

Meta-analysis

The effect of fish meal replacement by soyabean products on fish growth: a meta-analysis

James Sales*

Research Institute of Fish Culture and Hydrobiology, University of South Bohemia, Zatisi 728,
38925 Vodnany, Czech Republic

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Meta-analysis was applied to quantify the effect of replacement of fish meal by soyabean products in diets on fish growth. Measurement of growth in different units among studies required the use of a standardised effect size (Hedges' *d*). From a total of ninety-nine studies concentrating on fish meal replacement by defatted soyabean meal, 53 % were eliminated due to, among others, absence of a fish meal control diet (*n* 18), or no statistical differences or measurement of dispersion (*n* 6) indicated. Replacement of 4 to 40 % fish meal by soyabean meal (inclusion levels of 71–366 g/kg) resulted in a mean effect size of -0.1142 (95 % CI $-0.4665, 0.2382$) obtained in forty-eight comparisons evaluated with seventeen different fish species. However, at higher fish meal replacement levels the 95 % CI calculated for combined effect sizes did not overlap with zero. With soya protein concentrate replacing 25 to 100 % of fish meal in diets for seven fish species, methionine supplementation (mean -2.4373 (95 % CI $-3.9004, -0.9742$); *n* 10) did not have a substantial influence on the magnitude of cumulative effect sizes relative to no supplementation (mean -2.7306 (95 % CI $-3.7991, -1.6620$); *n* 16). Information on other soyabean products (full-fat soyabeans, soya flour) used as protein sources in fish diets was found as too limited for analysis and definite conclusions. The present study contributes by putting a numerical value to the magnitude of growth differences in fish when replacing dietary fish meal by soyabean products.

Fish meal: Soyabean products: Fish diets: Growth: Meta-analysis

Reviews on the future of aquaculture production⁽¹⁾ and development of fish diets^(2–9) have centred around the replacement of fish meal, on which most fish diets are based, by economically viable and environmentally friendly plant protein alternatives. Defatted soyabean meal has received considerable attention due to a high protein content, reasonably balanced amino acid profile, consistent availability, cost effectiveness and palatability to most fish species^(4,6).

Soyabeans, although evaluated after heat treatment in the whole form in fish diets, are predominantly processed into defatted soyabean meal with or without hulls, but also into soya flour, soya protein concentrate and soya protein isolate. However, costs hamper the use of the latter processed products for effective replacement of fish meal in fish diets⁽⁶⁾.

As with all potential plant protein sources, the nutrient and antinutritional profiles of soyabean meal are currently not ideal for inclusion in fish diets^(6,10). Furthermore, as summarised by, among others, Chou *et al.*⁽¹¹⁾ and Lim *et al.*⁽¹²⁾, there generally appears to be large variability among fish species in the maximum dietary levels of soyabean meal tolerated, indicating different sensitivities to soyabean meal inclusion. Barrows *et al.*⁽¹³⁾ concluded that the upper dietary inclusion levels of soyabean meal before fish performance or health

will be deteriorated is 10–15 % (25 % fish meal replacement) for carnivorous species such as Atlantic salmon (*Salmo salar*), rainbow trout (*Onchorynchus mykiss*), sea bass (*Dicentrarchus labrax*) and yellowtail (*Seriola quinqueradiata*). However, Heikkinen *et al.*⁽¹⁴⁾ stated upper inclusion levels of 20–30 % for carnivorous salmonids. In contrast, omnivorous and carnivorous freshwater fish such as common carp (*Cyprinus carpio*), tilapia (*Oreochromis* spp.), blue catfish (*Ictalurus furcatus*) and channel catfish (*I. punctatus*) seem to grow well on high percentages (70–100 %) of fish meal replaced by soyabean meal^(11,15). Factors causing discrepancy among researchers on the use of soyabean meal as a protein source for fish might be related to quality, processing and inclusion levels of soyabean meal, variation in diet formulation, and differences in fish species, fish size and culture system^(3,16–17).

Although several reviews^(2–6,9–10) on feed ingredients for use in fish diets have included the replacement of fish meal by soyabean products, they were concentrated on summative descriptions of results obtained from research studies. These narrative reviews consider all studies with equal weight, without an account for measures of dispersion. Meta-analysis, the review of scientific literature with the emphasis on providing a quantitative synthesis of data, allows the

*Corresponding author: Dr James Sales, fax +420 383 382 396, email james_sales_1@hotmail.com

evaluation and integration of results from a group of studies, even those with seemingly contradictory results⁽¹⁸⁾.

The objective of the present study was to analyse, with the use of meta-analytic techniques, available published growth results obtained in fish due to the replacement of dietary fish meal by soyabean products. The outcome would provide a numerical measurement of the extent of growth differences.

Materials and methods

Selection of studies

A comprehensive literature search was conducted on the Internet with the use of several search engines and publishers' websites. Cook *et al.*⁽¹⁹⁾ concluded that unpublished results should not be completely excluded from meta-analysis, but be subjected to the same rigorous methodological evaluation than published peer-reviewed data, and results being presented with and without inclusion of unpublished results. However, inclusion of the latter can be problematic, especially when coming from interested sources. Willingness of investigators related to outcome, with favourable results being provided more easily, and hidden unpublished results even after extensive consultation, could result in an unrepresentative sample of unpublished studies. This causes doubt if the inclusion of unpublished studies increases or decreases bias in meta-analyses⁽²⁰⁾. Taking the above into consideration, studies have been selected for evaluation in the present study that: (1) had replaced fish meal in diets by soyabean products, (2) presented a measurement of fish growth, (3) appeared in peer-reviewed journals, and (4) been published in English in order to extract all relevant information. Studies fulfilling the above were further subjected to evaluation for inclusion in meta-analyses according to criteria presented in Table 1.

Whereas some studies included only one level of fish meal replacement, others contained multiple replacements. Furthermore, different products^(21–28), similar products subjected to different processing treatments^(23,25,27), supplementation with amino acids^(12,26,29–41), effects at different dietary protein levels^(29,42), and the influence on different fish species⁽⁴³⁾ and fish sizes^(26,37–38,44–46), were often evaluated in the same study. Due to the apparent effect of all of the above variables, data were not pooled for individual studies, but used in individual comparisons. Although this might caused dependence on one another for some effect sizes, exclusion of non-independent comparisons may bias results more than their inclusion^(47,48). The above resulted in a coding system based on trial identification numbers.

