Dietary nutrient composition affects digestible energy utilisation for growth: a study on Nile tilapia (*Oreochromis niloticus*) and a literature comparison across fish species

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Abstract

The effect of the type of non-protein energy (NPE) on energy utilisation in Nile tilapia was studied, focusing on digestible energy utilisation for growth (k_{gDE}). Furthermore, literature data on k_{gDE} across fish species were analysed in order to evaluate the effect of dietary macronutrient composition. A total of twelve groups of fish were assigned in a 2×2 factorial design: two diets ('fat' $v.$ 'starch') and two feeding levels ('low' $v.$ 'high'). In the 'fat'-diet, 125 g fish oil and in the 'starch'-diet 300 g maize starch were added to 875 g of an identical basal mixture. Fish were fed restrictively one of two ration levels ('low' or 'high') for estimating k_{gDE}. Nutrient digestibility, N and energy balances were measured. For estimating k_{gDE}, data of the present study were combined with previous data of Nile tilapia fed similar diets to satiation. The type of NPE affected k_{gDE} (0·561 and 0·663 with the 'starch' and 'fat'-diets, respectively; $P < 0·001$). Across fish species, literature values of k_{gDE} range from 0·31 to 0·82. Variability in k_{gDE} was related to dietary macronutrient composition, the trophic level of the fish species and the composition of growth (fat:protein gain ratio). The across-species comparison suggested that the relationships of k_{gDE} with trophic level and with growth composition were predominantly induced by dietary macronutrient composition. Reported k_{gDE} values increased linearly with increasing dietary fat content and decreasing dietary carbohydrate content. In contrast, k_{gDE} related curvilinearly to dietary crude protein content. In conclusion, energy utilisation for growth is influenced by dietary macronutrient composition.

Key words: Nile tilapia: Digestible energy: Dietary nutrient composition: Energy metabolism

Fish feeds will have to further diversify ingredient composition due to the limited availability of wild fishery-derived fishmeal and fish oil as dietary protein and lipid sources[1]. This diversification is already reflected by the substantial amount of ingredients from oilseeds, pulses and cereals in fish diets[2–5], which increases the variability in dietary nutrient composition. Inclusion of plant ingredients as a protein source inevitably increases the dietary carbohydrate content. Digestible carbohydrates (i.e. starch) constitute a partial alternative for the digestible energy (DE) supplied by fish oil or vegetable oils in diets, especially for species such as tilapia.

An adequate formulation of animal feed in terms of energy supply requires information on (1) nutrient digestibility of ingredients, (2) energy requirements for maintenance and (3) utilisation efficiency of DE or metabolisable energy (ME) for growth (respectively, k_{gDE} and k_{gME}). Most energy evaluation systems for farm animals, which have a net energy approach, take into account dietary macronutrient composition-induced differences in energy utilisation efficiency (k_{gDE} or k_{gME}) as seen in pigs[6,7]. For instance, in the net energy evaluation system for the Dutch pig, the utilisation efficiency of DE for growth is different between digestible protein, digestible fat and digestible starch, being, respectively, 0·46, 0·92 and 0·77[8]. In general, energy evaluation in fish-feed formulation is based on the DE of feeds/ingredients and thus does not consider differences in k_{gDE} induced by dietary macronutrient composition.

In fish, energy utilisation efficiency for growth has been estimated on either a DE (k_{gDE}) or ME (k_{gME}) basis and mostly using diets with relatively high inclusion levels of fishmeal and fish oil, e.g. rainbow trout (*Oncorhynchus mykiss*)[9,10], Atlantic cod (*Gadus morhua*)[11], African catfish (*Clarias gariepinus*)[12], European eel (*Anguilla anguilla*)[13], white grouper (*Epinephelus aeneus*)[14,15], gilthead sea bream (*Sparus aurata*)[14], European sea bass (*Dicentrarchus labrax*)[14,16]. Between studies and/or fish species, a large variability in k_{gDE} (and/or k_{gME}) has been present, with values ranging between 0·60 and 0·80. So far, little attention has been paid to fish nutrition research on the possible causes of this large variability between fish species as well as within a given species between studies. This variation in k_{gDE} (and/or k_{gME}) between studies and fish species can be due to differences in the composition of somatic growth, because the energetic efficiency of protein retention (k_p) in fish is lower than that of fat deposition (k_f)[14,17,18]. Moreover, the between-study variation in k_{gDE} can also be induced by differences in dietary nutrient composition as demonstrated, for example, in grass carp (*Ctenopharyngodon idella*)[19], rainbow trout (*O. mykiss*)[18,20] and European eel (*A. anguilla*)[13]. However, in these studies, dietary composition varied in crude protein, fat as well as starch (i.e. carbohydrate) content. Information on a one-by-one comparison between dietary fat and starch (i.e. the type of non-protein energy (NPE) fraction) on k_{gDE} (and k_{gME}) in fish is missing.

The present paper examines the hypothesis that a major part of the variability in k_{gDE} (and k_{gME}) between studies/fish species stems from differences in macronutrient composition between the experimental diets. First, a feeding trial with Nile tilapia (*Oreochromis niloticus*) assessed the effect of the NPE source (i.e. fat *v.* starch) on energy utilisation (k_{gDE} and k_{gME}). Nile tilapia was chosen here because of its position at a low trophic level, and thereby assumed to be able to digest and metabolise substantial amounts of starch in comparison with fish at higher trophic levels with more carnivorous feeding habits. Second, energy utilisation values from the literature were analysed in an attempt to explain the large variability in k_{gDE} across studies/fish species with a specific focus on (1) the proximate composition of the experimental diets used for estimating k_{gDE}, (2) the trophic level of the fish species and (3) the composition of growth (i.e. the ratio of fat:protein gain).

Materials and methods

Fish and housing

Male Nile tilapia of the Swansea Silver GMT (Genetically Male Tilapia) strain were obtained from a commercial breeder (Til-Aqua International, Velden, The Netherlands). The experiment was conducted in the fish energy metabolism unit of the Aquaculture and Fisheries group of Wageningen University. This unit consists of twelve 200-litre aquaria ($87 \times 58 \times 46$ cm), all connected to the same recirculation system (comprising a common water reservoir, a drum filter for solids removal, a trickling filter for gas exchange and nitrification of NH_4^+ and an oxygenating reactor). At the start of the 6-week experimental period, thirty-four fish were stocked per aquarium. Fish were randomly assigned to aquaria and had a mean body weight (BW) of 75·2 g. Initial stocking density was 12·8 kg/m³.

Water flow of each aquarium was kept constant at 7 litres/min (expect for the first week, when water flow was 6 litres/min). The oxygen concentration of the inlet water was increased until nine parts per million by the addition of pure oxygen to ensure that the dissolved oxygen concentration inside the tanks (i.e. outlet water) did not drop below four parts per million. Water quality was kept within the optimal range for tilapia and was measured weekly. Mean water pH, temperature, conductivity, NH_4^+-N, NO_2^--N and NO_3^--N were 7·0 (SD 0·2), 27·7 (SD 0·2)°C, 3·31 (SD 0·17) mS/cm, 0·0 (SD 0·0) mg/l, 0·2 (SD 0·1) mg/l and 168 (SD 45) mg/l, respectively. Renewal of water in the system was minimal during the whole experimental period. Consequently, NO_3^--N concentration increased from 100 to 225 mg/l during the experiment. A 12 h light–12 h dark photoperiod was maintained with daybreak set at 07.00 hours.

The experiment was approved by the Ethical Committee judging Animal Experiments of Wageningen University, The Netherlands, and carried out according to the Dutch law on animal experiments.

