

REVIEW

REVIEWS IN Aquaculture

Balancing protein and energy in Nile tilapia feeds: A meta-analysis

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Abstract

Farmed fish are increasingly grown in intensive and semi-intensive systems where most nutrients are provided by formulated feeds. These are formulated to minimise costs and optimise growth, notably the rate of muscle protein synthesis (*i.e.*, fillet). Protein synthesis depends on the availability of amino acids (*i.e.*, protein) and energy. Thus, fish feeds are often formulated to provide a balanced ratio of protein to energy (P:E). Above and below the optimal dietary P:E, absorbed protein and energy are expected to be under-utilised for body protein synthesis, respectively. Estimates of the optimal dietary P:E vary largely for Nile tilapia (*Oreochromis niloticus* (L., 1758)). Published values range from 13 to 26 g of dietary protein per MJ of energy. Here, we challenge the idea that growth, protein utilisation efficiency and body composition can all be simultaneously optimised, when Nile tilapia are fed an optimal dietary P:E. Through linear and non-linear regression meta-analyses, we quantitatively describe the effects of protein and energy intake on nutrient partitioning, feed efficiency and growth. We find linear relationships between the dietary P:E and protein retention efficiency. The lack of an inflection contradicts the existence of an optimal P:E. Our regressions of protein and energy intake versus protein gain, indicate that protein gain is often simultaneously limited by both protein and energy intake, and may be limited by other factors than protein and energy intake, such as a maximal protein deposition capacity (PD_{max}). We conclude that there is no physiological basis for an optimal P:E in Nile tilapia feeds.

KEYWORDS

aquafeed, bioenergetics, feed formulation, intermediary metabolism, nutritional requirements

1 | INTRODUCTION

Nile tilapia (*Oreochromis niloticus* (L., 1758)) is one of the main fish species farmed worldwide. Its production has increased rapidly over the past 40 years, accelerated by the intensification of farming practices.¹ Intensification of tilapia production intends to increase productivity by optimising resource-use efficiency (*e.g.*, water, feed, labour). Like for other

animal species, the intensification of Nile tilapia farming has been supported by an increasing use of formulated feeds.² Formulated feeds increase fish farms productivity, because they allow a regular and abundant nutrients supply that is higher than most ecosystems could provide. Yet, formulated feed is costly and usually accounts for more than half of the total operation costs in intensive tilapia farming systems.^{3,4} Thus, overall farm productivity is largely determined by feed efficiency.⁴

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The main product of fish farming is the fillet (*i.e.*, trunk muscle), which is mainly composed of water and protein in fresh water species like Nile tilapia.^{5,6} Hence, feed efficiency is largely determined by protein retention efficiency in muscle tissue. This is of prime importance to tilapia farming, because protein is usually the most expensive dietary macronutrient.² In addition, the excretion of non-retained dietary protein metabolites (*i.e.*, nitrogenous compounds) contributes to acidification and disruption of natural ecosystems. This is a major challenge in countries with large aquaculture production.^{7,8} Optimal use of dietary protein (*i.e.*, protein retention efficiency) can therefore improve profitability of fish farming, while reducing its environmental impact.⁹

Following digestion, dietary protein is absorbed in the form of free amino acids and small peptides.¹⁰ In growing animals, most amino acids are used for body protein synthesis (anabolism) or catabolised either to provide substrates for oxidative energy production, or *de novo* fatty acid synthesis.¹¹ Body protein synthesis is an energy-demanding process; to maximise protein efficiency, amino acids should be used as building blocks instead of energy substrates. This is often achieved by increasing non-protein energy supply (*i.e.*, lipids and carbohydrates), thereby sparing absorbed amino acids from being used as energy substrates. Fish nutritionists often refer to this phenomenon as the protein-sparing effect.¹² Thus, optimising protein efficiency in farmed fish primarily comes down to balancing the proportion of protein and energy in their feeds, known as the dietary protein-to-energy ratio (P:E).

With the increasing use of formulated fish feeds, nutritionists have sought to determine optimal dietary P:E for the growth of most commercially important aquaculture species, including Nile tilapia. From a biological perspective, the existence of an optimal dietary P:E presumes that protein deposition is limited by either protein or energy availability. In some terrestrial monogastric species (*e.g.*, pigs and poultry), this assumption is supported by the presence of distinct protein- and energy-dependent phases in protein deposition, separated by a clear inflection in the growth curve.^{13,14} In these species, growth (*i.e.*, protein deposition) is limited by the availability of protein at low protein intake, and by that of energy at high protein intake. The presence of distinct protein- and energy-dependent growth phases results in a linear-plateau relationship between protein intake and protein gain (Figure 1). This relationship supports the theory of a physiologically optimal dietary P:E at the inflection point, indicating the minimum protein intake required for its maximum deposition.¹⁵ In some fast-growing animals (*e.g.*, 20–80 kg pigs), this model shows a maximal protein deposition capacity (PD_{max}), above which any increase in protein or energy intake does not result in additional protein gain.¹⁶

To our knowledge, the existence of distinct protein- and energy-dependent growth phases has not yet been tested in fish. However, optimal dietary P:E estimates have been published for many farmed fish species. These were determined by means of feed trials,^{17–20} or via factorial modelling.²¹ In Nile tilapia, published estimates of the optimal dietary P:E derived from growth experiments vary from 13.3 for 40–200 g fish²² to 26.3 for 0.01–0.3 g juveniles²³ (Figure 2). Differences in published estimates may be attributed to: body size class²²; aspects of experimental design, such as feeding strategy (restricted or

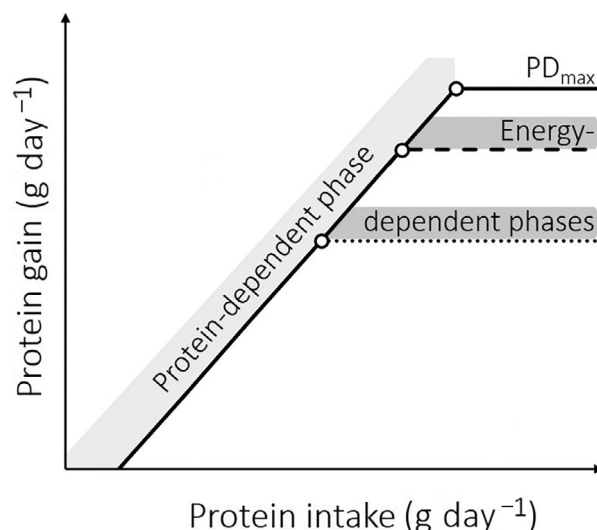


FIGURE 1 In terrestrial monogastric animals, protein gain is often described by a linear-plateau model. This model presumes that protein gain is either limited by protein intake (protein-dependent phase, light grey area) or by energy intake (energy-dependent phases, dark grey area). At low (.....) and high (---) energy intake, protein gain levels-off at different levels. This suggests the existence of an optimal protein-to-energy ratio at the inflection points of protein deposition (○). At even higher protein and energy intake, protein deposition is thought to be limited by a maximal protein deposition capacity (PD_{max})

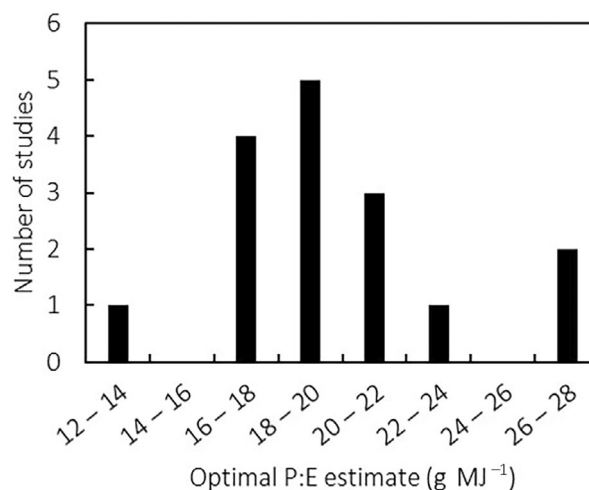


FIGURE 2 Published estimates ($n = 16$) of the optimal protein-to-energy ratio (P:E) for Nile tilapia feeds range from 13 to 26 g MJ⁻¹. Estimates were obtained or recalculated from 14 studies^{19,22–34}

to apparent satiation); dietary ingredients (nutrient content and digestibility); the range of dietary P:E tested; and adjustment of dietary P:E for differences in apparent digestibility of crude protein and gross energy (DP:DE). In addition, the outcome of these experiments also depends on the response variables used in determining optimal dietary P:E. Often, the optimal dietary P:E has been estimated based on differences in body mass gain and feed conversion ratio across a limited range of dietary P:E. If it exists, the optimal P:E is more likely

to be detected by a regression analysis across a large range of dietary P:E, rather than among a limited number of diets. Moreover, changes in dietary P:E primarily affect intermediary metabolism and more specifically amino acid partitioning towards body protein synthesis, oxidative catabolism and *de novo* fatty acid synthesis. Thus, the physiological validity of an optimal P:E should be tested in terms of nutrient partitioning, rather than whole-body mass gain. Following this approach, a recent study³⁵ showed a linear increase in protein retention efficiency in small Nile tilapia (body mass < 40 g), when feeding a fixed amount of protein and increasing amounts of non-protein energy (*i.e.*, decreasing dietary P:E). The authors concluded that the optimal dietary P:E was either lower than the lowest level tested in their experiment (16.6 g MJ⁻¹), or inexistent.

The objective of the present review is to determine if there is a physiological basis for an optimal P:E for Nile tilapia feeds, using two meta-analytical approaches. First, we examine the effect of dietary P:E on Nile tilapia growth, with a focus on nutrient partitioning. Then, we inspect the separate effects of protein and energy intake on protein and lipid gain in Nile tilapia. Finally, the biological significance of our results and implications for practical tilapia feed formulation are discussed.

