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Quantifying methionine requirement of juvenile African catfish (*Clarias gariepinus*)

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ABSTRACT

This study was conducted to estimate the methionine (Met) requirement of African Catfish (*Clarias gariepinus*). A basal diet was formulated to contain 32% crude protein, 12% lipids, and 0.44% cysteine using only protein from legume ingredients as intact protein. This diet was supplemented with graded levels of crystalline DL- methionine (0, 0.12, 0.24, 0.36, 0.48, 0.60, and 0.84%), which resulted in seven dietary methionine levels ranging from 12.2 to 36.0 g kg^{-1} crude protein. Triplicate groups of 40 fish (78 g) were restrictively fed one of the seven diets for six weeks. Dietary methionine level significantly affected growth rate, feed conversion ratio, retained nitrogen, methionine efficiency and body composition. All parameters were fitted to dietary digestible methionine content expressed per unit of digestible protein (dMetDP) to estimate the Met requirement using; the linear plateau model (LP), broken line model (BL), or quadratic regression model (QR). LP and BL recorded similar values for requirement estimates while QR evidently recorded a 57% higher requirement estimates across different parameters. The digestible methionine requirement of African catfish for growth (using LP) ranges between 18.7 and 21.4 g kg^{-1} per unit of digestible protein. This equates to a minimum dietary methionine level of 6.3 g kg^{-1} diet (19.2 g kg^{-1} Crude protein), which is lower than was has been previously reported for this species.

1. Introduction

African catfish (*Clarias gariepinus*) is considered an excellent aquaculture species predominantly cultivated in Africa and some other countries in the world. African catfish is interesting for aquaculture due to its fast growth, resistance to diseases and wide geographical distribution (Fagbenro et al., 1999). The euryphagic nature of the species (Bruton, 1979) enables it to utilize different ingredients efficiently (Fagbenro, 1998). However, the dearth of nutritional knowledge specific to this species is one of the major problems mitigating against its successful culture. The economic viability of its culture is dependent on achieving the development of least-cost feeds, particularly using sustainable ingredients such as plant proteins.

Recent studies have pointed out that fish have no definite requirement for protein but rather a specific requirement for essential amino acids (Miles and Chapman, 2007). This implies that, it is the essential amino acids in dietary protein that is most important for fish growth and development. However, the protein requirements (40–42%) of different life stages of African catfish has been previously reported (Uys, 1989), but the requirements for majority of the EAA are still unknown. Methionine is usually the first limiting amino acid in non-cereal plant products (Mai et al., 2006). Methionine is an indispensable sulphur amino acid that plays an essential functional role in initializing protein synthesis (Brosnan and Brosnan, 2006; Martinez et al., 2017; Wang et al., 2016). In addition, it participates in several metabolic processes including a precursor for S-adenosylmethionine (SAM) production, which serves as a principal methyl donor for ranges of molecules such as nucleic acids, choline, creatine and amines in vertebrates (Brosnan and Brosnan, 2006; Mato et al., 2002). Other methionine derivatives include cysteine, glutathione, taurine, sulphate, and some phospholipids (NRC, 2011). Methionine deprivation has been shown to cause growth reduction, decreased feed efficiency, reduced enzyme activities, intestinal development impairment, antioxidant degeneration and cataracts formation in various fish species (Espe et al., 2008; Harding et al., 1977; Jiang et al., 2017; Jiang et al., 2016; Poppi et al., 2017). To overcome this deficiency problem, crystalline methionine is usually

Abbreviations: Met, Methionine; $dMet_{DP}$, Dietary digestible methionine content expressed per unit of digestible protein; ADC, Apparent digestibility coefficient; BW, Body weight; DM, Dry matter

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supplemented in fish feed, based on the requirement of the species under culture.

The methionine requirements of commonly cultured fish species range from 13.0 to 45.3 g kg^{-1} crude protein (NRC, 2011; Zhou et al., 2011). For African catfish, Ovie and Eze (2010) and Fagbenro et al. (1999) reported a close range of dietary methionine requirement of 29.7 and 32.0 g kg^{-1} crude protein respectively. In these studies, highly digestible (e.g., fishmeal) and purified ingredients (e.g., casein and gelatine) supplemented with a large portion of synthetic (free) essential and non-essential amino acids were used for diet formulation, which may influence requirement outcome (Gorissen et al., 2016). There is debate about the validity of estimating amino acid requirement using purified ingredients with a large amount of synthetic amino acids to create the contrasts in the amino acid being studied. For practical reasons, the reliance on these expensive ingredients as protein sources in aquafeeds are being reduced and replaced with plant proteins. Therefore, it is imperative to investigate the effects of crystalline methionine inclusion to practical diets that are mostly deficient in methionine (Salze et al., 2017). In this study, we fed African catfish a plant based diet using only protein from legume ingredients, in which methionine is the first limiting amino acid. Crystalline methionine was supplemented to the diet in order to fill this knowledge gap on methionine requirements.

Generally, nutrient requirements are estimated using a dose-response approach, whereby mathematical models are fitted to a response variable (e.g., weight gain, nutrient deposition), against graded dietary levels of the nutrient (dose) under study (Salze et al., 2017). However, the selected mathematical model and the mode of expression can largely influence the final requirement estimate (NRC, 2011; Zhou et al., 2011). Different authors have employed a wide range of models in requirement studies, but the unavailability of a concerted model has left a doubt in the outcome of various studies. As a result, it is easy to associate differences in values among the same species to the method of analysis (Baker, 1986; Salze et al., 2017; Shearer, 2000). In spite of the widespread use of different analytical models, the present work compared three models commonly used in fish nutrition: the linear plateau model (LP), the broken-line model (BL), and the quadratic regression model (QR). LP presumes that the response parameter (e.g. growth) increases linearly below the requirement and is constant above the requirement. However, for some nutrients, the response above the requirement might alter and still be dose dependent [i.e., when becoming toxic at high doses (Shearer, 2000)]. Beyond breakpoint, the response can either increase or decrease, which can be clearly shown by the BL model. The QR is also used in fish requirement studies, which take into account the curvilinear decrease in performance caused by the imbalance effects of toxicity of the studied nutrient after the requirement is attained. Here we present the estimated methionine (in the presence of cystine) requirement of African catfish and explained the impact of using different analytical models on requirement estimates.

2. Materials and methods

2.1. Ethics statement and research facility

The study (project number 2018.W.0014.001) was carried out 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 juvenile African catfish (*Clarias gariepinus*) fingerlings were obtained from a commercial brood stock farm (Fleuren & Nooijen BV, Nederweert, The Netherlands). Upon arrival, fish were fed a commercial diet and adapted over 2 weeks to the experimental conditions. At the start of the experiment, 840 fish weighing on average 78 g were randomly assigned (40 fish per tank) into 21 experimental tanks. All tanks were connected to a common recirculation aquaculture system (RAS system). A trickling filter, sump and drum filter (Hydrotech 500®, Hydrotech Engineering, Italy) were connected to the RAS to help maintain the same water quality for all tanks. The water volume of the RAS system was 5 m^3 and water loss due to evaporation was continuously compensated with the addition of well water. Water refreshment was based on NO_3 removal from the system to keep NO_3 levels within limits (< 500 mg L^{-1}). Each tank was equipped with air stones and swirl separators (AquaOptima AS, column height 44 cm; diameter 24.5 cm) for collection of faeces and spilled pellet. Water quality parameters were monitored regularly and set at optimal levels for African catfish. Average (SD) measured values over the experimental period were as follows: water temperature 27.6 ± 0.11 °C; pH, 7.2 ± 0.26; ammonium, 0.27 ± 0.14 mg L^{-1}; nitrite, 0.21 ± 0.13 mg L^{-1}; nitrate, 494 ± 16.2 mg L^{-1}; conductivity, 3109 ± 415 μS; and dissolved oxygen concentration, 6.90 ± 0.409 mg L^{-1}. Photoperiod was kept at 12 h light: 12 h dark.