Diets and feeding

Triplicate aquaria were randomly assigned to one of four experimental treatments, which were arranged in a 2×2 factorial design: two diets ('fat' *v.* 'starch') and two feeding levels ('low' *v.* 'high'). The experimental diets were similar in composition regarding basal ingredients, but differed in the amount of gross energy originating from either fat or starch (Table 1) by exchanging fish oil and gelatinised maize starch. In the 'fat'-diet, 125 g fish oil was added to 875 g of a basal ingredient mixture, whereas in the 'starch'-diet, 300 g maize starch was added to 875 g of a basal ingredient mixture. The amount of gross energy in 125 g fish oil is approximately equal to that in 300 g gelatinised maize starch. Consequently, the ratio of basal ingredients (e.g. premix) and crude protein content to gross energy was similar between the two diets (Table 1). Based on the analysed dietary crude fat and total carbohydrate content (NFE; Table 1), 68 and 23 % of the dietary NPE content originated from crude fat and 32 and 77 % from NFE in the 'fat' and 'starch' diets, respectively. Digestibility was measured by using acid-insoluble ash as an inert marker. Diamol (Diamol GM; Franz Bertram, Hamburg, Germany) was added to both diets in order to increase the marker content of diets and collected faeces. The diets were produced by Research Diet Services (Wijk bij Duurstede, The Netherlands). The ingredients, excluding the oils, were mixed and thereafter hammer-milled through a 1 mm screen. The diets were processed by extrusion using a Clextral BC45 laboratory scale twin-screw extruder (Clextral, Firminy, France) with a 3 mm die, resulting in a sinking pellet of about 3 mm. In the 'starch' diet, all oils and in the 'fat' diet 2·5 % of the 13·5 % oils were added to the mixture before

Table 1. Ingredient (%) and analysed chemical composition (g/kg on a DM basis) of the experimental diets

	Diets	
	Fat	Starch
Test ingredients		
Fish oil*	12·50	–
Gelatinised maize starch†	–	25·53
Basal ingredients		
Soyabean meal	30·00	25·53
Fishmeal‡	25·00	21·27
Wheat gluten	15·00	12·77
Wheat bran	9·50	8·09
Soya oil	1·00	0·85
Pellet binder (Durabond)	2·00	1·70
Diamol§	2·00	1·70
$CaCO_3$	0·50	0·43
$CaPO_4$	1·50	1·28
Premix‖	1·00	0·85
Chemical composition		
DM	967	946
Crude protein	518	432
Crude fat	187	56
Ash	98	83
NFE	197	429
Starch and sugars	36	279
Gross energy (kJ/g DM)	22·8	19·7
Crude protein/gross energy (mg/kJ)	22·7	22·0
Digestible protein/digestible energy (mg/kJ)	24·0	23·1

NFE, nitrogen-free extract (total dietary carbohydrate content), calculated as DM − crude protein − crude fat − ash content.
* Triple Nine Fish oil, Esbjerg, Denmark.
† Merigel 100; Tate & Lyle, Amylum Europe NV (Aalst, Belgium).
‡ Fishmeal LT (90 % blue whiting and 10 % sprat; protein content 72 %); Triple Nine Fish protein.
§ Diamol GM; Franz Bertram, Hamburg, Germany.
‖ Mineral and vitamin composition of premix identical to Tran-Duy et al.[21].

extrusion. Following extrusion, the pellets were dried in a tray drier at 70°C for 3 h and thereafter cooled to ambient temperature. After cooling, the 'fat' diet pellets were coated and the remaining part of the oils and all diets were stored at 4°C. Before feeding, pellets were sieved to remove dust and small particles.

Fish were fed restrictively during the experiment. In order to estimate k_{gDE} and k_{gME} and the energy requirements for maintenance (DE_m and ME_m), two feeding levels were applied: the 'low' level aimed at about 30 % and the 'high' level at about 80 % of the 'satiation' intake. Tran-Duy et al.[21] found an apparent satiation feed intake of $16\cdot0$ g/kg^{08} BW per d in 50–160 g Nile tilapia fed comparable diets. Therefore, for the 'starch'-diet, feed ration was set at $6\cdot0$ and $13\cdot0$ g/kg^{08} BW per d at the 'low' and 'high' feeding levels, respectively. The created contrast between the 'fat' and 'starch' diets resulted in a difference in the nutrient (e.g. protein, energy) concentration between the diets (Table 1). In order to provide the same amount of energy and protein, fish were fed the same amount of basal ingredients at both diets. Therefore, for the 'fat'-diet, the amount of feed ration was set at $5\cdot1$ and $11\cdot1$ g/kg^{08} BW per d for the 'low' and 'high' feeding levels, respectively.

Fish were hand fed once per day. The feeding period started at exactly 13.00 hours and fish were given 1 h to consume their daily ration. During this feeding period, feed was gradually given (at 2–5 min intervals) and was adjusted to the demand of the fish, in order to minimise feed spillage. The daily feeding ratio per aquarium was calculated based on the mean initial fish weight, the feeding level of the treatment (in g/kg^{08} BW per d) and the expected growth of the fish. The daily growth for the feed ration calculation was estimated from the expected feed:gain ratio (FGR). At the 'high' feeding level, the FGR was assumed to be 1·1 for the prediction of daily growth. By definition, the FGR is dependent on the feeding level, due to the dilution of the amount of feed used for maintenance with increasing feeding level. Since the 'low' feeding level was expected to be slightly above the maintenance requirement, a FGR of 4 was assumed for the prediction of daily growth.

Measurements of nitrogen and energy balances

At the start and end of the 42 d experimental period, individual fish weights were measured after anaesthetising fish with tricaine methane sulfonate ($0\cdot2$ g/l buffered with $0\cdot4$ g sodium bicarbonate/l; MS-222; Crescent Research Chemicals, Phoenix, AZ, USA). From weight measurements, mean initial (BW_0) and final BW (BW_{42}) and the CV of BW_{42} were calculated per tank. Growth rate per metabolic weight unit (in g/kg^{08} BW per d) was calculated per tank as $(BW_{42} - BW_0) \times 42/MBW_m$, with MBW_m being the mean metabolic BW during the experimental period (in kg^{08}). From the feed ration, uneaten feed and feed spillage, which were recorded daily, feed intake was calculated and expressed as g/kg^{08} BW per d using MBW_m. Feed spillage was recorded by counting the number of feed pellets trapped in the faeces collectors during the feeding period. The FGR was calculated as feed intake divided by growth (both in g/kg^{08} BW per d).

A representative sample of each diet was taken and stored at 4°C. Initial body composition was determined in eighteen fish and final body composition in eight randomly selected fish per tank. Fish were euthanised by an overdose of MS-222 ($0\cdot8$ g/l buffered with $1\cdot6$ g sodium bicarbonate/l) and stored at −20°C. Faeces were daily collected per aquarium during the last 4 weeks of the experiment, according to the procedure described by Amirkolaie et al.[22] using settling tanks. Daily faecal collection started about 15 min after the end of the feeding period. Before starting feeding, faeces were collected, stored (daily) at −20°C and pooled per aquarium over the experimental period. Throughout the daily faecal collection period, the bottle trapping faeces was continuously submerged in ice water, to prevent bacterial decay. Before chemical analysis, the sampled fish were cut into small pieces, homogenised by grinding in a mincing machine through a $4\cdot5$ mm-screen grinder two times and subsequently freeze-dried. The collected faeces were freeze-dried. The freeze-dried faeces and the sampled feed were ground using a 1 mm-screen grinder.

Chemical analyses were done in triplicate. DM and ash were determined gravimetrically; DM after drying at 103°C for 4, 4 and 24 h until constant weight, respectively, for feed, freeze-dried faeces and fish samples (ISO 6496, 1983); ash

after incineration at 550°C for 4 h (ISO 5984,1978). Acid-insoluble ash was measured by dissolving the residue obtained after ash determination in HCl (ISO 5985, 1981). CP (N × 6·25) was determined by the Kjeldahl method (ISO 5983, 1979). Fat was quantified after petroleum–diethyl ether extraction (ISO 6492, 1999). Before fat analysis, feed and faecal samples were hydrolysed by boiling for 1 h with 3 M-HCl. Energy content was measured by direct combustion in an adiabatic bomb calorimeter (IKA-C-7000; IKA-analysentechnik, Weitersheim, Germany). Starch (including free sugars) was analysed according to the method described by Goelema *et al.*[23]. For feed and faeces, NFE (nitrogen-free extract; i.e. starch + free sugars + NSP) was calculated as DM − CP − fat − ash. Apparent digestibility coefficients of nutrients were calculated for each aquarium as in Amirkolaie *et al.*[22] using acid-insoluble ash as an inert marker.