2 | MATERIAL AND METHODS

2.1 | Literature search

A literature search was conducted on Nile tilapia growth and body composition data, focusing on variations in dietary protein and energy content. The following search query was submitted to the Scopus database: TITLE: 'protein' OR 'energy' OR 'carbohydrate' OR 'lipid' OR 'fat' AND TITLE: 'nile tilapia' OR 'oreochromis nilotic*'. The search query was voluntarily not specific to 'protein-to-energy ratio', because not all studies testing contrasts in dietary macronutrient profile mention the dietary P:E. Yet, any study testing contrasts in dietary macronutrient profile could be relevant to our two objectives. The relevance of collected publications was assessed based on their titles and abstracts, which led to the pre-selection of 51 studies (Appendix A). This list also included one unpublished, yet relevant, MSc thesis from our group.

2.2 | Data selection

A set of methodological criteria was defined in order to select publications meeting the two objectives of our meta-analysis (Table 1). All 51 pre-selected studies were reviewed and checked against these criteria, which led to the selection of 23 studies from which growth data were reported for 161 experimental dietary treatments (hereafter referred to as cases). Only 10 of these 23 studies reported apparent digestibility coefficients for dietary protein and energy. Thus, dietary crude protein and gross energy content were used for all data selection steps, calculations and analyses. Among the 23 selected studies, 20 reported growth data for an intra-study

TABLE 1 Selection criteria for pre-selected publications

Methodological characteristics	Selection criteria
<i>Experimental design</i>	
Species	Natural and selected strains of <i>Oreochromis niloticus</i>
Experiment duration	Precise duration available (in days)
Fish housing	In tanks, with water quality monitoring
Feed spillage monitoring	Hand feeding
<i>Data collection</i>	
Growth measurements	Initial and final body mass reported
Measurement error	Reported as SD, SE or pooled SE
Feed intake	Reported or re-calculable from feed efficiency indicators
Diet composition	Analysed ^a
Body composition (facultative) ^b	Analysed ^a
<i>Additional selection criteria</i>	
Intrastudy range in dietary P:E	>1.5 g MJ ⁻¹ in Subsets 1 and 2
Intrastudy variation in crude protein intake	>20% in Subset 3
Intrastudy variation in gross energy intake	>10% in Subset 4

Note: P:E = dietary crude protein-to-gross energy ratio.

^aIn accordance with the guidelines of the AOAC. Calculated compositions (*e.g.*, based on expected composition of dietary ingredients) were excluded.

^bObservations for which the sum of body crude protein, lipid and ash content (in g kg⁻¹) deviated from the reported DM content by more than 5% were excluded.

range of dietary P:E larger than 1.5 g MJ⁻¹ (Subset 1, Table 2). These were used to study the effect of dietary P:E on Nile tilapia growth performance and nutrient partitioning across 143 cases. Among the 20 studies of Subset 1, 10 presented initial and final body composition data for 72 cases, allowing the calculation of complete nutrient balances (Subset 2, Table 2). These 72 cases were used to investigate the effect of dietary P:E on nutrient retention efficiencies and on the nutritional composition of body mass gain. To test the effects of protein and energy intake on protein gain, studies in which the lowest and highest daily crude protein and gross energy intake differed by at least 20% and 10%, respectively, were selected (Table 1). The minimal intra-study range in crude protein and gross energy intake in the two subsets (Subsets 3 and 4) are presented in Table 2. The list of studies included in each of the four subsets is given in Appendix B.

2.3 | Experimental conditions in selected studies

The selected studies covered a large range of body size classes, dietary crude protein and gross energy content and dietary P:E (Table 2).

TABLE 2 Range and frequency of continuous and discrete experimental variables within the four subsets used in the meta-analysis

	Subset 1	Subset 2	Subset 3	Subset 4
Number of studies	20	10	12	12
Number of cases	143	72	69	82
Continuous variables				
Initial body mass (g)	3–385	6–60	6–76	6–76
Final body mass (g)	3–449	26–250	10–250	10–250
Dietary CP content (g kg ⁻¹)	133–565	172–565	172–565	172–565
Dietary GE content (MJ kg ⁻¹)	10–24	16–24	16–24	16–24
Dietary P:E ^a (g MJ ⁻¹)	8–34	9–34	9–34	9–26
Water temperature (°C)	23–30	27–28	27–29	27–29
Dissolved oxygen concentration (mg L ⁻¹) ^b	4.0–7.3	4.0–7.3	4.0–7.3	4.0–7.3
Duration of the experiment (days)	21–96	42–70	14–70	14–70
Daily dry matter intake (g kg ^{-0.8} day ⁻¹)	4–67	4–27	4–27	4–27
Discrete variables				
Strain				
GIFT	54	27	17	33
Other	89	45	52	49
Sex				
All-male	77	58	56	69
Mixed	9	9	9	9
Not reported	57	5	4	4
Feeding strategy				
Restricted	42	33	27	40
Apparent satiation	90	39	42	42
Not reported	11			

Abbreviations: CP, crude protein; GE, gross energy; GIFT, genetically improved farmed tilapia.

^aCrude protein-to-gross energy ratio corrected for the non-digestible energy content of purified cellulose sources included in the formulation of some experimental diets (98/161 across the 4 subsets).

^bDissolved oxygen content was not reported for all cases. Given ranges are based on $n = 100, 59, 56$ and 72 in Subsets 1, 2, 3 and 4, respectively.

Most experiments were conducted at temperatures close to the 28–30°C known optimum,³⁶ except for three early studies,^{28,29,37} which were conducted at sub-optimal water temperatures (21–24°C). Most studies were conducted on all-male selected populations of Nile tilapia, with the genetically improved farmed tilapia (GIFT) strain being used in many cases. All subsets included experiments conducted under restricted and apparent satiation feeding.

2.4 | Calculations

2.4.1 | Diet and body composition

Selected studies reported the analysed crude protein (*i.e.*, analysed nitrogen content $\times 6.25$), ether extract (*i.e.*, crude lipid) and ash content of experimental diets. For all diets, the total dietary carbohydrate fraction expressed per kg of dry matter (DM) was calculated as: $1000 - (\text{crude protein} + \text{crude lipid} + \text{ash})$. Analysed dietary gross energy content was reported in 82 of the 161 cases. In the remaining

cases, dietary gross energy content was estimated from the analysed dietary macronutrient composition and the corresponding energy equivalents³⁸: 17.2 kJ g⁻¹ for carbohydrates, 23.6 kJ g⁻¹ for crude protein and 39.5 kJ g⁻¹ for lipids. Purified cellulose and carboxymethylcellulose were included as dietary inert fillers in 98 of the 161 cases, at inclusion levels up to 200 g kg⁻¹. Although these ingredients contributed to dietary gross energy content, they are indigestible to Nile tilapia³⁹ and therefore never used in such proportions in practical feed formulation. The analysed or estimated dietary gross energy content was therefore adjusted to compensate for the induced overestimation of available dietary energy in these 98 diets. This was done by subtracting the share of gross energy corresponding to cellulose and/or carboxymethylcellulose. This share was estimated by multiplying the formulated level of cellulose and/or carboxymethylcellulose (in g kg⁻¹ of diet) with the average DM (953 g kg⁻¹) and gross energy contents (17.4 MJ kg⁻¹ DM) obtained for such products.⁴⁰

Fish body energy content was either taken as the analysed value reported by the authors (64/161 cases) or calculated as the sum of

body protein and lipid energy content, using the energy equivalents mentioned above (23.6 and 39.5 kJ g⁻¹, respectively). Body glycogen also contributes, to a small extent, to body energy content. Yet, none of the studies included in our analyses reported analysed body glycogen content. Most glycogen is stored in the liver and muscle tissue of Nile tilapia, where its content does not exceed 30 and 3 mg g⁻¹ of tissue, respectively.^{41,42} Thus, its energy content was regarded as neglectable in the calculation of total body energy content.

2.4.2 | Effect of dietary P:E on nutrient partitioning and growth

The first approach of the meta-analysis addressed the effects of dietary P:E on nutrient utilisation in Nile tilapia growth. Dietary P:E was used as single independent variable and was calculated for all diets, using the following equation:

$$\text{Diet P:E (g MJ}^{-1}) = \frac{CP_{diet}}{GE_{diet}}, \quad (1)$$

where CP_{diet} and GE_{diet} are the dietary crude protein (g kg⁻¹) and cellulose-adjusted gross energy (MJ kg⁻¹) content, respectively. Dependent variables were calculated at two scales: whole-body mass and individual nutrients. Whole-body growth and feed efficiency indicators included the daily body mass gain and the feed conversion ratio. These were calculated using the following equations:

$$\text{Daily body mass gain (g day}^{-1}) = \frac{(BM_f - BM_i)}{d}, \quad (2)$$

$$\text{Feed conversion ratio (g g}^{-1}) = \frac{DMI}{(BM_f - BM_i)}, \quad (3)$$

where BM_i and BM_f are the initial and final fish body mass (kg), d is the duration of the experiment in days and DMI is the total individual dry matter intake (kg). At the scale of individual macronutrients, nutrient partitioning was expressed in the form of gain-to-intake ratios and the nutrient content of body mass gain. Nutrient gain-to-intake ratio was calculated for protein, lipid and energy. For protein, this was calculated as:

$$\text{Protein gain: protein intake (\%)} = \frac{100 \times (BM_f \times BP_f - BM_i \times BP_i)}{DMI \times CP_{diet}}, \quad (4)$$

where BM_i and BM_f are expressed in kg, BP_i and BP_f are the initial and final fish body protein content (g kg⁻¹) and DMI is expressed in kg. For the calculation of lipid and energy gain-to-intake ratios, BP_i , BP_f and CP_{diet} were replaced by their lipid (BL_i , BL_f and CL_{diet}) and energy (BE_i , BE_f and GE_{diet}) equivalents, expressed in g and kJ kg⁻¹, respectively. The nutrient content of body mass gain (g kg⁻¹, as is) was calculated for body water, protein, lipid, ash and energy. For protein, this was calculated as:

$$\text{Protein gain: body mass gain (g kg}^{-1}, \text{ as is)} = \frac{BM_f \times BP_f - BM_i \times BP_i}{BM_f - BM_i}. \quad (5)$$

Again, BP_i and BP_f were replaced by their water (in g kg⁻¹), lipid (in g kg⁻¹), ash (in g kg⁻¹) and energy (in MJ kg⁻¹ as is) equivalents for the calculation of their respective contributions to body mass gain.

