A Meta-Analysis of Essential Amino Acid Requirements of Fish

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Abstract

There are wide variations in the published estimates of essential amino acid (EAA) requirements. Variations are thought to originate from different choices in mode of expression, response variable, and mathematical model. Here we conduct a meta-analysis of the growth-based, dose-response trials of 10 EAA in 22 teleost species: 249 studies were reviewed. Published data were entered in a spreadsheet and re-calculated across in a standard and systematic manner to allow comparisons. The considered unit of requirement were percentage of dry diet, g of EAA per MJ of digestible energy (DE), and g of ingested EAA per kg of metabolic body weight (MBW) per day. Response variables included growth in g per kg MBW per day and thermal-unit growth coefficient (TGC). Four mathematical models were also compared: broken-lime model (BLM), quadratic model (QM), broken-quadratic model (BQM), and saturation kinetic model (SKM). Results first indicate important differences in study quality, as 54% of the reviewed papers were excluded from the meta-analysis, often times because of poor growth or missing information. Additionally, the final dataset was greatly fragmented: 31% of the studied concerned rainbow trout, and lysine was the focus of 29% of all studies, leaving some species and EAA poorly covered. Comparisons of the requirement estimates show important variations between studies, even within species. With such variability there was no difference in requirement estimates calculated with different response variables. Similarly, this variability was not different between the three modes of expression, nor was it between mathematical models. However, there were significant effects of experimental design on the quality of fit of the models. Specifically, experiments that failed to produce a clear, plateauing dose-response curve had greatly increased probability to yielding absurd results (e.g. negative requirement). Finally, the present study emphasizes the critical need of a global, standard and systematic system to report and capture results from nutrition trials.

Keywords: Meta-analysis, amino acid, requirement

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1 Introduction

Aquafeed formulations are constantly refined, updated, and adjusted to reflect progress in nutrition science, as well as the variability in ingredients price and availability. Feeds must remain cost-effective and deliver nutrients efficiently to the animal in order to promote growth and health while minimizing nutrient discharge in the effluent. As a result, fish feed are increasingly formulated at the nutrient level, as is commonly done in other livestock, e.g., poultry. Naturally, the first prerequisite to this goal is a precise knowledge of essential amino acid (EAA) requirement.

However, a number of reviews have identified significant gaps in our understanding of EAA requirements of teleosts and highlighted the great variability in estimates of EAA requirements amongst and within species (Bureau and Encarnação, 2006; Cowey, 1994; Lall and Anderson, 2005; NRC, 1993, 2011; Wilson, 1989). For example, in rainbow trout *Oncorhynchus mykiss* lysine requirement range from 1.3 to 2.9% of the diet (3.7-7.3% of crude protein, CP), and arginine requirement estimates span from 1.4 to 2.2% of the diet. Genetic variations likely account for but a fraction of such different estimates. Rather, reasons explaining these discrepancies can be found in different methodological approaches. Calculation of a requirement estimate requires choosing a mode of expression, a response variable, and a mathematical model to describe their relationship. Modes of expression include percentage of the diet or crude protein, or in g/MJ digestible energy. The choice of mode of expression is a matter of much debate, and reflects the various assumptions authors make when considering what affects the requirements or not (Bureau and Encarnação, 2006; Bureau, 2008). Because these assumptions contradict each other, the same diet can be deemed deficient or not depending on the mode of expression of EAA requirement. Similarly, the choice of the response variable likely influences the estimates of requirements, as it introduces another level of variability between experiments. Responses commonly used in this context include expressions of weight gain (absolute weight gain, specific growth rate, percentage increase, thermal-unit growth coefficient, etc.), feed efficiency, nitrogen retention, or, more rarely, blood EAA concentration. Most studies consider growth as the primary response, but they do not all use the same starting weight in a given species. This is also problematic, since biological processes governing growth in fish are quite dynamic and

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change significantly during the production cycle (Dumas *et al*. 2007), and EAA requirements likely change as well. Mathematical models complete the triptych of the analytical aspect of nutrient requirement estimation. Different models have been used to describe the fish biological response, which typically follows the law of "diminishing return". The broken-line model (BLM) has been heavily used for the past two decades. However, when compared to other non-linear models, the BLM consistently returns lower estimates (Hernandez-Llamas, 2009). In contrast, Shearer (2000) noted that non-linear models could yield requirement estimates up to five times the published requirement.

Clearly, there is a dire need to re-visit fish EAA requirements. Thus, the present study first extensively reviewed the existing literature on EAA requirements in fish and systematically standardized the relevant data. The resulting dataset was then analyzed using various modes of expression, responses and mathematical models to re-evaluate the EAA requirement in these commercially important fish species.

2 Methods and calculations

2.1 Search of the literature and selection criteria

An extensive search of the EAA requirement literature was conducted using online databases, peer-reviewed journals, and books. At this point, the sole selection criterion was to only keep studies on commercially-relevant species (e.g., salmonidae, cychlidae, cyprinidae), resulting in 286 articles. There was among these a great variety of objectives, experimental designs, and analytical methodologies employed. Therefore, additional selection criteria were applied to the dataset to eliminate studies unsuitable to the present meta-analysis.

Since growth was by far the most studied type of response, only studies based on growth trials in response to graded dietary levels of a unique AA were kept. Thus experiments based on the measurement of plasma AA levels, or on the ideal protein theory, were not included (37 studies out of the initial 286).

Other selection criteria were as follow: husbandry practices must be adequate for the species of interest, including water quality. Information on temperature and experiment duration must be provided. All experimental diets must be adequately characterized and me*et al* l other

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known nutritional requirements. Studies must use five or more experimental diets in order to ensure the accuracy of the non-linear regressions analyses. Data, especially diet composition, must be reported on a dry-matter basis, or enough information must be provided to compute dry-matter composition of the diets. Studies where mortality was high and uncorrelated with EAA graded levels were not included. Finally, initial and final individual body weight, as well as feed intake, must be reported, or enough information must be provided to calculate them.

2.2 Variables and models combinations

The meta-analysis was conducted as a 3x2x4 array: three factor variables (percentage of EAA of interest in the dry diet, amount of EAA per MJ of digestible energy, and the amount of the EAA of interest ingested during the experiment), and two response variables (weight gain per kg of metabolic body weight and thermal-unit growth coefficient, TGC). Together, they form six pairs of variable. Finally, four regression models (broken-line, quadratic, broken-quadratic, and saturation-kinetic models) were used to estimate the EAA requirements for each of the variable pair and for each selected study.

2.2.1 Calculated factor and response variables

Weight gain, on a metabolic body weight-basis (WG_{MBW}, in g/kgMBW/d)

$$WG_{MBW} = \frac{FBW - IBW}{\left[\left(\frac{FBW^{1/3} + IBW^{1/3}}{2}\right)^{3} \times 10^{-3}\right]^{0.8} \times d}$$

Thermal-unit growth coefficient (TGC, dimensionless)

$$TGC = 100 \times \frac{FBW^{1/3} - IBW^{1/3}}{t \times d}$$

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AA dietary level to digestible energy (AA_{DE}, in g/MJ_{DE})

$$AA_{DE} = \frac{AA_D \times 10}{GE_D \times ADC_{GE}}$$

AA ingested (AA_I, in g)

$$AA_I = \frac{AA_D}{100} \times FI$$

Where IBW and FBW are the individual initial and final body weight (in g) respectively; d is the duration of the experiment (in days), t is the average temperature during the experiment (in ^oC); AA_D is the diet content of the amino acid of interest (% dry matter); GE_D is the gross energy content of the diet (in $MJ.kg^{-1}$); ADC_{GE} is the apparent digestibility coefficient of gross energy (%); FI is the feed intake during the experiment (in g, dry matter basis).

