Evaluation of the net energy value of glucose (cerelose) and maize starch in diets for rainbow trout (*Salmo gairdneri*)

BY JOHN W. HILTON[1], JAMES L. ATKINSON[2] AND STANLEY J. SLINGER[1]

[1] *Department of Nutritional Sciences and* [2] *Department of Animal and Poultry Science, University of Guelph, Guelph, Ontario N1G 2W1, Canada*

(*Received 11 September 1986 – Accepted 8 June 1987*)

1. Quadruplicate groups of rainbow trout (*Salmo gairdneri*) (mean body-weight 24·9 g) were reared on six dietary treatments (practical-type diets) in a modified paired-feeding experiment for 12 weeks at 15° to determine the net energy (NE) value of starch and glucose to rainbow trout.

2. Three test diets were prepared to contain (g/kg): 0 supplemented carbohydrate (diet 1), 250 maize starch (diet 2) and 250 glucose (diet 3) and were given *ad lib.* to the trout with the feeding rate of the glucose- and starch-fed groups being monitored after each feeding. The remaining three treatments involved controlled feeding of the trout with diet 1 at 75% of the feed intake of trout reared on diets 2 and 3, so as to provide the same levels of protein and lipids without carbohydrate, and with diet 2 at 100% of the feed intake of trout reared on diet 3.

3. The difference in the final carcass energy of the *ad lib.*-fed group and the respective controlled-fed group divided by the amount of dietary glucose or starch energy consumed by the trout is the NE value for that carbohydrate.

4. The determined NE value of glucose was 3·99 kJ/g and starch 2·17 kJ/g, which is 24·6 and 12·6% respectively of the gross energy values of these carbohydrates in rainbow trout.

5. The results indicate that digestible energy and calculated metabolizable energy values for carbohydrates in rainbow trout overestimate the utilizable energy content of the diet.

6. The determined NE values for glucose and starch in the present study should be used with caution since various factors (such as the feeding rate determined in the present study) may affect the utilization of dietary carbohydrates in rainbow trout.

The natural diet of a carnivorous fish such as the rainbow trout (*Salmo gairdneri*) contains little carbohydrate and therefore the ability of the trout to utilize this source of dietary energy may be limited. Nevertheless, when formulating commercial diets, it has been deemed necessary, and desirable as a cost-effective measure, to have a significant amount of carbohydrate in the diet. However, there is considerable controversy regarding the optimum level of digestible carbohydrates in a salmonid diet. Research in our laboratory (Hilton & Atkinson, 1982) has indicated that digestible carbohydrates in excess of 140 g/kg diet cannot be efficiently utilized by salmonids, which is consistent with the results of Phillips *et al.* (1948). However, this conclusion is in contrast to the conclusions of Buhler & Halver (1961), Luquet (1971) and Bergot (1979) who suggest that levels of 200 g/kg diet or higher are effectively utilized.

There is also some debate as to the energy value assigned to carbohydrates in a fish diet (Jobling, 1983). For salmonids the digestibility of different types of carbohydrates varies with complexity of carbohydrate structure (Singh & Nose, 1967; Phillips, 1969; Smith, 1971), concentrations in the diet (Takeuchi *et al.* 1979; Bergot & Breque, 1983; Spannhof & Plantikow, 1983), source and diet processing (Hilton *et al.* 1981; Hilton & Slinger, 1983), and the species of fish (Jobling, 1983). Furthermore, test diets with approximately the same digestible energy (DE) content and level of protein, but with varying amounts of glucose or fat, do not always produce the same growth results and carcass composition in trout (Hilton & Atkinson, 1982; Beamish *et al.* 1986). Therefore, the use of DE values as they pertain to carbohydrates may overestimate the utilizable or productive energy content of the diet. Similarly, the use of calculated metabolizable energy (ME) values of different

carbohydrates is fraught with errors and assumptions which makes their use very unreliable (Jobling, 1983). Furthermore, the procedures used in determining actual ME values for feedstuffs or diets (force-feeding, restraint, etc.) may result in stress and negative nitrogen balance which reduces the usefulness of this measurement (Cho *et al.* 1982). In addition, the use of fish metabolism chambers to determine ME values of feedstuffs can only be applied to large fish (165–530 g, Smith, 1971). Nevertheless, there is a need to determine the productive energy value of different types of carbohydrates in commercial trout diets.