2.4.3 | Separate effects of protein and energy intake on protein and lipid gain

The second approach of the meta-analysis addressed the separate effects of daily crude protein and gross energy intake on daily protein gain in Nile tilapia. To allow comparison across body mass classes, dependent and independent variables were expressed relative to fish geometric mean metabolic body mass, calculated as:

$$MBM_{mean}(\text{kg}^{0.8}) = \sqrt{BM_i^{0.8} \times BM_f^{0.8}}, \quad (6)$$

where BM_i and BM_f are the initial and final fish body mass (in kg). The metabolic exponent of 0.8 corresponds to a mean value observed across several fish species.⁴³ Daily relative crude protein intake was calculated as:

$$\text{Daily protein intake (g kg}^{-0.8} \text{ day}^{-1}) = \frac{DMI \times CP_{diet}}{d \times MBM_{mean}}, \quad (7)$$

where DMI is expressed in kg and CP_{diet} in g kg⁻¹. The same equation was used to calculate daily energy intake (in kJ kg^{-0.8} day⁻¹), with CP_{diet} being replaced by the cellulose-adjusted dietary gross energy content (GE_{diet} , in kJ kg⁻¹). Similarly, daily relative protein gain was calculated as:

$$\text{Daily protein gain (g kg}^{-0.8} \text{ day}^{-1}) = \frac{BM_f \times BP_f - BM_i \times BP_i}{d \times MBM_{mean}}, \quad (8)$$

where BM_i and BM_f are expressed in kg and BP_i and BP_f are expressed in g kg⁻¹. Daily lipid gain was calculated as in Equation 8, with BP_i and BP_f being replaced by their lipid equivalent (in g kg⁻¹).

2.5 | Statistical analyses

All statistical analyses were performed using SAS software version 9.4 (SAS Institute Inc., Cary, NC, USA).

2.5.1 | Effect of dietary P:E on nutrient partitioning and growth

For a given dependent variable, an optimal dietary P:E only exists if this variable reaches a maximal or minimal value at a given dietary P:E.

This implies the existence of a curvilinear, rather than linear, relationship between dietary P:E and the variable of choice. In the first approach of the meta-analysis, this was tested by fitting the following simple linear and quadratic mixed models to selected response variables:

$$Y_{ij} = a + Sa_j + (b + Sb_j) \times P:E_{ij} + e_{ij}, \quad (9)$$

$$Y_{ij} = a + Sa_j + (b + Sb_j) \times P:E_{ij} + (c + Sc_j) \times (P:E_{ij})^2 + e_{ij}, \quad (10)$$

where Y_{ij} is the value of the dependent variable (*i.e.*, response variables) for the i^{th} case of the j^{th} study, a is the intercept, Sa_j is the random j^{th} study effect on the intercept, b is the linear regression coefficient (*i.e.*, slope), Sb_j is the random j^{th} study effect on the slope, $P:E_{ij}$ is the dietary P:E for the i^{th} case of the j^{th} study and e_{ij} is the unexplained residual error. In the quadratic model (Equation 10), c is the quadratic regression coefficient and Sc_j is the random j^{th} study effect on the quadratic regression coefficient.

Other factors than the dietary P:E may affect the response (*i.e.*, dependent) variables that were tested in our meta-analysis. For example, factors like fish body size, water temperature and dissolved oxygen concentration may affect nutrient partitioning,⁴⁴ maintenance requirements⁴⁵ and voluntary feed intake,⁴⁶ respectively. However, the fixed effects of these factors were not tested in our meta-analysis. Our data set was built to test the effects of contrasts in dietary protein and energy content, which resulted in large intra-study and, to a lesser extent, inter-study variations in dietary P:E, in Subsets 1 and 2 (Figure 3a). On the contrary, intra-study variation in other experimental factors, like starting body mass and daily relative DM intake, was very small (Figure 3b,c), and even null in the case of water temperature and dissolved oxygen concentration which were fully fixed within studies. Thus, the fixed effects of these variables were

confounded with a virtually infinite number of other hidden study-specific effects. In addition, inter-study differences in factors such as starting body mass and daily relative DM intake were not homogeneously distributed, with a few studies reporting much higher values than the rest (Figure 3b,c). The lack of intra-study variability and the heterogeneous distribution of other variables than dietary P:E in our data set, meant that their fixed effects could not be properly estimated in our regression models. This is a common issue in meta-analyses of literature data, which can be tackled by adding random study effects to the regression models.^{61,62} Here, the inclusion of random study effects allowed us to test the pooled effect of either explicit (*e.g.*, water temperature) or hidden (*e.g.*, health status of the fish) study-specific factors on the estimated relationships (intercept, slope and their interaction) between dietary P:E and dependent variables. The regression models were fitted using the MIXED procedure of SAS 9.4. The significance of fixed effects and random study effects was assessed by *t*- and *Z*-tests, respectively, using critical values of $p = 0.05$ for fixed effects and $p = 0.10$ for random effects.⁶¹ Non-significant random study effects ($p > 0.10$) were removed from the regression models. In studies where data were reported from separate experiments, these were treated as separate studies in the estimation of random study effects.

2.5.2 | Separate effects of protein and energy intake on protein and lipid gain

The existence of an optimal P:E ratio may also be indicated when increasing protein intake results in distinct protein- and energy-dependent phases of protein gain (Figure 1). The second approach of the meta-analysis tested the existence of such phases by fitting simple linear and linear-plateau models to daily body protein and lipid gain data, using daily crude protein and gross energy intake as independent variables. Inter-study contrasts in fish size resulted in scale differences

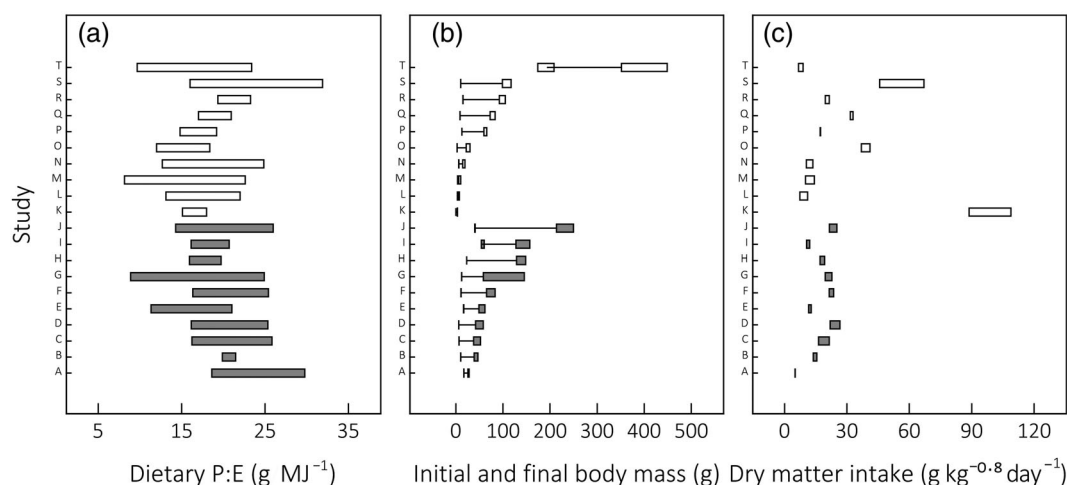


FIGURE 3 Ranges of (a) dietary crude protein-to-gross energy ratio (P:E), (b) initial and final body mass and (c) daily dry matter intake per unit of metabolic body mass reported in the 10 studies included in subset 1 only (□) and in the 10 studies included in both subset 1 and 2 (■). Studies are labelled from A to S, by order of increasing final body mass within each subset. Corresponding ranks in the reference list are as follows: A⁴⁷; B⁴⁸; C³⁵; D⁴⁹; E³³; F⁵⁰; G⁵¹; H⁵²; I⁵³; J⁵⁴; K²⁸; L^{29(Exp. 1)}; M^{29(Exp. 2)}; N⁵⁵; O⁵⁶; P⁵⁷; Q⁵⁸; R⁵⁹; S⁶⁰; T³²

in both intake and gain of protein, lipid and energy. Scale differences were partially corrected for, by expressing nutrient intake and gain relative to fish geometric mean metabolic body mass ($\text{kg}^{0.8}$). Yet, inter-study scale differences also arose from the fact that feed intake capacity varies with body size. Because most studies provided data for a single fish size class, there were only slight intra-study variations in protein, lipid and energy gain and intake. These inter-study differences are inherent to fish biology, and to the approach employed, and could not be corrected for. For this reason, the regression models used in the second approach (separate effects of protein and energy intake on protein and lipid gain) did not include random study effects. The simple linear model took the same form as in Equation 9, except that random study effects parameters S_{aj} and S_{bj} were excluded. This model was fitted using the MIXED procedure of SAS 9.4, while linear-plateau regression models were fitted using the NLMIXED procedure.⁶³ Fixed effects significance was tested as in the first approach. Model quality of fit was evaluated using the corrected Akaike information criterion (AICc).⁶⁴

2.5.3 | Weighting scheme

Across studies, measurement accuracy of input data (e.g., initial and final body mass, body composition) was reported in three ways: either as the SD of the mean of each case (i.e., dietary treatment); or as the SE of the mean of each case; or as the pooled standard error (PSE) of all cases' means. The SE of input data was calculated – if not reported – for each case, using the following equation:

$$SE_i = \frac{SD_i}{\sqrt{n_i}}, \quad (11)$$

where SE_i and SD_i are the SE and the SD of the i^{th} case mean, respectively, and n_i is the square root of the number of replicates for the i^{th} case. Case SEs were used to calculate intra-study PSEs as:

$$SE_j = \sqrt{\frac{\sum SE_{ij}}{n_j}}, \quad (12)$$

where SE_j is the PSE common to all the i^{th} cases of the j^{th} study, SE_{ij} is the SE of the mean for the i^{th} case of the j^{th} study and n_j is the number of cases tested in the j^{th} study. The accuracy of calculated variables was estimated by applying uncertainty propagation laws for additions and multiplications,⁶⁵ under the assumption that there was no correlation between operands. Finally, individual weights were calculated as follows:

$$W = \left(\frac{1}{SE_j^2} \right)^{0.1}, \quad (13)$$

where the inverse of the squared pooled SE_{ij} was raised to the power 0.1 to narrow the range of calculated weights. Weights were calculated for all response variables and used in all regression analyses, using the WEIGHT statement of the MIXED and NLMIXED

procedures. This ensured that data obtained with greater accuracy contributed more to the fitted model.