2.3. Diet preparation

The ingredient composition and proximate analysis of the seven experimental diets are given in Tables 1 & 2. These experimental diets were formulated to be identical regarding ingredient composition and thus macro-nutrient content except for the amount of crystalline DL-methionine supplementation and cellulose. The basal diet (Diet A), without methionine supplementation was formulated to have as low as possible methionine content using commonly used plant protein ingredients. Protein originated solely from soy protein concentrate and faba beans and resulted in a methionine content of 12.2 g kg^{-1} crude protein (CP). Between the experimental diets, cellulose was exchanged by crystalline DL-methionine in a dose-response manner: 0, 0.12, 0.24, 0.36, 0.48, 0.60, and 0.84%, respectively. This resulted in an analyzed methionine content ranging between 12.2 and 36.0 g kg^{-1} CP. This range was chosen based on the expected methionine requirement being

Table 1
Ingredient composition of the basal diet (Diet A) without methionine supplementation.

Basal Ingredients	%
Faba beans	15.00
Soy protein concentrate	35.76
Wheat	20.00
Wheat starch	15.00
Cellulose[1]	0.84
Fish oil	2.00
Rapeseed oil	8.00
Moisture loss[2]	0.50
Calcium carbonate	0.50
Monocalcium phosphate	2.50
Mineral premix	0.10
Vitamin premix	0.10
Yttrium oxide	0.10
DL-Methionine supplementation[1]	0.00
L-Lysine	0.40
L-Threonine	0.20

[1] The ingredient composition of the other 6 experimental diets (Diet B to G) was identical to the basal diet except for the content of cellulose and DL-methionine. In Diet B, C, D, E, F and G, respectively, 0.12, 0.24, 0.36, 0.48, 0.60, and 0.84% cellulose was replaced by crystalline DL-methionine.

[2] The pellet production was targeted at a dry matter content of 93%, which resulted in an expected water loss during extrusion of 0.5%.

Table 2
Analyzed proximate composition of experimental diets (on dry matter basis).

	Diets code						
	Diet A	Diet B	Diet C	Diet D	Diet E	Diet F	Diet G
Methionine level (g kg^{-1})	4.0	5.4	6.3	8.0	9.2	10.3	12.0
EAA (g kg^{-1})							
Arginine	23.2	23.2	23.7	23.2	23.1	23.3	22.9
Histidine	8.8	8.6	8.8	8.6	8.7	8.6	8.5
Isoleucine	14.2	14.2	14.4	14.5	14.3	14.5	14.1
Leucine	24.2	24.3	24.3	24.3	24.2	24.4	23.9
Lysine	22.0	22.0	21.9	21.9	21.5	22.1	21.8
Methionine	4.0	5.4	6.3	8.0	9.2	10.3	12.0
Phenylalanine	16.3	16.1	16.2	16.3	15.6	15.8	15.1
Threonine	13.9	13.9	14.0	13.8	13.7	13.8	13.5
Valine	14.4	14.4	14.5	14.7	14.3	14.5	14.3
NEAA (g kg^{-1})							
Alanine	13.7	13.7	13.8	13.8	13.6	13.6	13.5
Aspartic acid	35.6	35.7	35.5	35.2	35.2	35.3	34.8
Glutamic acid	60.1	59.9	60.6	59.3	59.9	60.9	59.1
Cystine	4.4	4.4	4.4	4.4	4.4	4.5	4.3
Glycine	13.4	13.5	13.5	13.4	13.3	13.5	13.2
Proline	16.6	16.5	16.4	16.8	16.7	17.2	16.3
Serine	15.8	16.0	16.1	16.1	15.8	16.1	15.6
Tyrosine	9.7	9.9	9.8	9.7	9.9	10.1	9.4
Sum of AA	310	312	314	314	313	319	312
Nutrients							
Dry matter (g kg^{-1})	932	930	933	929	932	929	932
Crude protein (g kg^{-1})	326	333	331	332	335	337	332
Crude fat (g kg^{-1})	129	128	131	131	132	129	132
Ash (g kg^{-1})	61.1	61.5	60.0	61.5	60.3	60.1	60.7
Energy (kJ g^{-1})	21.1	21.2	21.2	21.2	21.1	21.3	21.1

Amino acid composition was determined by the Skretting (ARC) laboratory Norway.
EAA: Essential amino acid.
NEAA: Non-essential amino acid.
SAA is without tryptophan since tryptophan was analyzed, Amino acid composition was determined by the Skretting (ARC) laboratory Norway.

around 25 g kg^{-1} CP, of other species summarized by NRC (2011). The diets were formulated to meet the nutrient requirements of African catfish (NRC, 2011), except for dietary methionine. As cysteine synthesis is dependent on the level of methionine in the diet and interferes with the methionine requirement, the cysteine levels were kept constant in all the seven diets. The analyzed dietary cysteine content was 0.42% (4.4 g kg^{-1}; Table 2). Yttrium oxide was added as a marker for the determination of apparent nutrient digestibility (ADC). The extruded floating pellets with sizes ranging from 3 to 3.5 mm, were produced by Skretting ARC Norway using a twin-screw extruder (Wenger, Sabetha, KS, U.S.A). Diets were sealed and stored at 4 °C throughout the experimental period.

2.4. Feeding and sampling

At the start of the experiment, 20 fish were selected at random and euthanized by an overdose phenoxy-ethanol (1.0 mL L^{-1}) for initial body composition analysis. During stocking of the tanks, total biomass and number of fish per tank were recorded while being sedated (0.25 mL L^{-1} phenoxy-ethanol). Each diet was randomly assigned to the experimental tanks in triplicate. During the 42-day experimental period, fish were fed restrictively in order to provide the same amount of CP to all fish across all diets. This is to minimize the variation in response parameters due to variability in feed intake. Feeding level was fixed at 19.8 g kg$^{-0.8}$ d^{-1} (about 90% of satiation) based on the mean initial weight over all diets. Daily feed ration per tank was increased based on an expected growth using a FCR of 1 for all diets, again to ensure that the feeding levels per fish were equal at all diets. In the case of mortality, the daily feeding rations was adjusted for the number of fish in the tank. Daily feed portions were hand-fed twice a day at 8:00 h and 16:00 h. During the first three days, feeding level was gradually increased from 20% to 100% of the intended ration. After each meal, the uneaten feed was weighted and the spilled pellets counted per tank, which were collected by the swirl separators 15 min after feeding was finished. For proximate analysis of the feed, a representative sample from each diet was taken and stored at 4 °C weekly.

Faeces were collected overnight (17.00 h – 7.30 h) for digestibility studies from week 2 onwards (Monday - Friday), using detachable collection bottles (250 mL) connected to settling tanks. The faecal collection bottles were submerged in ice-filled styrofoam box, to reduce microbial degradation. Faeces were pooled every week and per tank using aluminum trays, then stored at −20 °C for further analysis. Faeces from weeks 2, 4 and 6 were pooled per tank for analysis. Throughout the experiment, fish behavior was monitored, and fish were visually inspected for discernible signs of cataract and deformity that may arise from methionine deficiency. Mortality was checked twice a day, 30 min prior to feeding. At the end of the experiment, fish were batch weighed per tank for final weight under mild sedation and 10 fish per tank were randomly selected and killed by an overdose of phenoxy-ethanol (1.0 mL L^{-1}) for final body composition analysis.