2.2.2 Models

The broken line model (BLM) is defined as follow:

$$Y = a_1X + b_1 + (a_2 - a_1)\delta X + (b_2 - b_1)\delta + \varepsilon$$

δ=0 if $X \leq X_{bp}$, or δ=1 if $X \geq X_{bp}$

Where Y and X are the response and independent variables, respectively; X_{bp} is the abscissa of the breaking point thus defining the AA requirement level; b_1 and b_2 are the intercepts, and a_1 and a_2 the slopes, of the 2 lines, respectively. Note that a_2 was not constrained to zero. The quadratic model (QM) is defined as follow:

$$Y = aX^2 + bX + c + \varepsilon$$

Where Y and X are the response and independent variables, respectively, while a, b, and c are equation parameters. The amino acid requirement is the abscissa of the smaller root that solve the parabola's equation for Y=95% of the maximum response.

The following equation defines the broken-quadratic model (BQM):

$$Y = aX^2 + bX + c + a\left(X_{jp}^2 - X^2\right)\delta + b\left(X_{jp} - X\right)\delta + \varepsilon$$

δ=0 if $X \leq X_{jp}$, or δ=1 if $X \geq X_{jp}$

Where *Y* and *X* are the response and independent variables, respectively, and *a*, *b*, and *c* are equation parameters. X_{jp} is the abscissa of the junction point thus defining the AA requirement level. Note that since X_{jp} is a constant, the curve becomes a line of slope zero for amino acid levels above the estimated requirement.

Finally, the saturation-kinetic model (SKM) is defined as follow:

$$Y = \frac{b \times c + aX^d}{c + X^d} + \varepsilon$$

Where *Y* and *X* are the response and independent variables, respectively; *a* is the asymptote to the maximum response; *b* is the intercept; *c* is a coefficient; *d* is a kinetic order. The amino acid requirement is defined as the abscissa where the response variable reaches 95% of its predicted maximum (i.e. 95% of *a*).

Estimations of EAA requirements were computed for each of the independent/dependent variable couples and for each of the 4 models. Outputs were classified into the following categories: “no fit” indicates that the model failed to converge, and therefore no estimate of requirement was generated. “Within” means that the model converged and the estimated requirement fell within the range of experimental EAA levels tested in a given study. “Outside” signifies that the model successfully converged, but the estimate requirement fell outside of the EAA range of the experimental diets. “No break”, for the BLM and BQM, indicates that only one of the two curves calculated by the model fitted, hence no breaking point could be determined. Finally, the quadratic-based models (BQM and QM) sometimes converged to a positive *a*, thus causing the model to have a minimum instead of a maximum. This behaviour was noted as “minimum”.

2.3 Statistical analysis

Models were programmed and run using SAS 9.4 (SAS Institute). Qualities of fit and coefficient of variation of requirement estimates were tested by logistic tests and a two-way ANOVA (variable pairs, models), respectively.

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3 Results and discussion

3.1 Screening results

A total of 348 peer-reviewed articles spanning 52 years (1964 to 2016) were found during the literature search, out of which 48 were not growth-based trials – e.g. based on ideal-protein formulations, or on changes in plasma AA levels, review articles – hence could not be included in the meta-analysis. However, other selection criteria rejected over half (52%) of the 300 remaining studies from the meta-analysis (Figure 1). The main reasons for rejection included poor growth, missing information (e.g. dry matter data, feed intake), or too few graded levels (

Table **1**). The final dataset (Table 2) 145 studies which covered 37 species of fish, and 11 EAA. About 72% of the studies used 6 or 7 graded levels of the EAA of interest. One study used 24 graded levels. The variables most commonly used for the estimation of the requirement are the EAA content of the diet (% dry matter) and weight gain (as SGR or % of IBW) as the independent and response variable, respectively. The most commonly used models are the BLM and QM.

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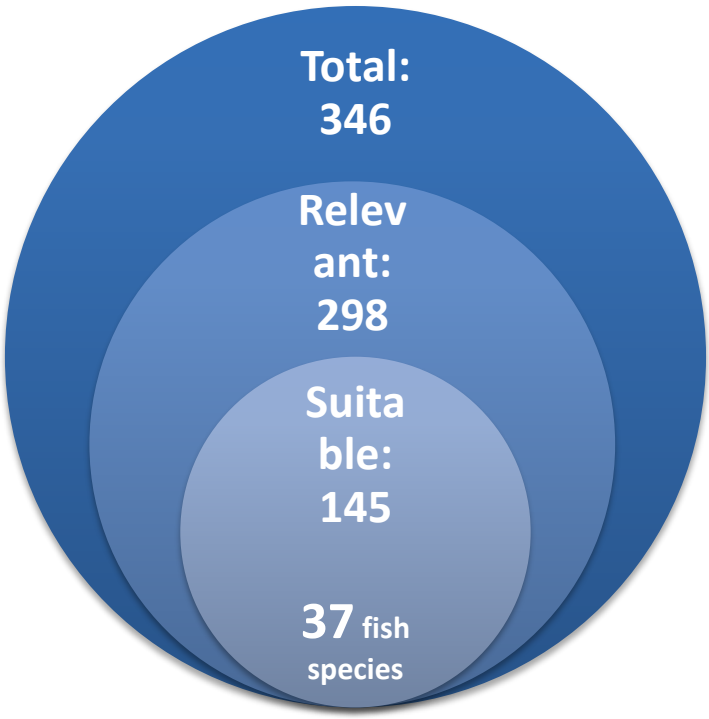

Figure 1: Diagram illustrating the screening of studies, from the total number found to the number of suitable studies that constituted our working data set

Table 1: Study rejection

Reason for rejection	% of rejected studies
Restricted feeding	3%
Poor growth	34%
Too few dietary levels	10%
Lack of intake data	44%
Lack of dry matter data	17%
Lack of raw growth data	2%

The number of excluded studies is striking. It highlights the uneven quality of the research effort in this area. It also exacerbated the fragmentation of the final dataset, both in terms of species and EAA. For instance, 25% of the studies focused on rainbow trout, whereas studies on other species such as European seabass, Japanese flounder, or Nile tilapia each represent 3% or less of the dataset. Similarly, lysine is by far the most-studied EAA (27% of the studies), while only five studies (3% of the dataset, Figure 2) describe the requirement of

phenylalanine. Moreover, these five studies were performed on five different species. Such fragmentation constitutes a major challenge for the understanding and definition of EAA requirements, l*et al* one their rationalization.

3.2 Estimates of EAA requirements

There were least two studies in 13 of the possible combinations of species and EAA (Table 3). These 13 combinations reflect the most-studied EAA (arginine, isoleucine, lysine, methionine, threonine, and tryptophan) and species (channel catfish, hybrid striped bass, rainbow trout, and Chinook, Coho and Atlantic salmons). Estimations are generally well in agreement with those reported by the NRC (2011): e.g., in % of diet dry matter, 1.66% arginine in rainbow trout, 1.53% lysine in channel catfish, or 0.61% methionine in hybrid striped bass.