In the past, poultry nutritionists have used productive energy measurements as an estimate of the net energy (NE) in order to describe the utilizable energy in feedstuffs ((US) National Research Council, 1981). Fraps & Carlyle (1942) defined productive energy as the energy stored as fat and protein from the portion of the ration eaten which exceeds the quantity used for maintenance purposes. Presumably, the amount of energy stored as glycogen is not considered to be a significant store by this definition, and in any case would also be measured by bomb calorimetry of the final carcass. Therefore, it should be possible to estimate the NE value of carbohydrates by measuring the extra energy retained by fish consuming diets which have the same nutrient composition with the exception of the carbohydrate content.

The purpose of the present study was to estimate the NE value of glucose (cerelose) and raw maize starch in a trout diet when included at a level of 250 g/kg diet.

METHODS

Experimental design

A modified paired-feeding experiment involving six dietary treatments was conducted using quadruplicate groups of rainbow trout reared at 15° in a completely randomized design. Three test diets formulated to contain (g/kg): 0 supplemented carbohydrate (diet 1), 250 raw maize starch (diet 2) and 250 glucose (cerelose, diet 3), were given *ad lib.* to the trout with the feed intake of trout reared on the starch (diet 2) and glucose (diet 3) diets being monitored after each feeding. The remaining three dietary treatments involved controlled feeding of trout at levels derived from the *ad lib.* intakes. Thus, diet 1 was also given to trout at 75% of the feed intake of those reared on either diet 2 (starch, diet 1A) or diet 3 (glucose, diet 1B) thereby giving the trout similar amounts of protein and lipid as fish fed on diets 2 and 3, but without additional carbohydrate. In addition, diet 2 (starch) was also given to trout at the same feed intake as those reared on diet 3 (glucose, diet 2A). After 12 weeks on the test diets the growth variables, liver:body-weight, liver glycogen content, carcass composition and NE value of the starch and glucose were determined.

Diet formulation, processing and analysis

Three test diets were formulated as described in Table 1 and processed by steam pelleting on a laboratory pellet mill. After processing, the test diets were analysed for ash, energy, moisture and protein content as described by Horwitz (1980), lipid content as described by Bligh & Dyer (1959), glucose content as described by Hilton *et al.* (1983) and starch content as described by Clegg (1956).

Supply and maintenance of fish

Rainbow trout were obtained from a commercial fish farmer and adjusted to laboratory conditions for approximately 10 weeks. After the adjustment period, 720 fish were transferred to a twenty-four tank aquatic system. The aquaria were circular enamel-lined metal tanks (capacity 65 litres), individually aerated and thermostatically maintained at

15·3 ± 0·4° on a biological filtration system with 5–20% daily replacement water. The pH varied between 7·6 and 7·8, dissolved oxygen between 6·2 and 8·1 mg/l and total ammonia (Nessler's reagent) between 0·01 and 1·10 mg/l throughout the study. The initial weight of the fish was 2·9 g and the fish were fed three to four times per d either to satiety, as described by Hilton & Slinger (1981), or to the previously described modified paired-feeding rate. Before the start of the growth study, two fish were removed from each tank, anaesthetized with tricaine methane sulphonate (MS 222), killed, ground, freeze-dried and stored at −20° until required for analysis.

DE determination

A digestibility study was conducted as described by Hilton & Slinger (1986) in which triplicate groups of thirty fish/tank (mean body-weight 65 g) were reared on the test diets. Faeces were collected by the gravity faeces collection technique as described by Cho *et al.* (1982), freeze-dried, ground and stored in a cooler at −50° until required for analysis. The acid-insoluble ash contents for the test diets and faeces were determined as described by Atkinson *et al.* (1984) and the moisture and energy contents of the diet and faeces determined by bomb calorimetry as described by Horwitz (1980).