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

Analyses were performed on the diets, whole fish samples, and faeces 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). A portion of the minced fish were freshly sampled for CP analysis. Minced fish samples for AA, crude fat and energy determination were freeze-dried before further analysis. Faecal samples were freeze-dried, then manually pulverized through a 1 mm screen sieve. Feed pellets were grinded by a grinding machine. Fish, faeces, 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). Ash content by incineration in a muffle furnace at 550 °C overnight (ISO 5984, 1978). The Kjeldahl method was used for CP analysis (ISO 5983, 1979). Crude fat analysis was determined using the Soxhlet method (ISO 6492, 1999). Energy was measured using adiabatic bomb-calorimeter (C7000 IKA®, IKA analysentechnik, Weitershem, Germany; ISO 9831, 1998). Yttrium, phosphorus, calcium and magnesium in feed and faeces 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. Calculation

Daily weight gain (g d^{-1}) was calculated as the differences between average initial (W_i) and final (W_f) body weight of fish divided by the duration of the experiment (t). Feed conversion ratio (FCR; g g^{-1}) on dry matter (DM) basis was calculated as (feed intake × dry matter content of the feed)/(final weight of fish – initial weight of fish). Feed intake (FI; % BW d^{-1}) was calculated as feed intake divided by mean body weight x (100%). Specific growth rate (SGR; % BW day^{-1}) was calculated as (LnWf − LnWi × 100)/t, where t is the duration of the experiment in days (d). Fish survival (%) was calculated as number of fish at the beginning of the experiment divided by the number fish at the end of the experiment x 100.

The apparent digestibility coefficient (ADC) of AA and 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 faeces)/(Yttrium concentration in the faeces × concentration nutrient in feed)]. The concentrations of Yttrium and nutrients were expressed on DM basis.

Methionine (Met), nitrogen (N) and energy balance parameters were calculated per treatment and expressed as; mg kg^{-1} d^{-1}, mg kg^{-1} d^{-1} and kJ kg^{-1} d^{-1}, respectively (summarized in supplementary Table A). Detailed description of calculation of balance parameters have been previously described by Saravanan et al. (2012). Met and protein efficiency ratio was calculated as the amount of nutrients retained as percentage of the digestible nutrient intake.

2.7. Statistical analysis

All measured parameters were tested for the effect of diet using one-way ANOVA. If significant ($p < 0.05$), means were compared by Tukey's honest significant difference (HSD, using IBM SPSS Statistics, version 23.0 for Windows (IBM Corp., Armonk, NY, USA). Thereafter, all parameters were subjected to regression analysis, to test for the linear and quadratic effect of dietary level of digestible methionine content expressed per unit of digestible protein ($dMet_{DP}$; X-variable). Estimation of methionine requirements was done by three different regression models: Linear plateau (LP), broken-line regression (BL) and quadratic regression (QR). These regression analyses were done for different response parameters (weight gain, retained nitrogen, etc.) against $dMet_{DP}$ (X-variable). Estimation of methionine requirements by LP and BL models were done by the procedure NLIN of SAS. All statistical analysis were performed using the Statistical Analysis System (SAS) statistical software package version 9.2 (SAS institute, Cary, NC, USA).

3. Results

The mean values of each experimental diet and the one-way ANOVA analysis all parameters are presented in Tables 3-6. In supplementary tables B and C, the regression analysis of all parameters in relation to the dietary digestible methionine (Met) content expressed per unit of digestible protein ($dMet_{DP}$) are presented. When being significant, the linear or quadratic relationship is given. At visual inspection, no cataracts or any other pathological sign were observed in fish fed Met-deficient diet in this study. Met supplementation did not affect the survival of African catfish, as survival rate averaged at 96% over treatments (Table 3; $p > 0.05$).

Fish were fed restrictively, the same ration, which resulted in minimal differences in feed intake among diets. Despite the equal feed intake, final body weight (BW) and growth increased with increasing Met supplementation, and were curvilinearly related to $dMet_{DP}$ (Fig. 1C; $P < 0.01$). Consequently, FCR reduced with $dMet_{DP}$ (Table 3 and Fig. 1F).

In this study, the impact of $dMet_{DP}$ on efficiency parameters were quantified. Growth and efficiency data paralleled each other. Feed conversion ratio (FCR), Met efficiency, protein efficiency ratio (PER) were all quadratically related to $dMet_{DP}$ ($p < 0.001$). Fish fed the diet without DL- Met supplementation had the highest FCR (0.99), which differ significantly from all other treatments (Table 3). Relatable effect of low dietary Met was seen in the PER values as fish fed the Met-deficient diet recorded the lowest PER. In contrast, Met efficiency was reduced with increasing $dMet_{DP}$.

Digestibility of macro nutrients was not influenced by the dietary treatments (One-way ANOVA; $P > 0.10$, Table 4). However, almost all AA ADC were affected by $dMet_{DP}$. This was seen in the result of Met ADC, which increased with increasing Met supplementation (Fig. 2). Except for Met ADC, no consistent pattern (dose-response) was present for AA ADC values between diets (Table 4 and Supplementary fig. A).

Regarding body composition, both protein and fat content were linearly affected by $dMet_{DP}$ ($P < 0.001$; Table 5 and Supplementary table B). Protein content in African catfish was low at low $dMet_{DP}$ and high at high $dMet_{DP}$. Body Met content paralleled the pattern of body protein content and increased linearly with increasing $dMet_{DP}$ ($P < 0.001$). In contrast, body fat content declined with increasing $dMet_{DP}$, which resulted to leaner African catfish at high $dMet_{DP}$.

Energy retention (ER), nitrogen retention and Met retention were measured as alternative growth indices (Table 6). The nitrogen retention showed a similar response as growth. It increased with $dMet_{DP}$ at low levels and the increase in response levelled off at higher levels of $dMet_{DP}$ (Fig. 1I; quadratically $P < 0.001$). In contrast, ER and Met retention were unaffected by $dMet_{DP}$ ($P > 0.1$; Table 6).

Table 7 highlights the digestible Met requirements of African catfish, which was estimated by fitting three different analytical models to the dose-response relationship: linear plateau (LP), broken line (BL) and quadratic regression (QR). Dietary digestible Met content expressed per unit of digestible protein ($dMet_{DP}$) was used as independent variable, while different outcome parameters as shown in Table 7 were applied as dependent variables. Fig. 1 shows the relationship between $dMet_{DP}$ and growth (panel A, B and C), FCR (panel D, E and F) and retained nitrogen (panel G, H and I) respectively, and the respective estimated methionine requirements.

In Table 7, the estimated methionine requirement of African catfish based on selected parameters using different analytical models (linear plateau, LP; Broken line, BL; Quadratic regression QR) is given.

Table 3
Growth performance and feed utilization of African catfish fed the experimental diets.

	Diet codes								
	Diet A	Diet B	Diet C	Diet D	Diet E	Diet F	Diet G		
Methionine level (g kg^{-1})	4.0	5.4	6.3	8.0	9.2	10.3	12.0	SEM	*P*-value
Experimental period (d)	42	42	42	42	42	42	42		
Tanks (n)	3	3	3	3	3	3	3		
Fish per tank (n)	40	40	40	40	40	40	40		
Survival (%)	95.8	98.3	95.8	97.5	97.5	94.2	94.2	2.02	ns
Initial BW (g)	78.8	78.4	78.2	77.9	77.6	77.2	77.4	0.52	ns
Final BW (g)	196^{a}	207^{ab}	211^{b}	211^{b}	210^{b}	210^{b}	208^{b}	2.45	**
Feed Intake (g d^{-1})	2.77	2.77	2.77	2.77	2.77	2.77	2.77	–	–
Growth (g d^{-1})	2.80^{a}	3.06^{ab}	3.15^{b}	3.17^{b}	3.16^{b}	3.16^{b}	3.12^{b}	0.06	**
SGR (% d^{-1})	2.18^{a}	2.31^{ab}	2.36^{b}	2.37^{b}	2.37^{b}	2.38^{b}	2.36^{b}	0.03	**
FCR (g g^{-1})	0.99^{b}	0.91^{a}	0.88^{a}	0.87^{a}	0.88^{a}	0.88^{a}	0.89^{a}	0.02	**

SGR, specific growth rate; FCR, feed conversion ratio; BW, body weight; SEM, standard error of means; ns, not significant $p < 0.1$; ** $p < 0.01$; values in the row with different superscripts are significantly different ($p < 0.05$) according to Tukeys' multiple comparison test.

