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Dietary Carbohydrate Level Influencing Feed Intake, Nutrient Utilisation and Plasma Glucose Concentration in the Rainbow Trout, *Oncorhynchus mykiss*

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Abstract: A twelve-week nutrition trial was carried out to evaluate the efficacy of different carbohydrate levels on digestibility, feed intake, growth performance, carcass and muscle composition and plasma glucose level in the rainbow trout, *Oncorhynchus mykiss*. Three test diets were formulated using extruded wheat meal (15.3, 32.2 and 43.5%) as the carbohydrate source and fed rainbow trout (Initial Body Weight: 33.8 ± 0.46 g.). These were either restricted: LCR (Low Carbohydrate Restricted), MCR (Medium-Carbohydrate-Restricted) and HCR (High-Carbohydrate Restricted); or satiation: LCS (Low-Carbohydrate Satiation), MCS (Medium-Carbohydrate Satiation) and HCS (High-Carbohydrate Satiation) respectively. The aim of applying different feeding regimes was to quantify the protein sparing effect of carbohydrate and to determine the response of rainbow trout to different levels of carbohydrates. The results showed that protein, energy and carbohydrate digestibility was reduced with increasing dietary carbohydrate levels, whilst lipid digestibility was similar all the groups. There was a significant energy substitution from the carbohydrate source in the restricted groups. However, since the maximum feeding rate was reached in the restricted regimes (MCR and HCR), a similar sparing action of carbohydrate was observed in the satiation groups (MCS and HCS). All the groups displayed good growth performance, and exhibited growth that was LCS exhibited the highest ($P< 0.05$) growth rate. MCR and MCS trout exhibited growth that was superior ($P< 0.05$) to that of the LCR, HCR and HCS treatments. The feed efficiency of all the treatments was close to 100%. The quantities of carcass and muscle protein, lipid and ash were not found to be different ($P> 0.05$). Transient hyperglycaemia was observed in trout fed a high carbohydrate diet. These results are discussed with respect to the protein sparing action of dietary carbohydrate in practical trout diets and the possible physiological effects of carbohydrates on feed intake regulation.

Key Words: Feed intake, carbohydrate, nutrient utilisation, plasma glucose level, digestibility, rainbow trout

Gökkuşağı Alabalıklarında (*Oncorhynchus mykiss*) Yem Karbonhidrat Oranının Yem Alımı, Nutrient Kullanımı ve Plazma Glukozu Üzerine Etkileri

Özet: Gökkuşağı alabalıklarında, *Oncorhynchus mykiss*, yem karbonhidrat oranlarının sindirilebilirlik, yem tüketimi, büyüme performansı, karkas ve et kompozisyonu ve plasma glukoz konsantrasyonu üzerine etkilerini araştırmak için on iki hafta süren bir besleme denemesi düzenlenmiştir. Farklı oranlarda (% 15.3, 32.2 ve 43.5) extrude buğday ununun karbonhidrat kaynağı olarak kullanıldığı üç test yemi formüle edilmiş ve alabalıklara (Başlangıç Ortalama Ağırlığı: 33.8 ± 0.46 g.) sınırlı miktarlarda [DKS (Düşük Karbonhidrat Sınırlı), OKS (Orta Karbonhidrat Sınırlı) ve YKS (Yüksek Karbonhidrat Sınırlı)] veya doyuncaya kadar [DKD (Düşük Karbonhidrat Doyum), OKD (Orta Karbonhidrat Doyum) ve YKD (Yüksek Karbonhidrat Doyum)] yedirilmiştir. Farklı besleme rejimlerinin seçilme amacı, karbonhidratların hangi oranlarda enerji kaynağı olarak kullanıldığını anlamak ve karbonhidratların protein yedekleme etkisini saptamaktı. Sonuçlara göre; protein, enerji ve karbonhidrat sindirimi yemdeki karbonhidrat oranına bağlı olarak azalmış, ancak lipid sindiriminde bütün deneme gruplarında benzer sonuçlar elde edilmiştir. Sınırlı beslenen gruplarda yem karbonhidratının önemli derecede enerji takviyesi yaptığı belirlenmiştir. Bununla beraber, sınırlı olarak beslenen balıklarda (OKS ve YKS) yem alımı maksimum yem alımına çok yakın olduğu için, doyuncaya kadar yemlenen alabalıklarda da (OKD ve YKD) benzer karbonhidrat enerjisi takviyesi gözlenmiştir. Bütün gruplar iyi bir büyüme ortaya koymuşlar; DKD balıkları maksimum büyüme oranı ($P< 0.05$), OKS ve OKD balıkları da DKS, YKS ve YKD balıklarından daha yüksek ($P< 0.05$) bir büyüme performansı sergilemişlerdir. Yem kullanım randımanı bütün gruplarda % 100 civarında gözlenmiş, karkas ve et protein, lipid ve kül değerleri de birbirine yakın olarak saptanmıştır ($P> 0.05$). Yüksek karbonhidrat içeren yemlerle beslenen alabalıklarda, plazma glukoz seviyesinin normale dönme süresinde bir gecikme belirlenmiştir. Bu sonuçlar, ticari yemlerde karbonhidratların protein enerjisini takviye olarak kullanılma avantajları ve fizyolojik yem tüketimi kontrolünde olası etkileri bakımından tartışılmıştır.