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Table 2: Summary of studies included in the final dataset

Studied EAA	Species	# of diet	EAA range in diets (% DM)	Av. water temp.	Av. IBW (g)	$\frac{maxFBW}{IBW}$	$\frac{minFBW}{maxFBW}$	Exp. Duration (days)	Reference
Arg	*Clarias hybrid*	6	1.06-2.28	26.0	6.71	6.23	0.49	28	(Singh and Khan, 2007)
	Ictalurus punctatus	8	0.20-1.60	26.7	200	2.21	0.51	56	(Robinson *et al.* 1981)
	Heteropneustes fossilis	6	0.85-2.13	23.5	4.83	3.77	0.49	56	(Ahmed, 2013)
	Labeo rohita	6	0.47-1.77	28.0	0.62	5.00	0.37	56	(Abidi and Khan, 2009)
	Cirrhinus mrigala	6	1.00-2.25	26.5	0.61	4.21	0.55	56	(Ahmed and Khan, 2004b)
	Ctenopharyngodon idella	6	0.93-2.41	27.8	3.85	5.53	0.50	70	(Gao *et al*. 2015)
	Catla catla	6	098-2.23	27.9	0.60	12.41	0.46	84	(Zehra and Khan, 2013a)
	Micropterus salmoides	6	1.70-3.01	28.0	24.93	2.77	0.88	56	(Zhou *et al*. 2012)
	Oncorhynchus kisutch	6	1.20-3.60	10.0	2.90	3.30	0.65	70	(Klein and Halver, 1970)
	Oncorhynchus tshawytscha	6	1.60-3.60	10.0	3.48	2.89	0.79	70	(Klein and Halver, 1970)
	Oncorhynchus kisutch	6	0.00-5.27	15.0	0.91	5.90	0.87	56	(Luzzana *et al.* 1998)
	Oncorhynchus mykiss	12	0.5-2.38	17.0	50.67	3.54	0.54	51	(Rodehutscord *et al*. 1995a)
	Oncorhynchus mykiss	8	0.47-2.50	15.0	12.60	3.35	0.64	42	(Kim *et al.* 1992b)
	Oncorhynchus mykiss	6	0.69-3.83	15.0	2.50	14.08	0.65	84	(Cho *et al.* 1992)
	Oncorhynchus mykiss	7	0.69-3.00	15.0	1.30	19.46	0.72	84	(Cho *et al.* 1992)
	Oncorhynchus mykiss	5	0.80-2.80	10.0	7.30	4.08	0.60	84	(Walton *et al.* 1986)
	Oncorhynchus mykiss	5	1.72-3.61	17.0	9.30	9.46	0.74	84	(Fournier *et al.* 2003)
	Salmo salar	5	1.13-2.86	6.5	385	1.50	0.91	126	(Berge *et al.* 1997)
	Salmo salar	5	1.10-3.20	15.0	111.9	5.00	0.87	98	(Lall *et al.* 1994)
	Psetta maxima	5	1.79-4.19	17.0	7.40	8.03	0.43	84	(Fournier *et al.* 2003)

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	Sparus macrocephalus	6	1.85-3.46	28.0	10.51	4.87	0.84	56	(Zhou et al. 2010b)
	Lates calcarifer	6	0.73-3.73	29.0	2.56	7.96	0.64	84	(Murillo-Gurrea *et al.* 2001)
	Rachycentron canadum	6	1.76-3.75	30.5	3.38	13.11	0.76	63	(Ren *et al.* 2014)
	Paralichthys olivaceus	6	1.25-3.25	20.6	1.85	4.25	0.53	40	(Alam *et al.* 2002)
His	*Ictalurus punctatus*	9	0.10-0.80	26.7	200	3.31	0.39	56	(Wilson *et al.* 1980)
	Cirrhinus mrigala	6	0.25-1.50	27.5	0.61	3.95	0.60	56	(Ahmed and Khan, 2005b)
	Catla catla	6	0.25-0.96	28.1	0.65	14.33	0.03	84	(Zehra and Khan, 2016c)
	Heteropneustes fossilis	6	0.90-1.95	27.9	5.27	14.33	0.08	84	(Khan and Abidi, 2014)
	Oncorhynchus mykiss	12	0.26-1.35	17.0	40.25	4.22	0.71	53	(Rodehutscord *et al.* 1997)
	Oncorhynchus keta	7	0.13-1.92	14.8	1.58	2.46	0.64	28	(Akiyama *et al.* 1985)
	Oncorhynchus kisutch	5	0.50-1.30	10.0	3.17	3.00	0.88	70	(Klein and Halver, 1970)
Ile	*Ictalurus punctatus*	7	0.30-1.30	26.7	200	2.95	0.47	56	(Wilson *et al.* 1980)
	Labeo rohita	6	0.75-1.99	26.7	0.62	4.46	0.62	56	(Khan and Abidi, 2007b)
	Cirrhinus mrigala	6	0.50-1.75	27.7	0.61	4.18	0.52	56	(Ahmed and Khan, 2006)
	Catla catla	6	0.51-1.73	27.8	0.61	11.69	0.43	84	(Zehra and Khan, 2013c)
	Oncorhynchus mykiss	12	0.50-1.53	16.0	45.65	3.43	0.55	59	(Rodehutscord *et al.* 1997)
	Salvelinus namaycush	7	0.54-1.26	8.3	3.20	4.00	0.94	84	(Hughes *et al.* 1983)
	Oncorhynchus tshawytscha	6	0.50-1.50	10.0	0.82	4.26	0.68	70	(Chance *et al.* 1964)
	Oncorhynchus tshawytscha	6	0.50-1.50	10.0	0.79	4.14	0.61	70	(Chance *et al.* 1964)
Leu	*Ictalurus punctatus*	8	0.60-2.00	26.7	200	2.87	0.76	56	(Wilson *et al.* 1980)

	Labeo rohita	6	0.74-1.99	26.7	0.59	4.00	0.46	56	(Abidi and Khan, 2007)
	Cirrhinus mrigala	6	0.75-2.00	27.7	0.64	4.08	0.55	56	(Ahmed and Khan, 2006)
	Oreochromis niloticus	6	0.53-1.81	29.0	1.94	10.77	0.70	56	(Gan *et al.* 2016)
	Salvelinus namaycush	7	0.96-2.24	8.3	3.20	4.03	0.84	84	(Hughes *et al.* 1983)
	Oncorhynchus tshawytscha	6	1.00-3.10	10.0	2.49	2.11	0.60	56	(Chance *et al.* 1964)
	Oncorhynchus mykiss	12	1.00-4.20	15.7	49.33	3.77	0.66	53	(Rodehutscord *et al.* 1997)
	Lateolabrax japonicus	6	0.90-3.88	29.0	8.00	4.90	0.85	56	(Li *et al.* 2015)
Lys	*Hybrid striped bass*	6	1.14-2.37	24.5	7.0	6.76	0.78	63	(Keembiyehetty and Gatlin, 1992)
	Hybrid striped bass	8	1.20-2.60	28.0	5.5	10.46	0.85	77	(Griffin *et al.* 1992)
	Ictalurus punctatus	6	0.75-2.00	26.7	202	2.50	0.66	44	(Wilson *et al.* 1977)
	Ictalurus punctatus	6	0.70-2.00	26.7	201	2.69	0.51	56	(Robinson *et al.* 1980b)
	Pelteobagrus fulvidraco	6	1.73-4.19	28.0	1.50	8.00	0.70	56	(Cao *et al.* 2012)
	Lepomis macrochirus	7	1.19-3.29	22.3	26.93	1.50	0.83	56	(Masagounder *et al.* 2011)
	Ctenopharyngodon idella	6	0.71-3.16	28.7	3.15	4.49	0.64	90	(Wang *et al.* 2005)
	Cirrhinus mrigala	6	1.48-2.78	26.5	0.66	3.95	0.59	40	(Ahmed and Khan, 2004a)
	Catla catla	6	1.22-2.47	26.8	0.59	12.32	0.45	84	(Zehra and Khan, 2013b)
	Piaractus mesopotamicus	6	0.82-2.10	29.6	6.67	7.08	0.61	90	(Abimorad *et al.* 2010)
	Oncorhynchus keta	8	0.38-4.00	14.8	1.27	3.66	0.57	28	(Akiyama *et al.* 1985)