Table 4
Apparent digestibility coefficient (ADC) of AA and nutrients in African catfish fed the experimental diets.

	Diet codes								
	Diet A	Diet B	Diet C	Diet D	Diet E	Diet F	Diet G		
Methionine level (g kg^{-1})	4.0	5.4	6.3	8.0	9.2	10.3	12.0	SEM	*P*-VALUE
<u>EAA (%)</u>									
Arginine	95.0^{ab}	95.0^{ab}	95.5^{b}	94.9^{ab}	95.2^{ab}	95.7^{b}	94.2^{a}	0.37	*
Histidine	92.1^{ab}	92.4^{ab}	92.9^{b}	92.0^{ab}	92.3^{ab}	93.2^{b}	90.9^{a}	0.56	*
Isoleucine	90.5^{ab}	90.6^{ab}	91.4^{ab}	90.5^{ab}	90.2^{ab}	91.8^{b}	89.4^{a}	0.60	*
Leucine	90.8^{ab}	91.2^{ab}	91.8^{ab}	90.8^{ab}	90.9^{ab}	92.2^{b}	90.0^{a}	0.54	*
Lysine	93.9^{ab}	94.0^{ab}	94.4^{ab}	93.6^{ab}	93.7^{ab}	94.6^{b}	92.9^{a}	0.49	#
Methionine	89.9^{a}	92.6^{b}	94.0^{bc}	94.7^{cd}	95.3^{cde}	96.4^{e}	96.1^{de}	0.50	***
Phenylalanine	91.1^{ab}	91.6^{b}	92.2^{b}	91.3^{ab}	91.0^{ab}	92.2^{b}	89.7^{a}	0.55	**
Threonine	90.1^{ab}	90.3^{ab}	90.9^{b}	89.8^{ab}	89.7^{ab}	91.4^{b}	88.6^{a}	0.61	*
Valine	88.6^{ab}	89.0^{ab}	89.7^{ab}	88.7^{ab}	88.4^{ab}	90.1^{b}	87.4^{a}	0.69	*
<u>NEAA (%)</u>									
Alanine	87.6^{ab}	87.7^{ab}	88.4^{ab}	87.4^{ab}	87.1^{ab}	88.9^{b}	85.8^{a}	0.87	#
Aspartic acid	93.2^{ab}	93.3^{ab}	93.8^{b}	92.9^{ab}	93.0^{ab}	94.0^{b}	92.0^{a}	0.49	*
Glutamic acid	95.6^{ab}	95.8^{ab}	96.1^{b}	95.5^{ab}	95.7^{ab}	96.2^{b}	94.9^{a}	0.31	*
Cystine	86.9^{ab}	87.5^{ab}	88.3^{b}	87.0^{ab}	87.0^{ab}	89.0^{b}	85.6^{a}	0.76	*
Glycine	87.4^{ab}	87.6^{ab}	88.5^{b}	87.2^{ab}	87.3^{ab}	89.0^{b}	85.5^{a}	0.88	*
Proline	92.1^{ab}	92.3^{ab}	92.8^{b}	92.3^{ab}	92.2^{ab}	93.5^{b}	90.9^{a}	0.46	**
Serine	91.4^{ab}	91.7^{ab}	92.3^{b}	91.5^{ab}	91.4^{ab}	92.7^{b}	90.3^{a}	0.53	**
Tyrosine	92.9^{ab}	93.2^{ab}	93.6^{ab}	92.5^{ab}	93.2^{ab}	93.8^{b}	91.5^{a}	0.66	#
Sum of AA	92.3^{ab}	92.5^{ab}	93.0^{ab}	92.2^{ab}	92.3^{ab}	93.4^{b}	91.4^{a}	0.50	*
<u>Nutrients (%)</u>									
Dry matter	74.1	73.45	75.71	73.54	76.35	74.11	72.33	1.14	ns
Protein	87.69	88.04	88.37	88.04	89.11	87.98	87.42	0.53	ns
Fat	93.93	94.19	94.77	94.39	95.13	94.08	94.01	0.29	ns
Ash	39.53	39.10	41.56	37.69	38.71	37.82	31.72	4.93	ns
Phosphorus	60.40	59.87	60.96	59.68	61.26	60.15	56.94	2.03	ns
Energy	79.68	79.40	80.89	79.63	82.15	80.20	78.48	0.84	ns

AA, amino acid; DM, dry matter; SGR, specific growth rate; FCR, feed conversion ratio; BW, body weight; SEM, standard error of means; NS, not significant $p < 0.1$; * $p < 0.05$; ** $p < 0.01$; ***, $p < 0.001$; values in the row with different superscripts are significantly different ($p < 0.05$) according to Tukeys' multiple comparison test.

Irrespective of the dependent variables, the LP and BL models resulted in similar values obtained for the Met requirement for African catfish. On the other hand, QR model estimated consistently higher values for all parameters. Only parameters such as Met ADC and Met efficiency showed disparity in their outcome, with high values recorded when all three models were applied. Growth parameters displayed similar values for optimal Met requirement, averaging at a $dMet_{DP}$ content of 19.7 g kg^{-1}, when LP model was applied. Quadratic regression analysis indicated a $dMet_{DP}$ requirement of 30.8 g kg^{-1} for SGR, 29.2 g kg^{-1} for daily growth, and 31.3 kg^{-1} for retained nitrogen. As for the efficiency indices, Met efficiency depicted larger variation compared to other parameters. Similarly, ADC Met projected high values for $dMet_{DP}$ breakpoints: LP, 31.0 g kg^{-1}; BL, 31.9 g kg^{-1}; and QR, 39.0 g kg^{-1}. In contrast, protein efficiency followed a similar pattern as growth, which plateaued at a $dMet_{DP}$ value of 19.4 g kg^{-1}, for Met requirement. Based on the R^2 values of the three models and the model, which depicted the least SSE, the BL model was shown to estimate the dose-response relationship more accurately, but the difference in R^2 was marginal compared to the LP model. In contrast to the LP model, the outcomes of the NLIN procedure of SAS were not fully stable for the BL model. Small differences in the starting values of the NLIN procedure affected the estimated breakpoint by BL for growth and retained nitrogen. Therefore, LP seemed to be the most appropriate model due to its stability. Using this LP model, the estimated digestible methionine requirement for optimal growth in African catfish is a $dMet_{DP}$ value ranging between 18.7 and 21.4 g kg^{-1}.

4. Discussion

In this study, methionine (Met) supplementation resulted in improved growth and better feed utilization of juvenile African catfish. The results suggest that Met was the first limiting amino acid in the basal diet used in the current study (a leguminous plant-based diet). This reflected in the poor feed efficiency, and reduced growth reported for fish fed the low-Met diet. However, African catfish were able to utilize crystalline Met, as supplementation improved growth performance. The general observation that low dietary Met level can limit growth performance has been demonstrated in various studies (Fagbenro et al., 1999; Liang et al., 2016; Yan et al., 2007; Zhou et al., 2011). Reduced growth in fish fed Met-deficient diets may be attributed to: reduced feed intake; reduced protein deposition (i.e., N-retention); and increased fat deposition (i.e., enhanced deamination of non- Met AA). All these factors contribute to an altered FCR, PER and protein utilization efficiency.