Anahtar Sözcükler: Yem tüketimi, karbonhidrat, nütrient kullanımı, plazma glukoz seviyesi, sindirilebilirlik, gökkuşağı alabalığı

Introduction

Since the rainbow trout, *Oncorhynchus mykiss,* being a carnivorous species, does not have any carbohydrate requirement per se (1, 2), carbohydrate nutrition has been considered less important than lipid nutrition in the practical diets of this fish species. However, the inclusion of a reasonable carbohydrate level in rainbow trout diets as a filler component is unavoidable. The utilisation of energy-dense diets in relation to high levels of dietary lipid (i.e. surplus lipid retention) will probably maintain the carbohydrate value because they are the only alternative source of energy. For 3 decades, there were conflicting interpretations in this field, in that some researchers (3, 4, 5, 6) recommended no more than 20% carbohydrate, whereas others claimed that 30% carbohydrate did not produce inferior growth or deterioration in health (7, 8, 9, 10). The technological improvement of carbohydrate availability by increasing the digestibility of carbohydrate ingredients plays a significant role in this, but there has been little work attempting to show to what extent energy from carbohydrate can spare protein for growth or how much lipid can be substituted to decrease the visceral fat accumulation under restricted and satiation feeding conditions. Therefore, the conflicting data and different interpretations regarding carbohydrate nutrition prompted this investigation of the influence of different dietary carbohydrate levels (15.3, 32.2 and 43.5% of extruded wheat meal) on feed intake, growth performance, carcass and muscle proximate composition and plasma glucose concentration in rainbow trout fed semi-practical diets.

Materials and Methods

Experimental Fish and Maintenance Facilities

Five hundred rainbow trout, Onchorynchus mykiss, were supplied by a local fish farm (Mill Leat Trout Farm, Ermington, Devon, UK) and were acclimatised to aquarium conditions for 3 weeks prior to the experiment. Graded batches of 40 trout (Initial Body Weight: 33.8 ± 0.46 g) were placed into duplicate 400-l fiberglass tanks within a closed, freshwater recirculation system with a parallel flow through the tanks of 6.8 l per minute at a temperature of 15 ± 0.2ºC. Approximately 20% of the system water was changed weekly to ensure the physico-chemical conditions were at an optimum level. The photoperiod was set at 12 hours light/12 hours dark (8:00 am: 8:00 pm) using fluorescent discharge lamps (480 lux) with daylight simulation.

Feeding and performance indicators

Three diets containing 15.2% (LC, low carbohydrate), 32.2% (MC, medium carbohydrate) and 43.5% (HC, high carbohydrate) extruded wheat meal were formulated (Table 1) and produced as described previously (11). Fish were fed either a restricted (LCR, low-carbohydrate-restricted; MCR, medium-carbohydrate-restricted; and HCR, high-carbohydrate-restricted) or a satiation diet (LCS, low-carbohydrate satiation; MCS, medium-carbohydrate satiation and HCS, high-carbohydrate satiation) by hand three times (09.00, 13.00 and 17.00 h) per day. The restricted regimes were designed to provide a set protein intake relative to the live weight of the fish. Therefore, allowance was made for the dilution effect of the increasing carbohydrate level in these diets. The feed provision was recorded daily throughout the 84-day trial. The trout (not anaesthetized) were weighed individually every two weeks following a 24-hour feed-deprivation period. Parameters relevant to growth, feed utilisation efficiency, and dress out and hepatosomatic index were calculated as explained in (11):