Oncorhynchus mykiss	24	0.45-5.80	15.7	50.9	3.65	0.32	55	(Rodehutscord *et al*. 1997)
Oncorhynchus mykiss	6	1.60-2.42	14.5	14.9	6.00	0.83	56	(Cheng et al., 2003)
Oncorhynchus mykiss	8	1.37-3.66	15.0	5.0	16.38	0.65	84	(Wang *et al*. unpublished)
Oncorhynchus mykiss	6	1.27-2.32	15.0	24.0	7.20	0.69	84	(Encarnacao *et al.* 2004)
Oncorhynchus mykiss	6	1.33-2.49	15.0	24.0	7.54	0.75	84	(Encarnacao *et al.* 2004)
Oncorhynchus mykiss	8	0.72-1.60	15.0	13.7	3.52	0.65	42	(Kim *et al.* 1992b)
Oncorhynchus mykiss	8	0.21-3.01	11.5	17.0	4.61	0.40	71	(Pfeffer *et al.* 1992)
Oncorhynchus mykiss	6	1.03-2.02	15.0	0.92	5.15	0.57	42	(Nang Thu *et al.* 2007)
Oncorhynchus mykiss	6	1.00-2.03	15.0	0.92	3.64	0.58	42	(Nang Thu *et al.* 2007)
Oncorhynchus mykiss	6	1.06-2.05	15.0	0.92	5.18	0.54	42	(Nang Thu *et al.* 2007)
Oncorhynchus mykiss	6	1.03-2.02	15.0	0.92	5.32	0.49	30	(Nang Thu *et al.* 2009)
Oncorhynchus mykiss	6	0.63-1.43	15.0	0.92	3.79	0.48	30	(Nang Thu *et al.* 2009)
Oncorhynchus mykiss	6	1.03-2.02	15.0	0.92	4.51	0.47	30	(Nang Thu *et al.* 2009)
Oncorhynchus mykiss	7	1.04-2.60	15.0	5.19	10.87	0.50	84	(Walton *et al.* 1984b)
Salmo salar	7	1.24-2.94	15.0	4.72	2.71	0.61	70	(Anderson *et al.* 1993)
Salmo salar	12	1.20-2.38	15.2	42.12	2.63	0.64	76	(Hauler *et al.* 2007)
Salmo salar	7	1.26-4.15	8.0	643	1.60	0.89	85	(Espe *et al.* 2007)
Lates calcarifer	6	1.00-2.50	27.0	13.12	4.58	0.70	84	(Murillo-Gurrea *et al.* 2001)
Sparus macrocephalus	6	2.08-4.05	18.0	9.12	4.14	0.85	56	(Zhou *et al.* 2009, 2010a)
Pagrus major	6	1.19-3.44	22.5	1.7	8.06	0.58	56	(Forster and Ogata, 1998)
Paralichthys olivaceus	6	1.04-3.16	19.5	3.0	5.33	0.45	63	(Forster and Ogata, 1998)

	Psetta maxima	7	1.20-3.11	18.0	18.1	1.82	0.88	56	(Peres and Oliva-Teles, 2008)
	Rachycentron canadum	6	1.15-3.25	28.5	1.26	19.09	0.66	56	(Zhou et al. 2007)
	Sciaenops ocellatus	7	1.00-2.50	27.0	6.72	4.57	0.68	56	(Craig and Gatlin, 1992)
	Dicentrarchus labrax	5	1.33-2.72	25.5	0.85	7.73	0.65	70	(Tibaldi and Lanari, 1991)
Met	*Hybrid striped bass*	6	0.38-1.60	24.0	4.8	5.12	0.20	56	(Keembiyehetty and Gatlin, 1993)
	Hybrid striped bass	5	0.60-1.00	24.0	8.6	5.38	0.51	56	(Keembiyehetty and Gatlin, 1993)
	Oreochromis niloticus	5	0.56-0.80	26.2	1.31	6.05	0.78	43	(Furuya *et al.* 2008)
	Megalobrama amblycephala)	6	0.39-1.54	28.5	3.34	7.33	0.73	63	(Liao *et al.* 2014)
	Catla catla	6	0.56-1.81	26.9	0.65	14.77	0.39	84	(Zehra and Khan, 2016b)
	Ictalurus punctatus	7	0.25-1.75	26.7	202	2.39	0.71	44	(Harding *et al.* 1977)
	Ictalurus punctatus	7	0.35-1.55	24.8	14.36	2.34	0.85	42	(Cai and Burtle, 1996)
	Myxocyprinus asiaticus	6	0.64-1.89	27.9	1.72	6.10	0.67	56	(Chu *et al.* 2014)
	Pseudobagrus ussuriensis	6	0.49-2.08	24.0	0.60	6.16	0.72	56	(Wang *et al.* 2016)
	Oncorhynchus mykiss	12	0.22-1.09	16.8	43.04	3.25	0.43	49	(Rodehutscord *et al.* 1995b)
	Oncorhynchus mykiss	12	0.21-1.10	16.8	43.46	3.59	0.42	49	(Rodehutscord *et al.* 1995b)
	Oncorhynchus mykiss	7	0.43-2.07	15.0	3.13	15.48	0.80	112	(Cowey et al. 1992)
	Oncorhynchus mykiss	6	0.23-0.80	15.0	15.4	3.08	0.67	42	(Kim *et al.* 1992a)
	Salvelinus alpinus	6	0.43-0.91	12.0	20.55	5.04	0.34	112	(Simmons *et al.* 1999)
	Salmo salar	6	0.69-1.29	8.5	495	2.44	0.87	85	(Espe et al., 2008)

	Paralichthys olivaceus	6	0.53-2.03	21.6	2.8	4.45	0.34	40	(Alam et al. 2000)
	Sparus macrocephalus	6	0.75-2.35	28.0	14.22	4.36	0.84	56	(Zhou et al. 2011)
	Sciaenops ocellatus	7	0.35-1.85	27.0	0.9	14.39	0.19	56	(Moon and Gatlin III, 1991)
	Trachinotus ovatus	6	0.86-1.45	29.5	12.40	6.58	0.64	56	(Niu et al. 2013)
	Rachycentron canadum	6	0.61-1.68	29.0	11.62	7.37	0.62	56	(Zhou et al. 2006)
	Lates calcarifer	6	0.62-1.26	26.5	2.61	11.10	0.86	84	(Coloso et al. 1999)
Phe	*Ictalurus punctatus*	7	0.20-0.80	26.7	199	2.50	0.60	56	(Robinson et al. 1980a)
	Cirrhinus mrigala	6	0.50-1.75	26.2	0.56	3.88	0.54	56	(Ahmed, 2009)
	Labeo rohita	6	0.39-1.64	26.0	0.58	4.14	0.60	56	(Khan and Abidi, 2007a)
	Catla catla	6	0.39-1.62	27.2	0.68	13.10	0.43	84	(Zehra and Khan, 2014)
	Oncorhynchus mykiss	7	0.26-1.75	15.0	12.7	2.91	0.55	42	(Kim, 1993)
Thr	*Ictalurus punctatus*	7	0.30-1.20	26.7	200	2.79	0.61	56	(Wilson *et al.* 1978)
	Hybrid striped bass	6	0.49-1.75	26.5	9.8	4.11	0.73	49	(Keembiyehetty and Gatlin, 1997)
	Hybrid striped bass	6	0.49-1.25	26.5	3.0	6.46	0.69	56	(Keembiyehetty and Gatlin, 1997)
	Cirrhinus mrigala	6	1.00-2.25	26.7	0.52	4.05	0.56	56	(Ahmed *et al.* 2004)
	Labeo rohita	6	0.71-2.15	27.0	0.58	4.14	0.49	56	(Abidi and Khan, 2008)
	Catla catla	6	0.74-1.93	26.9	0.60	14.32	0.40	84	(Zehra and Khan, 2016a)
	Oreochromis niloticus	6	0.71-2.46	28.8	6.00	3.49	0.63	56	(He *et al.* 2016)
	Oreochromis niloticus	5	0.89-1.54	28.5	563.30	1.47	0.92	28	(Michelato *et al.* 2016)
	Megalobrama amblycephala	5	0.61-2.49	27.0	3.01	4.30	0.70	63	(Habte-Tsion *et al.* 2015)
	Oncorhynchus keta	8	0.24-2.80	14.8	1.19	2.55	0.53	28	(Akiyama *et al.* 1985)
	Oncorhynchus mykiss	11	0.50-2.18	14.7	1.84	3.52	0.50	24	(Bodin *et al.* 2008)