Data analysis

Fish growth in studies selected for inclusion in the meta-analysis has been presented in different units: total weight gain (g), weight gain (%), specific growth rate (%), and daily and thermal growth coefficients. This necessitated the use of a common metric independent of differences in unit measurements. Effect size was measured with Hedges' d ⁽⁴⁷⁾, based on the difference between the means (\bar{X}) for treatment (T) and control (C) groups, standardised by dividing by the pooled standard deviation (s_p), and corrected for bias (J) for

Table 1. Selection of studies for inclusion in meta-analysis

Study	<i>n</i>
Defatted soyabean meal	
Fish meal together with other protein sources replaced	5
Soyabean meal together with other protein sources used for replacement	9
Lack of control group without soyabean meal	18
No measures of <i>P</i> values or dispersion	6
Results presented in graphs	6
Results combined for different treatments	1
Ornamental fish species evaluated	1
Only final live weight values reported	2
Variation in dietary crude protein contents among diets	4
Data duplication	1
Suitable studies	46
Soyabean meal only	31
Differences indicated as <i>P</i> <0.05	4
Pooled variance of means presented	13
Variance for individual means presented	16
Soyabean meal with amino acid supplementation	23
Differences indicated as <i>P</i> <0.05	1
Pooled variance of means presented	7
Variance for individual means presented	15
Soya protein concentrate	
Fish meal together with other protein sources replaced	1
Soya protein concentrate together with other protein sources used for replacement	2
Lack of control group without soya protein concentrate	2
No measures of <i>P</i> values or dispersion	1
Results presented in graphs	2
Results combined for different treatments	1
Variation in dietary crude protein contents among diets	1
Suitable studies	13
Soyabean protein concentrate only	10
Differences indicated as <i>P</i> <0.05	2
Pooled variance of means presented	5
Variance for individual means presented	3
Soya protein concentrate with amino acid supplementation	8
Differences indicated as <i>P</i> <0.05	3
Pooled variance of means presented	2
Variance for individual means presented	3
Full-fat soyabeans	
No measures of <i>P</i> values or dispersion	3
Results presented in graphs	2
Results combined for different treatments	1
Only final live weight values reported	1
Suitable studies	8
Full-fat soyabeans only	8
Differences indicated as <i>P</i> <0.05	2
Pooled variance of means presented	3
Variance for individual means presented	3
Full-fat soyabeans with amino acid supplementation	1
Pooled variance of means presented	1
Soya flour	
Soya flour only	4
Differences indicated as <i>P</i> <0.05	2
Soya flour with amino acid supplementation	4
Differences indicated as <i>P</i> <0.05	2
Pooled variance of means presented	2

Table 2. Highest levels of defatted soyabean meal inclusion and fish meal replacement at which growth obtained did not differ from that with a fish meal control diet ($P > 0.05$), and trials where soyabean meal inclusion could not maintain a similar growth to a fish meal control diet ($P < 0.05$)

Trial number	Fish species		Feeding habit	Water type	Water temperature	Initial size (g)	Period (d)	Dietary crude protein (g/kg)	Soyabean inclusion level (g/kg)	Fish meal replacement level (%)	Soyabean (g/kg)	Fish meal (%)	Unit	Reference
	Common name	Scientific name												
$P > 0.05$														
1*	Asian seabass	<i>Lates calcarifer</i>	C	S	N	1.3	70	395	210	38	210	38	WG	27
2	Cobia	<i>Rachycentron canadum</i>	C	S	W	32.3	56	461–494	71, 143, 214, 286, 357, 429	10, 20, 30, 40, 50, 60	286	40	TWG	11
3	European sea bass	<i>Dicentrarchus labrax</i>	C	S	W	188	100	503–507	250	27	250	27	SGR	41
4	Gilthead seabream	<i>Sparus aurata</i>	C	S	W	1.6	84	441–451	366	35	366	35	SGR	25
5*	Gilthead seabream	<i>Sparus aurata</i>	C	S	CO	40	60	594–612	107, 221, 334	10, 18, 27	334	27	WG	52
6	Hybrid striped bass	<i>Morone saxatilis/chrysops</i>	C	F	W	9.6	70	360	150, 300, 450	22, 42, 63	300	42	WG	26
7	Hybrid striped bass	<i>Morone saxatilis/chrysops</i>	C	F	W	12	70	360	200 250, 300	31, 37, 43	300	43	WG	26
8	Japanese flounder	<i>Paralichthys olivaceus</i>	C	S	W	4.6	56	499–521	400	47	400	47	WG	15
9	Japanese flounder	<i>Paralichthys olivaceus</i>	C	S	W	5	56	503	88, 177	10, 20	177	20	SGR	38
10	Japanese flounder	<i>Paralichthys olivaceus</i>	C	S	CO	45.5	70	500	88, 177, 265	10, 20, 30	177	20	SGR	38
11	Korean rockfish	<i>Sebastes schlegeli</i>	C	S	W	2.5	56	480	115, 153, 229, 344	15, 20, 30, 45	153	20	SGR	12
12	Mozambique tilapia	<i>Oreochromis mossambicus</i>	O	F	W	N	56	305–323	176, 352, 528, 704	25, 50, 75, 100	704	100	SGR	53
13	Murray cod	<i>Maccullochella peeli</i>	C	F	W	3.2	70	531–541	92, 179, 271, 346	4, 14, 20, 31	346	31	SGR	54
14	Nile tilapia	<i>Oreochromis niloticus</i>	O	F	W	10.6	70	312–326	524	83	524	83	SGR	21
15*	Rainbow trout	<i>Oncorhynchus mykiss</i>	C	F	CO	38	84	394–402	213	30	213	30	SGR	23
16*	Rainbow trout	<i>Oncorhynchus mykiss</i>	C	F	CO	38	84	402–408	236	30	236	30	SGR	23
17	Rainbow trout	<i>Oncorhynchus mykiss</i>	C	F	CO	38	45	419–421	316	40	316	40	SGR	55
18	Rainbow trout	<i>Oncorhynchus mykiss</i>	C	F	CO	99	84	400	296	37	296	37	TWG	43
19	Red drum	<i>Sciaenops ocellatus</i>	C	S	CO	7.4	56	371–376	194, 388, 582	25, 50, 75	388	50	WG	56
20	Red seabream	<i>Pagrus auratus</i>	C	S	W	24	42	458–485	300	38	300	38	SGR	57
21	Red snapper	<i>Lutjanus argenti-maculatus</i>	C	S	W	5.0	133	503–523	120, 240, 360, 480	13, 25, 38, 50	480	50	SGR	58
22	Rohu	<i>Labeo rohita</i>	O	F	W	4.1	70	345	143	20	143	20	SGR	37