Growth and biochemical analysis

The trout were weighed after every 4-week period and recounted and weighed at the end of 12 weeks. Mortalities and feed consumption were noted daily and feed:gain ratios determined at the end of each period. At the end of 12 weeks and 18 h after the last feeding, four fish were sampled at random from each tank of diets 1, 2 and 3 and immediately anaesthetized with MS 222. The fish were then individually weighed, livers removed and weighed and the livers then frozen in liquid N_2 and stored at −20° until required for analysis. The glycogen content of the liver was determined as described by Murat & Serfaty (1974). A further four fish that were fasted for 24 h were then sampled at random from each tank, anaesthetized with MS 222, killed by severing the spinal cord behind the head, ground and reground with a meat grinder, frozen, freeze-dried and reground in a Waring blender and stored at −20° until required for analysis. The initial and final fish carcasses were analysed for ash, moisture, protein and energy content as described by Horwitz (1980), and lipid content as described by Bligh & Dyer (1959).

Calculation of NE

On the basis of final body-weight (Table 2), carcass composition and energy content (Table 3), the total carcass energy of the fish on the different dietary treatments was determined. The difference in total carcass energy of the trout reared on the starch or glucose diets (diets 2 and 3) and the total carcass energy of the fish in the respective modified pair-fed groups reared on the control diet (diet 1) is that supplied by the glucose or starch supplement. The amount of starch and glucose energy consumed by the fish was determined by multiplying the measured feed consumption per fish (mean of four replicates) by the starch and glucose contents of test diets 2 (starch) and 3 (glucose, Table 1) as appropriate. The assigned energy value of glucose was 15·9 kJ/g and the starch 17·2 kJ/g. The NE of glucose or starch was then calculated as described in Table 4.

Statistical analysis

The results were subjected to analysis of variance and, where applicable, differences determined at $P < 0·05$ using Tukey's Honestly Significant Difference Procedures as outlined by Steel & Torrie (1980).

Table 1. *Formulation, composition and digestible energy value of the test diets*

Diet no....	1	2	3	
Ingredients (g/kg)				
Capelin meal	466	350	350	
Soya-bean meal	267	200	200	
Wheat gluten	67	50	50	
Wheat middlings	40	30	30	
Bentonite	26	20	20	
Vitamin premix†	26	20	20	
Mineral premix†	13	10	10	
Cerelose (D-glucose)	—	—	250	
Maize starch	—	250	—	
Fish oil	95	70	70	
Analyses‡ (g/kg)	Mean	Mean	Mean	SE
Protein	493	388	385	—
Lipid	136	106	111	—
Ash	121	91	92	—
Glucose	trace	trace	242	—
Starch	51	281	31	—
Digestible energy (kJ/g)	19·8[a]	18·1[b]	18·7[c]	0·12
Calculated metabolizable energy§ (kJ/g)	12·9	11·7	14·0	—

[a, b, c] Values in horizontal rows with unlike superscript letters were significantly different: $P < 0.05$.
† As described in Hilton & Slinger (1981).
‡ Values are the means of three samples per diet expressed on a dry matter basis.
§ Based on (kJ/g) 16·3 protein, 33·4 lipid, 6·7 starch, 15·7 glucose.

RESULTS

Digestibility study

The digestibility study indicated that all three diets had significantly different DE values (Table 1) with diet 1 having the highest DE (19·8 kJ/g) and diet 2 the lowest DE (18·1 kJ/g). However, it should be noted that the determined DE value of the starch diet (diet 2) was higher than would be predicted on the basis of the theoretical digestibility of starch in this fish (16·5 kJ/g). The faeces in the present study were collected by the gravity faeces collection system and therefore the faeces could potentially be in contact with water for up to 18 h. As noted by Windell *et al.* (1978) this can significantly increase the leaching losses from the faeces, particularly if the faeces contain large amounts of partially digested carbohydrates (Spannhof & Plantikow, 1983) as in diet 2. Therefore, the determined DE value of the starch diet (diet 2) in the present study may not be an accurate assessment.