Weight Gain (%) = final weight - initial weight / initial weight x 100

Feed Efficiency (%) = weight gain (g) / feed intake x 100

Feed Intake (FI) (%) = daily feed intake (g) x 100 / biomass (g)

Specific Growth Rate (SGR) (%day^{-1}) = ln (final mean weight) - ln (initial mean weight) / experimental days x 100

Apparent Net Protein Utilisation (ANPU) (%) = final retained protein (g) - initial retained protein (g) / DP intake (g) x 100

Apparent Net Energy Utilization (ANEU) (%) = final retained energy (MJ) - initial retained energy (MJ) / DE intake (MJ) x 100

Protein Utilised kg^{-1} Growth (g) = protein intake (g) / weight gain (g) x 1000

(DE) Utilised kg^{-1} Growth (MJ) = digestible energy (DE) intake (g) / weight gain (g) x 1000

Table 1. Diet Formulation (dry matter %) and chemical composition of experimental diets.

Ingredients	Diets[1]		
	LC	MC	HC
LT Fish Meal[a]	52.6	42.8	35.0
Poultry Meat Meal[b]	12.0	9.6	8.0
Blood Meal[c]	3.0	2.4	2.0
Extruded Wheat Meal[d]	15.3	32.2	43.5
Fish Oil[e]	10.81	8.65	7.2
Vitamin/Mineral Premix[f]	2.0	2.0	2.0
∝-cellulose[g]	1.89	-	-
$Cr_2 O_3$[g]	0.4	0.4	0.4
Binderg (CMC)*	2.0	2.0	2.0
Nutrient Analysis			
Protein (% DM)	48.7	41.7	37.3
Lipid (% DM)	20.5	17.5	15.2
Ash (% DM)	10.4	8.9	7.7
Carbohydrate (% DM)	13.2	22.0	30.5
Digestible Protein (DP) (%)	43.6	34.0	30.7
Digestible Energy (DE) ($MJ\ kg^{-1}$)	20.2	17.3	16.4
DP/DE Ratio (g DP MJ^{-1} DE)	21.6	19.7	18.7

1 LC (low carbohydrate), MC (medium carbohydrate) and HC (high carbohydrate)

a. Low-Temperature Fish Meal, Norsea Mink, LT 94. Donated by Trouw Aquaculture, Wincham, Cheshire, UK.

b. Int. Feed Number, 5-03-798, Trouw Aquaculture, Wincham, Cheshire, UK.

c. Int. Feed Number, 5-00-381, Trouw Aquaculture, Wincham, Cheshire, UK.

d. Int. Feed Number, 4-05-205, Trouw Aquaculture, Wincham, Cheshire, UK.

e. Atlantic Herring Oil (7-08-048), Seven Seas, Marfleet, Hull, UK.

f. (Closed Formulation), Trouw Aquaculture, Wincham, Cheshire, UK.

g. Sigma Chemical Company, Poole, Dorset, UK.

*: Carboxymethyl cellulose

Protein Efficiency Ratio = weight gain (g) / digestible protein (DP) intake (g)

Condition Factor (%) = fish weight (g) / (fish length)^3 (cm) x 100

Dress-Out (%) = fish weight (g) - gut weight (g) / fish weight (g) x 100

Hepatosomatic Index (%) = liver weight (g) / fish weight (g) x 100

Sampling and Analytical Procedures

After the completion of experimental feeding, the fish were starved for one day and, following feeding, faecal material was stripped (12) and stored at -25ºC for further analysis. Then 10 fish were removed and stored for subsequent carcass and muscle analysis. At the end of each period, blood samples (approximately 2 ml) were obtained and the anaesthetized fish were killed quickly before and after feeding, and their length, weight, gut weight and stomach contents were recorded. The blood samples were immediately centrifuged at 6000rpm in order to obtain clear plasma. Each sample was kept frozen at -70ºC until analysis. Plasma glucose reagent was supplied by Sigma Diagnostics (Sigma Chemical Co. Ltd., Poole, Dorset, UK), and spectrophotometric assays were performed.