3 | RESULTS

3.1 | Effect of dietary P:E on nutrient partitioning and growth

3.1.1 | Nutrient gain-to-intake ratio

Nutrient gain-to-intake ratios indicate the extent to which ingested nutrients are retained in body tissues. For protein and energy, these

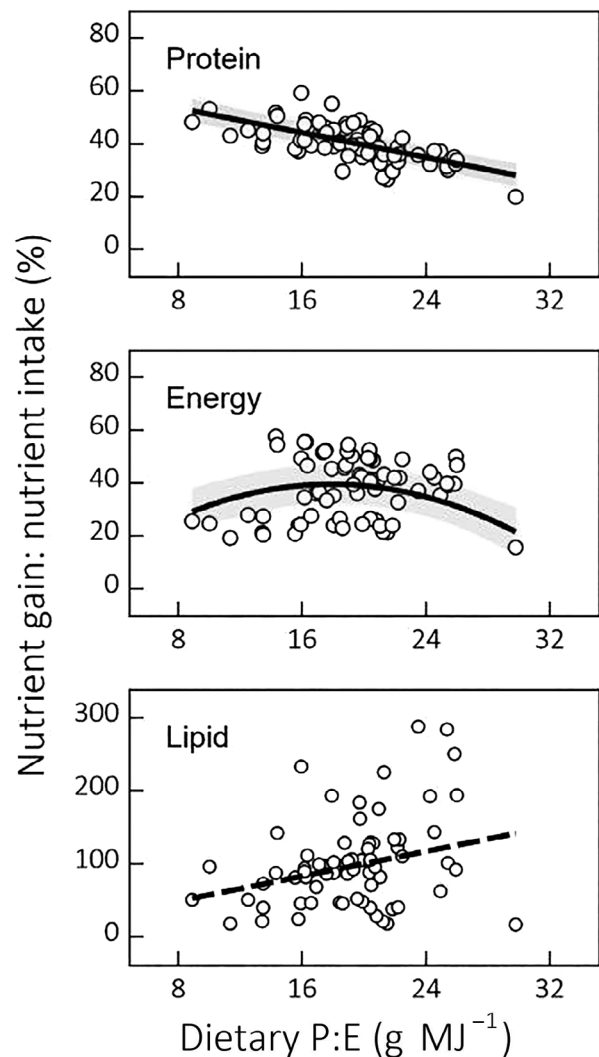


FIGURE 4 Effects of the crude protein-to-gross energy ratio (P:E) on the gain-to-intake ratio of crude protein, gross energy and lipid in Nile tilapia ($n = 72$). Solid lines (—) represent simple linear and quadratic relationships with estimates different from zero at $p < 0.05$. Dash lines (---) represent relationships for which model's residuals were not normally distributed, but for which parameter estimates differed from zero at $p < 0.05$. Grey areas represent the 95% confidence interval of model predictions for models with normally distributed residuals

ratios correspond to retention efficiencies because body protein and energy gain can only result from protein and energy ingestion, respectively. This is not the case for lipid gain, which can result from deposition of ingested lipids, but also from *de novo* fatty acid synthesis. In the latter case, lipid gain-to-intake ratio may exceed 100%, as shown on Figure 4.

With an R^2 of 0.67 (Table 3), the clearest effect of increasing dietary P:E was a linear decrease in protein efficiency. For every increase in dietary P:E of 1 g MJ⁻¹, protein retention efficiency decreased by 1.2% (Equation 14, Table 3). The absence of a significant ($p > 0.10$) random study effect on the linear regression estimate indicates that the linear relationship was consistent across studies. Unlike protein efficiency, energy retention efficiency increased in a quadratic way with increasing dietary P:E (Equation 15, Table 3); with a maximal retention efficiency of 39.6%, estimated at a dietary P:E of 17.9 g MJ⁻¹ (Figure 4). Changes in dietary P:E also affected the lipid gain-to-intake ratio. However, with an R^2 of 0.08 (Table 3), the predictive value of this model was lower than those obtained for protein

and energy efficiency. This was also reflected in the presence of significant random study effects on the model's intercept and slope, and their interaction.

3.1.2 | Nutrient composition of body mass gain

Expressing nutrient gain relative to body mass gain allows for comparison of nutrient deposition across studies. This is irrespective of differences in scale, originating from contrasts in fish size or feeding level. Body water, crude protein, lipid and ash content are interdependent and an increase in the relative gain of one, or several, of them inevitably results in a decrease in the relative gain of one, or several, of the others.

With an R^2 of 0.41 (Table 4), the clearest effect of changes in dietary P:E was on the protein content of body mass gain. For every increase of 1 g MJ⁻¹ in dietary P:E, the protein content of body mass gain increased by 1.2 g kg⁻¹ (Equation 18, Table 4). Similarly, the water content of body mass gain increased linearly with increasing dietary P:E (Figure 5). On the

TABLE 3 Linear and quadratic effects of dietary crude protein-to-gross energy ratio (P:E, in g MJ⁻¹) on the body gain-to-intake ratio of crude protein, lipid and gross energy in Nile tilapia ($n = 72$)

Dependent variable (Y, in %)	Equation	$P_{lin.}^a$	$P_{quad.}^a$	R^2	Eq. number
<i>Simple linear models</i>					
Protein gain: protein intake	$Y = 62.9^b (SE = 2.80) - 1.2 (SE = 0.10) \times P:E$	<0.001		0.67	(14)
Energy gain: energy intake	$Y = 48.0^b (SE = 9.11) - 0.5 (SE = 0.35) \times P:E$	0.19		0.001	(15)
Lipid gain: lipid intake	$Y = 15.5 (SE = 24.28) + 4.2^b (SE = 1.51) \times P:E$	0.021		0.08	(16)
<i>Quadratic models</i>					
Energy gain: energy intake	$Y = -1.21.4^b (SE = 0.898) + 4.6 (SE = 0.89) \times P:E - 0.13 (SE = 0.023) \times P:E^2$	<0.001	<0.001	0.35	(17)

SE = standard error.

^a p -Value for significance of simple linear ($p_{lin.}$) and quadratic ($p_{quad.}$) parameter estimates tested by a t -test.

^bParameter estimates followed by this sign were affected by a random study effect which differed from zero at $p < 0.10$.

TABLE 4 Linear and quadratic effects of dietary crude protein-to-gross energy ratio (P:E, in g MJ⁻¹) on the nutritional composition of body mass gain in Nile tilapia ($n = 72$)

Dependent variable (Y, in g kg ⁻¹)	Equation	$P_{lin.}^a$	$P_{quad.}^a$	R^2	Eq. number
<i>Simple linear models</i>					
Protein gain: body mass gain	$Y = 132^b (SE = 3.9) + 1.2 (SE = 0.18) \times P:E$	<0.001		0.41	(18)
Water gain: body mass gain	$Y = 628^b (SE = 26.6) + 4.5^b (SE = 0.95) \times P:E$	0.001		0.08	(19)
Lipid gain: body mass gain	$Y = 192^b (SE = 34.8) - 4.7^b (SE = 1.22) \times P:E$	0.004		0.04	(20)
Energy gain: body mass gain ^c	$Y = 10.8^b (SE = 1.38) - 0.2^b (SE = 0.05) \times P:E$	0.009		0.02	(21)
Ash gain ^d : body mass gain	$Y = 39.8^b (SE = 3.13) - 0.09 (SE = 0.116) \times P:E$	0.44		0.00	(22)
<i>Quadratic models</i>					
Ash gain ^d : body mass gain	$Y = 66.8^b (SE = 6.86) - 3.0 (SE = 0.68) \times P:E + 0.08 (SE = 0.018) \times P:E^2$	<0.001	<0.001	0.24	(23)

SE = standard error.

^a p -Value for significance of simple linear ($p_{lin.}$) and quadratic ($p_{quad.}$) parameter estimates tested by a t -test.

^bParameter estimates followed by this sign were affected by a random study effect which differed from zero at $p < 0.10$.

^cExpressed in MJ kg⁻¹.

^d $n = 72$.