	Oncorhynchus mykiss	11	0.50-2.10	17.0	50.74	3.25	0.61	51	(Rodehutscord *et al*. 1995a)
	Salmo salar	7	0.00-0.75	14.5	0.80	2.90	0.46	36	(Rollin *et al.* 2006)
	Salmo salar	11	0.50-2.18	14.7	0.80	3.00	0.47	36	(Bodin *et al.* 2008)
	Dicentrarchus labrax	6	0.81-2.63	21.1	7.50	2.80	0.89	65	(Tibaldi and Tulli, 1999)
	Sciaenops ocellatus	6	0.49-1.25	27.0	2.80	8.09	0.41	56	(Boren and Gatlin III, 1995)
Trp	*Ictalurus punctatus*	6	0.05-0.40	26.7	200	2.87	0.47	56	(Wilson *et al.* 1978)
	Heteropneustes fossilis	8	0.10-0.39	27.9	6.66	10.90	0.29	72	(Farhat and Khan, 2014)
	Heteropneustes fossilis	6	0.04-0.54	22.8	4.45	3.59	0.50	56	(Ahmed, 2012)
	Labeo rohita	6	0.22-0.44	25.8	0.36	6.81	0.31	84	(Abidi and Khan, 2010)
	Cirrhinus mrigala	6	0.06-0.56	27.7	0.62	3.77	0.47	56	(Ahmed and Khan, 2005a)
	Catla catla	6	0.10-0.34	27.7	0.60	16.44	0.31	84	(Zehra and Khan, 2015)
	Oncorhynchus mykiss	12	0.13-0.56	15.5	49.72	3.54	0.58	64	(Rodehutscord *et al.* 1997)
	Oncorhynchus mykiss	5	0.01-0.43	9.0	5.45	4.60	0.24	112	(Poston and Rumsey, 1983)
	Oncorhynchus mykiss	6	0.08-0.60	15.0	13.76	3.82	0.36	84	(Walton *et al*. 1984a)
Val	*Ictalurus punctatus*	7	0.40-1.60	26.7	201	2.77	0.61	56	(Wilson *et al.* 1980)
	Labeo rohita	6	0.75-2.00	26.0	0.16	2.69	0.70	42	(Abidi and Khan, 2004)
	Cirrhinus mrigala	6	0.75-2.00	27.7	0.62	4.13	0.54	56	(Ahmed and Khan, 2006)
	Oncorhynchus mykiss	12	0.62-3.42	15.7	49.36	3.55	0.43	53	(Rodehutscord *et al*. 1997)
	Oncorhynchus tshawytscha	6	0.65-1.90	10.0	0.67	3.90	0.78	70	(Chance *et al.* 1964)

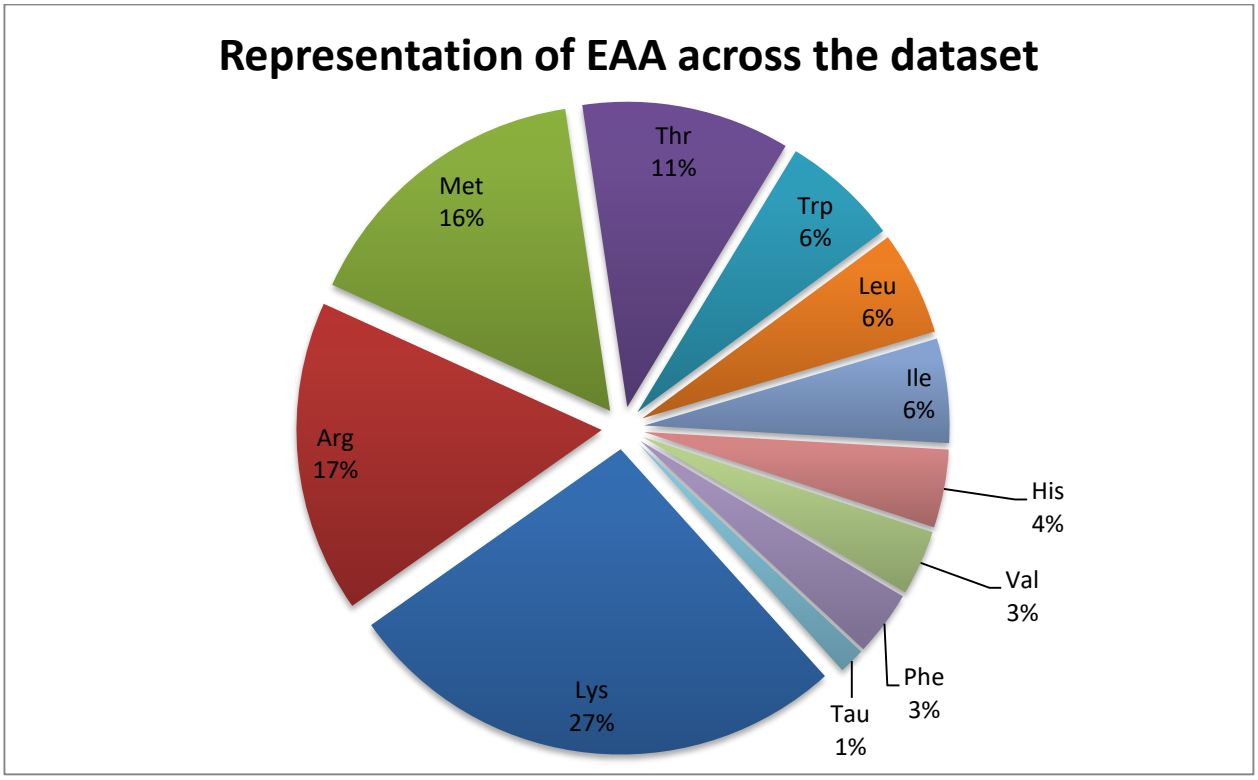

Figure 2: Representation of the eleven EAA in the dataset

In Table 3 we also see the requirements estimated with different variable pairs. While the influence of different response variable can be observed directly, it is meaningless to compare requirements computed with different modes of expression, since they are expressed in different units. Instead, the coefficient of variation (CV) of requirement estimates provides a good indication of the estimate consistency between variable pairs. Regardless of the species and EAA, CVs hover around 22% (median is 15% across the full dataset), which is comparable to the 34% found by Hauler and Carter (2001), but far less than the 100%+ described by Shearer (2000). Bodin *et al*. (2009) found that the choice of response variable impacts the relative lysine requirement in rainbow trout, but not the absolute (i.e. lysine intake) requirement. In our dataset, absolute requirement estimates are indeed numerically closer between response variables, than the relative requirement estimates. However, the variability of the estimates precludes us from establishing statistical significance. By focusing on the requirements for rainbow trout, shows that none of the four mathematical models results in more precise estimates. As well, and contrary to the conclusions of Hauler and Carter (2001) or Bodin *et al*. (2009), we did not observe a significant difference in estimate deviation between the two relative (% diet dry matter and g/MJDE) and the absolute (g ingested/kg MBW/d) modes of