Growth and biochemical analysis

The final feed intakes of the modified pair-fed groups, diets 1A and 1B, were 75·4 and 74·8% respectively of the feed intake of trout fed on diets 2 and 3. After 12 weeks on the test diets, the trout reared *ad lib.* on the control (diet 1) and starch (diet 2) diets had a significantly higher final body-weight than trout reared on the glucose diet (diet 3, Table 2). Trout reared on the modified pair-fed control diet (diets 1A and 1B) had a significantly lower final body-weight than trout reared on the corresponding starch or glucose diets. However, trout reared on the starch-based diet fed at the same rate as that of the glucose-fed trout (diet 3) had essentially the same final body-weight (Table 2). There was no significant difference in the feed:gain ratios of the trout reared on any of the control diets (diets 1, 1A and 1B) and these were significantly lower than the feed:gain ratios of trout

Table 2. *Final body-weight, feed intake, feed:gain ratios and mortalities of rainbow trout* (Salmo gairdneri) *after 12 weeks on the test diets*†

(Values are expressed as the means of four replicates, initial body-weight 24·9 g/fish)

Diet no....	1	2	3	1A	1B	2A	SE
Nominal feeding rate	*Ad lib.*	*Ad lib.*	*Ad lib.*	75% of diet 2	75% of diet 3	100% of diet 3	—
Actual feed intake (g/tank)	4696[a]	5743[a]	4334[b]	4331[b] (75·4% of diet 2)‡	3242[c] (74·8% of diet 3)	4323[b] (99·7% of diet 3)	52·89
Final body-weight (g/fish)	179[a]	184[a]	156[b]	174[c]	142[d]	151[b]	1·86
Feed:gain	1·01[a]	1·21[b]	1·10[c]	0·98[a]	0·92[a]	1·15[b, c]	0·01
Mortalities (%)	0	1·5	2	1	0	1	—

[a, b, c, d] Values in horizontal rows with unlike superscript letters were significantly different: $P < 0·05$.
† For details of diets, see p. 454 and Table 1.
‡ Numbers in parentheses refer to the determined feeding rate of the group compared with the respective *ad lib.*-fed group.

Table 3. *Final carcass composition (g/kg), liver:body-weight (× 100) and liver glycogen content of the rainbow trout* (Salmo gairdneri) *after 12 weeks on the test diets*†

(Results expressed on a dry matter basis and are means of four replicate samples)

Diet no....	1	2	3	1A	1B	2A	SE
Feeding rate	*Ad lib.*	*Ad lib.*	*Ad lib.*	75% of diet 2	75% of diet 3	100% of diet 3	—
Protein	512	542	520	532	545	533	13·4
Lipid	404	377	409	379	374	381	16·3
Ash	47	56	47	51	50	50	2·7
Moisture	688	696	697	701	697	702	7·0
Energy (kJ/g)	29·4[a]	28·1[b]	28·8[ab]	28·2[ab]	28·3[ab]	28·4[ab]	0·26
Liver:body-weight (× 100)	1·0[a]	1·1[a]	1·5[b]	nd	nd	nd	0·05
Liver glycogen	4·2[a]	7·4[b]	13·7[c]	nd	nd	nd	0·61

[a, b, c] Values in horizontal rows with unlike superscript letters were significantly different: $P < 0·05$.
nd, not determined.
† For details of diets, see p. 454 and Table 1.

Table 4. *Calculation of the net energy (NE) of consumed starch and glucose in rainbow trout* (Salmo gairdneri)