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Table 2. Continued

Fish species														
Trial number	Common name	Scientific name	Feeding habit	Water type	Water temperature	Initial size (g)	Period (d)	Dietary crude protein (g/kg)	Soyabean inclusion level (g/kg)	Fish meal replacement level (%)	Soyabean (g/kg)	Fish meal (%)	Unit	Reference
23	Sharpsnout seabream	<i>Diplodus puntazzo</i>	O	S	W	48.3	64	417	200, 400, 600	230, 450, 680	680	68	SGR	46
24	Sharpsnout seabream	<i>Diplodus puntazzo</i>	O	S	W	196	91	474	200, 400, 600	230, 450, 680	680	68	SGR	46
25	Southern catfish	<i>Silurus meridionalis</i>	C	F	W	23.8	56	478–487	116, 231, 347, 463, 579	13, 26, 39, 52, 65	347	39	SGR	39
26	Tilapia hybrid	<i>Oreochromis niloticus/ aureus</i>	O	F	W	1.2	60	229–236	174	30	174	30	WG	29
27	Tilapia hybrid	<i>Oreochromis niloticus/ aureus</i>	O	F	W	4.5	56	225–238	184, 373, 557	33, 67, 100	373	67	WG	30
28	Tilapia hybrid	<i>Oreochromis niloticus/ aureus</i>	O	F	W	5.1	56	255	189	29	189	29	WG	22
29	Tilapia hybrid	<i>Oreochromis niloticus/ aureus</i>	O	F	N	120	33	250	200	40	200	40	TWG	59
30	Tilapia hybrid	<i>Oreochromis niloticus/ aureus</i>	O	F	W	196	54	288–311	200, 550	34, 100	200	34	TWG	60
31	Tin foil barb	<i>Barbodes altus</i>	O	F	W	0.9	56	422–433	267, 365, 515	25, 34, 51	365	34	SGR	16
<i>P</i> < 0.05														
32	Atlantic salmon	<i>Salmo salar</i>	C	F	CO	207	84	400	296	37	296	37	TWG	43
33	Rainbow trout	<i>Oncorhynchus mykiss</i>	C	F	CO	2.6	182	414–419	780	100	780	100	TWG	24
34*	Rainbow trout	<i>Oncorhynchus mykiss</i>	C	F	CO	5.3	42	433–438	585	60	585	60	WG	61
35	Rainbow trout	<i>Oncorhynchus mykiss</i>	C	F	CO	50.7	63	488	600	63	600	63	SGR	32
36	Rainbow trout	<i>Oncorhynchus mykiss</i>	C	S	CO	307	63	378	249	40	249	40	TGC	17
37	Rohu	<i>Labeo rohita</i>	O	F	W	2.6	56	344	616	100	616	100	SGR	37
38	Sunshine bass	<i>Morone chrysops/saxatilis</i>	C	F	W	9.2	56	400	559	75	559	75	WG	33
39	Tilapia hybrid	<i>Oreochromis niloticus/ aureus</i>	O	F	W	1.2	60	312–317	231	30	231	30	WG	29

C, carnivorous; S, salt water; N, not indicated; WG, weight gain (%); W, warm water ($\geq 20^{\circ}\text{C}$); TWG, total weight gain (g); SGR, specific growth rate (%); CO, cold water ($< 20^{\circ}\text{C}$); F, fresh water; O, omnivorous; TGC, thermal growth coefficient.

* Not included in meta-analysis.

small sample sizes (n):

$$d = \frac{\bar{X}_T - \bar{X}_C}{s_p} J$$

with

$$J = 1 - \frac{3}{4(n_T + n_C) - 9}$$

and

$$s_p = \sqrt{\frac{(n_T - 1)s_T^2 + (n_C - 1)s_C^2}{n_T + n_C - 2}}$$

The asymptotic SE of the effect size was estimated by Hedges⁽⁴⁹⁾:

$$SE = \sqrt{\frac{n_T + n_C}{n_T n_C} + \frac{d^2}{2(n_T + n_C - 2)}}$$

Precision of d was illustrated with the 95 % CI:

$$d - 1.96 SE \text{ to } d + 1.96 SE.$$

Summary statistics were calculated using a random-effects model⁽⁵⁰⁾, which takes into account between-trial variability (true heterogeneity) as well as within-trial variability (sampling error).

A fail-safe number (N_{fs})⁽⁵¹⁾ has been calculated to indicate the number of unpublished comparisons with null effects needed to reduce the observed d to a negligible level:

$$N_{fs} = n \frac{\bar{d} - \bar{d}_s}{\bar{d}_s - \bar{d}_{fs}},$$

where n is the number of treatment v. control comparisons, \bar{d} is the weighted mean d of comparisons, \bar{d}_s is the desired minimal mean d and \bar{d}_{fs} is the mean d of additional comparisons.

Results and discussion

Soyabean meal

Of ninety-nine studies presenting information on the influence of replacement of dietary fish meal by defatted soyabean meal on fish growth, 47% were found suitable for inclusion in a meta-analysis (Table 1). Absence of a diet without any soyabean meal, which could serve as a true control group for calculation of an effect size, was the single factor resulting in the highest amount (n 18) of rejected studies.

Comparisons of the replacement of fish meal by soyabean meal at different levels without dietary supplementation of amino acids, extracted from different studies and coded as trials, are presented in Table 2^(11,12,15–17,21–27,29,30,32,33,37–39,41,43,46,52–61).

