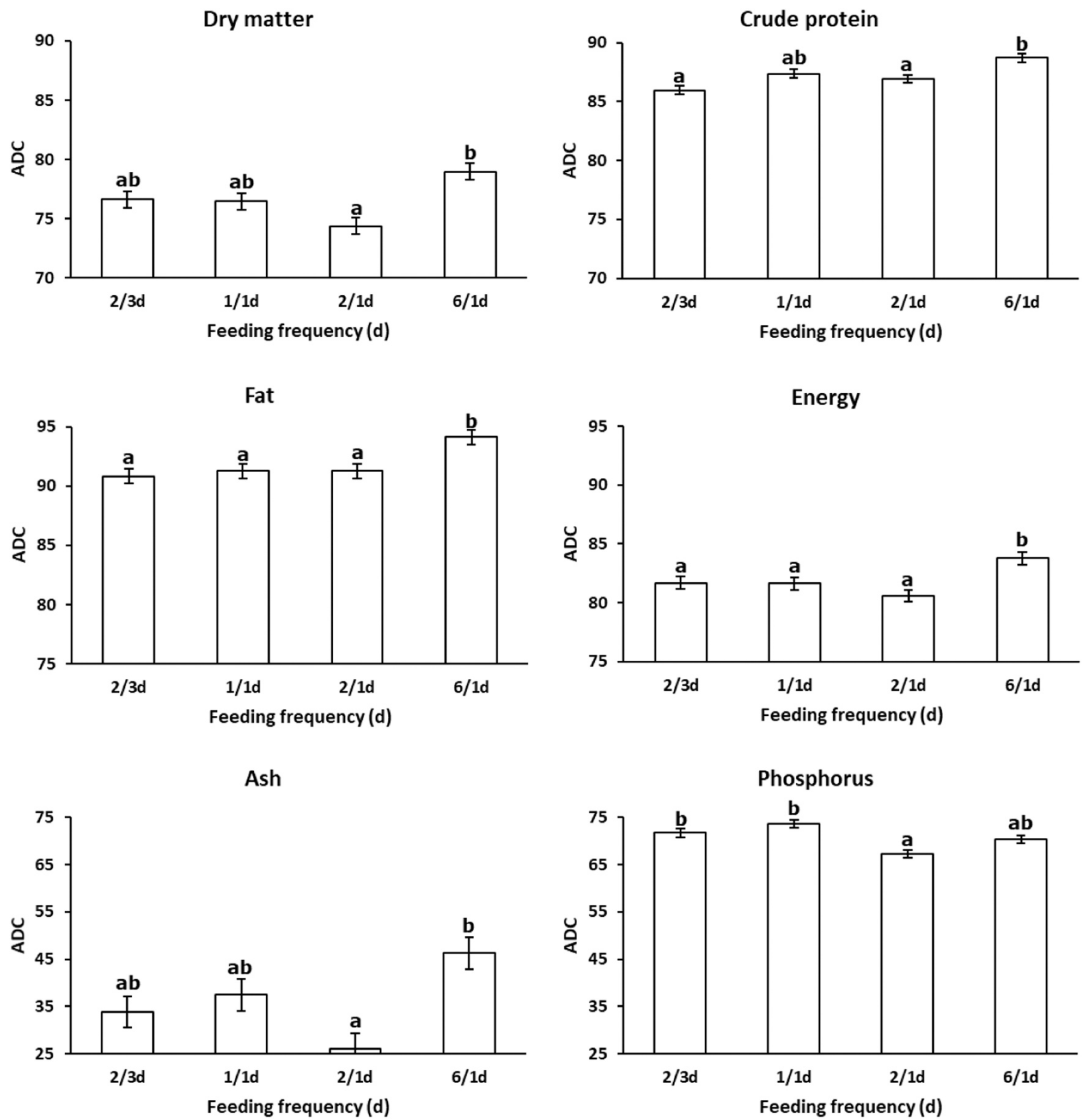

Fig. 2. Effect of feeding frequency (FF) on apparent digestibility coefficient (ADC) of nutrients in African catfish. Presented values are means over both experimental diets. Means lacking a common letter differ significantly ($P < 0.05$). The applied feeding frequencies were: 2/3d, feeding two times in three days; 1/1d, feeding one time per day; 2/1d, feeding two times per day; 6/1d, feeding six times per day.