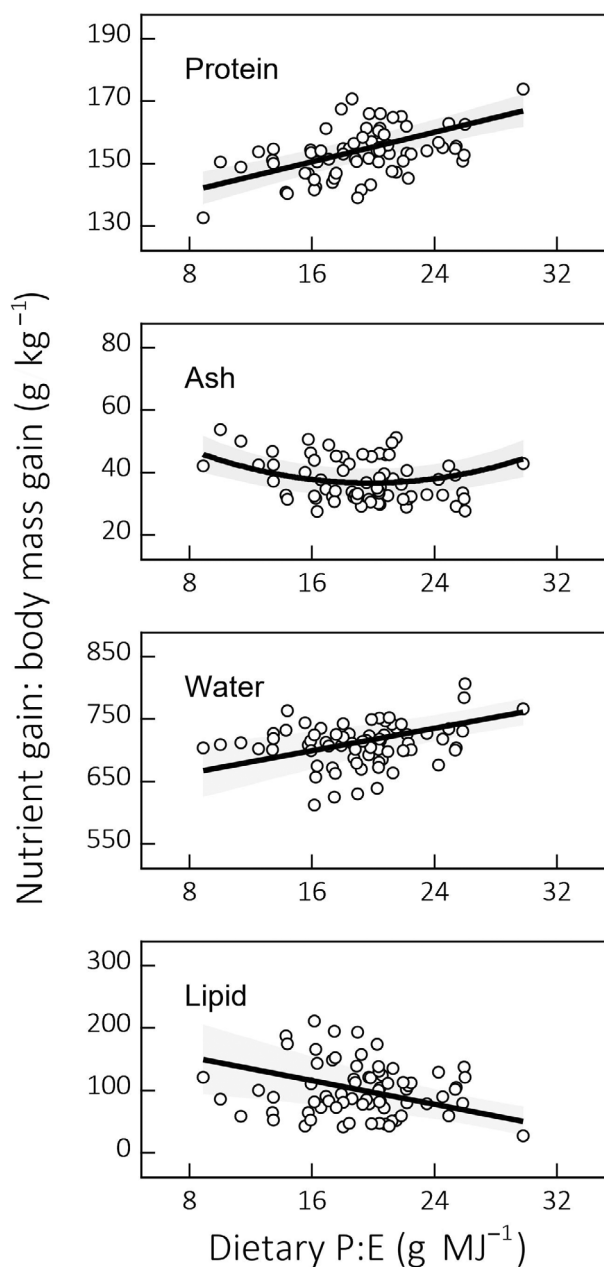


FIGURE 5 Effects of dietary protein-to-gross energy ratio (P:E) on the protein, ash, water and lipid content of body mass gain in Nile tilapia ($n = 72$). Black lines represent simple linear and quadratic relationships with estimates different from zero at $p < 0.05$. Grey areas represent the 95% confidence interval of predictions for models with normally distributed residuals

contrary, the lipid and energy content of body mass gain decreased by 4.7 g and 0.2 MJ kg⁻¹ for every dietary P:E increase of 1 g MJ⁻¹ (Equations 20 and 21, Table 4). Only the relative ash gain was affected in a quadratic way by changes in dietary P:E (Equation 23, Table 4), with a minimal ash-to-body mass gain ratio of 36.7 g kg⁻¹, estimated at a dietary P:E of 19.8 g MJ⁻¹ (Figure 5). The absence of significant random study effects on the slope of Equation 18, and on the first- and second-order quadratic parameter of Equation 23, indicated that the relationships were consistent across studies. This was not the case for the models obtained

for water, lipid and energy content of body mass gain, which was in line with the larger variability observed for these nutrients (Figure 5).

3.1.3 | Whole-body mass growth indicators

Practical growth and feed efficiency indicators are often based on whole-body mass. This is the case for daily body mass gain and the feed conversion ratio. The effects of dietary P:E on these indicators were tested across 143 cases, reported in 20 studies. Dietary P:E had significant ($p < 0.05$, Table 6) curvilinear effects on both indicators. However, the distribution of the two models' residuals clearly deviated from a normal distribution. This indicated that the response variables were under- or over-estimated in many cases and that the equations obtained had a limited predictive value, as indicated by the low R^2 obtained for both models (Table 5). The two models predicted that maximal body mass gain and minimal feed conversion ratio, were achieved at dietary of 20.0 and 21.5 g MJ⁻¹, respectively (Figure 6a,b).

3.1.4 | Fillet yield and composition

Among the 51 studies initially collated, 5 reported the effect of dietary protein and energy balance on fillet yield.^{6,22,30-32} Dietary P:E was either expressed as P:E^{6,22,32} or DP:DE.^{30,31} Because it was impossible to express dietary P:E on a common basis across these five studies, the effect of dietary P:E on fillet yield was analysed per study.

Fillet yield increased linearly ($p < 0.01$, Table 6) with dietary P:E in two of the five selected studies (Equations 30 and 32, Table 6) and tended ($p = 0.08$) to do so in a third one. In the study of Haidar (2017b), fillet yield increased in a quadratic way (Equation 33) with dietary P:E. There were clear inter-study differences in fillet yield (Figure 7), with one study²² reporting fillet yield ranging from 44% to 52%, a much higher range than that reported by the other four studies (23%–37%).

3.2 | Separate effects of protein and energy intake on protein and lipid gain

3.2.1 | Effect of CP intake on protein and lipid gain

The effect of crude protein intake on Nile tilapia protein and lipid gain were investigated using growth data from 69 experimental cases, tested in 12 studies. Crude protein intake ranged from 1.8 to 12.5 g kg^{-0.8} day⁻¹ (Figure 8a,b), corresponding to absolute intakes of 0.1–2.0 g day⁻¹. Daily protein and lipid gains ranged from 0.5 to 4.4 and from 0.1 to 6.0 g kg^{-0.8} day⁻¹, which corresponded to absolute gains of 0.02 to 0.64 and 0.001 to 0.82 g day⁻¹, respectively.

Body protein gain followed a two-phased response to increasing crude protein intake (Figure 8a). Accordingly, the relationship was

TABLE 5 Linear and quadratic effects of dietary crude protein-to-gross energy ratio (P:E, in g MJ⁻¹) on daily body mass gain and feed conversion ratio of Nile tilapia (*n* = 143)

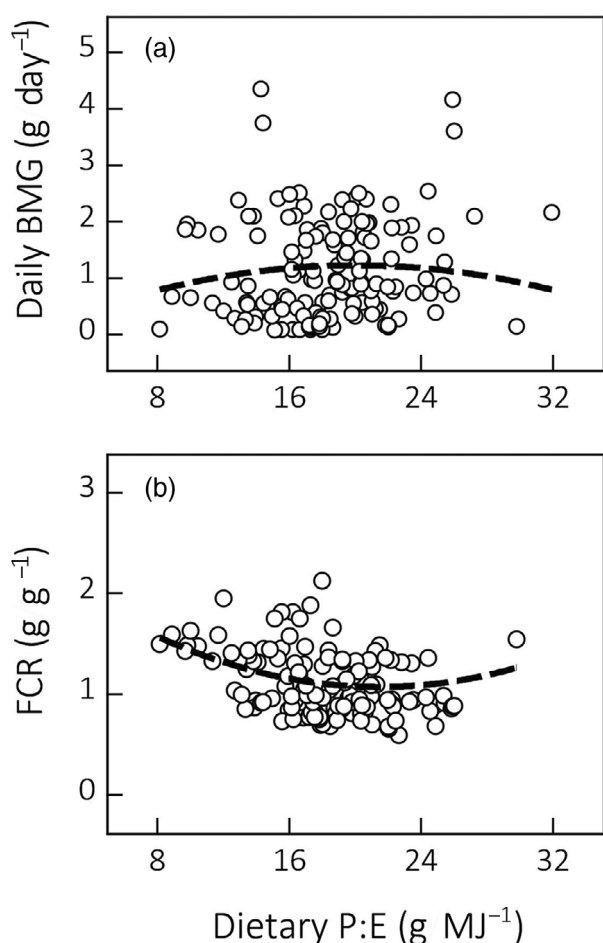
Dependent variable (Y)	Equation	<i>p</i> _{lin.} ^a	<i>p</i> _{quad.} ^a	R ²	Eq. number
<i>Simple linear models</i>					
Body mass gain (g day ⁻¹)	$Y = 1.1^b (SE = 0.28) + 0.01^b (SE = 0.009) \times P:E$	0.40		0.00	(24)
Feed conversion ratio ^c (g g ⁻¹)	$Y = 1.4^b (SE = 0.13) - 0.01^b (SE = 0.007) \times P:E$	0.041		0.00	(25)
<i>Quadratic models</i>					
Body mass gain (g day ⁻¹)	$Y = 0.01^b (SE = 0.343) + 0.12^b (SE = 0.027) \times P:E - 0.003 (SE = 0.0007) \times P:E^2$	<0.001	<0.001	0.10	(26)
Feed conversion ratio ^c (g g ⁻¹)	$Y = 2.3^b (SE = 0.19) - 0.12^b (SE = 0.020) \times P:E + 0.003 (SE = 0.0005) \times P:E^2$	<0.001	<0.001	0.20	(27)

SE = standard error.

^a*p*-Value for significance of simple linear (*p*_{lin.}) and quadratic (*p*_{quad.}) parameter estimates tested by a *t*-test.

^bParameter estimates followed by this sign were affected by a random study effect which differed from zero at *p* < 0.10.

^c*n* = 140.

**FIGURE 6** Quadratic effects of crude protein-to-gross energy ratio (P:E) on (a) body mass gain (BMG, *n* = 143) and (b) the feed conversion ratio (FCR, *n* = 140) of Nile tilapia. Dashed lines represent relationships for which model's residuals were not normally distributed, but for which parameter estimates differed from zero at *p* < 0.05

better described by a linear-plateau model, than by a simple linear model, as indicated by its lower AICc value (28 vs. 87, Table 7). Protein gain increased linearly, with a marginal efficiency of 52%

(Equation 35, Table 7), up to 3.9 g kg^{-0.8} day⁻¹. This model indicated a levelling-off of protein deposition, for crude protein intake levels higher than 8.4 g kg^{-0.8} day⁻¹ (Table 7). Body lipid gain also increased with crude protein intake. However, the nature of this relationship was less evident, due to an increase in lipid gain variability in fish fed more than 6 g kg^{-0.8} day⁻¹ of crude protein (Figure 8b). This relationship was slightly better described by a simple linear model, rather than a linear-plateau model (AICc = 181 vs. 183, respectively, Table 7).

3.2.2 | Effect of gross energy intake on protein and lipid gain

The effect of daily gross energy intake on protein and lipid gain was evaluated from 82 experimental cases, tested in 12 studies. Daily gross energy intake ranged from 95 to 611 kJ kg^{-0.8} day⁻¹ (Figure 8c,d), corresponding to absolute intakes of 5–83 kJ day⁻¹. Body protein and lipid gain were expressed in g kg^{-0.8} day⁻¹, as in Table 7. This facilitated a comparison between the effects of crude protein and gross energy intake. The ranges of daily protein and lipid gain were as in the previous section (0.5–4.4 and 0.1–6.0 g kg^{-0.8} day⁻¹, respectively).