A total of 67% of trials evaluated carnivorous species, with separation according to water type (fresh v. salt) and water temperature (cold v. warm). Only one saltwater omnivorous species (sharpnout seabream; *Diplodus puntazzo*)⁽⁴⁶⁾ has been included, and all omnivorous species had been reared in warm ($\geq 20^\circ\text{C}$) water. Evaluation periods varied from

33 to 182 d, although 80% of trial periods were between 8 and 12 weeks. Dietary crude protein levels, converted, if possible, to dry weight when presented on a wet weight basis, varied from 250 to 612 g/kg. Fish meal replaced included brown, Chilean, menhaden, Norwegian, Peruvian and white sources. However, information on the processing status of soyabean meal evaluated was extremely limited. Available data indicated the ranges of crude protein and lipid of fish meal evaluated as 614–750 and 35–152 g/kg, respectively, with 448–544 and 10–141 g/kg, respectively, reported for soyabean meal. In trials 14 and 15 replacement of fish meal by soyabean meal presented higher ($P < 0.05$) specific growth rate values than the fish meal control diet. This could probably be related to the quality of the fish meal used^(21,23).

In the calculation of Hedges' d , referred to as effect size hereafter, at individual replacement levels (Fig. 1), comparisons from trials 1, 5, 15, 16 and 34 (Table 2) were excluded due to the absence of a measurement of dispersion of the means. Although effect size can be calculated from P values if the direction of the finding is known, P values in the above five trials were reported as less or more than a number. Such significance levels are often treated as if they were an exact P value (0.05) if $P < 0.05$, with effect size set to zero if results are reported as non-significant ($P > 0.05$). However, doing this causes poor estimates⁽⁶²⁾, and so was omitted in the present study. An additional trial⁽⁶³⁾, which evaluated inclusion (76, 117, 153, 194, 270 g/kg) of toasted solvent-extracted soyabean meal as replacement (12, 18, 24, 30, 42%) for low-temperature dried fish meal in diets (958–962 g/kg crude protein) with Atlantic salmon (fish size: 280 g) over a 60 d period, was included in the meta-analysis. Although this trial did not indicate significance levels among individual replacements, it presented a pooled SEM. Estimation of an effect size failed in trial 20 due to SD values of 0.0.

Limited values and overlapping of 95% CI demonstrated no gain in separation of species according to feeding habits (Fig. 1). Furthermore, dietary crude protein levels, which could be categorised according to feeding habit (carnivorous, 360–612 g/kg; omnivorous, 225–433 g/kg; Table 2) in the present study, were not linearly related to effect size (Fig. 2), as illustrated by a weighted Pearson correlation coefficient (r) of 0.1334 (95% CI $-0.0934, 0.3471$; $P = 0.2474$).

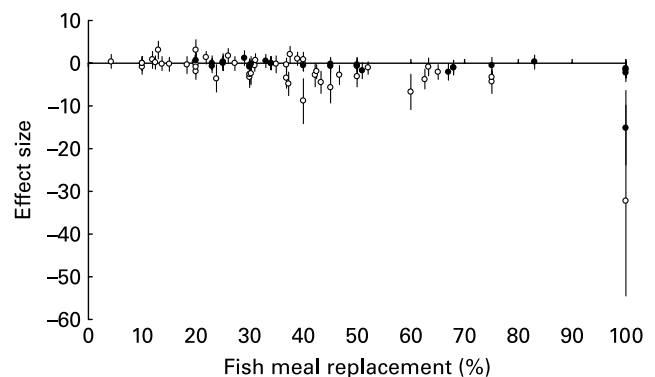


Fig. 1. Effect sizes (Hedges' d , as defined in the Data analysis section) for growth with 95% CI as influenced by level of fish meal replacement by defatted soyabean meal for carnivorous (○; n 52) and omnivorous (●; n 25) fish species.

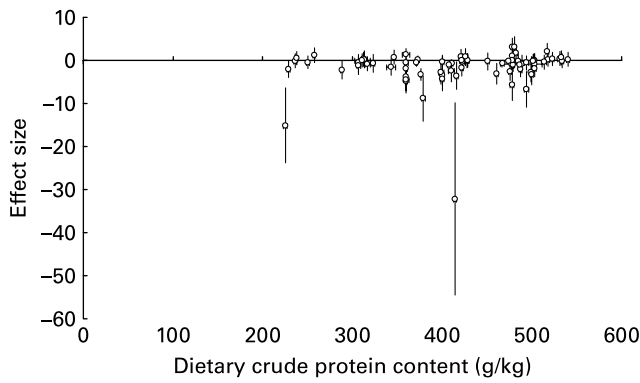


Fig. 2. Effect sizes (Hedges' *d*, as defined in the Data analysis section) for growth with 95% CI when replacing fish meal by defatted soyabean meal as influenced by dietary crude protein levels (*n* 77).

This eliminated the suggestion⁽³⁾ that dietary crude protein level, despite some contradictory results, might have an influence on the effect of replacement of fish meal by soyabean meal.

Figure 1 illustrates that the influence of fish meal replacement level prevented the calculation of a cumulative mean effect size across all levels. In addition, effect sizes did not follow a distinct trend with increasing replacement levels. The absence of a strong linear relationship was displayed by a weighted Pearson *r* of -0.4271 (95% CI -0.5943 , -0.2246 ; $P=0.0001$). This presented an R^2 value of 0.1824 , with little of the variation explained by a linear model, and little predictive value.

However, according to their distribution (Fig. 1), effect sizes tended to be grouped into three replacement level categories: 4–40%, with several mean effect sizes higher than 0 and most 95% CI overlapping with zero; 42–83%, with all mean values less than 0 and limited overlapping of 95% CI with zero; and 100% with values, although limited (*n* 5), including extremes. Trials presenting effect sizes that deviated to a large extent from zero in the 4–40% replacement category included: 37% fish meal replacement evaluated with hybrid striped bass in trial 7 (-4.8717 ; 95% CI -7.6320 , -2.1115), and 40% replacement with rainbow trout in trial 36 (-8.8314 ; 95% CI -14.0782 , -3.5847).

As mentioned above, factors related to ingredients, diet, fish species and rearing might have an influence on the outcome of dietary fish meal replacement by soyabean meal. With information on these sources of variability seldom reported, and all sources of variation most often unidentified, the logic of the analysis in the present study was that effect sizes have been sampled from a distribution of effect sizes with a true

effect that could vary from study to study. Therefore a random-effects model was the appropriate model to compute the mean of the effect sizes⁽⁶⁴⁾. Mean effect sizes for different fish meal replacement categories are presented in Table 3. To be compatible with further comparisons, categories were classified as 4–40, 41–95 and 100%. This strategy should not be confounded with subgroup analysis, which can be described as an analogue of the ANOVA⁽⁶⁵⁾, and is used to identify heterogeneity among studies when fitting a fixed-effects model.