Energy and N balance parameters were calculated per aquarium and expressed as, respectively, kJ/kg^{08} BW per d and mg/kg^{08} BW per d. N balance calculations were as follows: N intake as the product of feed intake and dietary N content; digestible N intake as N intake times the N digestibility coefficient; retained N as the difference between the final and initial N body mass; branchial urinary N losses as digestible N intake minus retained N. Energy balance calculations were as follows: energy intake as the product of feed intake and dietary energy content; DE intake as energy intake times the energy digestibility coefficient; branchial urinary energy losses as branchial urinary N losses times the energy concentration of NH_3−N (24·9 kJ N/g$^{[24]}$, assuming that all N was excreted as NH_3−N); ME intake as DE minus branchial urinary energy losses; retained energy (RE) as the difference between the final and initial body energy quantities; heat production as ME minus RE.

Fat retention efficiency was calculated as fat deposited (in g/kg^{08} BW per d) divided by the digestible fat intake (in g/kg^{08} BW per d).

Statistical analysis

The data were analysed using the Statistical Analysis Systems statistical software package version 9.1 (SAS Institute, Cary, NC, USA). The mean value of variables such as BW, feed intake, FGR, body composition, digestibility coefficients, N balance and energy balance of the treatment groups were subjected to two-way ANOVA using the procedure general linear model followed by multiple comparison of means using Tukey's multiple range test. DE_m and k_{gDE} were derived from RE and DE intake values using linear regression analyses of the general linear model, and ME_m and k_{gME} were estimated from RE and ME intake using the data of the present study. To increase the power of the statistical analysis (i.e. the range of DE and ME intake), these estimates were also done combining the data of the present study and a previous study on Nile tilapia[21]. In that previous study[21], measurements were identical and the contrast in diets was similar (replacing 125 g fat by 300 g starch), except that fish were fed to satiation in that study. In all tests, the difference between the treatment groups was considered significant when $P < 0.05$.

From the literature, a database across fish species was created containing estimates of k_{gDE} and the proximate composition of the diets on a DM basis used in these studies (energy, CP, fat, ash and NFE). Between literature sources, large variability exists in the way of estimating k_{gDE} in the mode of expression of data on DE intake and RE (kJ/fish per d, kJ/kg BW per d, kJ/kg^{08} BW per d, kJ/kg^{082} BW per d, etc.). From the data in the original papers, k_{gDE} were estimated by the linear regression of DE intake on RE expressing data as kJ/kg^{08} BW per d. When the data on NFE (i.e. the total carbohydrate fraction) were not reported, this was calculated either as DM − CP − fat − ash or from the gross energy, CP and fat content using 23·7, 39·5 and 17·6 kJ/g as the energetic values for CP, fat and carbohydrates, respectively. To classify the fish species in the database, regarding natural feeding behaviour (herbivorous *v.* carnivorous), the trophic level reported in FishBase[25] was added to the database. Spearman's correlation coefficients were calculated between k_{gDE} and the different parameters (dietary proximate composition and trophic level). Additional regression analyses between k_{gDE} and these parameters were performed.

Results

Performance data are shown in Table 2. Averaged over treatments, 4·9 % of the rations were not eaten. This was caused by the presence of uneaten feed during the first 2 weeks of the experiment, especially at the 'starch' diet ($P < 0.05$) and at the 'high' feeding level ($P < 0.001$). As planned with the experimental design, the realised feed intake was affected by both diet ($P < 0.001$) and feeding level ($P < 0.001$). However, due to the higher level of uneaten feed at the 'starch' diet, the intended difference in feed intake (in g/kg^{08} BW per d) was slightly small between the 'starch' and 'fat' diets (Table 2). Consequently, both energy and N intakes were slightly different between the two diets ($P < 0.05$; Table 4). However, growth (in g/kg^{08} BW per d) was not different between the diets ($P > 0.1$), but increased with the feeding level ($P < 0.001$; Table 2). The FGR was affected by both dietary energy source and feeding level ($P < 0.001$) and tended to be affected by the interaction effect ($P = 0.061$). The highest FGR (1·16) was registered in fish fed the 'starch' diet at the 'low' feeding level.

Final body composition is shown in Table 3. The final protein content of fish was similar between the treatments ($P > 0.1$). Fat content was influenced by both dietary energy source and feeding level ($P < 0.01$) and tended to be affected by the interaction effect ($P < 0.10$). Fish fed the 'fat' diet at the 'high' feeding level had the highest fat content (Table 3). A similar pattern, as observed for the fat content, was present for final DM and energy contents.

Apparent digestibility coefficients of all nutrients were affected by both feeding levels ($P < 0.05$) and by the dietary energy source ($P < 0.01$) (Table 3). For all nutrients, digestibility was higher at the 'low' feeding level compared with the 'high' feeding level. Except for fat digestibility, the digestibility of all nutrients was higher in fish fed the 'starch' diet than in fish fed the 'fat' diet. The digestibility of fat was highest for

Table 2. Effect of dietary energy source ('starch' v. 'fat') and feeding level ('low' v. 'high') on feed intake and growth performance of Nile tilapia (*Oreochromis niloticus*)
(Mean values with their standard errors)

| | Diet | | | | | P | | |
| | Fat | | Starch | | | | | |
Feeding level	Low	High	Low	High	SEM*	D	FL	D×FL
Growth period (d)	42	42	42	42	–	–	–	–
Fish per tank (n)	34	34	34	34	–	–	–	–
Tanks (n)	3	3	3	3	–	–	–	–
Survival (%)	99	100	100	99	–	–	–	–
Initial BW (g)	75·6	75·6	74·5	74·9	0·73	0·246	0·859	0·770
Final BW (g)	103·4[a]	168·3[b]	101·0[a]	168·1[b]	2·68	0·638	<0·001	0·692
CV of final BW (%)	19·7	18·1	21·4	18·9	1·56	0·459	0·236	0·795
Feed intake (g/kg$^{0.8}$ BW per d)	4·5[a]	9·9[c]	5·1[b]	11·0[c]	0·16	0·001	<0·001	0·173
Growth (g/kg$^{0.8}$ BW per d)	4·6[a]	12·0[b]	4·4[a]	12·1[b]	0·22	0·904	<0·001	0·608
FGR	0·99[a]	0·83[b]	1·16[c]	0·91[a,b]	0·020	<0·001	<0·001	0·061

D, main effect of dietary non-protein energy source ('starch' v. 'fat'); FL, main effect of 'low' v. 'high'; D × FL, interaction effect between diet and feeding level; BW, body weight; FGR, feed:gain ratio.
[a,b,c] Mean values with unlike superscript letters within a row were significantly different (*P*<0·05).
* *n* 3 per experimental treatment group.

the 'fat' diet. The digestibility of DM, fat and carbohydrates (i.e. NFE) was influenced by the interaction effect of feed level and dietary energy source. For these nutrients, the difference in digestibility between the 'low' and 'high' feeding levels was higher at the 'fat' diet.