Starch	
Final total carcass energy of starch group (diet 2)	1571·8 kJ/g
Final total carcass energy, diet 1A (modified pair-fed)	1467 kJ/g
Difference in retained carcass energy (carcass energy gained diet 2 − diet 1A)	104·8 kJ
Amount of feed consumed per fish	193 g
Amount of starch consumed per fish	48·3 g
Amount of starch-energy consumed per fish	830 kJ
NE of starch = (104·8 ÷ 830) × 100	
= 12·6% of starch energy	
= 2·17 kJ (0·52 kcal)/g starch	
Glucose	
Final total carcass energy of glucose group (diet 3)	1361·3 kJ/g
Final total carcass energy, diet 1B (modified pair-fed)	1217·6 kJ/g
Difference in retained carcass energy (carcass energy gained diet 3 − diet 1B)	143·7 kJ
Amount of feed consumed per fish	145 g
Amount of glucose consumed per fish	36·1 g
Amount of glucose-energy consumed per fish	584 kJ
NE of glucose = (143·7 ÷ 584) × 100	
= 24·6% of glucose energy	
= 3·99 kJ (0·95 kcal)/g glucose	

reared on the glucose or starch diets (diets 2, 3 and 2A). Trout reared on the starch diet (diet 2) had a significantly higher feed:gain ratio than trout reared on the glucose diet (diet 3). Trout reared on the starch diet pair-fed to the feeding rate of trout fed on the glucose diet had a feed:gain ratio intermediate to that of the *ad lib.* starch- or glucose-fed fish (diet 2A, Table 2). Mortalities were uniformly low (< 2%) and were not apparently related to either dietary treatment or feeding regimen.

Final carcass analysis indicated that the fish fed on the various test diets had essentially the same carcass composition and energy content with the exception that trout reared on the starch diet (diet 2) had a significantly lower final carcass energy than trout reared on diet 1 (Table 3). Trout reared on the glucose diet (diet 3) had a significantly higher liver weight:body-weight ratio and liver glycogen content than trout reared on either the control or starch diets (Table 3). In addition, trout reared on the starch diet (diet 2) had a significantly higher liver glycogen content than trout reared on the control diet (diet 1).

NE of starch and glucose

The calculations used to determine the NE for starch and glucose are outlined in Table 4. Based on the assumption that the gross energy of glucose was 16·2 kJ/g and starch 17·2 kJ/g, the calculated NE for glucose was 3·99 kJ/g glucose and for starch 2·17 kJ/g starch.

DISCUSSION

The results of the present study indicate that the NE of glucose and raw maize starch in a practical-type diet provided 3·99 and 2·17 kJ/g respectively to rainbow trout when included at 250 g/kg diet. The NE values were approximately 25% of the glucose and 13% of the starch gross energy values for these compounds in trout (Table 5). This partially explains

Table 5. *The gross energy (GE), digestible energy (DE), metabolizable energy (ME) and net energy (NE) of glucose and raw maize starch in rainbow trout* (Salmo gairdneri) (*kJ/g*)

	Glucose	Maize starch
GE	15·9	17·2
DE*	15·7	6·9
ME†	15·7	6·7
NE‡	3·99	2·17
NE as a percentage of GE	25·1	12·6

* Assuming that the digestibility of glucose is 0·99 and raw maize starch is 0·40.
† Phillips (1972).
‡ Present study.

why the present and previous studies in this laboratory have indicated that the determined DE or calculated ME levels of test diets, as indicated in Table 1, containing significant levels of carbohydrates overestimates the productive energy value of those diets (Hilton *et al.* 1981, 1982; Hilton & Atkinson, 1982; Beamish *et al.* 1986). Why the absorbed glucose from cerelose or raw maize starch does not supply the expected source of energy cannot be determined from the present study. However, oral glucose tolerance in the trout is poor and this indicates a poor or impaired metabolic utilization of the absorbed glucose by the trout (Palmer & Ryman, 1972; Hilton, 1982).