Random samples of 10 initial and experimental fish carcasses and muscle were dried at 105ºC in order to determine the moisture content. Crude protein was determined using the Kjeldahl method after acid digestion. Lipid analysis was performed according to (13). Ash was determined by the ignition of samples in a muffle furnace at 550ºC overnight (12 hours). These procedures were all applied according to official AOAC methods (14). Carbohydrate in the feed and in the faeces was determined according to (15). Digestibility was determined by the Cr_2O_3 technique (16) and calculated as given by (17). The energy content of the freeze-dried samples was determined in an adiabatic bomb calorimeter (Gallenkamp) (11).

Statistical Analysis

The data were subjected to an analysis of variance (ANOVA) and the multiple range test ($P<0.05$) of Duncan (18) using the statistical software package Statgraphics (Manugistics Incorporated, Rockville, MD, U.S.A.). Percentage data were arcsin transformed prior to comparison. Allometric analysis of the carcass and muscle of the experimental fish was performed using multiple regression analysis to compare the slopes as outlined by (19).

Results

Digestibility: The apparent digestibility coefficients of dry matter, protein, energy, lipid and carbohydrate were calculated for each group (Table 2) after the digestibility trial. Low-carbohydrate groups (LCR and LCS) displayed relatively high dry matter digestibility coefficients. A correlation between the low-carbohydrate and high carbohydrate treatments was also observed in that protein, energy and carbohydrate digestibility was reduced with increasing carbohydrate level. However, lipid digestibility exhibited a similar pattern in all the treatments. Some differences were detected in the restricted and satiation groups in fish fed the same diet; however, no statistical evaluation was possible since the samples were pooled from each dietary treatment.

Feed Intake: Fish fed to apparent satiation (LCS, MCS and HCS) displayed a feed intake which was more uniform and closer to the 2% bw fixed feeding level (Table 3). The low-carbohydrate-satiation (LCS) group consumed more feed than the LCR grup, but fish receiving this diet reduced their feed intake following the tenth week of the trial. Similarly, the MCS and HCS fish their decreased their feeding rate after the tenth week of the feeding trial. When the overall mean feed intake was taken into account, MCR, MCS, HCR and HCS fish fed the same exhibited similar feeding responses. It can also be stated that apart from the LCR and MCR groups, the mean feed intake of other treatments (HCR, LCS, MCS and HCS) were found to be similar (Table 3).

Growth and Nutrient Utilisation: Although the feeding responses of the above-mentioned groups were very similar, these groups displayed significant differences in growth rate (Table 4). The LCS trout showed the highest performance ($P< 0.05$). The MCR and MCS groups displayed the next-highest performance and they exhibited significantly superior growth ($P< 0.05$) in comparison to the LCR, HCR and HCS treatments. However, there was no statistical significance in the growth of LCR, HCR and HCS fish ($P> 0.05$). The specific growth rate (SGR) also supported the same view that the growth performance of the rainbow trout used in this experiment was LCS > MCR = MCS > HCR = HCS = LCR. The feed efficiency of all the groups except for HCR and HCS was found to be more than 100%, and the feed efficiency of the LCR group was the highest. This parameter was 91.6 and 92.2% for the HCR and HCS trout, respectively. The digestible protein (DP) utilised per kg^{-1} growth was between 312 (MCR) and 420 g (LCS). It was determined that apparently more protein was utilised per kg^{-1} growth in the groups fed a high protein diet (LCR and LCS). The digestible energy (DE) utilised per kg^{-1} growth lay between 15.9 (MCR) and 19.5 MJ (LCS). The apparent net protein utilisation (ANPU) of the MCR trout was the highest (53.7%), while the LCS group exhibited the lowest ANPU (41.5%). The apparent net energy utilisation (ANEU) was found to be in accordance with the ANPU parameter, in that the ANEU of the MCR fish displayed the highest value, whilst that of the LCR fish was the lowest (38.3%) (Table 4). The condition factor of the LCS trout was significantly higher ($P< 0.05$) than the other groups, but there was a significant difference between the condition factor of the LCS and MCR fish. The dress-out (%) of the rainbow trout ranged from 86.7 to 88.1%; however, no significance ($P> 0.05$) was evident. The hepatosomatic index (HSI) increased significantly ($P< 0.05$) with the carbohydrate level. Feeding strategy did not affect liver size (e.g., the HSI of the LCR and LCS fish was 1.1%, while the HSI of the MCR and MCS fish was 1.4%) (Table 4).