In line with the design of the experiment, all parameters of the N and energy balances were strongly affected by the feeding level (*P*<0·001; Table 4). Due to the higher amount of uneaten feed with the 'starch' diet, the daily N intake and energy intake were slightly lower for the 'starch' diet (*P*<0·001). However, due to both the higher digestibility of energy and the lower amount of energy loss via urine and branchia, the difference in ME intake between the diets was

not significant (*P*=0·063) and was smaller than the difference in energy intake, 5·5 *v.* 9·0 kJ/kg$^{0.8}$ BW per d (Table 4). However, the difference in RE between fish fed both diets increased compared with the difference in ME intake, 8·5 *v.* 5·5 kJ/kg$^{0.8}$ BW per d. Consequently, RE was significantly affected by dietary NPE source (*P*<0·05). The higher RE for the 'fat' diet compared with the 'starch' diet was caused by a higher fat deposition (*P*<0·01; Table 4). Protein retention (i.e. N retention) was similar for both diets (*P*>0·1). Regarding the fat retention, the effect of dietary energy source was dependent on the feeding level, indicated by the significant interaction effect (*P*<0·05; Table 4). At the 'low' feeding level, energy retained as fat was equal between the diets,

Table 3. Effect of dietary energy source ('starch' v. 'fat') and feeding level ('low' v. 'high') on body composition (on fresh weight basis) and apparent digestibility of nutrients in Nile tilapia (*Oreochromis niloticus*)
(Mean values with their standard errors)

| | Diet | | | | | P | | |
| | Fat | | Starch | | | | | |
Feeding level	Low	High	Low	High	SEM*	D	FL	D×FL
Final body composition†								
DM (g/kg)	274[a]	294[b]	267[a]	275[a]	2·8	0·002	<0·001	0·073
Protein (g/kg)	153	149	152	152	1·2	0·405	0·057	0·111
Fat (g/kg)	78[a]	99[b]	72[a]	79[a]	3·2	0·003	0·002	0·070
Ash (g/kg)	40	39	38	36	0·9	0·041	0·150	0·666
Energy (kJ/g)	6·5[a]	7·3[b]	6·3[a]	6·7[a]	0·11	0·009	<0·001	0·080
Apparent digestibility								
DM (%)	85·3[d]	84·0[a]	87·9[b]	87·1[c]	0·09	<0·001	<0·001	0·029
Protein (%)	95·3[a,c]	95·1[a]	96·0[b]	95·5[c]	0·07	<0·001	0·004	0·156
Fat (%)	98·2[c]	96·4[a]	96·4[a]	95·8[b]	0·08	<0·001	<0·001	<0·001
NFE (%)	58·9[d]	55·1[a]	83·6[b]	82·2[c]	0·23	<0·001	<0·001	<0·001
Ash (%)	61·0[b]	59·3[a]	62·8[c]	62·5[b,c]	0·33	<0·001	0·018	0·069
Energy (%)	90·8[b]	89·4[a]	91·4[c]	90·5[b]	0·09	<0·001	<0·001	0·061

D, main effect of dietary non-protein energy source ('starch' v. 'fat'); FL, main effect of 'low' v. 'high'; D × FL, interaction effect between diet and feeding level; NFE, nitrogen-free extract (total dietary carbohydrates).
[a,b,c,d] Mean values with unlike superscript letters within a row were significantly different (*P*<0·05).
* *n* 3 per experimental treatment group.
† Initial body composition (on fresh weight basis) was as follows: DM 294 g/kg; protein 155 g/kg; fat 101 g/kg; ash 34 g/kg; energy 7·4 kJ/g.

Table 4. Effect of dietary energy source ('starch' v. 'fat') and feeding level ('low' v. 'high') on nitrogen and energy balance of Nile tilapia (*Oreochromis niloticus*)

(Mean values with their standard errors)

| | Diet | | | | | P | | |
| | Fat | | Starch | | | | | |
Feeding level	Low	High	Low	High	SEM*	D	FL	D×FL
N balance (mg/kg$^{0.8}$ BW per d)								
N intake	361[a]	797[c]	333[a]	721[b]	10·8	0·001	<0·001	0·057
Digestible N intake	344[a]	758[c]	319[a]	689[b]	10·5	0·002	<0·001	0·065
Branchial urinary N losses	235[a]	481[c]	217[a]	399[b]	5·4	<0·001	<0·001	<0·001
Retained N	109[a]	276[b]	102[a]	289[b]	6·6	0·631	<0·001	0·178
Energy balance (kJ/kg$^{0.8}$ BW per d)								
Energy intake	99[a]	219[c]	95[a]	205[b]	3·1	0·016	<0·001	0·157
Digestible energy intake	90[a]	196[b]	87[a]	186[b]	2·8	0·036	<0·001	0·249
Branchial urinary energy losses	5·8[a]	12·0[c]	5·4[a]	9·9[b]	0·14	<0·001	<0·001	<0·001
Metabolisable energy intake	84[a]	184[b]	81[a]	176[b]	2·7	0·063	<0·001	0·347
Heat production	67[a]	98[b]	68[a]	102[b]	2·9	0·419	<0·001	0·528
Retained energy	17[a]	86[b]	13[a]	73[c]	2·5	0·012	<0·001	0·107
Retained energy as fat	0·8[a]	45[b]	− 2[a]	30[c]	2·4	0·007	<0·001	0·039
Retained energy as protein	16[a]	41[b]	15[a]	43[b]	1·0	0·631	<0·001	0·178

D, main effect of dietary non-protein energy source ('starch' v. 'fat'); FL, main effect of 'low' v. 'high'; D × FL, interaction effect between diet and feeding level; BW, body weight.
[a,b,c] Mean values with unlike superscript letters within a row were significantly different (*P*<0·05).
* *n* 3 per experimental treatment group.

whereas at the high feeding level, energy retained as fat in fish fed the 'starch' diet was 33·3 % lower compared with fish fed the 'fat' diet. The observed pattern in energy retained as fat between the treatments paralleled the pattern in fat retention efficiency (i.e. fat gain as a percentage of digestible fat intake; Fig. 1), which was also affected by the interaction effect of diet and feeding level (*P*<0·05). The difference in fat retention efficiency between the two diets also increased with the feeding level. At the 'high' feeding level, the fat retention efficiency was 67 and 134 % for fish fed the 'fat' and 'starch' diets, respectively. This in combination with the similar protein retention (i.e. retained N, Table 4) indicates that *de novo* lipogenesis from carbohydrates occurred at the 'starch' diet at the 'high' feeding level.

Within the present study, the linear relationship between DE intake and RE (both expressed as kJ/kg$^{0.8}$ BW per d) was

$$RE = -40\cdot7\,(\text{SE }5\cdot00) + 0\cdot636\,(\text{SE }0\cdot031) \times DE \quad \text{for the 'fat' diet,} \tag{1}$$

$$RE = -37\cdot6\,(\text{SE }5\cdot03) + 0\cdot586\,(\text{SE }0\cdot033) \times DE \quad \text{for the 'starch' diet,} \tag{2}$$

with a combined R^2 of 98·9 %. The linear relationship between ME intake and RE (in kJ/kg$^{0.8}$ BW per d) was

$$RE = -40\cdot3\,(\text{SE }5\cdot00) + 0\cdot675\,(\text{SE }0\cdot033) \times ME \quad \text{for the 'fat' diet,} \tag{3}$$

$$RE = -36\cdot7\,(\text{SE }5\cdot01) + 0\cdot614\,(\text{SE }0\cdot035) \times ME \quad \text{for the 'starch' diet,} \tag{4}$$

with a combined R^2 of 98·9 %. Both the estimate of k_{gDE} and k_{gME} were numerically higher in tilapia fed the 'fat' diet compared with those fed the 'starch' diet. To increase the power of the statistical analysis (i.e. the range of DE and ME intake),
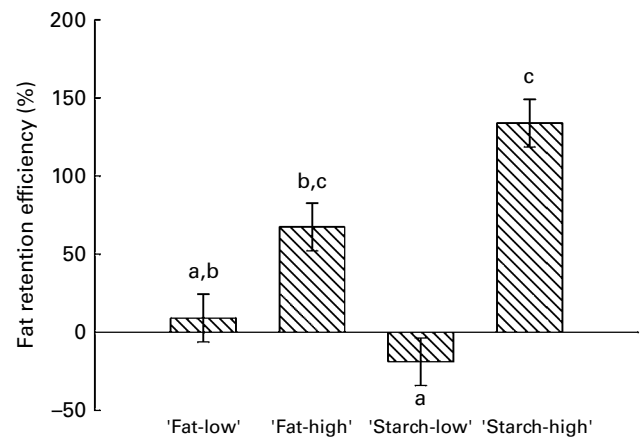
k_{gDE} and k_{gME} were estimated combining the data of the present study and a previous study on Nile tilapia[21] in which fish were fed to apparent satiation. On this combined dataset, the linear relationship between DE intake and RE (in kJ/kg$^{0.8}$ BW per d) was (Fig. 2)

$$RE = -44\cdot5\,(\text{SE }4\cdot98) + 0\cdot663\,(\text{SE }0\cdot021) \times DE \quad \text{for the 'fat' diet,} \tag{5}$$

$$RE = -34\cdot7\,(\text{SE }5\cdot41) + 0\cdot561\,(\text{SE }0\cdot026) \times DE \quad \text{for the 'starch' diet,} \tag{6}$$