Salze, G. et al., 2017.A Meta-Analysis of Essential Amino Acid Requirements of Fish. En: Cruz-Suárez, L.E., Ricque-Marie, D., Tapia-Salazar, M., Nieto-López, M.G., Villarreal-Cavazos, D. A., Gamboa-Delgado, J., López Acuña, L.M. y Galaviz-Espinoza, M. . (Eds), Investigación y Desarrollo en Nutrición Acuícola Universidad Autónoma de Nuevo León, San Nicolás de los Garza, Nuevo León, México, pp. 303-337. ISBN 978-607-27-0822-8.

expression. However, the relationship between lysine requirement in rainbow trout and IBW depended on the unit of requirement (Figure 3): there is a significant linear regression when the requirement is expressed as g/kg MBW/d (P=0.0041, R^2=0.482), but not when express as percent of diet dry matter (P=0.3170, R^2=0.077). Therefore, differences in fish size account for some of the observed deviation, and can be better quantified using an absolute, intake-based mode of expression. Overall, the variability in requirement estimates limits our interpretation of EAA requirements in fish. It also underscores the need to better identify factors governing EAA requirements. This is clearly reflected by the debate over the mode of expression of EAA requirement. The three modes of expression chosen for this meta-analysis represent three, fundamentally different approaches which are based on mutually-exclusive assumptions. Therefore, there is a great need to further investigate the determinants of EAA requirements, and notably their dependence to dietary ingredients or feed intake.

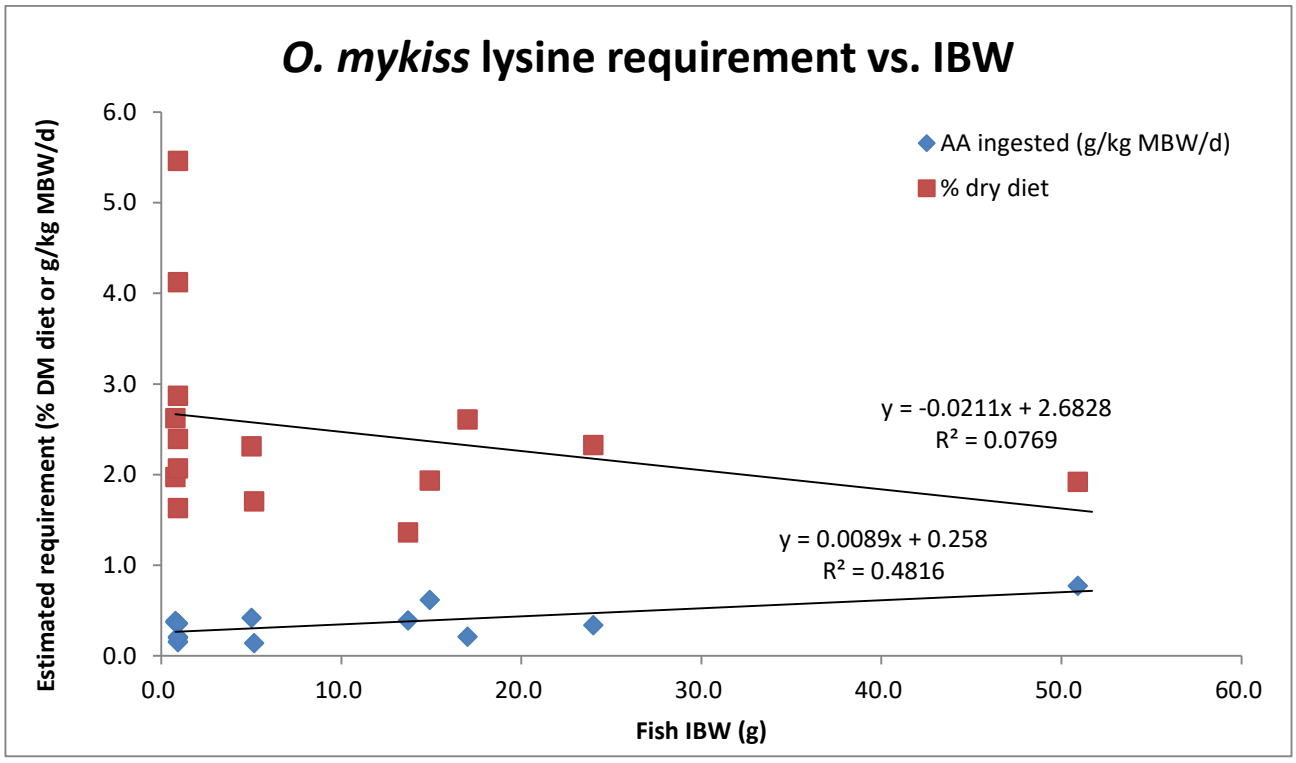

Figure 3: Relationship between initial body weight (IBW, g) and lysine requirement of *O. mykiss* expressed as a percent of diet dry matter or as an ingested amount.

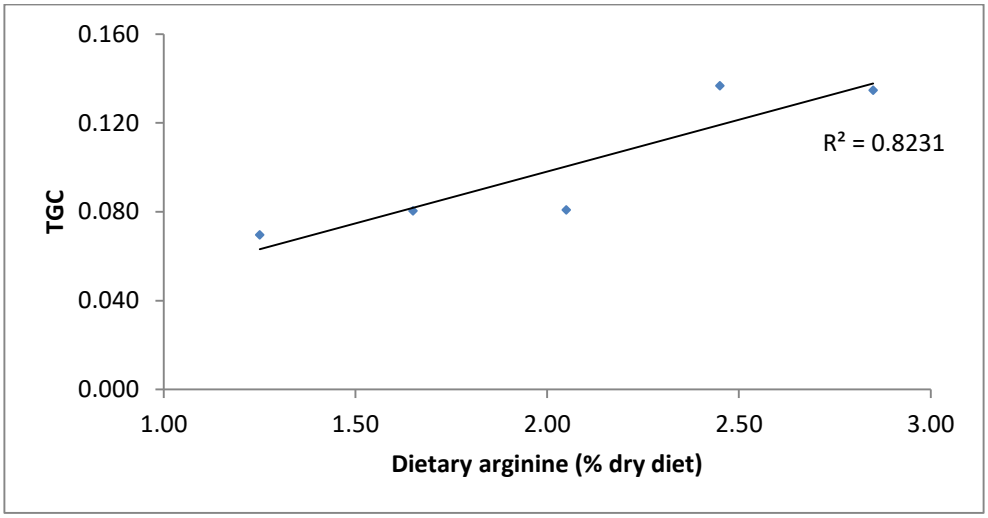

Figure 4: Growth response of turbot (*Psetta maxima*) to graded levels of dietary arginine, illustrating a linear pattern. Adapted from Fournier *et al*. (2003).