Protein gain followed a two-phased response to gross energy intake (Figure 8c), as indicated by the lower AICc value of the linear-plateau model (96, Equation 39, Table 7), compared with that of the simple linear model (116, Equation 38). The second phase (plateau) depended on a limited number of cases (*n* = 8); its inflection, at 502 kJ kg^{-0.8} day⁻¹ (Table 8), was close to the highest gross energy intake level (611 kJ kg^{-0.8} day⁻¹). Lipid gain increased linearly (*p* < 0.05) with gross energy intake (Equation 40, Table 8).

Daily protein and lipid gain increased with gross energy intake at a rate of 8 and 10 g MJ, respectively (Equations 38 and 40, Table 8). Once converted to their energy equivalents (multiplication by 23.6 and 39.5 kJ g for protein and lipid, respectively), these rates equalled 18.5% for protein and 40.3% for lipid, corresponding to an overall incremental efficiency of 58.7% for gross energy deposition.

TABLE 6 Intra-study linear and quadratic effects of dietary crude protein-to-gross energy (CP:GE, in g MJ⁻¹) or digestible protein-to-digestible energy (DP:DE, in g MJ⁻¹) ratios on the fillet yield (Y, in %) observed in five Nile tilapia studies

Study ^a	n ^b	Equation	p _{lin.} ^c	p _{quad.} ^c	R ²	Eq. number
<i>Simple linear models</i>						
6	8	$Y = 20.2 (SE = 4.06) + 0.3 (SE = 0.19) \times CP:GE$	0.15		0.31	(28)
22	18	$Y = 42.8 (SE = 0.5) + 0.5 (SE = 0.27) \times CP:GE$	0.08		0.18	(29)
30	12	$Y = 26.4 (SE = 0.95) - 0.2 (SE = 0.05) \times DP:DE$	0.002		0.62	(30)
31	10	$Y = 31.6 (SE = 2.45) + 0.2 (SE = 0.12) \times DP:DE$	0.13		0.26	(31)
32	19	$Y = 25.8 (SE = 0.92) + 0.3 (SE = 0.05) \times CP:GE$	<0.001		0.61	(32)
<i>Quadratic models</i>						
6	8	$Y = -36.5 (SE = 20.13) + 5.9 (SE = 1.96) \times CP:GE - 0.1 (SE = 0.05) \times CP:GE^2$	0.030	0.036	0.74	(33)

SE = standard error.

^aSee list of references for publication details.

^bNumber of test diets from which regression equations were obtained.

^cp-Value for significance of simple linear (p_{lin.}) and quadratic (p_{quad.}) parameter estimates tested by a t-test.

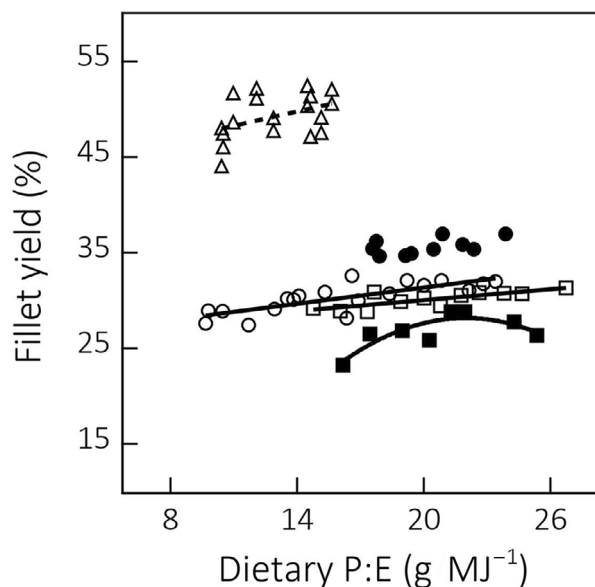


FIGURE 7 Relationship between dietary protein-to-energy ratio (P:E) and Nile tilapia fillet yield. Solid and dotted lines represent simple linear and quadratic relationships for which parameter estimates significantly differed from zero at $p < 0.05$ and $p < 0.10$, respectively. Data were derived from five independent studies^{6,22,30–32} for which the following symbols were used: ○³² ($n = 19$); ●³¹ ($n = 10$); □³⁰ ($n = 12$); ■⁶ ($n = 8$); △²² ($n = 18$)

4 | DISCUSSION

Fish growth depends on dietary nutrient availability, of which energy and protein (*i.e.*, amino acids) are of prime importance. In fish feed formulation, the balance between dietary protein and energy, expressed as the dietary P:E, is often regarded as a parameter that can be optimised. This assumption relies on the hypothesis that a balanced supply of non-protein energy substrates (*i.e.*, lipids and carbohydrates) can spare ingested amino acids from oxidative catabolism, thus

maximising their use for body protein synthesis. In the present review, and meta-analysis, we investigated if nutrient partitioning and growth can be optimised via adjustments in the P:E of Nile tilapia feeds. Our analyses showed that changes in dietary P:E have linear effects on nutrient partitioning in Nile tilapia, and that protein gain is often simultaneously limited by both protein and energy intake.

4.1 | The optimal dietary P:E is context dependent

From a fish farming perspective, the concept of an optimal dietary P:E implies that one or several production variable(s) have an optimal value at a constant dietary P:E, above or below which no further improvement can be achieved. This should result in a non-linear relationship between dietary P:E and the variable(s) of interest. However, regression analyses are not always applied to estimate the optimal dietary P:E of farmed fish species. In some published studies, the optimal P:E was derived from post hoc pairwise comparison of means, whereby one treatment is appointed as the optimal value. For example, the authors of a study on 17–60 g Nile tilapia fed to apparent satiation stated that the optimal dietary P:E was 19 g MJ⁻¹.³³ Post hoc analysis showed that body mass gain was higher in fish fed this level of dietary P:E than at any other level tested in the experiment (ranging from 11 to 21 g MJ⁻¹). However, reanalysis of the published data³³ shows that there are no significant linear or quadratic relationships between dietary P:E and body mass gain.

Our meta-analysis indicated that the most prominent effects of changes in dietary P:E were on protein partitioning. These effects were linear, and as such, did not reach plateau (*i.e.*, optimal) values. With 67% of explained variability, the clearest effect of increasing dietary P:E was a linear decrease in protein retention efficiency (Table 3). The second clearest effect of increasing dietary P:E was a linear increase in the protein content of body mass gain, of which 41% of the variability was explained by the regression model (Table 4). The marginal water and protein gains (*i.e.*, slopes of 4.4 and 1.2 g,

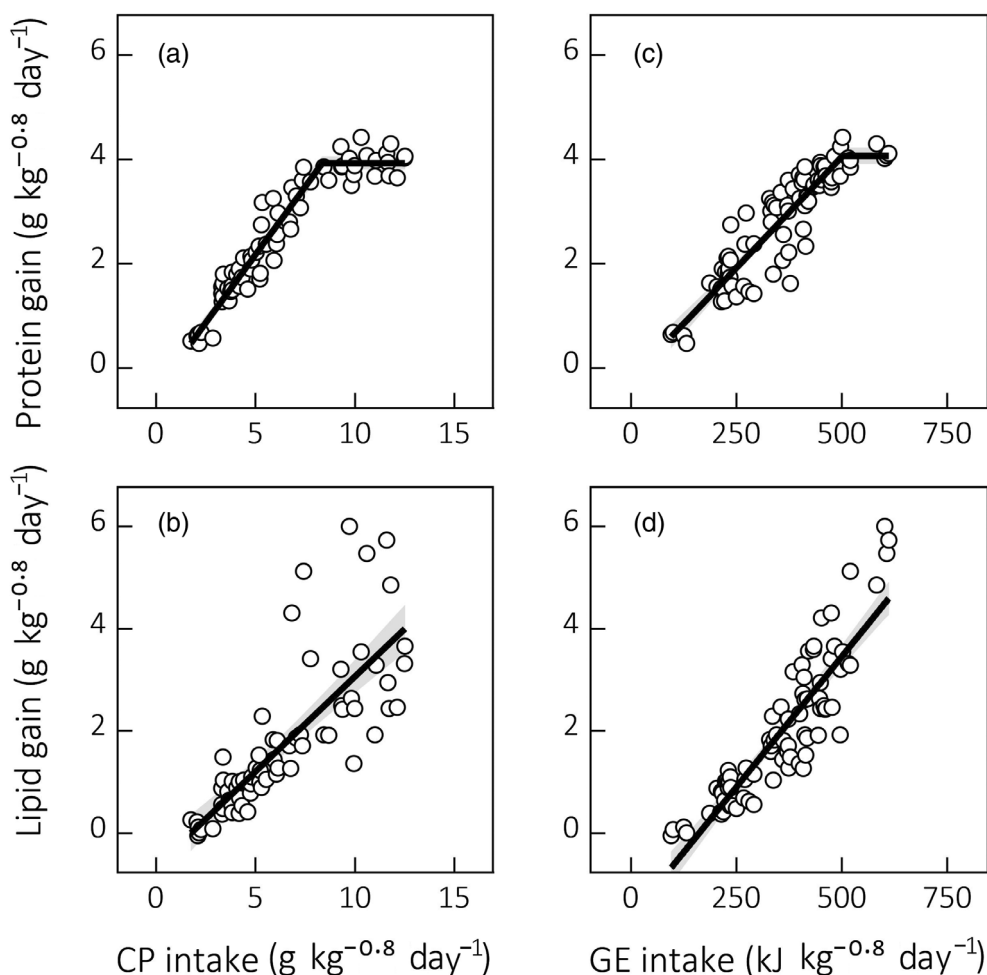


FIGURE 8 Effects of (a,b) daily intake of crude protein (CP, $n = 69$) and (c,d) gross energy (GE, $n = 82$) on (a,c) daily protein and (b,d) lipid gain in Nile tilapia. Solid black lines represent selected linear–plateau and simple linear models for which parameter estimates significantly differed from zero ($p < 0.05$). Grey areas represent the 95% confidence interval of model predictions for models with normally distributed residuals

TABLE 7 Linear and linear–plateau relationships between daily crude protein intake (CPI, in $\text{g kg}^{-0.8} \text{ day}^{-1}$) and daily protein and lipid gain in Nile tilapia ($n = 69$)

Dependent variable (Y, in $\text{g kg}^{-0.8} \text{ day}^{-1}$)	Model	First segment equation	CPI at inflection (in $\text{g kg}^{-0.8} \text{ day}^{-1}$)	Plateau (in $\text{g kg}^{-0.8} \text{ day}^{-1}$)	AICc ^a	Eq. number
Protein gain	Linear	$Y = 0.30 \text{ (SE = 0.115)} + 0.35 \text{ (SE = 0.016)} \times \text{CPI}$			87	(34)
	Linear–plateau	$Y = -0.44 \text{ (SE = 0.250)} + 0.52 \text{ (SE = 0.025)} \times \text{CPI}$	8.4 (SE = 0.23)	Y = 3.9 (SE = 0.06)	28	(35)
Lipid gain	Linear	$Y = -0.65 \text{ (SE = 0.229)} + 0.37 \text{ (SE = 0.033)} \times \text{CPI}$			181	
	Linear–plateau	$Y = -0.80 \text{ (SE = 0.432)} + 0.41 \text{ (SE = 0.037)} \times \text{CPI}$	10.6 (SE = 0.01)	Y = 3.5 (SE = 0.19)	183	(37)

SE = standard error.