efficiency of digested protein of African catfish when fed the Adq-Met diet (Table 6). This observation was supported by the absence of an interaction effect between FF and dietary CAA supplementation for all tested parameters. Furthermore, at the lowest FF (2/3d-FF with a feeding interval of 36 h), none of the growth, energy and nitrogen balance parameters were improved by the supplementation of Met with the exception of utilization efficiency of digested protein, which tended to be higher for the High-Met diet. This implies that synchrony of dietary CAA with protein-bound AA is not affected by feeding frequency in African catfish. In contrast to the current observation, positive effects of feeding frequency on nutrient synchronization have been demonstrated in other farmed animals (Batterham, 1974; Van den Borne et al., 2006). For example, increasing feeding frequency increased the efficiency of digestible protein in pre-ruminant calves when fed non-clotting protein sources under varying feeding frequencies (Van den Borne et al., 2006). In pigs, increasing the feeding frequency from one time per day to six times per day improved their response to a lysine-deficient diet supplemented with free lysine (Batterham, 1974). Similar to the results of the current study, channel catfish and common carp have shown that feeding frequency did not improve utilization efficiency of supplemental lysine and methionine respectively (Nwanna et al., 2012; Zarate et al., 1999).

Fish in the present study received similar amounts of feed in all treatments except for the 2/3d-FF treatment group. Feed rejection was observed in this group during the first part of the experiment. In the original design, it was planned to give the same ration across treatments and thus, the amount of feed per feeding moment (i.e., meal) increases as FF reduces. Consequently, this increased meal size may have led to the overloading of the gut by the bulk supply of feeds. The current feed intake results of African catfish (Fig. 1) suggest that feed intake is hampered when the FF is reduced below once daily. This was unexpected beforehand, as fish are known to be capable of adjusting their stomach volume to accommodate food in order to compensate for the period of starvation (Jobling, 1982), when fish are fed to apparent satiation under different regimes. Feeding fish less frequently for a longer time can lead to an increased gut capacity, which will result in hyperphagia (Jobling, 1982; Rouhani, 1993). This is a situation whereby fish try to adapt and adjust to reduced access to feed by consuming more feed per meal (Eyo and Ekanem, 2011). To which extent hyperphagia may occur varies among species, ages and sizes (Okomoda et al., 2019). Furthermore, the response of fish to varying feeding frequencies has been linked to the size of its stomach (Pillay and Kutty, 2005). This is because smaller fish size with a smaller stomach do require more frequent feeding for maximum growth to be attained compared to larger fishes (Okomoda et al., 2019). The fact that juvenile fish were used in the current study might explain the feed rejection at the low FF. Studies conducted on other fish species have shown that feed consumption and growth generally increased with feeding frequency up to a given limit (Aderolu et al., 2010; Basçinar et al., 2001; Lanna et al., 2016; Wang et al., 1998). However, more studies are required to investigate the interaction between feed intake and feeding frequency in fish.