In the current study, the decline in performance was not related to feed intake (FI) because fish were fed restrictively. In literature, the majority of requirement studies often apply satiation feeding strategy. This may lead to variation in nutrient intake, as loss of appetite has been reported in species fed Met-deficient diets under satiation feeding (Alam et al., 2000; Elmada et al., 2016; Wu et al., 2017). Methionine is known to play a crucial role in the modification of the gene and endocrine pathways responsible for appetite regulation (Fontagné-Dicharry et al., 2017; Sourabié et al., 2018). Restricted feeding prevents differences in FI between treatments, therefore, variations in growth rate will only result from differences in metabolic efficiency (Cowey, 1994). Moreover, the total consumption of feed supplied will improve the use of available nutrients, thereby increase feed efficiency (Coloso et al., 1999). Our results suggest that the major reason for the hampered performance in the low-Met group was the reduction in N retention (protein deposition) coinciding with a reduced protein efficiency. However, methionine efficiency was higher in fish fed the Met-deficient diets. This high efficiency (slightly higher but not different from 100%) is an indication that Met was the first limiting AA. Consequently, the available Met was completely utilized. This is because the first limiting

Table 5
Whole body composition (g kg $^{-1}$) of African catfish fed with the experiemntal diets.

	Initial	Diets code								
		Diet A	Diet B	Diet C	Diet D	Diet E	Diet F	Diet G		
Methionine level (g kg^{-1})		4.0	5.4	6.3	8.0	9.2	10.3	12.0	SEM	*P*-value
EAA (g kg^{-1} dry matter)										
Arginine	37.8	30.5^{a}	33.2^{b}	34.2^{b}	32.8^{b}	33.6^{b}	33.8^{b}	33.7^{b}	0.40	***
Histidine	16.0	11.9^{a}	13.6^{b}	14.2^{bc}	14.1^{bc}	14.3^{bc}	14.4^{c}	14.4^{c}	0.16	***
Isoleucine	24.8	19.3^{a}	21.2^{b}	22.0^{b}	21.4^{b}	21.8^{b}	22.0^{b}	22.0^{b}	0.25	***
Leucine	43.4	33.6^{a}	37.0^{b}	38.4^{b}	37.3^{b}	38.0^{b}	38.5^{b}	38.4^{b}	0.44	***
Lysine	47.6	37.9^{a}	41.8^{b}	44.0^{b}	42.6^{b}	43.3^{b}	44.1^{b}	44.1^{b}	0.53	***
Methionine	15.2	11.8^{a}	13.2^{b}	13.6^{b}	13.4^{b}	13.7^{b}	13.7^{b}	13.7^{b}	0.15	***
Phenylalanine	24.9	20.2^{a}	22.0^{ab}	22.7^{b}	21.8^{ab}	22.5^{b}	22.6^{b}	22.7^{b}	0.39	**
Threonine	25.8	20.2^{a}	22.1^{b}	22.7^{b}	22.2^{b}	22.5^{b}	22.7^{b}	22.6^{b}	0.26	***
Valine	27.0	21.5^{a}	23.5^{b}	24.5^{b}	23.7^{b}	24.2^{b}	24.3^{b}	24.4^{b}	0.31	***
NEAA (g kg^{-1} dry matter)										
Alanine	38.8	31.9^{a}	34.7^{b}	35.5^{b}	33.8^{ab}	34.7^{b}	34.7^{b}	34.4^{b}	0.44	**
Aspartic acid	57.8	45.4^{a}	50.0^{b}	52.2^{b}	50.4^{b}	51.4^{b}	52.0^{b}	51.4^{b}	0.71	***
Glutamic acid	84.1	66.2^{a}	73.1^{b}	76.3^{b}	73.4^{b}	75.0^{b}	75.6^{b}	74.7^{b}	0.92	***
Cystine	5.39	4.25^{a}	4.60^{ab}	4.69^{b}	4.53^{ab}	4.68^{b}	4.74^{b}	4.75^{b}	0.08	**
Glycine	49.1	43.5	46.5	46.5	43.4	44.7	44.3	44.0	0.93	ns
Proline	30.6	26.0	27.8	28.0	26.6	27.2	26.9	26.9	0.56	ns
Serine	24.8	19.7^{a}	21.4^{b}	21.8^{b}	21.1^{b}	21.5^{b}	21.7^{b}	21.7^{b}	0.26	***
Tyrosine	15.1	11.8^{a}	12.8^{ab}	13.6^{b}	12.6^{ab}	13.1^{ab}	13.2^{ab}	13.4^{b}	0.30	**
Sum of AA	568	456^{a}	499^{b}	515^{b}	495^{b}	506^{b}	509^{b}	507^{b}	5.2	***
Proximate composition (g kg^{-1} dry matter)										
Dry matter	243	293	282	285	287	290	287	287	3.0	ns
Crude protein	645	501^{a}	547^{b}	546^{b}	553^{b}	557^{b}	551^{b}	560^{b}	5.9	***
Crude fat	236	387^{b}	353^{a}	351^{a}	350^{a}	345^{a}	353^{a}	347^{a}	6.7	***
Ash	121	105	99.0	99.1	100	102	100	105	3.1	ns
Phosphorus	17.0	18.6	15.7	15.8	17.7	17.3	17.2	18.2	1.0	ns
Energy (kJ g^{-1} DM)	24.9	28.1^{b}	27.1^{ab}	27.0^{ab}	26.9^{ab}	26.8^{a}	27.0^{ab}	26.9^{ab}	0.3	*

Amino acid composition was determined by the Skretting (ARC) laboratory Norway. EAA, essential amino acid; NEAA, non-essential amino acid; SAA, sum of amino acid (SAA is without tryptophan, no tryptophan was detected after acid hydrolysis); DM, dry matter; SEM, standard error of means; NS, not significant $p < 0.1$; * $p < 0.05$; ** $p < 0.01$; ***, $p < 0.001$; values in the row with different superscripts are significantly different ($p < 0.05$) according to Tukeys' multiple comparison test.

Table 6
Effect of methionine inclusion on nitrogen and energy balances of African catfish.

	Diets code								
	Diet A	Diet B	Diet C	Diet D	Diet E	Diet F	Diet G		
Methionine level (g kg^{-1})	4.0	5.4	6.3	8.0	9.2	10.3	12.0	SEM	*P*-value
Methionine balance (mg d^{-1})									
Gross methionine intake (MNI)	11.06^{a}	14.96^{b}	17.60^{c}	22.28^{d}	25.68^{e}	28.51^{f}	33.25^{g}	0.01	***
Digestible methionine intake (MNI)	9.95^{a}	13.86^{b}	16.54^{c}	21.10^{d}	24.48^{e}	27.49^{f}	31.95^{g}	0.07	***
Retained methionine	10.79	12.02	12.60	11.64	13.08	11.77	12.37	0.61	ns
Methionine efficiency	108.47^{d}	86.73^{c}	76.19^{bc}	55.19^{ab}	53.44^{a}	42.81^{a}	38.71^{a}	4.43	***
Nitrogen balance (mg d^{-1})									
Gross nitrogen intake	144.7^{a}	147.9^{c}	146.8^{b}	148.3^{d}	148.8^{e}	149.5^{f}	147.8^{c}	0.05	***
Digestible nitrogen intake	126.9^{a}	130.2^{ab}	129.7^{ab}	130.5^{ab}	132.6^{b}	131.5^{b}	129.2^{ab}	0.80	**
Branchial urinary losses	63.9^{b}	55.2^{ab}	51.4^{a}	49.3^{a}	49.6^{a}	50.9^{a}	47.9^{a}	1.62	***
Retained nitrogen	63.0^{a}	75.0^{b}	78.4^{bc}	81.3^{bc}	83.0^{c}	80.7^{bc}	81.3^{bc}	1.86	***
Protein efficiency	49.6^{a}	57.6^{b}	60.4^{b}	62.3^{b}	62.6^{b}	61.3^{b}	63.0^{b}	1.33	***
Energy balance (KJ d^{-1})									
Energy intake	58.60^{a}	58.80^{b}	58.80^{b}	58.96^{c}	58.57^{a}	59.10^{d}	58.67^{a}	0.02	***
Digestible energy intake	46.69	46.69	47.57	46.95	48.12	47.40	46.05	0.50	ns
Branchial urinary losses	1.59^{b}	1.37^{ab}	1.28^{b}	1.23^{b}	1.24^{b}	1.27^{b}	1.19^{b}	0.05	***
Metabolizable energy intake	45.10	45.32	46.29	45.72	46.88	46.13	44.85	0.48	#
Heat production	17.85	18.97	18.89	18.10	19.24	18.53	17.67	1.04	ns
Retained energy	27.25	26.34	27.40	27.63	27.65	27.60	27.19	0.72	ns
Retained energy as protein	9.33^{a}	11.11^{b}	11.61^{bc}	12.03^{bc}	12.29^{c}	11.95^{bc}	12.05^{bc}	0.24	***
Retained energy as fat	17.92	15.23	15.79	15.59	15.36	15.65	15.14	0.66	ns
Maintenance energy requirement (kJ kg$^{-0.8}$ d^{-1})	41.94	40.67	37.56	31.75	36.66	34.42	29.83	6.23	ns