Fig. 1. Effect of dietary energy source ('starch' v. 'fat') and feeding level ('low' v. 'high') on fat retention efficiency (i.e. fat retention as a percentage of digestible fat intake) of Nile tilapia (*Oreochromis niloticus*). The *P* value of the effect of dietary energy source, feeding level and their interaction was 0·244, <0·001 and 0·015, respectively. Values are means and standard errors represented by vertical bars. [a,b,c] Mean values with unlike letters were significantly different (*P*<0·05).
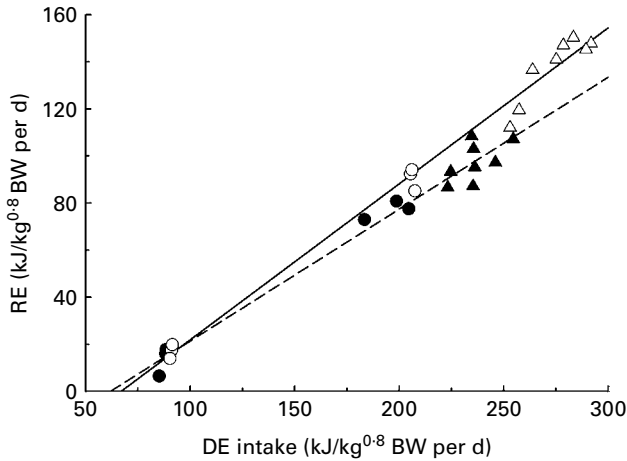
Fig. 2. Relationship between energy retention (RE) and digestible energy (DE) intake in Nile tilapia (*Oreochromis niloticus*) fed the 'fat' diet (○, △) and the 'starch' diet (●, ▲), combining the data of the present study (○, ●, being fish fed restrictively; *n* 12) and the study of Tran-Duy *et al.*[21] (△, ▲, being fish fed to satiation; *n* 16). The estimated regression lines at both diets are given in equations (5) and (6). BW, body weight.

with a combined R^2 of 98·5 %. The linear relationship between ME intake and RE (in kJ/kg^{08} BW per d) was

$$\text{RE} = -42\cdot4\,(\text{SE }4\cdot94) + 0\cdot689\,(\text{SE }0\cdot022) \times \text{ME}$$
$$\text{for the 'fat' diet,} \tag{7}$$

$$\text{RE} = -32\cdot9\,(\text{SE }5\cdot36) + 0\cdot579\,(\text{SE }0\cdot027) \times \text{ME}$$
$$\text{for the 'starch' diet,} \tag{8}$$

with a combined R^2 of 98·5 %. Preliminary analysis showed that estimated intercepts as well as the regression coefficients were not affected when a fixed effect of study was included in the model. Both k_{gDE} and k_{gME} were significantly different between the two diets ($P<0·001$), whereas the intercepts of the relationships (equations (5)–(8)) did not differ between the diets ($P>0·1$). DE_m were 67 and 62 kJ/kg^{08} BW per d for the 'fat' and 'starch' diets, respectively (derived from equations (4) and (5)). ME_m were 62 and 56 kJ/kg^{08} BW per d for the 'fat' and 'starch' diets, respectively (derived from equations (7) and (8)). The numerically lower estimated energy requirements for maintenance for the 'starch' diet group compared with the 'fat' diet group were not significant. At both diets, the curvilinear relationships between RE and DE intake as well as between RE and ME intake, described by a quadratic function, were not significant ($P>0·1$).

Discussion

The present study assessed the effect of the type of NPE source (fat *v.* starch) on energy utilisation in Nile tilapia (*O. niloticus*). The composition of NPE affected the efficiency of DE utilisation for growth (k_{gDE} (Fig. 2) and k_{gME}), but led only to a small numerical difference in the energy requirement for maintenance (both DE_m and ME_m). When fish are fed near the maintenance level, the available DE (and/or ME) is predominantly used for ATP production to sustain vital live processes. Theoretically, the energetic efficiency of ATP

production is higher from glucose compared with fat[26]. This might be the cause for the 9–10 % numerically lower DE_m and ME_m in tilapia fed the 'starch' diet compared with those fed the 'fat' diet. Within fish species, considerable variation in maintenance requirements was present (see DE_m in Table 5), which was also the case for tilapia. For Nile tilapia, ME_m was 62 and 56 kJ/kg^{08} BW per d for the 'fat' and 'starch' diets, respectively, in the present study (at 28°C) and 57 kJ/kg^{08} BW per d in the study of Meyer-Burgdorff *et al.*[27] (at 26°C). In red tilapia[28], ME_m was 54 and 77 kJ/kg^{08} BW per d at 21 and 24°C, respectively. The large variability in maintenance requirements (DE_m and ME_m) within fish species is most probably due to differences in environmental conditions between studies. Environmental conditions such as water temperature[15,28,29], water oxygen concentration[30,31] and stocking density[32,33] have been demonstrated to alter the energy requirements for maintenance in fish. Also between fish species, a large variability in energy requirements for maintenance was present (Table 5). For example, the average DE_m values for rainbow trout, European sea bass, grass carp and Nile tilapia, given in Table 5, were 35, 42, 67 and 61 kJ/kg^{08} BW per d, respectively. Next to species-related factors, experimental conditions are likely to contribute to the variability in energy requirements for maintenance between fish species.

So far, little attention has been paid to the possible impact of dietary composition on DE_m (and ME_m). Glencross *et al.*[34] indicated that DE_m did not differ between a fishmeal-based diet *v.* diets with 15 or 30 % lupin kernel meal. However, for proper estimation of the effects of dietary composition on DE_m (and ME_m) by the regression of DE intake on RE, a starvation group as well as groups fed below maintenance should not be included in the dataset. This forces the intercept of the regression lines to be similar, leading to a possible biased estimation of the difference in DE_m (and ME_m). To demonstrate this impact, we recalculated the estimated DE_m based on the data reported by Glencross *et al.*[34] by the linear regression of DE on RE. With the inclusion of a common starvation group, DE_m of trout fed the fishmeal, the 15 % lupin kernel meal or the 30 % lupin kernel meal diet was 38, 42 and 41 kJ/kg^{08} BW per d, respectively, and excluding the common starvation group, DE_m was 28, 33 and 34 kJ/kg^{08} BW per d, respectively. With the increasing plant ingredient inclusion in fish feeds, effects of dietary ingredient composition on DE_m may occur. Since this inclusion can coincide with the introduction of (un)known anti-nutritional factors into the feeds. This aspect deserves further research.

Efficiencies of energy utilisation for RE (i.e. growth) reported in the literature vary regarding the estimation method. First, energetic efficiency can be based on DE (k_{gDE}) or ME (k_{gME}). The majority of recent studies on energetic efficiency report k_{gDE} values, being estimated by the regression of DE on RE using comparative carcass analysis together with digestibility measurements. Studies reporting k_{gDE} are relatively uniform in their approach. In contrast, studies reporting k_{gME} differ regarding the method of measuring (or calculating) ME intake: (1) by both measuring energy digestibility and estimating the branchial and urinary energy losses through measuring the N balance (as done in the

Table 5. Estimates of utilisation efficiencies of digestible energy (DE) and metabolisable energy (ME) for growth (respectively, k_{gDE} and k_{gME}), DE requirements for maintenance (DE$_m$) and proximate composition of diets (on a DM basis) used for estimating k_{gDE} and k_{gME} in various fish species