In the current study, increasing the feeding frequency improved nutrient digestibility regardless of the dietary Met level. Usually, nutrient digestibility is improved when fish are exposed to challenging conditions, which often relates to a reduced feed intake. For example in Nile tilapia (*Oreochormis niloticus*), exposure to hypoxia (Tran-Duy et al., 2012) as well as exposure to brackish conditions (Tran-Ngoc et al., 2017) led to higher nutrient digestibility. However, in the current study, the opposite was found as feed intake was lower at the lowest feeding frequency, which coincided with the lowest macronutrient ADCs. In general, macronutrients ADCs were highest in the fish fed six times per day (Fig. 2). This may be due to close intervals in feed availability, which resulted in increased enzymatic activities for optimum digestion (Zhao et al., 2016). In line with our result, a study on common carp where two feeding frequencies (twice daily vs. continuous feeding) were applied observed increased protein digestibility under continuous feeding (Nwanna et al., 2012). Furthermore, Zhou et al. (2003) reported an improved protein and energy digestibility in gibel carp (*Carassius auratus gibelio*) when feeding frequency was increased from two to four meals per day. In contrast to these findings, there are several studies in which feeding frequency had no effect on nutrient digestibility (Amadou et al., 2019; Charles et al., 1984; Marian et al., 1982; Zhao et al., 2016). These discrepancies between studies can stem from both differences in the range of applied feeding frequencies or diet formulations. In contrast to macronutrients, the digestibility of minerals such as phosphorus and magnesium was lower at higher frequencies when compared to lower feeding frequencies (Fig. 2). The reason for this observation is unclear but may be due to changes in stomach pH induced by feeding frequency. Postprandial stomach pH declines with time after meal (Saravanan et al., 2013). Moreover, stomach pH is important in dissolving minerals for absorption. Therefore, it can be suggested that frequent feeding might result in higher average pH throughout the day. However, the impact of feeding frequency on stomach chyme characteristics of African catfish requires further assessment.

African catfish fed 2/3d-FF in this study showed low nutrient digestibility. This could be as a result of an overload of the digestive enzymes with bulk amounts of feed, resulting in a reduction of nutrient absorption capacity and digestibility (Staessen et al., 2020). Only a few studies have examined the effects of feeding fish less than once per day on nutrient digestibility (Li and Lucas, 2017). Feeding channel catfish every other day was not found to significantly influence nutrient ADC (Li and Lucas, 2017). In the current study, fish fed at 2/1d-FF had the lowest nutrient digestibility (average over both diets). This implies that feeding more frequently than 2/1d is optimal for a good digestibility. Earlier studies on performance in African catfish suggested that feeding twice or three times per day is optimum for growth (Aderolu et al., 2010; Eyo and Ekanem, 2011; Marimuthu, 2010). However, these studies were done by applying satiation feeding and no digestibility measurements were carried out. In addition, the frequencies applied in those studies ranged from one time to three times per day. Feeding intervals have been reported to strongly correlate with gastric evacuation time (Huebner and Langton, 1982; Liu and Liao, 1999; Zhao et al., 2016), as such, increasing the frequency would have probably influenced their final outcome by feed intake.

Although, a high FF improved digestibility, growth remained nearly unchanged (Table 4). Yet, fish doubled in size at the end of the experiment, indicating a favorable rearing condition for improved growth. The absence of a FF effect on growth might be due to the impact of FF on endogenous fecal losses. If endogenous fecal losses were higher in less frequently fed fish, the true nutrient ADC might have been similar to the ADC of frequently fish and consequently also growth. However, this hypothesis needs further testing. Lack of differences in growth among treatments may also be explained by the activities of the fish during feeding. At the lower FFs, African catfish were seldomly resting with their tail at the bottom of the tank, and this behavior was frequently recorded for fish in the 2/3d-FF group. Subjective observations during this experiment suggested that fish fed at the highest FF tended to be more active compared to fish from the other FFs. This might also explain the higher numerical values for maintenance energy requirement recorded for this group (Table 6), which is an indication that more nutrients and energy were diverted into physical activity rather than growth. Furthermore, heat production was higher for the fish fed on a 6/1d regime, which may be related to energy losses due to swimming activities (NRC, 1993). In line with this study, several studies have reported that increasing feeding frequency had no significant effect on the growth of fish (Amadou et al., 2019; Lanna et al., 2016; Sveier and Lied, 1998). Lanna et al. (2016) reported that feeding Nile tilapia (*Oreochromis niloticus*) a low-protein diet supplemented with CAA under different feeding regimes had no effect on growth. Similarly, Atlantic Salmon (*Salmo salar*) fed under two feeding regimes showed no differences in growth and feed efficiency (Sveier and Lied, 1998). In contrast, a GIFT Nile tilapia fed 6 times per day grew better than when fed only two times per day (Zhao et al., 2010). Likewise, Zarate et al. (1999) confirmed that increasing the feeding frequency from two to five times per day significantly increased the growth of channel catfish fed a diet supplemented with free lysine. Differences in the outcome of studies could be attributed to the differences in duration of the experiments and quantity of feed consumed in each frequency group (Lanna et al., 2016), but most likely due to differences across species and feed intake behavior (e.g., filter feeders versus prey swallowers).