^aThe lower the value of the corrected Akaike Information Criterion (AICc) is, the better the model fits the data.

respectively) estimated for every dietary P:E increase of 1 g MJ^{-1} are in line with the composition of Nile tilapia fillets, which contain 4 g of water for every 1 g of protein.⁵ Thus, it can be assumed that these linear increases in water and protein relative gains, reflect increases in muscle gain. This assumption is supported by an increase in fillet yield

with increasing dietary P:E, as illustrated in Figure 7. Protein retention efficiency and fillet yield respond in opposite ways to changes in dietary P:E. In other words, balancing dietary P:E for Nile tilapia feeds primarily involves a trade-off between protein retention efficiency and fillet gain.

TABLE 8 Linear and linear-plateau relationships between daily gross energy intake (GEI, in $\text{kJ kg}^{-0.8} \text{day}^{-1}$) and daily protein and lipid gain in Nile tilapia ($n = 82$)

Dependent variable (Y, in $\text{g kg}^{-0.8} \text{day}^{-1}$)	Model	First segment equation	GEI at inflection (in $\text{kJ kg}^{-0.8} \text{day}^{-1}$)	Plateau (in $\text{kJ kg}^{-0.8} \text{day}^{-1}$)	Eq. AICc ^a number
Protein gain	Linear	$Y = -0.01$ (SE = 0.150) + 0.008 (SE = 0.0004) \times GEI			116 (38)
	Linear-plateau	$Y = -0.21$ (SE = 0.220) + 0.009 (SE = 0.0004) \times GEI	502 (SE = 0.4)	$Y = 4.1$ (SE = 0.08)	96 (39)
Lipid gain	Linear	$Y = -1.64$ (SE = 0.207) + 0.010 (SE = 0.0006) \times GEI			170 (40)

SE = standard error.

^aThe lower the value of the corrected Akaike Information Criterion (AICc) is, the better the model fits the data.

An alternative way of determining the optimal dietary P:E is via the factorial models approach.⁴³ In this approach, the optimal dietary P:E is calculated, based on theoretical growth capacity and corresponding protein and energy requirements. Daily maintenance requirements, gain capacity, and utilisation efficiencies are empirically estimated for both protein and energy. These estimates are then used to calculate the minimal amount of dietary protein and energy needed to support growth and thus the optimal dietary P:E. This approach was applied to estimate optimal dietary P:E for Nile tilapia of 10 to 1000 g.⁶⁶ The authors of this study calculated optimal dietary P:E declining from 33 to 21 g MJ⁻¹ for fish of 10–1000 g. In the factorial approach, protein retention efficiency is fixed regardless of dietary P:E. In their calculations of the factorial approach, the authors of the tilapia study used a fixed protein retention value of 52%.⁶⁶ The current meta-analysis demonstrates an alternative view, whereby protein retention efficiency decreases linearly with increasing dietary P:E (Figure 4), within a 6–250 g range of body mass. Furthermore, the factorial model approach often uses fixed values for body protein and energy content. The authors of the study applying the factorial approach to Nile tilapia, used a fixed value of 159 g kg⁻¹ for body protein content and calculated energy content as a function of body mass, based on empirical observations.⁶⁶ The linear relationships between dietary P:E and the protein, lipid and energy content of body mass gain depicted in Figure 5 show that this assumption is not valid. Optimal dietary P:E values calculated via the factorial approach are dependent on the composition of the diets used to estimate maintenance requirements, protein and energy utilisation efficiencies, and body composition. For practical applications, studies on larger Nile tilapia will be needed to determine if our findings hold true over the whole commercial growth period. If so, accounting for these effects will require new growth modelling tools, which may give more flexibility in terms of diet composition, than the factorial approach does.

Balancing dietary P:E is also likely to depend on its effect on lipid gain. Higher lipid deposition, induced by feeding low dietary P:E, mainly occurs along the fish viscera. In a Nile tilapia in vivo trial, decreasing dietary P:E from 28.9 to 17.3 g MJ⁻¹, resulted in an increase in visceral lipid content from 162 to 479 g kg⁻¹. This caused

the proportion of body lipid stored along the viscera to increase from 19.0% to 49.3%, while whole body lipid content increased from 95 to 202 g kg⁻¹ (as is).⁶ This increase in visceral lipid gain in fish fed low-dietary P:E explains the reduction in fillet yield with decreasing dietary P:E. In addition, the authors reported that reducing dietary P:E caused an increase in hepatic lipid content from 15 to 57 g kg⁻¹.⁶ Hepatic steatosis (*i.e.*, abnormal lipid accumulation) is commonly seen in farmed fish and may in some cases represent a pathological condition.^{67,68} Again, balancing dietary P:E involves some trade-offs between dietary protein utilisation efficiency, fillet yield, and possibly fish health.

The practical implication of these findings is that the optimal dietary P:E is always context dependent. On the one hand, low-dietary P:E may be advantageous in situations in which high protein retention efficiency is required (*e.g.*, scarcity of affordable protein-rich ingredients, or compliance with restrictions on nitrogen emissions). On the other hand, farming systems in which fillet yield is remunerated may benefit from feeding higher dietary P:E.³⁴ Thus, adjusting dietary P:E towards an optimum requires context-specific cost-benefit analyses that go beyond the changes in nutrient partitioning induced.

4.2 | Interplay with other dietary factors

Besides the most evident effects of changes in dietary P:E on protein partitioning, our meta-analysis also showed effects on other response variables, although with a lower predictive value. These findings illustrate the limits of dietary P:E as a predictor of fish growth performance and diet utilisation. For example, our results suggest that dietary P:E is not a good predictor either of dietary lipid partitioning towards lipid gain (Figure 4), or of the lipid content of body mass gain (Figure 5), of which only 8% and 4% of the variability were explained by changes in dietary P:E, respectively (Tables 3 and 4). Unlike body protein, which can only be synthesised from ingested or recycled amino acids, body lipids can be synthesised from fatty acids, as well as sugars and amino acids (*de novo* fatty acid synthesis). Similarly, all absorbed fatty acids, sugars and amino acids may be oxidised to cover

the energy costs of maintenance and growth. However, the energy content and energy utilisation efficiencies of digested lipids, carbohydrates and proteins differ.⁶⁹ A meta-analysis of Nile tilapia energy retention data showed that 0.91 kJ of energy are retained by Nile tilapia for every kJ ingested in the form of digestible lipid, whereas 1 kJ of carbohydrate intake leads to only 0.66 kJ of energy retention.⁶⁹ Therefore, the extent to which ingested lipids, carbohydrates and proteins are directed towards energy production or lipid gain depends on their relative proportions, and especially on the composition of non-protein energy (*i.e.*, lipid-to-carbohydrate ratio), a distinction which is not made when evaluating a feed on the sole basis of its dietary P:E. Differences in the composition of non-protein energy may thus explain part of the variability in lipid gain, which differences in dietary P:E cannot. This probably contributed to the absence of a clear relationship between dietary P:E and feed conversion ratio in our meta-analysis. Our analysis also indicated that dietary P:E is not a good predictor of absolute growth indicators, such as daily body mass gain. This is due to the fact that absolute growth depends on many factors other than dietary P:E, of which feed intake is the one having the largest impact. Variability in feed intake, especially when fish are fed to apparent satiation, may be a main reason for variability in and/or absence of the relationship between growth and dietary P:E between studies. For example, there were neither linear nor quadratic relationship between dietary P:E and growth in two studies on 16–60³³ and 7–40⁷⁰ g Nile tilapia fed to apparent satiation. On the contrary, a recent study on 4–60 g Nile tilapia fed to apparent satiation⁴⁹ did show a linear increase in growth with decreasing dietary P:E.

The magnitude of the effects of changes in dietary P:E on nutrient partitioning is likely to depend on another factor: the dietary amino acid profile. Fish do not have a requirement for protein, but rather for amino acids, of which the profile determines protein utilisation.⁷¹ The closer the dietary amino acid profile is to the ideal, the more likely it is that changes in dietary P:E will affect nutrient partitioning, and especially protein retention efficiency. The dietary amino acid profile of the experimental diets included in our meta-analysis were not reported by the authors. However, most of them contained one or several purified protein ingredients such as casein or soy protein isolate. Such ingredients are usually not relevant for the formulation of practical Nile tilapia feeds. However, they are often used in experimental diets for their high nutrients' content and digestibility. In most diets included in our meta-analysis, the combination of such ingredients with crystalline amino acids supplementation prevented the risk of a deficiency in one or more essential amino acid(s).