Although the determined NE value of glucose was higher than that of starch, the final body-weight of trout given the starch diet *ad lib.* was significantly higher than that of trout fed on the glucose diet *ad lib.* In contrast, trout fed on the control or starch diets grew equally well (Table 2). To maintain this growth rate, the fish on the starch diet ate approximately 1000 g feed/tank more than the control group. Since the digestibility of raw maize starch would be no more than 40% (Phillips, 1969; Smith, 1971), the remainder of the undigested starch could act as a bulk factor, increasing the feed intake of the trout as they sought to achieve an appropriate level of nutrient intake. Previous studies in our laboratory have indicated that increasing the undigestible bulk or fibre content of diets increases feed consumption by trout (Hilton *et al.* 1983). Similarly, Kaushik & de Oliva Teles (1985) showed that trout fed on diets containing either gelatinized or natural starch were able to grow equally well as the result of increased feed intake in those fish fed on the less digestible natural starch diet. The poorer growth of trout fed on the glucose diet compared with both the control and starch-based diets in the present study (Table 2) implies that this compensatory intake response is not always operative. If it were, fish fed on the glucose-containing diet should have grown as well as the starch-fed fish, while consuming less diet. Although they did eat approximately 1400 g feed/tank less, this was also over 460 g/tank less than the control fish consumed (Table 2) and this reduced intake contributed to depressed growth rate. A reduced feeding response in consecutive daily feedings of trout reared on diets high in available carbohydrate has been observed consistently in both the present and previous studies conducted in our laboratory (Hilton *et al.* 1981; Hilton & Atkinson, 1982; Hilton & Slinger, 1983). It has been suggested that the poor glucose tolerance and prolonged hyperglycaemia induced by such diets may affect some sort of glucostatic receptors in the trout, thus reducing appetite or feeding response. Interestingly, recent work with channel catfish has indicated that a diet containing glucose as the carbohydrate source leads to growth depression when compared with a starch-based diet (Wilson & Poe, 1987). It is obvious that we require a greater understanding of the physiological role of dietary glucose in fish.

On the basis of the results of the present study, the utilization of the DE or calculated ME values of carbohydrates as a basis for determining or calculating the utilizable energy content of a trout diet should be discontinued. However, caution should be used in applying the NE values of the glucose and starch determined in the present study. Productive energy values in poultry are not always additive (Davidson *et al.* 1957; Hill & Andersen, 1958). Furthermore, the utilization of glucose as an energy source in trout appears to depend on a number of factors such as the alternative energy sources in the diet (Hilton *et al.* 1982) and the protein content of the diet (Bergot, 1979). For example, there was no significant difference in the final body-weight or carcass composition of trout reared on diet 3 (glucose) and diet 2A, the pair-fed starch group (Table 2). The calculated NE value of the starch using the results of the pair-fed group, diet 2A, was 3·85 kJ/g starch, which is much higher than that determined for starch in the *ad lib.*-fed group (diet 2, 2·17 kJ/g starch, Table 4). Therefore, the feeding rate of the fish must affect the NE value or the utilizable energy derived from dietary carbohydrates. In addition, since the level of complex carbohydrates in a diet, the type or amount of diet, and the processing of carbohydrate may affect carbohydrate digestibility (Takeuchi *et al.* 1979; Hilton *et al.* 1981; Hilton & Slinger, 1983; Spannhof & Plantikow, 1983), these factors would also affect the NE value of the carbohydrate. Therefore, it is probable that no fixed or universally applicable NE value can be assigned to glucose or starch in the trout diet. However, further studies on the effect of dietary energy and protein content and the level of different types of carbohydrates in the trout diet are required in order to produce a range of values for the NE of specific carbohydrates in the trout.

The authors wish to thank Ms Debbie Conrad and Mr Marty Hodgson for technical support, Hoffmann–La Roche Canada for generously donating the vitamins used in the study, and the Ontario Ministry of Agriculture and Food and the Natural Sciences and Engineering Research Council for financial support.