Table 2. Diğestibility coefficients of dietary nutrient components.*

	Restricted[1]			Satiation[2]		
	LCR	MCR	HCR	LCS	MCS	HCS
Dry Matter	83.8	76.9	77.1	86.0	71.0	69.1
Protein	88.2	84.5	85.7	90.8	78.6	79.0
Energy	89.8	84.8	78.0	91.5	82.4	76.6
Lipid	89.5	90.4	88.7	90.4	88.6	88.1
Carbohydrate	93.2	85.4	89.0	94.0	89.1	84.7

* Coefficients based on pooled sample material from each dietary treatment.

1. LCR (low-carbohydrate restricted), MCR (medium-carbohydrate restricted) and HCR (high-carbohydrate restricted).
2. LCS (low-carbohydrate satiation), MCS (medium-carbohydrate satiation) and HCS (high-carbohydrate satiation).

Table 3. Feed intake (FI) of rainbow trout (% body weight day^{-1}) fed for 12 weeks.

Week	Restricted			Satiation		
	LCR	MCR	HCR	LCS	MCS	HCS
0-2	1.7	2.0	2.3	2.4	2.3	2.2
2-4	1.7	2.0	2.2	2.1	2.2	2.0
4-6	1.5	1.6	1.9	1.9	1.9	1.8
6-8	1.5	1.8	2.1	1.9	1.8	2.1
8-10	1.4	1.6	1.9	1.9	1.8	2.1
10-12	1.4	1.7	2.0	1.6	1.4	1.6
Mean F.I.	1.5	1.8	2.1	2.0	1.9	2.0

Table 4. Growth performance of rainbow trout fed different levels of carbohydrate, either restricted or satiation for 84 days.

Parameters	Restricted			Satiation			±SEM*
	LCR	MCR	HCR	LCS	MCS	HCS	
Initial Mean Wt. (g)	34.0	33.8	33.7	33.3	33.8	33.9	0.46
Final Mean Wt. (g)	132.0^a	147.4^b	137.5^a	169.0^c	145.2b	132.7^a	5.21
Weight Increment (%)	289	337	307	407	330	292	2.80
Feed Efficiency (%)	116	108	92	104	102	92	2.73
SGR (%)	1.6	1.8	1.7	1.9	1.7	1.6	0.18
ANPU (%)	44.8	53.7	50.7	41.2	49.5	50.3	2.56
ANEU (%)	38.3	53.3	48.9	42.0	50.5	48.2	1.85
Feed Intake (bw %)	1.5	1.8	2.1	2.0	1.9	2.0	0.08
DP utilised per kg^{-1} growth (g)	374	312	335	420	333	332	3.28
DE utilised per kg^{-1} growth (MJ)	17.4	15.9	17.9	19.5	16.9	17.8	4.62
Condition Factor (%)	1.26^a	1.27^{ab}	1.26^a	1.31^b	1.23^a	1.24^a	0.02
Dress-Out (%)	88.1	87.4	86.7	88.1	86.7	87.3	0.32
Hepatosomatic Index (%)	1.1^a	1.4b	1.7c	1.1a	1.4b	1.6c	0.05

* Values in each row allocated common superscripts or without superscripts are not significantly different from each other ($P > 0.05$).

Carcass and Muscle Composition: The carcass and whole-fillet proximate compositions of rainbow trout fed different levels of carbohydrate are presented in Table 5. The carcass and muscle components (protein, lipid and ash) were not found to be significantly different between treatments ($P> 0.05$). Thus, it was observed that the body protein, lipid and ash contents of the trout were not affected by diets containing different carbohydrate concentrations or by different feeding regimes (Table 6). These results demonstrate that fish size is a necessary parameter to prevent contradictory results.

Plasma Glucose: Postprandial plasma glucose profiles displayed a characteristic example during the sampling phase. Since the glucose concentrations for the restricted and satiation regimes for each diet were almost identical, the data was pooled. The plasma glucose (mmol l^{-1}) level of the LC, MC and HC trout was elevated and reached maximum concentrations ($P<0.05$) at 4, 8 and 24h, respectively. Transient hyperglycaemia was observed in rainbow trout fed a high-carbohydrate diet, since the plasma glucose level remained significantly high ($P<0.05$) even 48 hours after alimentation.