Interpretation of effect sizes is controversial, but the most accepted opinion is that of Cohen⁽⁶⁶⁾, who proposed values of 0.2, 0.5 and 0.8 to be considered as indicative of small, medium and large standardised effect sizes, respectively, in social sciences. However, biological importance is more objective than practical or clinical importance in which subjective judgements are needed, and biologists should evaluate effect sizes according to their hypotheses⁽⁶⁷⁾. In the present study an effect size was considered as statistically significant from no effect (0) at the 5% level (two-tailed) if the 95% CI did not overlap with zero⁽⁶⁸⁾. According to the above, growth obtained with diets in which 4–40% of fish meal (inclusion levels of 150 to 756 g/kg) was replaced by soyabean meal (inclusion levels of 71 to 366 g/kg) did not differ from growth when feeding a fish meal control diet (Table 3). However, with an upper 95% CI of -1.1625 , fish meal replacement at 41–95% caused a cumulative effect size substantially different from zero. The effect size calculated for 100% fish meal replacement should be treated with caution, as it becomes impossible to estimate the between-trials variance with any precision when sample sizes become limited⁽⁶⁴⁾.

Due to most studies evaluating the effect of soyabean meal inclusion at several fish meal replacement levels, the occurrence of the tendency to only publish positive results causing publication bias, the so-called 'file drawer problem'⁽⁶⁹⁾, is unlikely to have had any importance in the present study. However, a N_{fs} was calculated to estimate the robustness of each cumulative effect size, with \bar{d}_s chosen as -0.2000 and \bar{d}_{fs} as 0 ⁽⁷⁰⁾. With a mean effect size of -0.1142 , calculation of the number of unpublished comparisons with null effects to reduce the observed effect size to -0.2000 was irrelevant for replacement of fish meal at 4–40%. However, with replacement of 41–95% fish meal, 183 additional studies with an effect size of 0 would reduce the mean effect size to -0.2000 . With N_{fs} considered as strong if greater than $5n + 10$, with *n* the original number of studies⁽⁷¹⁾, the above value illustrates the stability of the latter calculated mean effect size. Although seventy-four null effects would be

Table 3. Mean effect sizes* for fish meal replacement by defatted soyabean meal at different levels

Fish meal replacement (%)	Without amino acid supplementation			With amino acid supplementation		
	<i>n</i>	Effect size	95% CI	<i>n</i>	Effect size	95% CI
4–40	48	-0.1142	$-0.4665, 0.2382$	29	-0.1512	$-0.6125, 0.2354$
41–95	24	-1.7282	$-2.2942, -1.1625$	35	-0.8563	$-1.3287, -0.3838$
100	5	-3.1614	$-5.8824, -0.4405$	7	-3.4988	$-5.6241, -1.3735$

* Hedges' *d* (as defined in the Data analysis section).

Table 4. Highest levels of defatted soyabean meal inclusion and fish meal replacement, with dietary supplementation of methionine, at which growth obtained did not differ from that with a fish meal control diet ($P > 0.05$), and trials where soyabean meal inclusion together with supplemented methionine could not maintain a similar growth to a fish meal control diet ($P < 0.05$)

Fish species															
Trial number	Common name	Scientific name	Feeding habit	Water type	Water temperature	Initial size (g)	Period (d)	Dietary crude protein (g/kg)	Soyabean inclusion level (g/kg)	Fish meal replacement level (%)	Methionine (%)	Soyabean (g/kg)	Fish meal (%)	Unit	Reference
<i>P</i> > 0.05															
40	Atlantic cod	<i>Gadus morhua</i>	C	S	CO	534, 1750	84	549–570	246	21	1.60	246	21	SGR	72
41	Atlantic salmon	<i>Salmo salar</i>	C	F	CO	46.6	63	413–419	204, 273	25, 33	0.30, 0.50	273	33	TWG	73
42*	Atlantic salmon	<i>Salmo salar</i>	C	S	CO	923	300	391–426	170, 340	18, 38	2.00, 2.70	170	18	TWG	74
43	Common carp	<i>Cyprinus carpio</i>	O	F	W	307	51	276	186	43	0.16	186	43	TWG	75
44†	Hybrid striped bass	<i>Morone saxatilis/chrysops</i>	C	F	W	5	84	350	157, 340, 440	24, 51, 65	0.20, 0.30, 0.40	157	24	TWG	44
45	Hybrid striped bass	<i>Morone saxatilis/chrysops</i>	C	F	W	12	70	360	350, 400	47, 51	0.20, 0.30	400	51	WG	26
46†	Hybrid striped bass	<i>Morone saxatilis/chrysops</i>	C	F	CO	100–150	84	350	157, 340, 440	24, 51, 65	0.20, 0.30, 0.40	440	65	TWG	44
47†	Hybrid striped bass	<i>Morone saxatilis/chrysops</i>	C	F	W	200	98	350	340	51	0.30	340	51	TWG	44
48‡	Japanese flounder	<i>Paralichthys olivaceus</i>	C	S	W	5	56	498–504	177, 265, 354	20, 30, 40	0.50, 0.50, 0.50	265	30	SGR	38
49‡	Korean rockfish	<i>Sebastes schlegeli</i>	C	S	W	2.5	56	480–490	229, 344, 458	30, 45, 60	0.50, 0.50, 0.50	229	30	SGR	12
50	Milkfish	<i>Chanos chanos</i>	O	S	W	4	56	300	217, 441, 658	33, 67, 100	0.31, 0.43, 0.65	441	67	WG	42
51	Milkfish	<i>Chanos chanos</i>	O	S	W	4	56	400	290, 588, 877	33, 67, 100	0.29, 0.48, 0.87	588	67	WG	42
52§	Red drum	<i>Sciaenops ocellatus</i>	C	S	W	4.9	56	395–401	660, 696, 733	90, 95, 100	0.78, 0.81, 0.84	660	90	WG	45
53	Red drum	<i>Sciaenops ocellatus</i>	C	S	W	8.8	56	392–400	666, 740	90, 100	0.49, 0.54	666	90	WG	45
54	Rohu	<i>Labeo rohita</i>	O	F	W	4	70	345–350	261, 379, 497, 615	40, 60, 80, 100	0.30, 0.30, 0.50, 0.50	615	100	SGR	37
55	Silver seabream	<i>Rhabdosargus sarba</i>	C	S	W	1.5	60	398–405	210, 420, 630	26, 50, 74	0.50, 0.60, 0.80	210	26	SGR	76
56	Southern catfish	<i>Silurus meridionalis</i>	C	F	W	19.7	56	480–500	347, 463	39, 52	(0.12, 0.26), (0.21, 0.33)	463	52	SGR	39
57	Sunshine bass	<i>Morone chrysops/saxatilis</i>	C	F	W	9.2	56	400	559	75	0.30, 0.55	559	75	WG	33
58	Tilapia hybrid	<i>Oreochromis niloticus/aureus</i>	O	F	W	1.2	60	229–237	174	30	0.20	174	30	WG	29
59	Tilapia hybrid	<i>Oreochromis niloticus/aureus</i>	O	F	W	1.2	60	311	231	30	0.26	231	30	WG	29