At both applied dietary methionine supplementation levels, performance, nitrogen and energy balance parameters were unaffected by feeding frequency. However, feeding frequency had an impact on feed intake and nutrient digestibility in African catfish. A higher feeding frequency increased the digestibility of nutrients but this did not translate into increased growth, most likely due to increased maintenance energy requirements because of higher physical activity of the fish.

Funding

This experiment was partially funded by Skretting Aquaculture Research Centre, Norway and Wageningen University. Folasade E. Elesho received a scholarship from Tertiary Education Trust Fund, TETfund Nigeria (2013/2014 merged) for her PhD research.

CRediT authorship contribution statement

F.E. Elesho: Conceptualization, Methodology, Formal analysis, Investigation, Project administration, Writing – original draft, Visualization; **S. Kröckel:** Conceptualization, Resources, Writing – review & editing; **E. Ciavoni:** Formal analysis, Investigation; **D.A. H. Sutter:** Conceptualization, Resources, Writing – review & editing; **J.A.J. Verreth:** Conceptualization, Supervision, Funding acquisition, Writing – review & editing; **J.W. Schrama:** Conceptualization, Methodology, Formal analysis, Investigation, Project administration, Writing – review & editing Supervision Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

The authors wish to acknowledge the technical assistance rendered by Menno ter Veld and the staff of the aquaculture research facilities of Wageningen University in running the experiment. We would like to thank Ronald Booms and Tino Leffering for their support during the lab analysis, also thanks to the technical staff of Skretting Aquaculture Research Centre laboratory for amino acid analysis.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.anifeedsci.2021.115098.

References

Aderolu, A., Seriki, B., Apatira, A., Ajaegbo, C., 2010. Effects of feeding frequency on growth, feed efficiency and economic viability of rearing African catfish (Clarias gariepinus, Burchell 1822) fingerlings and juveniles. Afr. J. Food Sci. 4, 286–290.

Amadou, L.M., Farokh, N., Robane, F., Cheikh, B., 2019. Feeding Frequency Effect on Growth, Body Composition, Feed Utilization and Ammonia Excretion of Juvenile Grouper Epinephelus coioides.

Ambardekar, A.A., Reigh, R.C., 2007. Sources and utilization of amino acids in channel catfish diets: a review. North Am. J. Aquac. 69, 174–179.

Barroso, J.B., Peragon, J., Garcia-Salguero, L., de la Higuera, M., Lupianez, J.A., 1999. Variations in the kinetic behaviour of the NADPH-production systems in different tissues of the trout when fed on an amino-acid-based diet at different frequencies. Int. J. Biochem. Cell B 31, 277–290.

Basçinar, N., Okumus, I., Basçinar, N.S., Saglam, H.E., 2001. The influence of daily feeding frequency on growth and feed consumption of rainbow trout fingerlings (Oncorhynchus mykiss) reared at 18.5–22.5 C. Isr. J. Aquac. 53, 80–83.

Batterham, E., 1974. The effect of frequency of feeding on the utilization of free lysine by growing pigs. Br. J. Nutr. 31, 237–242.