Discussion

Digestibility: The present investigation has clarified certain aspects in the carbohydrate nutrition of rainbow trout in relation to feeding behaviour and physiology. Carbohydrate digestibility was effectively reduced by the incorporation of increasing carbohydrate levels in this experiment as previously reported by (20) and (21). This finding was also in good agreement with (22), who determined 82.1% carbohydrate and 88.5% energy digestibility coefficients in rainbow trout. However, carbohydrate digestibility was superior in the medium- (MCR and MCS) and high-carbohydrate (HCR and HCS) groups when compared to the results of (17), who determined 77.2% and 74.8% in rainbow trout diets containing 20 and 30% dextrin, respectively. Dry matter and energy digestibility also declined with the increasing level of extruded wheat in the diet. The relatively low digestibility of extruded wheat, with an approximately 30% inclusion level in rainbow trout, might be due to the absorption of amylase by starch and inhibition of the hydrolysis of the starch, as suggested by (23) and (24). It could also be explained by acceleration of the chyme transport through the intestine in order to obtain more digestible energy, thus reducing scope for hydrolysis and digestion (25, 26). In a similar manner to carbohydrate digestibility, the dietary carbohydrate level also influenced the rate of protein digestion inversely. The apparent protein digestibility coefficients were lower than those in the results reported by (8), (22), (27) and (28), who reported that the protein digestibility of wheat grain or wheat starch was between 90 and 98%. The apparent lipid digestibility, however, was not affected by the incorporation of different sources of dietary

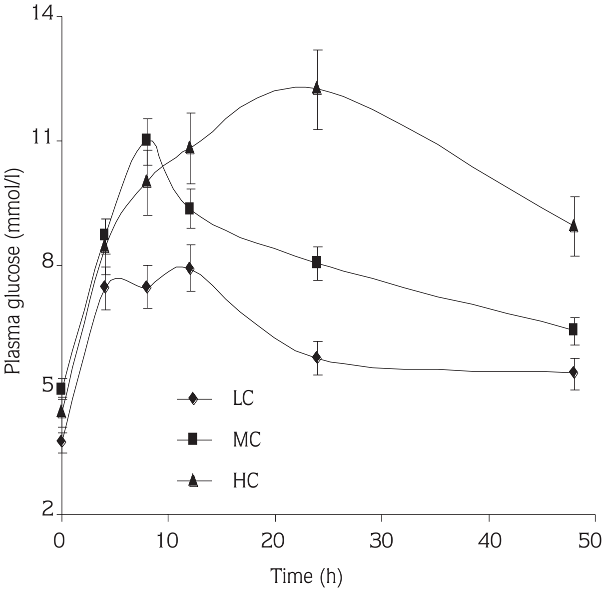

Figure 1. Postprandial plasma glucose concentration in rainbow trout fed different levels of carbohydrate.

carbohydrate as previously shown in trout (26). However, lipid digestibility was lower than in of (22).

Feed Intake: The relative feed consumption of rainbow trout fed up to 43.5% extruded wheat meal did not show a dramatic difference between satiation treatments. It also did not result in any negative effects on the physical health of fish as previously demonstrated by McLaren et al. (1974) (cited in 29) and (8). However, (30) reported that 250 g kg^{-1} extruded starch caused intracellular damage due to a surplus deposition of glycogen in the liver of rainbow trout. The hepatosomatic index increased proportionally to carbohydrate level, probably because of hepatic glycogen deposition (3, 31, 32, 33, 34, 35), although these workers did not describe the feeding response of the fish. However, it appeared that the feed intake of trout was not influenced by chemical alteration of the liver during the first ten weeks of the feeding trial. However, during the last two weeks of the experiment, the appetite of the fish could have been affected by intracellular damage due to surplus deposition of glycogen in the liver of rainbow trout as demonstrated by (30). Moreover, glucostatic receptors might have been affected in the long term (after the tenth week of the experiment). However, these factors need to be examined more closely. The mean feed intake was 2.0, 1.9 and 2.0% body weight day^{-1} in the low-carbohydrate-satiation (LCS), medium-carbohydrate-satiation (MCS) and high-carbohydrate-satiation (HCS) regimes, respectively. The similar apparent feed consumption of these groups may indicate that plasma glucose concentration may not be significantly elevated by carbohydrate level, or that plasma glucose level did not play a major role in the modulation of feed intake.