Fish meal replacement by soyabean products

Table 4. Continued

Fish species															
Trial number	Common name	Scientific name	Feeding habit	Water type	Water temperature	Initial size (g)	Period (d)	Dietary crude protein (g/kg)	Soyabean inclusion level (g/kg)	Fish meal replacement level (%)	Methionine (%)	Soyabean (g/kg)	Fish meal (%)	Unit	Reference
60	Tilapia hybrid	<i>Oreochromis niloticus/ aureus</i>	O	F	W	4.5	56	230–240	184, 373, 557	33, 67, 100	0.15, 0.30, 0.45	373	67	WG	30
<i>P</i> <0.05															
61	Atlantic salmon	<i>Salmo salar</i>	C	S	CO	186	68	470–471	175	21	0.13	175	21	SGR	77
62	Atlantic salmon	<i>Salmo salar</i>	C	F	CO	96.3	48	427–433	314	36	0.40	314	36	SGR	78
63	European sea bass	<i>Dicentrarchus labrax</i>	C	S	W	188	100	488–507	480	50	0.20	480	50	SGR	41
64	Hybrid striped bass	<i>Morone saxatilis/ chrysops</i>	C	F	W	9.6	70	360	600, 730	82, 100	0.20, 0.30	600	82	WG	26
65¶	Rainbow trout	<i>Oncorhynchus mykiss</i>	C	F	CO	50.7	63	490	600	63	0.50	600	63	SGR	32

C, carnivorous; S, salt water; CO, cold water (< 20°C); SGR, specific growth rate (%); F, fresh water; TWG, total weight gain (g); O, omnivorous; W, warm water (≥ 20°C); WG, weight gain (%).

* Fish meal control diet supplemented with 1.2% DL-methionine.

† Fish meal control diet supplemented with 0.1% DL-methionine.

‡ Plus L-lysine.

§ Fish meal control diet supplemented with 0.2% L-methionine.

|| Not included in meta-analysis.

¶ Plus arginine, histidine, L-lysine, threonine, tryptophan.

needed to reduce the effect size to -0.2000 at 100 % fish meal replacement, care should be practised with the interpretation of this calculated mean effect size, as described above.

Supplementation with amino acids

With defatted soyabean meal limiting in total sulfur amino acids when used in animal feeds, diets with high dietary inclusion levels of soyabean meal are often supplemented with methionine and other amino acids⁽⁶⁾. Trials that have evaluated this concept are summarised in Table 4 (12,26,29,30,32,33,37–39,41,42,44,45,72–78).

In general, methionine supplementation, varying from 0.12 to 2.70 %, has been applied at higher fish meal replacement levels (Table 4) than used when only soyabean meal was included. Five trials (trials 45, 48, 49, 54, 64) evaluated replacements with methionine supplementation together with lower replacement levels without supplementation (Table 2). Few trials included supplementation of lysine (trials 48, 49, 65) and other essential amino acids (trial 65). Methionine was included either in the DL (trials 40, 41, 42, 44, 46, 47, 56, 61, 62, 63) or L form (trials 45, 52, 53, 54, 55, 60, 64, 65), with some studies not reporting the form of methionine.

With the calculation of combined effect sizes, additional studies that did not present statistically significant differences, but supplied data suitable for meta-analysis, were included. Refstie *et al.*⁽⁷⁹⁾ replaced 68 % of fish meal by soyabean meal (inclusion level: 600 g/kg) in diets (447–467 g/kg crude protein) supplemented with 0.5 % DL-methionine to evaluate growth of 33.5 g rainbow trout over 56 d. Refstie *et al.*⁽⁸⁰⁾ included 339 g/kg hulled toasted soyabean meal together with 2.30 % DL-methionine to replace 39 % fish meal in diets (388–433 g/kg crude protein) for Atlantic salmon (fish size: 107 g) over a 55 d period. The above two studies presented growth parameters over different subperiods of the trial. However, variability associated with the dividing factor removed from standard deviations could be regained to get a pooled standard deviation over the entire period⁽⁶²⁾. Venou *et al.*⁽⁸¹⁾ presented data on the replacement (20, 30, 45 %) of fish meal by hulled soyabean meal (before and after extrusion) at inclusion levels ranging from 231–485 g/kg,

with DL-methionine supplementation at 0.20–0.30 %. These diets (470 g/kg crude protein) were evaluated with 9 and 50 g gilthead seabream over periods of 60 and 66 d, respectively.

Amino acid supplementation did not substantially change effect sizes in the 0–40 and 100 % fish meal replacement categories, compared with those obtained without supplementation. However, it caused a decrease in the mean effect size and 95 % CI in the 41–95 % group (Table 3). Trial 42, with an evaluation of growth in Atlantic salmon over a 300 d period, presented effect sizes of 1.9352 (95 % CI -0.4396 , 4.3100) and -6.3832 (95 % CI -11.2212 , -1.5452) at 18 and 38 % fish meal replacement levels, respectively.

Effect sizes obtained with and without amino acid supplementation in the same study are illustrated in Fig. 3. In trials 49, 56 and 57, with 30, 52 and 75 % fish meal replaced, respectively, methionine supplementation caused overlapping of 95 % CI with zero, compared with no overlapping without supplementation. Although 95 % CI still intersected with zero, methionine supplementation decreased the positive effect size found without supplementation in trial 56 at 39 % fish meal replacement, and to a lesser extent in trial 60 at 33 % replacement.