Fish species	k_{gME}	k_{gDE}	Proximate composition (on a DM basis) CP (g/kg)	Fat (g/kg)	NFE (g/kg)*	Ash (g/kg)	GE (kJ/g)	Initial BW (g)	Trophic level†	DE$_m$ (kJ/kg$^{0.8}$ BW per d)
Argyrosomus japonicus [29]	–	0·60	473	195	238	94	23·1	85	4·48	47
Salmo salar [42]	0·82	0·80	542	305	59	95	25·1	95	4·43	32
Oncorhynchus mykiss [34]	–	0·75‡(0·75)	483	210	59§	109	21·0§	96	4·42	38‡(41)
O. mykiss [34]	–	0·70‡(0·75)	479	215	121§	98	22·4§	96	4·42	42‡(41)
O. mykiss [34]	–	0·75‡(0·75)	476	231	170§	89	23·9§	96	4·42	41‡(41)
O. mykiss [10]	0·63	0·62‡(–)	435	235	239	91	24·2	160	4·42	20
O. mykiss [30]	–	0·62	507	186	209	98	23·1	55	4·42	44
O. mykiss [9]	0·61	0·55‡(–)	518	269	136	77	25·2	13	4·42	16
O. mykiss [36]‖	–	0·69‡(0·76)	471	216	74§	51	21·4§	97	4·42	45‡(39)
O. mykiss [36]‖	–	0·74‡(0·76)	501	218	52§	56	21·9§	96	4·42	37‡(39)
Lates calcarifer [38]	–	0·61	524	233	120	123	24·0	15	4·35	46
L. calcarifer [38]	–	0·76	539	206	131	124	23·7	410	4·35	35
Seriola lalandi [37]	–	0·57‡(0·55)	531	111	253	106	21·4	225	4·07	33‡(54)
S. lalandi [37]	–	0·60‡(0·55)	531	111	253	106	21·4	115	4·07	86‡(54)
S. lalandi [37]	–	0·56‡(0·55)	512	162	215	111	22·1	225	4·07	44‡(54)
S. lalandi [37]	–	0·57‡(0·55)	512	162	215	111	22·1	115	4·07	88‡(54)
Epinephelus aeneus [14]	–	0·69	630	198	0	171	22·6	180	4·02	34
Dicentrarchus labrax [14]	–	0·68	630	198	0	171	22·6	78	3·79	45
D. labrax [16]	–	0·68	604	195	18	183	21·5	45	3·79	44
D. labrax [32]	–	0·67	499	202	205	94	23·0	72	3·79	43
D. labrax [32]	–	0·64	499	202	205	94	23·0	72	3·79	51
D. labrax [35]	–	0·82‡(0·83)	453	153	308	86	22·8	22	3·79	27‡(–)
Gadus morhua [11]	–	0·78	542	305	58	95	25·1	250	3·73	42
G. morhua [11]	–	0·78	649	161	74	116	22·3	250	3·73	42
Anguilla anguilla [43]	0·76	0·72‡(–)	487	256	145¶	111	24·3	45	3·53	49‡(–)
Sparus aurata [14]	–	0·65	630	198	0	171	22·6	95	3·26	48
Pangasianodon hypophthalmus [39]	–	0·55	352	113	461	74	20·9	40	3·12	38
Cyprinus carpio [17]	–	0·76‡(–)	449	131	280	140	20·2	155	2·96	67‡(–)
Ctenopharyngodon idella [19]	0·63	0·50‡(–)	702	42	194¶	62	21·7	14	2·00	68‡(–)
C. idella [19]	0·60	0·47‡(–)	353	21	579¶	47	19·4	14	2·00	71‡(–)
C. idella [19]	0·69	0·66‡(–)	495	165	330¶	10	24·1	14	2·00	49‡(–)
C. idella [44]	–	0·31‡(–)	358	31	464	147	17·8	12	2·00	78
Oreochromis niloticus [27]	0·67	0·62(–)**	401	113	372	114	21·9	7	2·00	53(–)**
O. niloticus (present study)	0·69	0·66	518	187	197	98	22·8	75	2·00	67
O. niloticus (present study)	0·58	0·56	432	56	429	83	19·7	75	2·00	62
Correlation coefficient with k_{gDE}	–	–	0·31	0·66	−0·60	0·07	0·52	0·38	0·46	–
P value correlation coefficient	–	–	0·072	0·001	0·001	0·698	0·001	0·024	0·006	–

CP, crude protein; NFE, nitrogen-free extract (total dietary carbohydrates); GE, gross energy; BW, body weight.

* NFE was calculated as DM − CP − fat − ash content, except for values marked with § or ¶.

† Trophic level given by FishBase [25].

‡ Values of k_{gDE} and DE$_m$ without being marked with ‡ are reported in the literature from regression of retained energy on DE intake both expressed per metabolic BW (kg$^{0.8}$). Values of k_{gDE} and DE$_m$ being marked with ‡ are recalculated from the reported data expressing retained energy and DE intake in kJ/kg$^{0.8}$ BW per d. Values between brackets are original k_{gDE} and DE$_m$ values reported in the literature.

§ Diets in Glencross *et al.* [34,36] contained cellulose as the inert diet filler. NFE and GE values from these studies are corrected for the amount of cellulose included in the diets.

‖ The estimates of k_{gDE} of the reference diet in Glencross *et al.* [36] were excluded from the dataset, because this reference group was fully identical to the reference diet in Glencross *et al.* [34].

¶ NFE value was calculated from gross energy, CP and fat content using 23·7, 39·5 and 17·6 kJ/g as the energetic values for CP, fat and carbohydrates.

** In the study of Meyer-Burgdorff *et al.* [27], only the ME values were reported. For the estimation of k_{gDE} and DE$_m$, DE intake was estimated assuming that branchial urinary energy losses were 6·5 % of the ME intake.

present study) or (2) by measurements of heat production of fish by either direct or indirect calorimetry. Since the majority of (recent) reported studies estimated k_{gDE}, we restricted ourselves to comparing the current estimated values in the present study on the basis of k_{gDE}. In Table 5, an overview of reported values in different fish species is given, in which k_{gDE} is reported and also studies from which k_{gDE} could be estimated from the reported data. In the majority of studies reported, k_{gDE} was estimated by expressing DE and RE per unit of metabolic BW using a power of 0·8, but some studies deviated from this; for example, Carter & Brafield[19] expressed DE and RE as kJ/d per fish and Peres & Oliva-Teles[35] as kJ/kg BW per d. Values of k_{gDE} reported in Table 5 are recalculated all on the basis of kJ/kg$^{0·8}$ BW per d. The present study assessed the impact of NFE composition on energetic efficiency. Therefore, an analysis of k_{gDE} in relation to dietary nutrient composition was made over fish species. Several studies[34,36,37] given in Table 5 assessed the impact of ingredient/diet composition on k_{gDE}, but none of them found significant differences in k_{gDE}. Unfortunately, only the pooled k_{gDE} over diets were given in these studies. For these studies, the original k_{gDE} for each diet was calculated from the reported data.