4.3 | Protein deposition in Nile tilapia: energy dependence

In its first approach, our meta-analysis indicated that changes in dietary P:E affect protein partitioning in a linear way. The linear increase in protein retention efficiency, with decreasing dietary P:E, illustrates the protein-sparing effect of rising dietary lipid and/or carbohydrate content (*i.e.*, non-protein energy). This phenomenon has already been

reported for *Oreochromis niloticus* × *O. aureus* hybrid tilapia,⁷² Nile tilapia³⁵ and other fish species.^{12,73,74} The data analysed here suggest that there is no limit to the increase in protein retention efficiency, when reducing dietary P:E from 30 to 9 g MJ⁻¹ in Nile tilapia feeds. This relationship was shown to be independent of feeding level in two subsequent studies in which the protein retention efficiency of Nile tilapia increased linearly with decreasing dietary P:E (16–25 g MJ⁻¹) under both restricted³⁵ and apparent satiation⁴⁹ feeding conditions. Altogether, these results suggest that energy availability is always a limiting factor in protein deposition in Nile tilapia. In our meta-analysis, protein gain increased linearly over most of the range of gross energy intake (Figure 8c). However, the increase in gross energy intake cannot be distinguished from a concomitant increase in protein intake, in the present meta-analysis. It is therefore not possible to distinguish between the effects of protein or energy intake on protein gain, as shown in Figure 1.

4.4 | Protein deposition in Nile tilapia: maximal capacity

The second approach of our meta-analysis revealed a two-phased response of protein gain to increasing protein intake (Figure 8a). This finding suggests that protein deposition is dependent on protein intake, until the latter reaches 8.4 g kg^{-0.8} day⁻¹. Similar non-linear responses of protein gain to increasing protein intake were reported for Mozambique tilapia (*Oreochromis mossambicus*),⁷⁵ mixed-sex Nile tilapia,²⁴ red tilapia (*Oreochromis mossambicus* × *Oreochromis homorum*),⁵¹ *Oreochromis niloticus* × *Oreochromis mossambicus* hybrids⁵¹ and all-male Nile tilapia.⁵¹ Yet, in these three studies, as in most of the studies included in our meta-analysis, increased protein intake was achieved by gradually exchanging dietary protein for lipids and/or carbohydrates, thereby often leading to simultaneous changes in protein and energy intake. Thus, the separate effects of energy and protein availability on protein gain cannot be distinguished, neither in previous tilapia studies,^{24,51,75} nor in the present meta-analysis. The levelling-off in protein deposition at a level close to 4 g kg^{-0.8} day⁻¹, as reported here, may correspond either to the effect of a limiting energy intake, or to a physiological limit to daily protein gain. The second hypothesis seems more plausible. First, if the plateau was caused by a shortage of energy, lipid gain would likely level-off too. Instead, lipid gain increased linearly with crude protein intake, implying that energy was abundant. This increase resulted from either dietary lipid deposition or *de novo* fatty acid synthesis. The latter can lead to high fat deposition in Nile tilapia, resulting in body fat gain exceeding dietary fat intake.⁵⁴ Moreover, lipid gain showed increased variability at high protein intake levels, with daily gain ranging from 1.4 up to 6.0 g kg^{-0.8} day⁻¹ for those diets where protein gain levelled-off. The observation that the variability in lipid, but not protein, gain increased with crude protein intake, suggests that lipid gain was acting as an 'adjustment variable' in cases where energy was either limiting or in excess. Second, protein deposition also seemed to level-off at high levels of gross energy intake. Although the plateau included a limited number of cases ($n = 8$), compared with that found in response to daily protein intake ($n = 20$), the co-occurrence of a plateau,

at high protein and energy intake, suggests that protein deposition was limited by a factor other than protein and energy availability, in at least 8 out of the 91 cases in our meta-analysis.

Dissolved oxygen availability and water temperature influence fish bioenergetics¹² and are, thus, potent modulators of feed intake and growth in Nile tilapia.^{76,77} Reported dissolved oxygen concentrations for the eight cases where protein gain levelled-off in the absence of a protein and energy limitation, ranged from 6.0 to 7.2 mg L⁻¹. These levels are in line with recent recommendations³⁶ and it is therefore unlikely that protein deposition was limited by oxygen availability in these cases. The same applies to the water temperature ranges applied to these eight cases (27.5–28°C), which were close to the 28–30°C recommended for Nile tilapia.³⁶ The same oxygen and temperature ranges also applied to the 20 observations where protein gain levelled-off at high crude protein intake. Thus, at the highest end of the daily protein and energy intake ranges, protein gain was neither limited by protein (20 cases), energy intake (8 cases), dissolved oxygen availability or water temperature. Instead, the plateau observed in daily protein gain may reflect the presence of a genetically determined maximal potential for protein gain, similar to that coined PD_{max} by pig nutritionists.¹⁶ The existence of a PD_{max} has not been established yet for fish, although some studies indicated that the concept might apply to rainbow trout (*Oncorhynchus mykiss*)⁷⁸ and Australian snapper (*Pagrus auratus*).⁷⁹ In pigs, PD_{max} is often assumed to be reachable in a limited range of body mass only (e.g., 20–80 kg), due to constraints in voluntary feed intake in smaller and larger animals.⁸⁰ Here, the eight cases where protein gain levelled-off, in the apparent absence of a protein or energy limitation, were obtained in fish with a body mass ranging from 6 to 60 g,⁴⁹ 12 to 145 g⁵¹ and 40 to 240 g.⁵⁴ Aside from body mass, the extent of genetic selection for growth may determine the presence or absence of a PD_{max}. Selective breeding for growth performance is likely to result in selected tilapia populations with a higher feed intake capacity and/or a higher PD_{max}, when compared with their non-selected counterparts, thereby making the PD_{max} more (or less, respectively) evident to observe.

4.5 | Implications for future research

There does not seem to be a single optimal balance between dietary protein and energy in Nile tilapia. This is regardless of whether protein and energy are expressed relative to each other (dietary P:E) or evaluated separately (absolute intakes). The high dietary protein requirements of most fish species have often been explained by a lack of endogenous regulation of amino acid catabolism.⁸¹ The fact that fish excrete excess nitrogen, the waste product of amino acid catabolism, in the form of ammonia (i.e., at lower energetic costs) means that amino acids are more efficient energy substrates to fish than they are to mammals.⁸² Under this hypothesis, the preferential use of a large proportion of ingested amino acids as an energy substrate likely leads to low protein retention efficiency in fish, regardless of energy intake levels. This is possibly even truer for carnivorous fish species, for whom protein makes up an even

larger part of the natural diet than for Nile tilapia. Optimal dietary P:E and distinct protein- and energy-dependent phases have been observed in several terrestrial farm species, including lambs,¹³ pigs¹⁵ and broiler chickens.⁸³ However, distinct protein- and energy-dependent phases are absent in pre-ruminant calves,⁸⁴ in which this absence is believed to reflect low absolute (<50%) and marginal (<40%) protein utilisation efficiencies.⁸⁵ Gross protein retention efficiency ranged from 20% to 60% in our data set (Figure 4), while simple linear and linear-plateau regression showed marginal protein retention efficiency of 35% and 52%, respectively (Table 7). Here, faecal protein losses were not accounted for, and therefore these estimates probably underestimated both net and marginal protein utilisation efficiencies. Thus, protein utilisation efficiency does not seem to be as low in Nile tilapia as it is in pre-ruminant calves. In other words, the apparent absence of distinct protein- and energy-dependent phases in protein deposition cannot be solely explained by the extent to which amino acids are diverted from body protein synthesis (i.e., catabolised). This absence indicates that protein gain may be simultaneously limited by protein and energy availability in Nile tilapia, a hypothesis which, to our knowledge, has not been tested yet. Finally, most data used in our meta-analysis were obtained from fish weighing less than 200 g. Nutrient balance data for large Nile tilapia are still not available for testing concepts like the PD_{max} and the absence of distinct protein- and energy-dependent growth phases across body size classes. Obtaining such data would increase the relevance of this kind of meta-analytical work since most of the feed consumed on tilapia farms is given to fish heavier than those used in laboratory studies until now.

5 | CONCLUSION

Our meta-analysis demonstrated that there is no physiological optimal P:E for Nile tilapia feeds. Changes in dietary P:E primarily affect nutrient partitioning, especially that of protein. These effects are linear and, as such, do not indicate an optimum. Through its effects on protein partitioning, dietary P:E also influences fillet yield and to some extent lipid gain. The benefits of some of these effects being contradictory, determining an optimal dietary P:E is always context dependent. Fitting linear-plateau models to protein and lipid gain data did not provide strong evidence of distinct protein- and energy-dependent phases in protein deposition. This finding also contradicts the existence of a single optimal dietary P:E, above and below which growth would be limited by energy and protein availability, respectively. Our findings also indicate that protein deposition may be limited by factors other than protein and energy intake, such as a maximal genetic potential for daily protein gain.

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AUTHOR CONTRIBUTIONS

Gauthier D.P. Konnert: Conceptualisation, Investigation, Formal analysis, Visualisation, Writing – Original draft, Review & Editing; **Walter J.J. Gerrits:** Conceptualization; Writing – review and editing. **Sander W.S. Gussekloo:** Conceptualization; Writing – review and editing. **J. W. Schrama:** Funding acquisition, Project administration, Conceptualisation, Writing – Original draft, Review & Editing.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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APPENDIX A: LIST OF THE 51 PRE-SELECTED STUDIES

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APPENDIX B: NUMBER OF CASES (i.e., DIETARY TREATMENTS) PER STUDY INCLUDED IN EACH DATA SUBSET USED IN THE META-ANALYSIS

Study reference number ^a	Number of cases			
	Subset 1	Subset 2	Subset 3	Subset 4
51	10	10	10	10
33	9	9	9	9
49	8	8	8	8
53	7	7	7	7
50	7	7	7	7
54	4	4	4	4
52	3	3	3	3
35	16	16		16
47	3	3	3	
86			6	6
48	5	5		
46			4	4
87			4	4
88			4	4
32	19			
55	11			
29 (Exp. 2)	10			
29 (Exp. 1)	7			
28	6			
60	6			
56	4			
58	4			
57	2			
59	2			
Total	143	72	69	83

^aNumbers correspond to those used in the list of references of the article.