Different supplementation levels of DL-methionine at a constant fish meal replacement level presented similar results with Southern catfish (trial 56). However, supplementation with multiple amino acids resulted in a significantly higher growth than supplementation of only methionine and lysine in rainbow trout (trial 65). In trial 57, Keembiyehetty & Gatlin⁽³³⁾ evaluated different forms (L-, DL-, acetyl-, DL-hydroxyl analogues) of methionine at the same fish meal replacement level with sunshine bass, but did not find any significant growth differences among L-, DL- and acetyl-methionine, and a fish meal control diet.

The 95 % CI (-2.7177 , -0.3164) of the cumulative mean effect size (-1.5171) with non-supplemented diets (n 12) of trials indicated in Fig. 3 did not include zero. However, when amino acids were supplemented, the 95 % CI moved to -1.2308 to 0.0015 , with a mean effect size of -0.6146 . It should be stressed that supplemented crystalline amino acids are suggested to be prone to faster uptake and catabolism⁽⁸²⁾, and to leaching in aquatic environments⁽⁸³⁾, compared with those in intact protein.

Soya protein concentrate

Trials that replaced dietary fish meal by soya protein concentrate, produced through aqueous ethanol or methanol extraction of defatted soya flakes, with a typical crude protein content of 650–700 g/kg⁽⁸⁴⁾, are presented in Table 5 (24,25,31,34–36,40,85–89). However, crude protein content of the product used in trial 74⁽⁸⁶⁾ was indicated as approximately 900 g/kg. Soya protein concentrate has been evaluated with only seven fish species, of which two (Atlantic halibut, white sturgeon) were omnivorous, and twenty-nine from thirty-six comparisons used it to replace ≥ 50 % of fish meal.

Replacement of fish meal by soya protein concentrate caused a significant growth decrease in most trials. However, its value as a fish meal substitute was substantially increased when supplemented with amino acids (Table 5). Deng *et al.*⁽⁴⁰⁾ evaluated a mixture of amino acids, included as

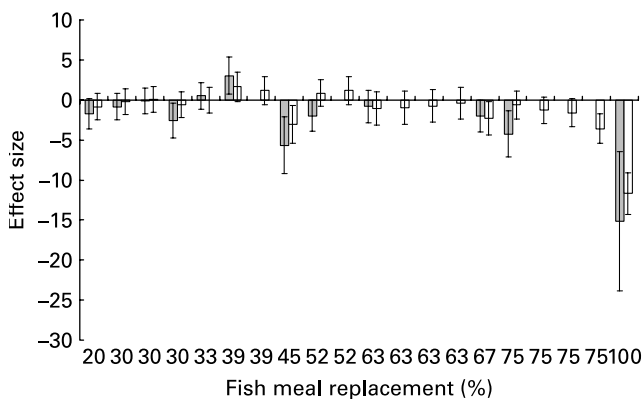


Fig. 3. Effect sizes (Hedges' d , as defined in the Data analysis section) for growth with 95 % CI when replacing fish meal by defatted soyabean meal for amino acid supplementation (\square ; n 17) compared with non-supplementation (\blacksquare ; n 12) evaluated in the same study.

Table 5. Highest levels of soya protein concentrate (SPC) inclusion and fish meal replacement, with and without dietary supplementation of methionine, at which growth obtained did not differ from that with a fish meal control diet ($P>0.05$), and trials where soya protein concentrate could not maintain a similar growth than a fish meal control diet ($P<0.05$)

Fish species														
Trial number	Common name	Scientific name	Feeding habit	Water type	Water temperature	Initial size (g)	Period (d)	Dietary crude protein (g/kg)	SPC inclusion level (g/kg)	Fish meal replacement level (%)	SPC (g/kg)	Fish meal (%)	Unit	Reference
Without methionine supplementation														
$P>0.05$														
66*	Rainbow trout	<i>Oncorhynchus mykiss</i>	C	F	CO	83	84	458–465	220	35	22	35	DGC	31
67*	Rainbow trout	<i>Oncorhynchus mykiss</i>	C	F	CO	97	33	393–419	N	50, 75, 100	N	75	SGR	34
68	Rainbow trout	<i>Oncorhynchus mykiss</i>	C	F	CO	106	90	393–420	320, 490, 635	52, 76, 100	320	52	DGC	35
$P<0.05$														
69	Gilthead seabream	<i>Sparus aurata</i>	C	S	W	1.6	84	441–452	236	35	236	35	SGR	25
70	Gilthead seabream	<i>Sparus aurata</i>	C	S	W	12.1	56	491–507	200, 400, 725	27, 54, 100	200	27	TWG	85
71	Japanese flounder	<i>Paralichthys olivaceus</i>	C	S	W	2.4	54	489–495	159, 318, 477, 557, 635	25, 50, 75, 88, 100	159	25	SGR	40
72	Rainbow trout	<i>Oncorhynchus mykiss</i>	C	F	CO	2.6	182	402–419	570	100	570	100	TWG	24
73	Turbot	<i>Scophthalmus maximus</i>	C	S	CO	7.4	56	500	441	60	441	60	SGR	36
74	White sturgeon	<i>Acipenser transmontanus</i>	O	F	CO	2	56	421–445	406	100	406	100	WG	86
With methionine supplementation														
$P>0.05$														
75	Atlantic halibut	<i>Hippoglossus hippoglossus</i>	O	S	CO	631	84	472–482	280	39	280	39	SGR	87
76	Atlantic salmon	<i>Salmo salar</i>	C	S	CO	231	84	496–508	500	75	500	75	SGR	88
77*†	Rainbow trout	<i>Oncorhynchus mykiss</i>	C	F	CO	12	70	466–494	159, 318, 477, 637	25, 50, 75, 100	318	50	WG	89
78*	Rainbow trout	<i>Oncorhynchus mykiss</i>	C	F	CO	83	84	427–465	420, 620	67, 100	620	100	SGR	31
79	Turbot	<i>Scophthalmus maximus</i>	C	S	CO	13	56	500	185, 365, 550, 735	25, 50, 75, 100	185	25	SGR	36
$P<0.05$														
80‡	Japanese flounder	<i>Paralichthys olivaceus</i>	C	S	W	2.5	54	496	447	77	447	77	SGR	40
81§	Japanese flounder	<i>Paralichthys olivaceus</i>	C	S	W	2.5	54	496	447	77	447	77	SGR	40
82*	Rainbow trout	<i>Oncorhynchus mykiss</i>	C	F	CO	97	33	393–422	N	100	N	100	SGR	34
83	Rainbow trout	<i>Oncorhynchus mykiss</i>	C	F	CO	106	90	393–420	634	100	634	100	DGC	35
84¶	Rainbow trout	<i>Oncorhynchus mykiss</i>	C	F	CO	106	90	393–420	634	100	634	100	DGC	35

C, carnivorous; F, fresh water; CO, cold water (< 20°C); DGC, daily growth coefficient; N, not indicated; SGR, specific growth rate (%); S, salt water; W, warm water (≥ 20°C); TWG, total weight gain (g); O, omnivorous; WG, weight gain (%).