The k_{gDE} values estimated in the present study for Nile tilapia (equations (7) and (8)) are in line with the calculated value of k_{gDE} from the study of Meyer-Burgdorff et al.[27] on Nile tilapia (Table 5). When comparing estimated k_{gDE} values in different fish species, the large variability is striking, ranging from 0·31 to 0·82 (Table 5). Often one of the reasons suggested for this large variation between fish species is the difference in natural feeding ecology (i.e. carnivorous, omnivorous or herbivorous). The reported studies (Table 5) seem to confirm this, since a positive correlation between k_{gDE} and the trophic level (obtained from FishBase[25]) was present (r 0·46, $P < 0·01$). Estimated k_{gDE} in fish at a lower trophic level (i.e. more herbivorous) are lower than those estimated for fish at a higher trophic level (i.e. more carnivorous) (Fig. 3). However, it should be noted that with changing trophic level, there is also a change in diet composition used in the studies to estimate k_{gDE}. The protein and fat contents of diets used in studies on low-trophic-level fish species are lower than in studies on higher-trophic-level fish species (Table 5), whereas the dietary content of carbohydrates shows the reverse pattern. The proximate composition of diets used in studies to estimate k_{gDE} correlates with the estimated k_{gDE} values, except for the dietary ash content (Table 5 and Fig. 3). The residuals of k_{gDE} of the linear regression of k_{gDE} on the dietary fat content were not correlated with the trophic level (r 0·00; $P > 0·1$). This observation, together with the impact of dietary composition within species (Nile tilapia,
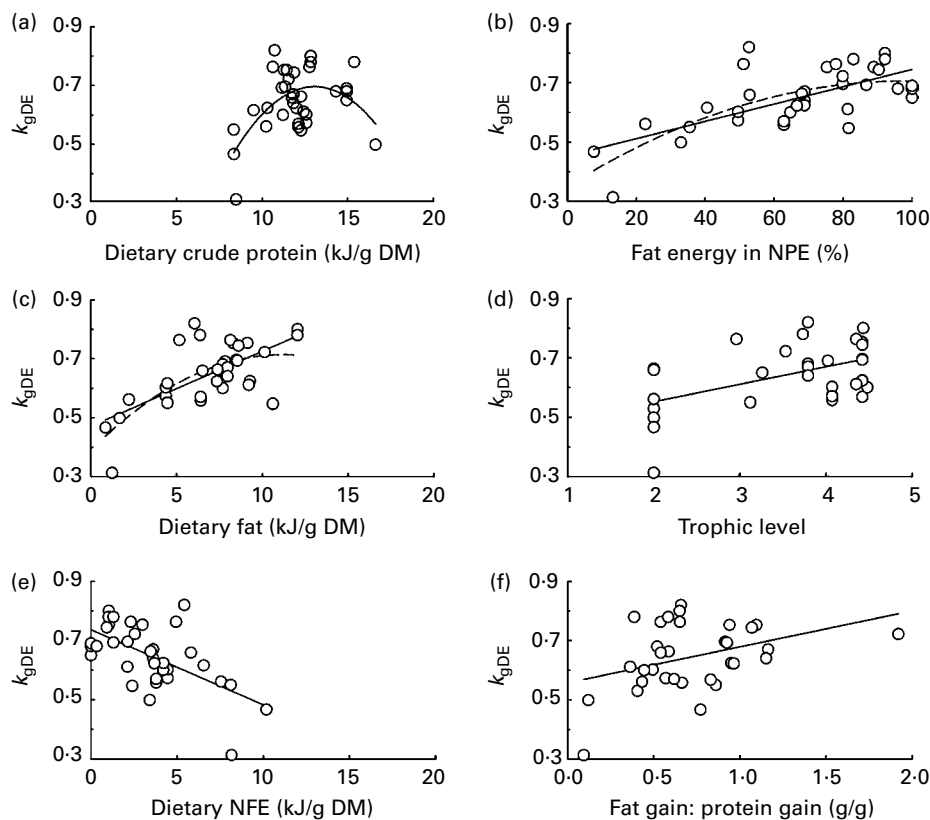
Fig. 3. Relationships between the efficiency of digestible energy utilisation for growth (k_{gDE}) reported/derived from the literature in various fish species (given in Table 5) with the following: dietary crude protein content; dietary fat content; dietary total carbohydrate content (nitrogen-free extract; NFE); fat energy as a percentage of dietary non-protein energy (NPE) content; the trophic level of the fish species (derived from FishBase[25]); the ratio of fat:protein gain (in g/g) at the highest feeding level applied within each treatment group of the respective literature source. Solid lines indicate a significant relationship (either linear or quadratic) with k_{gDE} ($P < 0·05$) and broken lines indicate a tendency for a significant relationship ($P < 0·10$). Equations are presented in Table 6.

present study, Fig. 2; trout[18]), suggests that the observed positive relationship between k_{gDE} and the trophic level of fish species is rather diet-induced than species-specific.

Both the experimental data on Nile tilapia and the between-study comparison of k_{gDE} demonstrate that dietary nutrient composition is an important factor explaining variability in k_{gDE} values between studies. The between-study comparison showed that both dietary fat and NFE contents were linearly related to k_{gDE} ($P<0.001$), but for the fat content, the curvilinear function (i.e. quadratic function) tended also to be significant ($P<0.10$; Fig. 3 and Table 6). Despite the fact that, theoretically, the formation of ATP is most efficient in animals if glucose is used as the substrate[26], k_{gDE} decreases with increasing dietary NFE content (Figs. 2 and 3). This is due to the fact that at higher inclusion levels of NFE, more carbohydrates are used as substrates for *de novo* fatty acid synthesis. This use of dietary NFE for the synthesis of fatty acids is illustrated by the fat retention efficiency being above 100 % in Nile tilapia fed the 'starch' diet at the 'high' feeding level (Fig. 1). The synthesis of body fat from NFE (synthesis of fatty acids from glucose units) requires more ATP than the synthesis of body fat from fatty acids originating from dietary fat, which causes the decline in k_{gDE}. This phenomenon also explains the increase in k_{gDE} with increasing dietary fat content, as well as with the increase in the percentage of fat energy within the NPE fraction (Fig. 3 and Table 6). Since dietary fat and NFE contents between studies were strongly negatively correlated ($r -0.74$; $P<0.001$), the curvilinear trend between k_{gDE} and dietary fat content is also reflected in a curvilinear trend between k_{gDE} and the percentage of fat energy within the NPE fraction ($P<0.10$; Table 6).

Using the linear equations from the comparison of k_{gDE} between studies, k_{gDE} increases by 0.025 units for every increase in the dietary fat content of 1 kJ/g DM and decreases by 0.025 units for every increase in the dietary NFE content of 1 kJ/g DM (Table 6). Applying these estimates of the effect of

fat and NFE contents on k_{gDE} from Table 6 on the difference in dietary fat and NFE contents in the present study on Nile tilapia (respectively, 131 and 232 g/kg DM; Table 1) predicts a difference in the k_{gDE} of 0.129 and 0.102 units between the 'starch' and 'fat' diets, respectively. This is well in line with the observed difference of 0.102 between the two diets (equations (5) and (6)). The impact of dietary fat content obtained from the between-species comparison of 0.025 units increase in k_{gDE} per 1 kJ/g DM increase in dietary fat content is identical to the estimated impact from a pooled analysis of experiments in rainbow trout[18]. This rainbow trout study reported an increase in k_{gDE} by 0.025 units if dietary fat content increased by 1 kJ/g DM.

Next to dietary fat and NFE contents, also the dietary CP content explained part of the variability in k_{gDE} between studies (Table 5 and Fig. 3). This relationship was curvilinear, i.e. the quadratic component was significant ($P<0.01$; Table 6). At both low and high dietary CP levels, k_{gDE} was reduced. The reduced k_{gDE} at low CP levels is most probably due to the fact that low dietary CP levels coincided with high NFE levels (when the fat level is constant; Table 5), which reduces k_{gDE} as demonstrated in the present study on Nile tilapia. The reduction in k_{gDE} at high dietary CP levels is probably caused by the fact that CP provided in excess to the fish leads to an increased energy demand for ammoniogenesis and for the formation of fatty acids, similarly to the reduced efficiency at high NFE when fat is synthesised from glucose. The analysis of the CP:GE ratio in the diets revealed no relationship with k_{gDE} ($P>0.05$; data not shown). This confirms the aforementioned suggestion that both dietary fat and NFE contents predominantly determine k_{gDE}.