SEM, standard error of means; NS, not significant $p < 0.1$; * $p < 0.05$; ** $p < 0.01$; ***, $p < 0.001$; values in the row with different superscripts are significantly different ($p < 0.05$) according to Tukeys' multiple comparison test.

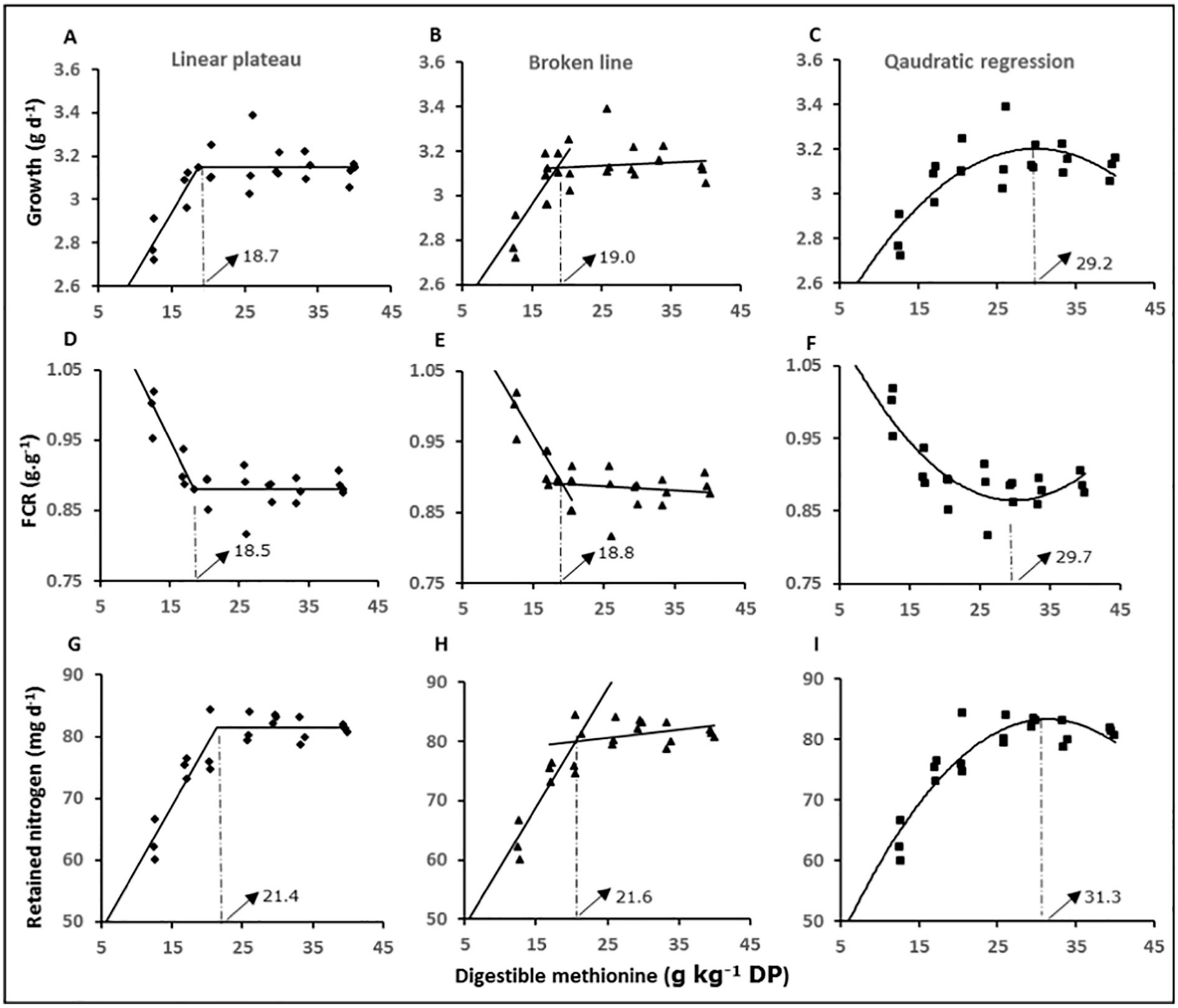

Fig. 1. The relationship between dietary digestible methionine content (expressed in g kg^{-1} digestible protein [DP]) and growth (panel a, b and c); FCR (d, e and f); retained nitrogen (g, h and I), in African catfish. Three models for estimating the optimal dietary digestible methionine content were compared: linear plateau model (panel a, d and g); broken line model (panel b, e and h); and quadratic regression (panel c, f and i). The estimated equations and R^2 for the linear plateau and broken line model are provided in supplementary table E and for quadratic regression in supplementary table B and C.

AA in a diet often has the highest marginal efficiency leading to the complete utilization of available AA rather than being catabolized (Gerrits et al., 1998).

As expected, body protein contents (on DM basis) increased with increasing Met level and lowest values were recorded for fish fed the Met-deficient diet (Table 5). High body protein contents in the Met-balance and Met-excess group is an indication of increased nitrogen retention due to the supply of diets with balanced AA. Nwanna (2016) and Ovie and Eze (2010) reported that DL-methionine inclusion in the diet resulted in a significant increase of African catfish carcass protein, which improved carcass quality. The body fat content showed an opposite trend to the body protein content as it decreased with increasing Met supplementation. This inverse response of body protein and fat to Met deficiency have been reported in some studies (Powell et al., 2017). Since protein synthesis and accretion depend on a balanced AA supply (De la Higuera et al., 1998), a deficiency in one essential amino acid (EAA) will disrupt this process. As a result, shortage of Met (e.g., in Met-deficient diet) at the synthesis sites will cause the remaining AA to be catabolized and or used as energy source for fat synthesis. This explains the higher fat content of fish fed the Met-deficient diet. Our results show a positive contribution of Met inclusion on overall protein efficiency (PER). Improved PER in response to Met supplementation has been observed in other fish species (Alam et al., 2000; Luo et al., 2005; Ruchimat et al., 1997; Wu et al., 2017; Yan et al., 2007). The retained nitrogen supports the growth trend reported in this study, suggesting increased protein synthesis due to Met inclusion (Elmada et al., 2016).