Growth and Nutrient Utilisation: Superior growth performance was observed in the low-carbohydrate-satiation regime probably because the protein and energy density of the diet was adequately balanced and, consequently, the scope for growth in the fish was near optimum (SGR: 1.9%). However, the medium-carbohydrate-satiation (MCS) and high-carbohydrate-satiation (HCS) groups grew 16.4 and 27.3% less than the LCS fish, respectively. This is probably because all the groups were fed for gastric fullness, but the carbohydrate diluted diets provided less digestible energy for maximum growth (2). This is also in agreement with the common view that high levels of carbohydrate in trout diets decrease carbohydrate digestibility (1). For example, the medium-carbohydrate (220g kg^{-1} DM) groups grew more than the high-carbohydrate (305g kg^{-1} DM) groups despite similar digestibility coefficients. It may therefore be suggested that a content of approximately 32.2% extruded wheat meal in rainbow trout diets provides a good growth performance (1.7-1.8 SGR), nutrient and energy utilisation (50% ANPU or ANEU) and digestibility under a near-to-satiation feeding regime. The growth performance (SGR) of the HCR and HCS groups fed 43.5% extruded wheat meal was superior when compared to that of (8), who fed rainbow trout diet containing 38% extruded wheat at 18ºC for 18 weeks and obtained 1.3% day^{-1} SGR. However, the dietary lipid level in that study was 8.7%, whereas that of the fish fed the high-carbohydrate diet (HC) in the present investigation was 15.2%. Therefore, dietary lipid level or lipid-carbohydrate interaction may play a role in the growth performance of trout (9). Moreover, protein and energy retention was 34.0 and 33.3% respectively in the aforementioned study, whilst the HCS group of the present investigation displayed 50.3 and 48.2% protein and energy retention efficiency, respectively. Thus, it may be suggested that optimum growth and nutrient utilisation is achieved by adjusting the dietary lipid and carbohydrate levels according to the digestible energy

Table 5. Proximate composition of the pooled carcass and muscle of experimental animals presented as a percentage of the whole fish.

Carcass	Initial	LCR	MCR	HCR	LCS	MCS	HCS	±SEM*
Moisture	72.0	70.5	70.4	68.9	69.0	70.4	70.1	0.41
Protein	15.3	16.4	16.5	16.6	16.9	16.2	16.4	0.24
Lipid	10.4	10.1	11.2	11.6	11.8	11.4	11.4	0.25
Ash	2.4	2.5	2.4	2.4	2.3	2.5	2.4	0.05
Muscle								
Moisture	77.9	72.6	72.7	72.5	72.1	72.4	72.1	0.28
Protein	16.7	18.7	18.2	17.9	18.6	17.4	18.5	0.17
Lipid	4.3	7.5	7.8	8.0	7.8	8.4	8.4	0.24
Ash	2.3	1.9	1.9	2.0	1.9	2.0	2.0	0.04

*± standard error of the pooled means (n=10). Values in each row are not significantly different from each other (P > 0.05) (see Table 6).

Table 6. Allometric analysis of carcass and muscle components of rainbow trout as explained by (19).

	Log (body protein)= a + b* Log (wt) R^2= 0.97		Log (body lipid)= a + b* Log (wt) R^2= 0.94		Log (body ash) = a + b* Log (wt) R^2= 0.78		Log (muscle pro.)= a + b* Log (wt) R^2= 0.98		Log (muscle lipid) = a + b* Log (wt) R^2= 0.82		Log (muscle ash) = a + b* Log (wt) R^2= 0.91	
	a	b	a	b	a	b	a	b	a	b	a	b
LCR	-0.76	0.99	-1.07	1.03	-1.42	0.90	-0.71	0.99	-1.44	1.16	-1.58	0.94
MCR	-0.76	0.99	-1.04	1.03	-1.42	0.90	-0.72	0.99	-1.43	1.16	-1.58	0.94
HCR	-0.76	0.99	-1.03	1.03	-1.42	0.90	-0.73	0.99	-1.41	1.16	-1.58	0.94
LCS	-0.74	0.99	-1.01	1.03	-1.42	0.90	-0.71	0.99	-1.44	1.16	-1.58	0.94
MCS	-0.77	0.99	-1.03	1.03	-1.42	0.90	-0.74	0.99	-1.39	1.16	-1.58	0.94
HCS	-0.76	0.99	-1.03	1.03	-1.42	0.90	-0.72	0.99	-1.39	1.16	-1.58	0.94
	S F= 5.1	NS F= 1.4	S F= 2.7	NS F= 0.5	S F= 0.9	NS F= 2.0	S F= 7.5	NS F= 0.7	S F= 2.5	NS F= 2.3	S F= 0.8	NS F= 0.6

S, significant; NS, non-significant

(DE) requirement of the fish under examination. The specific growth rate (SGR) of the MCR (1.8) and MCS (1.7) group in this study was lower than that (2.2%) reported by (10), who fed trout a diet which contained 33% wheat midlings. One possible reason for this is the difference in dietary lipid levels as mentioned previously.