Bureau, D.P., Kaushik, S.J., Cho, C.Y., 2003. Bioenergetics, Fish Nutrition. Academic Press,, San Diego, pp. 1–59.
Charles, P.M., Sebastian, S.M., Raj, M.C.V., Marian, M.P., 1984. Effect of feeding frequency on growth and food conversion of Cyprinus carpio fry. Aquaculture 40, 293–300.
Cheng, Z.J.J., Hardy, R.W., 2002. Apparent digestibility coefficients and nutritional value of cottonseed meal for rainbow trout (Oncorhynchus mykiss). Aquaculture 212, 361–372.
Council, E., 2009. Commission Regulations (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed. Off. J. Eur. Comm. 26 February 2009, L54: 1. 130.
Elesho, F., Sutter, D., Swinkels, M., Verreth, J., Kröckel, S., Schrama, J., 2021. Quantifying methionine requirement of juvenile African catfish (Clarias gariepinus). Aquaculture 532, 736020.
Eyo, V., Ekanem, A., 2011. Effect of feeding frequency on the growth, food utilization and survival of African catfish (Clarias gariepinus) using locally formulated diet. Afr. J. Environ. Pollut. Health 9, 11–17.
Fagbenro, O.A., Balogun, A.M., Fasakin, E.A., 1999. Dietary methionine requirement of the African catfish, Clarias gariepinus. J. Appl. Aquac. 8, 47–54.
Huebner, J.D., Langton, R.W., 1982. Rate of gastric evacuation for winter flounder, Pseudopleuronectes americanus. Can. J. Fish. Aquat. Sci. 39, 356–360.
Jobling, M., 1982. Some observations on the effects of feeding frequency on the food-intake and growth of plaice, Pleuronectes-Platessa L. J. Fish. Biol. 20, 431–444.
Lanna, E.A.T., Bomfim, M.A.D., Ribeiro, F.B., Quadros, M., 2016. Feeding frequency of Nile tilapia fed rations supplemented with amino acids. Rev. Caatinga 29, 458–464.
Li, M.H., Lucas, P.M., 2017. Effects of feeding frequency on apparent energy and nutrient digestibility/availability of channel catfish, Ictalurus punctatus, reared at optimal and suboptimal temperatures. J. World Aquac. Soc. 48, 132–136.
Liu, F.-G., Liao, I.C., 1999. Effect of feeding regimen on the food consumption, growth, and body composition in hybrid striped bass Morone saxatilis× M. chrysops. Fish. Sci. 65, 513–519.
Lumbard, L., 1997 (Master of Science thesis). Utilization of Crystalline Lysine by Palmetto Bass, Morone saxatalis Female X Morone Chrysops Male. Louisiana State University,, Baton Rouge, Louisiana.
Mai, K.S., Wan, J.L., Ai, Q.H., Xu, W., Liufu, Z.G., Zhang, L., Zhang, C.X., Li, H.T., 2006. Dietary methionine requirement of large yellow croaker, Pseudosciaena crocea R. Aquaculture 253, 564–572.
Marian, M.P., Ponniah, A., Pitchairaj, R., Narayanan, M., 1982. Effect of feeding frequency on surfacing activity and growth in the air-breathing fish, Heteropneustes fossilis. Aquaculture 26, 237–244.
Marimuthu, K.A.C.C., 2010. Effect of different feeding frequency on the growth and survival of African catfish (Clarias gariepinus) fingerlings. Adv. Environ. Biol. 187–194.
Nose, T., Lee, D.L., 1974. A Note on Amino Acids Essential for Growth of Young Carp.
NRC, 1993. Nutrient Requirements of Fish. Committee on Animal Nutrition, 114. Board on Agriculture.
NRC, 2011. Nutrient Requirements of Fish and Shrimp. The National Academies Press,, Washington, DC.
Nwanna, L.C., Lemme, A., Metwally, A., Schwarz, F.J., 2012. Response of common carp (Cyprinus carpio L.) to supplemental DL-methionine and different feeding strategies. Aquaculture 356, 365–370.
Okomoda, V.T., Aminem, W., Hassan, A., Martins, C.O., 2019. Effects of feeding frequency on fry and fingerlings of African catfish Clarias gariepinus. Aquaculture 511, 734232.
Pillay, T.V.R., Kutty, M.N., 2005. Aquaculture: Principles and Practices. Blackwell publishing,.