REFERENCES

Atkinson, J. L., Hilton, J. W. & Slinger, S. J. (1984). *Canadian Journal of Fisheries and Aquatic Sciences* **41**, 1384–1386.
Beamish, F. W. H., Hilton, J. W., Niimi, E. & Slinger, S. J. (1986). *Fish Physiology and Biochemistry* **1**, 85–92.
Bergot, F. (1979). *Aquaculture* **18**, 157–167.
Bergot, F. & Breque, J. (1983). *Aquaculture* **34**, 203–212.
Bligh, E. G. & Dyer, W. G. (1959). *Canadian Journal of Biochemistry and Physiology* **37**, 911–917.
Buhler, D. R. & Halver, J. E. (1961). *Journal of Nutrition* **74**, 307–318.
Cho, C. Y., Slinger, S. J. & Bayley, H. S. (1982). *Comparative Biochemistry and Physiology* **73B**, 25–41.
Clegg, K. M. (1956). *Journal of the Science of Food Agriculture* **7**, 40–44.
Davidson, J., McDonald, I. & Williams, R. B. (1957). *Journal of the Science of Food Agriculture* **8**, 173–182.
Fraps, G. S. & Carlyle, E. C. (1942). *Texas Agriculture Experimental Station Bulletin* **625**, 1–51.
Hill, F. W. & Andersen, D. L. (1958). *Journal of Nutrition* **64**, 587–603.
Hilton, J. W. (1982). *Journal of Fish Biology* **20**, 69–78.
Hilton, J. W. & Atkinson, J. L. (1982). *British Journal of Nutrition* **47**, 597–607.
Hilton, J. W., Atkinson, J. L. & Slinger, S. J. (1982). *Canadian Journal of Fisheries and Aquatic Sciences* **39**, 1229–1234.
Hilton, J. W., Atkinson, J. L. & Slinger, S. J. (1983). *Canadian Journal of Fisheries and Aquatic Sciences* **40**, 81–85.
Hilton, J. W., Cho, C. Y. & Slinger, S. J. (1981). *Aquaculture* **25**, 185–194.
Hilton, J. W. & Slinger, S. J. (1981). *Canadian Special Publication Fisheries and Aquatic Sciences* **55**, 1–15.
Hilton, J. W. & Slinger, S. J. (1983). *Aquaculture* **35**, 201–210.
Hilton, J. W. & Slinger, S. J. (1986). *Canadian Journal of Fisheries and Aquatic Sciences* **43**, 1149–1155.
Horwitz, W. (1980). *Official Methods of Analysis of the Association of Analytical Chemists*, 13th ed. Washington, DC: Association of Official Analytical Chemists.
Jobling, M. (1983). *Journal of Fish Biology* **23**, 685–703.
Kaushik, J. & de Oliva Teles, A. (1985). *Aquaculture* **50**, 89–101.

Luquet, P. (1971). *Annales Hydrobiologique* **2**, 685–703.
Murat, J. C. & Serfaty, A. (1974). *Clinical Chemistry* **20**, 1576–1577.
National Research Council (1981). *Nutritional Energetics of Domestic Animals and Glossary of Energy Terms*. Washington, DC: National Academy of Sciences.
Palmer, T. N. & Ryman, B. E. (1972). *Journal of Fish Biology* **4**, 311–319.
Phillips, A. M. Jr (1969). In *Fish Physiology*, vol. 1, pp. 18–69 [N. S. Hoar and D. J. Randall, editors]. New York: Academic Press.
Phillips, A. M. Jr (1972). In *Fish Nutrition*, pp. 1–28 [J. E. Halver, editor]. New York: Academic Press.
Phillips, A. M. Jr, Tunison, A. V. & Brockway, D. R. (1948). *Fisheries Research Bulletin* **11**, 1–44.
Singh, R. P. & Nose, T. (1967). *Bulletin Freshwater Fisheries Research Laboratory* **17**, 21–25.
Smith, R. R. (1971). *Progressive Fish-Culturist* **33**, 132–134.
Spannhof, L. & Plantikow, H. (1983). *Aquaculture* **30**, 95–108.
Steel, R. G. D. & Torrie, J. H. (1980). *Principles and Procedures of Statistics: A Biometrical Approach*, 2nd ed, p. 633. Toronto: McGraw-Hill Book.
Takeuchi, T., Watanabe, T. & Ogino, C. (1979). *Bulletin Japanese Society of Scientific Fisheries* **45**, 977–982.
Wilson, R. P. & Poe, W. E. (1978). *Journal of Nutrition* **177**, 280–286.
Windell, J. T., Folty, J. W. & Sarakon, J. A. (1978). *Progressive Fish-Culturist* **40**, 51–55.

Printed in Great Britain