Results of various studies, which assessed the impact of diet composition on the energy utilisation for growth, are not always in line with each other. In Nile tilapia (the present study), grass carp[19], rainbow trout[18,20] and European eel[13], it was shown that diet composition (i.e. differences

Table 6. Linear and quadratic relationships* estimated from the studies reported in Table 5 and depicted in Fig. 3, explaining the energetic efficiency of digestible energy for growth (k_{gDE}; *Y*) by proximate dietary nutrient composition, trophic level of the fish species and the ratio of fat:protein gain measured at the highest feeding level applied in these studies

Equation	R^2 (%)	*P* (linear component)	*P* (quadratic component)
Dietary crude protein (*X*, in kJ/kg DM)			
$Y=0.44$ (SE 0.111) $+ 0.017$ (SE 0.0092) $\times X$	9.5	0.072	–
$Y=-1.02$ (SE 0.431) $+ 0.262$ (SE 0.0708) $\times X - 0.010$ (SE 0.0029) $\times X^2$	34.4	–	0.002
Dietary fat (*X*, in kJ/kg DM)			
$Y=0.47$ (SE 0.038) $+ 0.025$ (SE 0.0050) $\times X$	43.4	<0.001	–
$Y=0.39$ (SE 0.056) $+ 0.058$ (SE 0.0178) $\times X - 0.0026$ (SE 0.0014) $\times X^2$	49.2	–	0.067
Dietary NFE (*X*, in kJ/kg DM)			
$Y=0.74$ (SE 0.025) $- 0.025$ (SE 0.0058) $\times X$	36.3	<0.001	–
Fat energy of dietary non-protein energy (*X*, in %)			
$Y=0.45$ (SE 0.039) $+ 0.0029$ (SE 0.0006) $\times X$	45.8	<0.001	–
$Y=0.35$ (SE 0.065) $+ 0.007$ (SE 0.0023) $\times X - 0.037$ (SE 0.019) $\times 10^{-3} \times X^2$	51.5	–	0.062
Trophic level of fish species (*X*)			
$Y=0.46$ (SE 0.068) $+ 0.053$ (SE 0.0181) $\times X$	20.8	0.006	–
Ratio of fat:protein gain (*X*, in g/g)			
$Y=0.57$ (SE 0.042) $+ 0.113$ (SE 0.053) $\times X$	13.1	0.042	–

NFE, nitrogen-free extract (total dietary carbohydrates).
* Only relationships (both linear and quadratic), which were significant or tended to be significant ($P<0.10$), are given.

in macronutrient composition by altering ingredient composition) affected the energy utilisation for growth, whereas in other studies on rainbow trout[34,36], yellowtail kingfish[37] and Atlantic cod[11], no differences between the diets were observed. Except for the Atlantic cod study[11], the absence of a dietary effect on k_{gDE} is probably due to the relatively small differences in proximate composition of the experimental diets[34,36,37] (Table 5). Moreover, some of these studies, which assessed the impact of the diet on k_{gDE}, included data from a group of fish which were starved[34,36,37]. In addition to affecting the estimation of maintenance requirements, inclusion of data from starved fish will also influence the slope of the regression line (i.e. k_{gDE}).

The comparison among fish species (Fig. 3 and Table 6) indicates that dietary macronutrient composition can explain up to 52% of the variation in k_{gDE}, which can be judged as considerable. However, still 48% of the variation in k_{gDE} could not be explained. The proportion of explained variability might increase by multiple regression analyses, combining various dietary nutrient contents into the model. However, with the dataset presented in Table 5, this was impossible due to the strong correlation between dietary CP, fat and NFE contents. The proportion of the explained variation in k_{gDE} would most probably increase if: (1) dietary starch content would be used instead of NFE content and (2) digestible dietary nutrient content were used instead of the crude chemical composition. However, due to the limited amount of data available in the literature, this could not be done. The comparison of k_{gDE} among fish species (Fig. 3 and Table 6) was made on k_{gDE} values obtained by the linear regression of DE on RE. In the present study, the relationship between DE and RE was best described by a linear relationship. However, in various studies, it has been shown that a curvilinear relationship better describes the relationship between DE and RE, e.g. in rainbow trout[34,36], barramundi[38] and Tra catfish[39]. This variability in the relationship between DE intake and RE (linear v. curvilinear) may have contributed to the unexplained variability in k_{gDE} estimated by linear regression (Fig. 3 and Table 6). Another possible factor contributing to the unexplained variability in k_{gDE} is the difference in the efficiency of protein deposition (k_p) between fish species. Azevedo et al.[40] compared salmon and trout fed similar diets and found that trout had a 21% higher k_p compared with salmon. Furthermore, the unexplained variation in k_{gDE} between studies might be due to the differences in BW of the fish. In barramundi[38] fed the same diet, k_{gDE} increased with BW of the fish. However, in other studies, it has been found that BW of the fish did not affect k_{gDE}, e.g. yellowtail kingfish[37]. The possible impact of BW on k_{gDE} is also suggested by the significant positive correlation between k_{gDE} among species and the initial BW in the studies reported in Table 5 (r 0·38; $P<0·05$). However, the residuals of k_{gDE} of the linear regression of k_{gDE} on dietary fat content only tended to be correlated with initial BW (r 0·30; $P<0·10$), whereas the residuals of the regression of k_{gDE} on dietary NFE content were not correlated with initial BW (r 0·23; $P>0·1$). This suggests that variation in BW between studies had only a minor contribution to the unexplained variation in k_{gDE}.

Moreover, the unexplained variability in k_{gDE} might be related to differences between fish species in the ratio of fat:protein gain, since the energetic efficiency for protein deposition (k_p) is lower than that for fat deposition (k_f) as observed in various fish species[14,17,18]. To examine this aspect on the collected dataset from the literature, the ratio of fat:protein gain (in g/g) at the highest feeding level within each treatment (diet) was calculated. As depicted in Fig. 3 and Table 6, this ratio significantly explained part (13·1%) of the variation in k_{gDE} ($P<0·05$), be it less than dietary fat ($>43%$) and NFE (36%) contents. The residuals of k_{gDE} of the linear regression k_{gDE} on dietary fat content were not correlated with the ratio of fat:protein gain (r 0·05; $P>0·1$). In contrast, the residuals of k_{gDE} of the linear regression of k_{gDE} on the fat:protein gain ratio were correlated with the dietary fat (r 0·53; $P<0·01$), protein (r 0·45; $P<0·01$) and NFE contents (r −0·59; $P<0·001$). This suggests that the variability in k_{gDE} between studies as well as between fish species is more likely to be induced by dietary macronutrient composition than by the growth composition. The relationship with growth composition might be due to the impact of dietary macronutrient composition on the ratio of fat:protein gain. In general, a better understanding of the causes of variability in energetic efficiency requires further research.

The finding of the present study that dietary macronutrient composition can have a large impact on the utilisation of DE for growth (both within and between species) has implications for practical diet formulations. In general, energy evaluation in fish-feed formulation is based on the DE of feeds/ingredients. Optimal DE contents of fish feeds are based on the optimal digestible protein (DP):DE ratio. The optimal DP:DE ratio for fish species is either obtained from experimental studies, as, for example, summarised in the National Research Council[41], or derived from factorial models which describe both energy and protein partitioning over somatic and non-somatic (i.e. maintenance) growth compartments[15,16,38]. Using the optimal DP:DE ratio as the basis for diet formulation is valid as long as the proximate composition (i.e. CP, fat and starch content) of the practical feeds is comparable with that of the experimental diets used to estimate/derive the optimal DP:DE ratio for a fish species. However, in light of the expected diversification in ingredients used in fish-feed formulations, also dietary ratios of, for example, starch:fat might vary. Based on the present findings, changes in the dietary NPE composition will alter the k_{gDE} values of these diets and thus estimations of the optimal DP:DE ratio of these diets. To overcome this impact of changes in dietary composition on the optimal DP:DE ratio, a transition from evaluating ingredients/diets on a DE basis towards an evaluation of net energy may be required in fish-feed formulations, as established in pig nutrition[6,7]. Such a net energy evaluation, although expected to be more relevant for herbivorous fish displaying a higher capacity to digest and utilise substantial amounts of starch, is also interesting from a comparative point of view (between fish species and between fish and terrestrial animals).

In conclusion, in Nile tilapia, the energy utilisation for growth depends on the type of NPE source. Exchanging fish

oil by maize starch (125 *v.* 300 g/kg of feed intake) reduced the DE utilisation for growth (k_{gDE}) from 0·663 to 0·561. Part of the variability in k_{gDE} across fish species reported in the literature relates to (1) the proximate composition of the experimental diets used for estimating k_{gDE}, (2) the trophic level of the fish species and (3) the composition of growth (i.e. the ratio of fat:protein gain). However, the across-species comparison suggests that variability explained by trophic level and by the composition of growth is predominantly induced by differences in dietary proximate composition. In line with the findings in the present study on Nile tilapia, increasing the dietary fat content increases k_{gDE} and increasing the dietary carbohydrate content (NFE) decreases k_{gDE}. Opposite to fat and carbohydrate, which are linearly related to k_{gDE}, the dietary CP content is curvilinearly related to k_{gDE}: both at a 'low' and a 'high' dietary CP contents, k_{gDE} is reduced.

Acknowledgements

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