Plakas, S.M., Katayama, T., 1981. Apparent digestibilities of amino acids from three regions of the gastrointestinal tract of carp (Cyprinus carpio) after ingestion of a protein and a corresponding free amino acid diet. Aquaculture 24, 309–314.
Rouhani, Q., 1993. Digestible Energy as a Criterion for the Development of Diets for the African Sharptooth Catfish, Clarias gariepinus (Pisces: Clariidae). Rhodes University,.
Saravanan, S., Geurden, I., Orozco, Z.G.A., Kaushik, S.J., Verreth, J.A.J., Schrama, J.W., 2013. Dietary electrolyte balance affects the nutrient digestibility and maintenance energy expenditure of Nile tilapia. Br. J. Nutr. 110, 1948–1957.
Saravanan, S., Geurden, I., Figueiredo-Silva, A.C., Kaushik, S.J., Haidar, M.N., Verreth, J.A., Schrama, J.W., 2012. Control of voluntary feed intake in fish: a role for dietary oxygen demand in Nile tilapia (Oreochromis niloticus) fed diets with different macronutrient profiles. Br. J. Nutr. 108, 1519–1529.
Staessen, T.W.O., Verdegem, M.C.J., Weththasinghe, P., Schrama, J.W., 2020. The effect of dietary non-starch polysaccharide level and bile acid supplementation on fat digestibility and the bile acid balance in rainbow trout (Oncorhynchus mykiss). Aquaculture 523, 735174.
Sveier, H., Lied, E., 1998. The effect of feeding regime on growth, feed utilisation and weight dispersion in large Atlantic salmon (Salmo salar) reared in seawater. Aquaculture 165, 333–345.
Teshima, S., 1990. Effects of methionine-enriched plastein supplemented to soybean protein based diets on common carp, Cyprinus carpio and tilapia Oreochromis niloticus. The Second Asia Fisheries Forum. The Asian Fisheries Society, pp. 279–282.
Tran-Duy, A., van Dam, A., Schrama, J.W., 2012. Feed intake, growth and metabolism of Nile tilapia (Oreochromis niloticus) in relation to dissolved oxygen concentration. Aquac. Res. 43, 730–744.
Tran-Ngoc, K.T., Schrama, J.W., Le, M.T.T., Nguyen, T.H., Roem, A.J., Verreth, J.A.J., 2017. Salinity and diet composition affect digestibility and intestinal morphology in Nile tilapia (Oreochromis niloticus). Aquaculture 469, 36–43.
Van den Borne, J.J., 2006. Nutrient Synchrony in Preruminant Calves. PhD thesis, Wageningen University, The Netherlands., pp. 2–7
Van den Borne, J., Verstegen, M., Alferink, S., Giebels, R., Gerrits, W., 2006. Effects of feeding frequency and feeding level on nutrient utilization in heavy preruminant calves. J. Dairy Sci. 89, 3578–3586.
Wang, N., Hayward, R.S., Noltie, D.B., 1998. Effect of feeding frequency on food consumption, growth, size variation, and feeding pattern of age-0 hybrid sunfish. Aquaculture 165, 261–267.
Yamada, S., Tanaka, Y., Katayama, T., 1981. Feeding experiments with carp fry fed an amino-acid diet by increasing the number of feedings per day. Nippon Suisan Gakkaishi 47, 1247. -1247.
Zarate, D.D., Lovell, R.T., Payne, M., 1999. Effects of feeding frequency and rate of stomach evacuation on utilization of dietary free and protein-bound lysine for growth by channel catfish Ictalurus punctatus. Aquac. Nutr. 5, 17–22.
Zhao, H., Jiang, R., Xue, M., Xie, S., Wu, X., Guo, L., 2010. Fishmeal can be completely replaced by soy protein concentrate by increasing feeding frequency in Nile tilapia (Oreochromis niloticus GIFT strain) less than 2 g. Aquac. Nutr. 16, 648–653.
Zhao, S., Han, D., Zhu, X., Jin, J., Yang, Y., Xie, S., 2016. Effects of feeding frequency and dietary protein levels on juvenile allogynogenetic gibel carp (C arassius auratus gibelio) var. CAS III: growth, feed utilization and serum free essential amino acids dynamics. Aquac. Res. 47, 290–303.
Zhou, Z., Cui, Y., Xie, S., Zhu, X., Lei, W., Xue, M., Yang, Y., 2003. Effect of feeding frequency on growth, feed utilization, and size variation of juvenile gibel carp (Carassius auratus gibelio). J. Appl. Ichthyol. 19, 244–249.