In the current study, the dietary digestible Met requirement expressed per digestible protein ($dMet_{DP}$) for the growth of African catfish (*Clarias gariepinus*) was 18.7 g kg^{-1} (based on the linear plateau model). This is equivalent to a dietary Met content of 6.3 g kg^{-1} diet and 19.2 g kg^{-1} crude protein (CP) (Supplementary table D). These estimates fall in the range of dietary Met values (4.9–7.1 g kg^{-1} diet) reported for different fish in literature, with no clear pattern across species (Espe et al., 2008; Furuya et al., 2001; Harding et al., 1977; Ren et al., 2017). Only few studies reported slightly lower dietary Met values ranging between 4.9 and 5.3 g kg^{-1} diet (Jackson and Capper, 1982; Kim et al., 1992; Nguyen and Davis, 2009). Quite a number of identified factors such as experimental design, method of analysis and the choice of statistical models, may largely influence the requirement estimate (Figueiredo-Silva et al., 2015; Shearer, 2000). Furthermore, cysteine and methionine make up the total sulphur amino acid (TSAA) of fish (Ahmed et al., 2003; Wilson, 1986; Zhou et al., 2011). Cysteine inclusion in the diet has been shown to spare up to 40–60% Met in meeting the requirements for TSAA (NRC, 2011). This will replace Met in the synthesis of cysteine and its derivatives (Brosnan and Brosnan, 2006; Fagbenro et al., 1999). High requirement for Met reported for

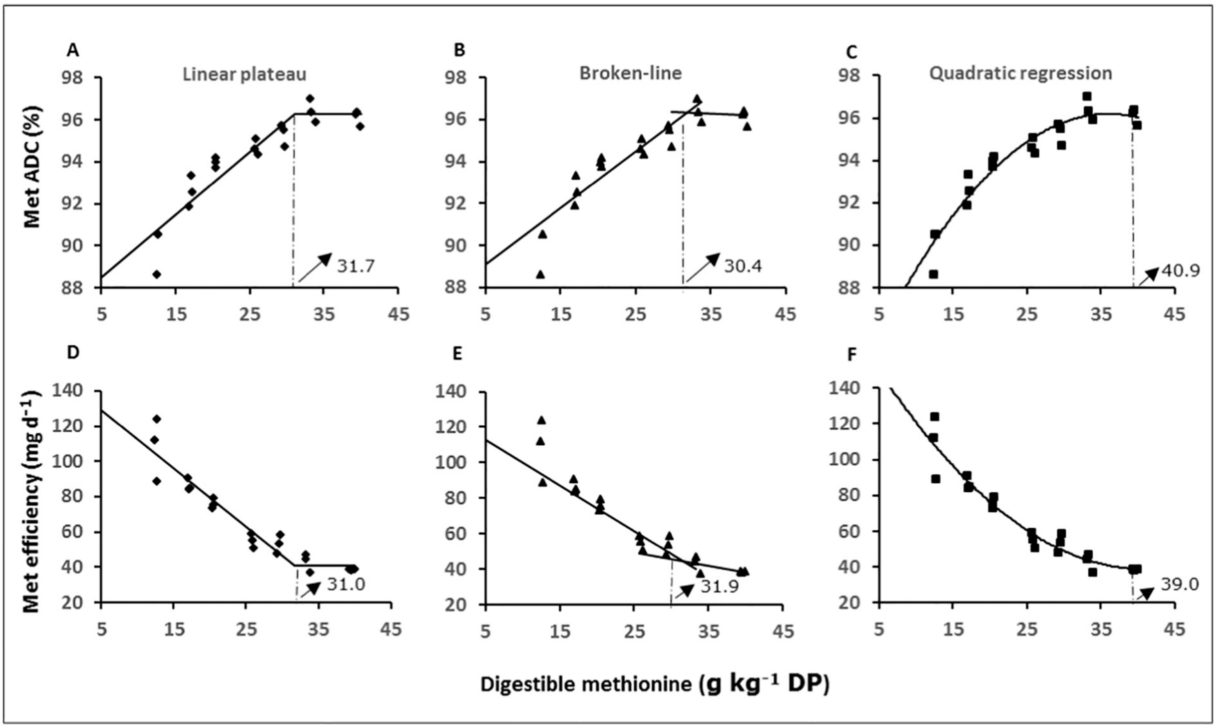

Fig. 2. The relationship between dietary digestible methionine content (expressed in g kg^{-1} digestible protein [DP]) and methionine (Met) digestibility (panel a. b and c) and methionine (Met) efficiency (d, e and f), in African catfish. Three models for estimating the optimal dietary digestible methionine content were compared: linear plateau model (panel a and d); broken line model (panel b and e); and quadratic regression (panel c and f). The estimated equations and R^2 for the linear plateau and broken line model are provided in supplementary table E and for quadratic regression in supplementary table B and C.

Table 7
Estimated methionine requirement of African catfish on selected parameters using different analytical models (linear plateau, LP; Broken line, BL; Quadratic regression QR).

Traits	Breakpoint LP (SE)	Breakpoint BL (SE)	Maximum QR
SGR (% d^{-1})	19.1 (1.46)	19.0 (1.66)	30.8
Growth (g d^{-1})	18.7 (1.35)	19.0 (1.57)	29.2
Retained nitrogen (mg d^{-1})	21.4 (0.91)	18.7 (1.06)	31.3
FCR (g g^{-1})	18.5 (1.16)	18.8 (1.35)	29.7
Methionine efficiency (mg d^{-1})	31.7 (1.55)	24.6 (2.57)	40.9
Protein efficiency (%)	19.4 (1.19)	18.8 (1.26)	32.0
ADC methionine (%)	31.0 (1.7)	31. 9 (2.03)	39.0
Branchial urinary losses (mg d^{-1})	19.8 (1.59)	19.0 (1.66)	33.3

SGR, specific growth rate; FCR, feed conversion ratio; ADC, apparent digestibility coefficient; SE, standard error of the estimated breakpoint is given between brackets. The full relationships for the models LP and BL are given in Supplementary table E and for the QR model in Supplementary table B and C.

some species could be attributed to low inclusion level of cysteine in the diet. In the current study, cysteine was supplied at a constant level (4.3–4.5 g^{-1} kg diet) in the diet.

The reported Met requirement value in the current study, though slightly lower, has a clear consistent pattern with the whole-body Met composition (24.4 g kg^{-1} CP) of African catfish (Table 5). In general, most essential AA have an estimated requirement by dose-response study that is lower than the body composition of the respective AA (NRC, 2011). Previously, amino acid requirements estimates were based on the amino acid profiles of the fish and whole-body protein (NRC, 2011; Wilson, 1986). This is still used when limited information is available for a new species.

In this study, 3 models; quadratic regression (QR), linear plateau (LP) and broken-line regression (BL), were applied to estimate the Met requirement of African catfish. Estimated requirements for $dMet_{DP}$ were similar for the LP and BL models whereas estimates for the QR, averaged over all parameter were 57% higher (Table 7). There is an ongoing debate in the field of fish nutrition as to which model is most appropriate for nutrient requirements estimation. Some authors argued that fish response to nutrient dosage is curvilinear (Figueiredo-Silva et al., 2015), whereby they often opt for quadratic regression (QR) model as method of analysis (Elmada et al., 2016; Zhou et al., 2006) (Supplementary table D). I.e., they believe that animals gradually (smoothly) transition from one state (deficiency) to another (balanced diet). On the contrary, others favour a sharp transition and therefore apply models with a distinct/sharp inflection point by using e.g. BL or LP analysis (Chi et al., 2020; Harding et al., 1977; Nguyen and Davis, 2009). A major down side of QR for estimating requirement is the fact that the estimated value is dependent upon the width of the dosing (x-variable) applied in the study. When we applied a stepwise reduction in the range (width) of Met doses in our study by excluding first diet G (with the highest dose), then diet F and G and finally E, F and G, this strongly reduced the estimated $dMet_{DP}$ requirement from 29.2 to 23.6 g kg^{-1} DP (see Fig. 3). The estimated requirement for growth by BL analysis altered the estimation only from 19 to 18.4 g kg^{-1} DP (data not shown), whereas LP remained nearly unchanged. This implies that using values below or above required nutrient needed for maximum response would have greater impact on predictions near maximal responses when QR is used (Pesti et al., 2009). Given this arbitrary sensitivity for the width of the applied dosing, favours us to choose LP or BL for Met requirement estimation.