3.3 Overall limitations in the dataset

One of the biggest missing pieces of information in the present dataset is digestibility. Indeed, only 15 of the 145 selected studies measured and reported protein digestibility, and none measured the digestibility of individual EAA. Additionally, experimental diets were formulated using a wide range of ingredients. In the selected studies, diets were typically formulated using a mix of natural proteins (fishmeal, soy and corn proteins, etc.) and crystal amino acids. Not only the EAA supplied by these ingredients likely have different digestibility, but there may be relevant interactions among amino acids as well as with other non-protein components. These interactions may affect the availability of EAA to the animal, hence the apparent EAA requirement. For example, phytate is known to bind to amino acids – especially basic amino acids such as lysine, histidine and arginine – which limits their bioavailability to the animal (Chowdhury et al., 2012; Selle et al., 2006; Storebakken et al., 2000). Therefore, data from studies using phytate-rich ingredients such as plant proteins should be carefully interpreted.

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Table 3: Estimates of essential amino acid requirements computed using the quadratic model (QM)

	Estimates of requirements					
Unit of requirement estimate	Percent of diet dry matter		g/MJ DE		g ingested AA/kg MBW/d	
Growth variable	g/kg MBW/d	TGC	g/kg MBW/d	TGC	g/kg MBW/d	TGC
Arg						
O. kisutch	1.83 ± 69.9%	1.94 ± 60.9%	1.17 ± 68.5%	1.24 ± 59.8%	0.09 ± 8.5%	0.10 ± 1.2%
O. mykiss	1.66 ± 19.0%	1.69 ±19.0%	0.95 ± 15.5%	0.97 ± 15.4%	0.23 ± 13.0%	0.23 ± 13.3%
S. salar	1.86 ± 4.8%	1.87 ± 3.5%	0.93 ± 10.3%	0.94 ± 11.6 %	0.11 ± 51.4%	0.11 ± 52.9%
Ile						
O. tshawytscha	0.97 ± 6.3%	0.98 ± 7.0%	0.65 ± 6.2%	0.66 ± 7.0%	0.02 ± 3.7%	0.02 ± 4.8%
Lys						
I. punctatus	1.53 ± 0.8%	1.55 ± 14.9%	1.44 ± 1.0%	1.46 ± 6.3%	0.35 ± 15.1%	0.36 ± 6.1%
O. mykiss	2.02 ± 24.0%	2.04 ± 27.9%	1.02 ± 25.7%	1.03 ± 36.6%	0.25 ± 26.9%	0.25 ± 36.4%
S. salar	1.95 ± 46.9%	1.96 ± 49.7%	0.97 ± 39.2%	0.98 ± 41.1%	0.10 ± 51.4%	0.11 ± 52.1%
Met						
Hybrid striped bass	0.96 ± 21.1%	0.97 ± 21.1%	0.75 ± 21.1%	0.76 ± 21.5%	0.24 ± 52.8%	0.25 ± 54.2%
I. punctatus	0.76 ± 21.1%	0.69 ± 40.0%	0.74 ± 17.7%	0.66 ± 36.4%	0.13 ± 46.1%	0.12 ± 61.4%
O. mykiss	0.61 ± 10.5%	0.63 ± 11.4%	0.37 ± 5.2%	0.37 ± 4.9%	0.09 ± 11.9%	0.09 ± 11.4%
Thr						
Hybrid striped bass	0.92 ± 0.5%	0.94 ± 0.0%	0.72 ± 0.8%	0.74 ± 1.0%	0.08 ± 9.6%	0.08 ± 9.0%
O. mykiss	1.26 ± 14.0%	1.27 ± 14.0%	0.70 ± 6.4%	0.70 ± 6.1%	0.16 ± 20.5%	0.16 ± 20.6%
Trp						
O. mykiss	0.29 ± 16.4%	0.29 ± 16.2%	0.17 ± 11.3%	0.17 ± 11.1%	0.03 ± 30.7%	0.03 ± 31.1%

Values are estimates of requirement in the indicated unit, ± the coefficient of variation.

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Table 4: Comparisons of lysine requirement estimates in rainbow trout *O. mykiss*, depending on choice of variables and mathematical model

Estimates of requirements (± coefficient of variation)												
Unit of requirement estimate	Percent of diet dry matter				g/MJ DE				g ingested AA/kg MBW/d			
Growth variable	g/kg MBW/d		TGC		g/kg MBW/d		TGC		g/kg MBW/d		TGC	
Broken line	1.66 21.6% *(n=14)*	±	1.73 22.5% *(n=12)*	±	0.81 21.4% *(n=13)*	±	0.94 43.5% *(n=9)*	±	0.19 30.9% *(n=14)*	±	0.18 42.0% *(n=12)*	±
Broken quadratic	1.69 17.1% *(n=11)*	±	1.72 20.2% *(n=11)*	±	0.86 17.6% *(n=11)*	±	0.88 33.4% *(n=11)*	±	0.20 19.7% *(n=11)*	±	0.21 33.2% *(n=11)*	±
Quadratic	1.83 22.9% *(n=12)*	±	1.86 28.0% *(n=12*	±	0.96 22.8% *(n=12)*	±	0.97 38.3% *(n=12)*	±	0.22 28.3% *(n=13)*	±	0.22 38.0% *(n=13)*	±
Saturation kinetic	1.99± 17.9% *(n=8)*		2.10 12.4% *(n=6)*	±	1.05 20.5% *(n=7)*	±	1.13 22.2% *(n=6)*	±	0.28 15.5% *(n=5)*	±	0.30 24.1% *(n=6)*	±

The starting body weight of fish also greatly varies between studies, from 0.16g to 662g, although 60% of the studies started with fish between 0.5 and 25g. However, nutrient utilization is known to change with fish size, including in early juvenile stages (Dumas *et al*. 2007). Therefore, considering EAA requirements across growth stanza likely introduces a source of variation in the requirement estimates.

Finally, only a minority of studies reported final proximate analysis of the fish, or protein deposition results. This precluded us from including variables such as protein retention in our analysis, which have also been debated as a possible way to express EAA requirements.

3.4 Choice of mathematical model and experimental design

Although the choice of mathematical model does not improve the variability of requirement estimates, there were significant differences in performances and quality of fit. The BLM, BQM and QM always converged, while the SKM failed to do so in 19.9% of the selected studies, all variable pairs combined. It should be noted here that a converged model does not sufficient to produce an accurate and/or precise estimation of the requrement. Table 5 shows which parameters of experimental design the models are most sensitive to, and the quality of the outcome beyond model convergence. Both BLM and SKM are sensitive to the number of experimental diets. For instance, each additional diet in the experimental design will make the BLM 0.23 times as likely to produce a minimum instead of a maximum (or 4.35 times as likely to produce a maximum, p=0.0028). The SKM model, although closer to biological reality, demands careful experimental design. Although a low number of diets will not significantly increase the risk of failed fit, each additional diet will make the SKM 0.89 times as likely to result in an estimate outside the experimental range (p=0.0059). This is problematic, and an “outside” fit should not be considered accurate.

Although fish growth was used as a screening criteria for constituting the working dataset, the maxFBW/IBW ratio still significantly affected how the QM and BQM models fitted the data (Table **5**). High weight gain in the best (presumably replete) treatments during the experiment resulted in a reduced risk of producing an estimate outside the experimental range – odds ratio of 0.63 (p=0.0111) and 0.71 (p=0.0084) for the BQM and QM, respectively. In other words, sufficient weight gain during the experiment contributes to estimates within the experimental range, and in that sense, contribute to the accuracy of the estimates. More surprisingly, the BQM also has a significant tendency to fit a minimum (i.e., negative slope of the quadratic segment) at high maxFBW/IBW

values. Upon closer inspection of such studies, fish growth response to the graded EAA levels is always quite linear, as illustrated in Figure 4. However, all models are based on the law of "diminishing returns", and thus are best suited for situations that illustrate such a pattern. In 50% of failed BQM cases (and 97% of failed BLM), the model could not fit one of the 2 lines. This highlights that models are sensitive to the presence of both the initial ascending portion of the curve, and the following plateau. Models' sensitivity to the former is well illustrated by the ratio between the lowest and highest fish weight at the end of the study (minFBW/maxFBW).