* Not included in meta-analysis.

† Plus L-lysine and L-threonine.

‡ Plus mixture of L-leucine, L-lysine, DL-methionine, L-threonine, L-valine as crystalline amino acids.

§ Plus mixture of L-leucine, L-lysine, DL-methionine, L-threonine, L-valine as cellulose acetate-encapsulated amino acids.

|| Supplemented with 0.22% DL-methionine.

¶ Supplemented with 0.42% DL-methionine.

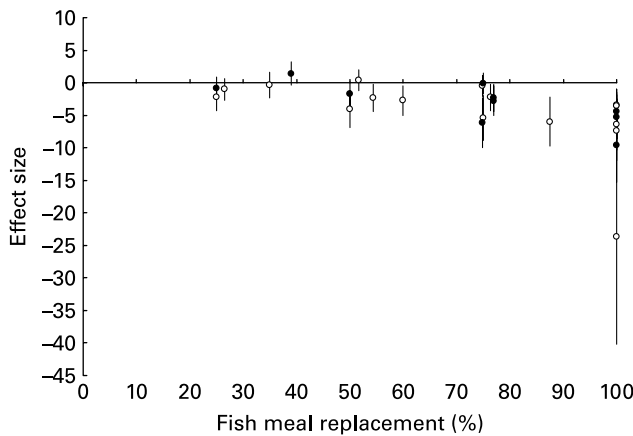


Fig. 4. Effect sizes (Hedges' d , as defined in the Data analysis section) for growth with 95% CI as influenced by level of fish meal replacement by soya protein concentrate without (\circ ; n 16) and with (\bullet ; n 10) amino acid supplementation.

either a crystalline amino acid mixture (trial 80) or encapsulated by cellulose acetate phthalate (trial 81), at a similar fish meal replacement level, but did not find any significant differences in the growth of Japanese flounder between treatments. Methionine supplementation at 100% fish meal replacement decreased ($P < 0.05$) growth compared with a fish meal control diet in rainbow trout (trials 82, 83, 84). However, at lower replacement levels in the latter studies soya protein concentrate without amino acid supplementation resulted in similar growth between diets (trials 67, 68).

The absence of a measurement of variance eliminated trials 66, 67, 77, 78 and 82 (Table 5) from the calculation of effect sizes (Fig. 4). An additional trial⁽⁹⁰⁾, which indicated statistical significance for differences in specific growth rate of Atlantic salmon (fish size: 106–111 g) over different phases of a 84 d period, was included in the calculation of effect sizes. In the latter study soya protein concentrate (inclusion level: 480 g/kg) replaced 75% of low temperature dried fish meal in diets with crude protein levels of 430–457 g/kg.

Limited values and non-significant weighted Pearson r 's between effect sizes and replacement levels found without ($r = -0.1055$; 95% CI $-0.5714, 0.4118$; $P = 0.6973$; n 16) and with ($r = -0.4541$; 95% CI $-0.8428, 0.2459$; $P = 0.1874$; n 10) amino acid supplementation eliminated any further evaluation of relationships. Cumulative mean effect sizes did not differ substantially between trials without (-2.7306 ; 95% CI $-3.7991, -1.6620$) and with (-2.4373 ; 95% CI $-3.9004, -0.9742$) amino acid supplementation, and 95% CI did not overlap with zero in either.

The evaluation of effect sizes obtained with fish meal replacement by other soyabean products, for example, full-fat soyabeans and soya flour, was prevented by a lack of studies presenting appropriate values, as illustrated in Table 1.

Conclusions

The present study quantified the magnitude and precision of the effect caused by the replacement of dietary fish meal by soyabean products on fish growth. The absence of

standardisation in units for measurement of growth in fish resulted in the application of Glassian meta-analysis, based on standardised effect sizes calculated between a control (fish meal) and treatment (fish meal replacement) diet. An important contribution from the study could be ascribed to the identification of deficiencies in reporting of results. Failure to report a measurement of variation, as found with numerous studies evaluated for inclusion, rendered results unsuitable for meta-analysis. Standardising in experimental protocol regarding, among others, replacement levels, evaluation period, measurement units and reporting of variance, are of utmost importance for evaluation of trends with information supplied by different studies.

Data used in the current study presented evidence that the effect of the replacement of fish meal by defatted soyabean meal did not display a definite trend with replacement level. However, replacement of up to 40% fish meal caused similar growth to that obtained with diets based solely on fish meal as a protein source, irrespective of dietary protein content, in a wide range of fish species. Amino acid supplementation of diets, mostly as crystalline methionine, aided in decreasing the negative effect caused by the replacement of fish meal at levels higher than 40%. Despite the fact that the above has been indicated by narrative reviews, it was based on summative results obtained with null hypothesis significance testing in individual studies. With limited replicates, as often is encountered in fish nutrition studies, the latter testing technique has low statistical power to detect differences, and gives no indication of the size of differences. The present study is the first to put numerical values to the above differences, and to indicate the direction of effects as obtained across studies.

Evaluation of the influence of fish species, and the influence of stratification of fish species according to feeding habit, water type and water temperature on growth differences due to the replacement of fish meal by soyabean products, are hampered by a lack of suitable values for analysis. A similar lack of values prevented searching of trends at replacement levels higher than 40%. Further research in order to provide results suitable for meta-analysis is urgently needed.

Baseline values are presented in the current study for the magnitude of effect sizes due to replacement of fish meal with soya products, which could be utilised not only in further meta-analyses, but also for comparative purposes in research on individual fish species. Furthermore, the present study illustrates the use of Glassian-based meta-analytic techniques to quantify responses in studies on fish nutrition.

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J. S. was the only contributor to all (research, analysis, writing, etc) of the paper.

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