As previously mentioned, LP and BP gave almost identical estimates in the current study. Regarding most criteria used, the slope of the regression above the breakpoint in BL analysis was never significantly different from zero (data not shown). This suggests that under the current experimental condition, the Met level (I.e., highest level; Diet G) did not affect the performance parameters. In literature, methionine has been reported to be toxic in excessive dosage (Choo et al., 1991; Murthy and Varghese, 1998). This toxicity is induced by the over

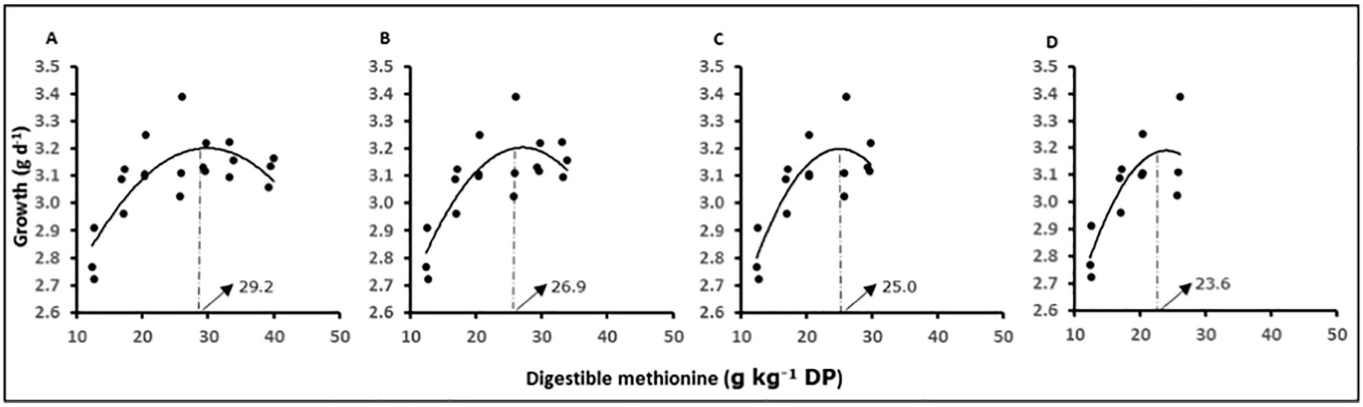

Fig. 3. Methionine requirement of African catfish in response to varying nutrient dose range, using quadratic regression A) All diets ($y = -0.0012 \times ^2 + 0.07\times + 2.1578$ $R^2 = 0.6161$), B) all diets excluding diet G, ($y = -0.0018 \times ^2 + 0.0968\times + 1.894$ $R^2 = 0.6641$) C) all diets excluding diet F and G, ($y = -0.0025 \times ^2 + 0.1248\times + 1.6383$ $R^2 = 0.6875$) D) all diets excluding diets E, F and G ($y = -0.003 \times ^2 + 0.1416\times + 1.4942$ $R^2 = 0.6813$).

accumulation of S-Adenosylmethionine SAM in the liver, a product formed in the Met metabolism pathway (Ahmed, 2014). In addition, excessive methionine intake has been reported to negatively affect FI in some fish species (NRC, 2011). At this toxic level, protein deposition is reduced and a fall in growth slope is often observed. Using LP model in such case will only display the linear component of the dose-response variables until a plateau is reached (Hermesch et al., 1998). However, it does not enable the means to deviate after the inflection point, which is generally not the case. Hypothetically, departure from linearity may occur, whereby the slope descends at higher dose concentration above the requirement. BL allows an ascending or descending slope, and show the clear fall in response to toxicity that may arise from excess nutrient dosage (Gonçalves et al., 2016; Shearer, 2000). This model depicts clearly the theoretical ideas of the pattern of nutritional responses exhibited by animals compared to LP (Pesti et al., 2009). However, for BL, instability of the estimated breakpoint was observed when the input values for growth and retained nitrogen of the NLIN procedure was alternated in this current study, whereas LP remained stable.

Comparing the X variables used differs among studies. In most studies (Supplementary table D), Met requirement are often expressed as g kg^{-1} diet or g kg^{-1} CP. AA requirements expressed as g kg^{-1} diet have the disadvantage that these requirements change if the applied dietary CP content alters. As an example, tilapia diets for pond culture where the natural food web contributes to the fish diet, have a lower optimal dietary protein content than tilapia diets for cage culture without a food web (Kabir et al., 2019). Applying the Met requirement of 19.2 g kg^{-1} CP found in the current study would imply a optimal dietary Met content of 5.8 and 7.7 g kg^{-1} feed for a diet having respectively a CP content of 300 and 400 g kg^{-1} feed. In the current study, we expressed Met requirement as dietary digestible methionine content in g kg^{-1} digestible protein (dMet g kg^{-1} DP) in order to make the estimated requirement independent upon differences in protein and AA digestibility. Although, AA are formed from protein hydrolysis, individual AA digestibility can differ from the overall protein digestibility (unpublished data). Since different diets are fed in different requirement studies, expressing estimates on digestible basis will reduce variability and ensure precise comparison of values among species (NRC, 2011). Moreover, estimating methionine requirement based on digestible methionine has been previously suggested (Figueiredo-Silva et al., 2015; NRC, 2011; Ren et al., 2017). Furthermore, evidences suggest that protein deposition may be a more robust and rational criterion for response (y) variables, compared to weight gain, FCR and SGR that are commonly used (NRC, 2011). Different parameters (Table 7) tested in this study, gave dMet estimates that fall within the same range (18.7–21.4 g kg^{-1} DP). This response is in line with literature as e.g. Zhou et al. (2011) observed only slight differences in the Met requirement for both SGR and protein productive value (PPV) in black sea bream (*Sparus macrocephalus*). Similar observations were made in blunt snout bream (*Megalobrama amblycephala*) (Liang et al., 2016) and Indian major carp (*Cirrhinus mrigala*) (Ahmed et al., 2003).

It is worthy to note that the existing Met requirement estimates; 32.0 g kg^{-1} CP (Fagbenro et al., 1999) and 29.7 g kg^{-1} CP (Ovie and Eze, 2010), for African catfish in literature are higher than what we found in the current study (19.2 g kg^{-1} CP). This may be due to a number of factors; firstly, both studies did not take the cysteine level into account. Secondly, variability in analytical models applied, e.g., Ovie and Eze (2010) employed QR for data analysis (Supplementary table D). Thirdly, choice of ingredients used in diet formulation. For example, Fagbenro et al. (1999), used casein and gelatine as intact protein, which are considered as highly digestible ingredients. Using such purified products in requirement studies may influence requirement values compared to when practical diets are used (Nguyen and Davis, 2009). For practical reasons, faba beans and soy protein concentrate were solely used as intact protein in our study. Lastly, the initial body weights of fish used in these studies greatly differ. it has been reported that the intestinal transporters capacity and whole-body activity of enzymes for AA catabolism vary with developmental stage (Segner and Verreth, 1995). Although, Fagbenro et al. (1999) used broken-line as the method of analysis, the differences in estimates may also arise from differing experimental duration, and genetic variation in African catfish used (Figueiredo-Silva et al., 2015; Shearer, 1995; Shearer, 2000).

In conclusion, the low-methionine plant-based diet (not supplemented with crystalline methionine) used in the present study resulted in methionine deficiency, indicated by poor feed efficiency and reduced growth of fish. However, crystalline methionine supplementation (0.12% to 0.84%) alleviated this deficiency problem. Based on linear plateau model, the digestible methionine requirement of juvenile African catfish (80–210 g) for growth ranges between 18.7 and 21.4 g kg^{-1} expressed per unit of digestible protein ($dMet_{DP}$), depending on the response criteria. This equates to a minimum dietary methionine level of 6.3 g kg^{-1} diet (19.2 g kg^{-1} crude protein) in the presence of 4.4 g kg^{-1} cysteine. Furthermore, the current study demonstrated that quadratic regression can lead to an overestimation of nutrient requirements.

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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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2020.736020.

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