Table 5: Odds ratio of maxFBW/IBW, minFBW/maxFBW, and number of diets affecting the fit outcome of the requirement models.

Parameter **Outcome**	**Broken Quadratic Model**	**Broken-Line Model**	**Quadratic Model**	**Saturation Kinetic Model**
maxFBW / IBW ratio				
Failed fit	NS	NS	NS	NS
Minimum	1.15	NS	NS	
No break point	NS	NS		
Outside estimate	0.63	NS	0.71	NS
minFBW / maxFBW ratio				
Failed fit	NS	NS	NS	30.73
Minimum	NS	83.75	NS	
No break point	106.40	26.28		
Outside estimate	NS	0.31	17.71	3.20
Number of Diets				
Failed fit	NS	NS	NS	NS
Minimum	NS	0.23	NS	
No break point	NS	NS		
Outside estimate	NS	NS	NS	0.89

Note: values odds ratio of an outcome occurring vs. a converged fit resulting in an estimate withing the experimental range. Minimum: the model has a minimum instead of a maximum; No break point: the breaking point could not be

determined; Failed fit: the model failed to converge; Outside estimate: the estimated requirement is outside of experimental range. NS: odd ratio non-significant different from 1; Empty cells indicate that this outcome is non-applicable to the model.

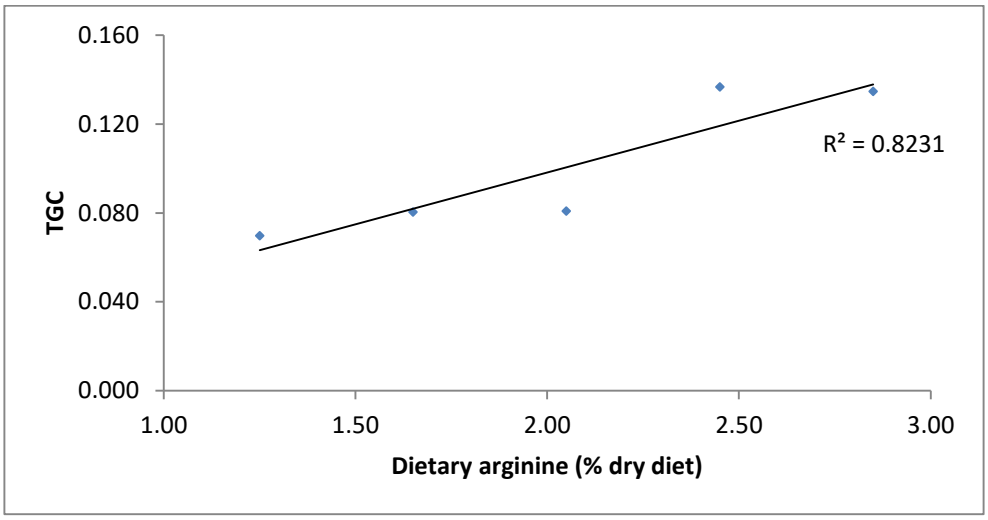

Figure 5: Growth response of turbot (*Psetta maxima*) to graded levels of dietary arginine, illustrating a linear pattern. Adapted from Fournier *et al*. (2003).

The minFBW/maxFBW was by far the most impactful metric regarding fit performance and quality in all models. If one could imagine a 1 unit increase in minFBW/maxFBW ratio, the BQM and the BLM would be 106.40 (p=0.0307) and 26.28 (p=0.0319) times as likely to fail in finding a breaking point, respectively. The BLM would also be 83.75 times as likely to fit a first line with a negative slope (p=0.0392). Finally, the SKM would be 3.20 (p=0.0170) and 30.73 (p<0.0001) times as likely to find an estimate outside the experimental range and fail altogether, respectively. Evidently, studies failing to maximize the difference in final average weights between treatments at the end of the experiment are in great risk of not being able to accurately estimate the requirement. This occurs when the basal diet is not deficient enough and/or amino acid contents in other experimental diets

do not reach a high enough level to produce a clear plateau. Consequently, particular attention should be given to diet formulations to create such a pattern.

The above discussion highlights factors which nutritionists should carefully consider when developing the experimental design and formulating experimental diets. Our results show that none of the four models result in a significantly improved precision of the requirement estimate. The BLM has been a model of choice for decades, due to its simplicity and ease of fit, especially at a time when computers were not as ubiquitous. However, the limitations of the BLM have been brought to light (Hernandez-Llamas, 2009) and other models have since been suggested. However, no model can be a priori assumed to be superior in all situations, and a careful model selection procedure should be implemented for each experiment as part of the statistical evaluation of the results. Several methods exist for this, although the Akaike Information Criterion (AIC) method (Akaike, 1974; Arnold, 2010), which balances goodness of fit and parsimony of the models, is practical and provides guidance to the nutritionist in his/her choice of model selection (Salze *et al.* 2017).

4 Conclusions and recommendations

The standardization of the current body of scientific literature, as presently conducted, did not lead to clearer, more precise requirement estimates. However, it did highlight some important points. First, the quality of published studies is highly variable: less than half of the relevant studies were deemed suitable for our final data-set. The main reasons for these rejections include poor growth and a lack of reported information such as diet moisture content.

Future investigations of EAA requirements should focus on non-salmonid species, different life stages, and less-studied EAA such as taurine, phenylalanine, leucine, or histidine in order to remedy the knowledge fragmentation. Trials should use no fewer than 6 experimental diets, more if available facilities and resources allow. The use of the BLM should be avoided, as it has now been clearly demonstrated that it underestimates the requirement. Choice of mathematical model should then follow a rational evaluation of fit quality. SKM, QM, or BQM are all valid models, and none should

Salze, G. et al., 2017.A Meta-Analysis of Essential Amino Acid Requirements of Fish. En: Cruz-Suárez, L.E., Ricque-Marie, D., Tapia-Salazar, M., Nieto-López, M.G., Villarreal-Cavazos, D. A., Gamboa-Delgado, J., López Acuña, L.M. y Galaviz-Espinoza, M. . (Eds), Investigación y Desarrollo en Nutrición Acuícola Universidad Autónoma de Nuevo León, San Nicolás de los Garza, Nuevo León, México, pp. 303-339.

be *a priori* assumed inherently superior to another. In addition, it is important that the number and range of graded dietary levels of the test EAA are chosen so that the ascending and plateau segments of the curve can be clearly established with the requirement falling approximately in the middle. Finally, the effective loss of it knowledge due to unreported information is very counterproductive. It is therefore most critical that fish nutritionists agree on a standard and systematic system to report and capture results of such trial.

Salze, G. et al., 2017.A Meta-Analysis of Essential Amino Acid Requirements of Fish. En: Cruz-Suárez, L.E., Ricque-Marie, D., Tapia-Salazar, M., Nieto-López, M.G., Villarreal-Cavazos, D. A., Gamboa-Delgado, J., López Acuña, L.M. y Galaviz-Espinoza, M. . (Eds), Investigación y Desarrollo en Nutrición Acuícola Universidad Autónoma de Nuevo León, San Nicolás de los Garza, Nuevo León, México, pp. 303-339.

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