In terms of protein sparing, the MCR (medium-carbohydrate-restricted) fish spared considerable protein for growth when compared to the LCR fish, growing 11.7% more than the LCR (low-carbohydrate-restricted) group despite having the same protein intake. Consequently, the MCR group utilised approximately 19.7% less digestible protein and 10% less digestible energy per kg^{-1} growth than the LCR group. The similar growth performances of the MCR and MCS, and of the HCR and HCS treatments may be explained by the fact that the restricted feeding regimes were near to satiation level and, consequently, these groups consumed similar amounts of feed. The final weights of the high-carbohydrate-restricted or satiation regimes and the low-carbohydrate-restricted group were not significantly different, even though the HCR and HCS groups consumed approximately 33% more feed than the LCR group. It should be noted that the low digestion efficiency of the high-carbohydrate diet resulted in higher feed intake and more faecal output (35). However, the apparent net protein utilisation of the LCR group was 12.7% lower than that of the HCR and HCS groups. This may thus suggest that a high level of dietary carbohydrate (305 g kg^{-1}) spared protein within the limits of this study. However, the protein-sparing action of such high levels of carbohydrate is open to discussion because of their reduced digestibility coefficients and utilisation efficiencies. The highest apparent net energy utilisation (ANPU) in the medium-carbohydrate-restricted groups supported this view, in that ANPU was lowest in the LCS fish, although the low-carbohydrate-satiation group exhibited the best growth performance. The estimation of the partitioning of dietary energy according to (36) suggested that non-faecal energy loss in the LCS group was the highest and contributed nearly one third of the gross energy consumed. This calculation is an indication of the lowest ANPU and ANEU of the low-carbohydrate-satiation group. However, the present estimates are not completely satisfactory. The apparent digestibility coefficients may not represent overall digestion ability since multiple meals may accelerate the chyme in the gastro-intestinal tract and consequently reduce the digestion efficiency (37, 25, 38, 23, 39).

Carcass and Muscle Composition: The dress-out (%) of the fish was not significantly different (P>0.05) between any of the groups. This was the first indication of the similar carcass composition of the experimental treatments. In contrast to (40), (33), (41) and (10), allometric analysis of the proximate composition of the carcass and muscles showed a very uniform picture in terms of the levels of protein, lipid and ash content in the all treatments. Therefore, it can be suggested that a level of complex digestible dietary carbohydrates of up to 43.5% does not affect the carcass and muscle proximate composition of trout under the present experimental conditions. It can be suggested that in general diets enriched with digestible carbohydrate and which have a digestible energy concentration of between 16.4 and 20.2 MJ kg^{-1} do not change the body composition of trout significantly.

Plasma Glucose: A temporary elevation in glucose concentration in the high-carbohydrate treatment was maintained for 48 hours following feeding. The same phenomenon in rainbow trout has also been reported widely trout (5, 42, 43). However, the glucose levels of the trout fed LC and MC diets returned to their initial values within 24 hours post feeding. Therefore, the scope of this prolonged hyperglycaemia was constant with the carbohydrate level. However, the rainbow trout did not show any lack of appetite during the study. It can be suggested that the plasma glucose level does not affect the appetite of trout in terms of the regulation of feed intake. This claim was tested in the laboratory and it was hypothesised that appetite in rainbow trout is mainly controlled by gastric evacuation rate as previously demonstrated by (44).

Regulation of Feed Intake: Regulation of the feed intake was observed in all the satiation treatments; however, a relative reduction of feed intake was observed in these groups after the tenth week of the trial. Feed intake regulation is very complex and it will be possible to improve our understanding only if we consider more factors under the same dietary conditions. Therefore, this study highlights the necessity of fully understanding the significance of dietary carbohydrate levels and energy in the regulation of voluntary feed intake in fish. For instance, a better understanding of postprandial circulating hormones could be useful to the practical fish production industry and lead to better control feed intake (45, 46, 47, 48).

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