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DIGESTIBILITY OF DIFFERENT CARBOHYDRATE SOURCES IN COD (*GADUS MORHUA*), AND ITS RELATION TO GLUCOSE CONTENT IN BLOOD AND URINE

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ABSTRACT

In a feeding experiment with cod the digestibilities of five carbohydrate containing meals were determined. Carbohydrate digestibility increased as processing of wheat meal increased, reaching a maximum level at 69 % for extruded wheat. A commercial prosessed potato-starch had about the same digestibility as raw milled wheat, while the carbohydrate of a fat-extracted soybean meal did not seem to be digested. The protein digestibility was reduced in fish fed fat-extracted soybean meal compared to other meal types. Blood glucose levels were related to the amount of carbohydrate digested, and the amount of glucose in the urine reflected blood glucose levels.

INTRODUCTION

The carbohydrate digestibility in fish depends on the dietary level and the type of complex carbohydrate. This was found for carp (*Cyprinus carpio*) by Chiou and Ogino (1975), for yellowtail (*Seriola quinqueradiata*) by Shimeno et al. (1978), for rainbow trout (*Salmo Gairdneri*) by Bergot and Breque (1983) and Ufodike and Matty (1984), and for cod (*Gadus morhua*) by Hemre et al. (1989). Several investigations have shown that wheat kernel contains amylase inhibitors which interfere with the enzyme activity in the intestine of fish (Silano et al., 1975; Hofer and Sturmbauer, 1985). Cooking and gelatinizing result in hydratization and shortening of the starch chains. Extrusion destroys the enzyme inhibitors and gives a mixture of limit-dextrins and short oligo- and disaccarides. Processing of starch by gelatinization (Bergot and Breque, 1983) or extrusion (Vens-Cappell, 1984) increases the carbohydrate digestibility in salmonids. These facts are taken in account by feed-producers either by processing the carbohydrate source alone or the complete pellet-mixture before oil is added. Slow hydrolysis of starch is found throughout the length of the intestine and glucose absorbtion takes place in the pyloric cecae and the intestine (Buddington and Diamond, 1987).

Limited availability of fish-meal for use in fish feeding may be a reality in the near future and research is in progress to evaluate alternative vegetable protein sources. However, the high fibre contents in these protein sources often may reduce the bioavailability of nutrients, particularly high contents of water-soluble fibre may reduce the digestibility of several nutrients including glucose (Utne et al., 1981; Isaksson et al., 1982; Krogdahl, 1987; Shiou et al., 1988).

Bergot (1979) found a 24 hour delay before blood glucose levels normalized in rainbow trout after a glucose load. Hilton et al. (1987) concluded, based on the amount of insulin secreted, that rainbow trout reacted diabetic-like after being fed a highly absorbable starch.

This paper reports a feeding experiment with cod, conducted to study the digestibilities of five types of carbohydrate meal produced by different processing methods. The levels of blood glucose 24 hours after feeding and the excretion of glucose in the urine were determined. The experiment was a follow-up of an earlier study on carbohydrate retention and digestibility in cod (Hemre et al., 1989).

MATERIALS AND METHODS

Fish and diets

Cod hatched and reared at the Aquaculture Station Austevoll (Institute of Marine Research, Bergen, Norway) were kept in five 1.5 m^3 tanks with 20 fish (mean weight = 150 g at start) in each tank. The fish were fed *ad lib* once a day for four weeks before sampling to ensure an active digestive process in all groups. Salinity and temperature were 30 gL^{-1} and 7° C, respectively.

Five experimental diets containing equal amounts of carbohydrate were used. The carbohydrate sources were: fat-extracted soybean meal, raw, gelatinized and extruded wheat and a commercially processed potato starch (Lygel F60 – Lyckeby International, Sweden). The fat-extracted soybean meal contained 40% carbohydrates, including water-soluble fibre and about 55% protein with active trypsin inhibitors present. The raw wheat used was milled before adding it to the rest of the feed. The diets were isonitrogenous by using squid mantle as a balancing protein source. Capelin oil was used as the lipid source, and Cr_2O_3 was added as an inert indicator for digestibility measurements. The composition of the diets is given in Table 1.

Table 1. Feed composition and analytical values (g/kg)

Group	I	II	III	IV	V
Squid mantle	59	803	803	803	847
Water	646	–	–	–	–
Capelin oil	28	27	27	27	26
Carbohydrate[a]	254	157	157	157	115
Guar-gum	10	10	10	10	10
Vitamin mixture[b]	1	1	1	1	1
Mineral mixture[c]	1	1	1	1	1
Cr_2O_3	1	1	1	1	1
Analysis					
Dry weight	370	337	347	352	297
Digestible carbohydrate	6	60	61	65	50
Protein	152	142	146	157	132
Total energy, MJ/kg	4.4	4.6	4.6	4.6	4.0

[a] I = Fat-extracted soybean meal containing 40% carbohydrates
II = Raw wheat meal (milled whole wheat)
III = Gelatinized/pre-cooked wheat meal
IV = Extruded wheat meal
V = Highly processed potato starch (Lygel F60 – Lyckeby International)
[b] Composition, see Lie et al (1988)
[c] Commercial mineral mixture for poultry and swine, Lied and Rosenlund (1984)

At the end of the experiment the cod were killed with a sharp blow on the head. For analysis of glucose, blood was collected as described by Sandnes et al. (1988) and urine was removed directly from the bladder by a medical syringe. The gastrointestinal tract was removed and divided into five segments by ligations immediately after the pyloric ceca, at the first caudal and the second cranial bend of the intestine, at the ileorectal valve, and at the anus. The segments were frozen at –20° C, and dissected for analyses of gut contents. Duplicate samples from 10 fish were prepared and analysed.

Analytical methods

Samples of feeds and gut contents were analysed for dry matter, protein and carbohydrate. Protein (Nx6.25) was analysed according to Crooke and Simpson (1971). The chromium concentration in diets and faeces samples were determined by atomic absorption spectrophotometry as described by Lied et al. (1982). Digestible carbohydrate in the diets and faeces were analysed using an enzymatic method as described by Hemre et al. (1989). Blood glucose in the urine were measured spectrophotometrically as NADPH at 340 nm after a hexokinase reaction using an automated analyser (Hemre et al., 1989). Digestibilities were calculated by the formula:

$$\text{Digestibility (\%)} = 100 - 100\ \frac{\text{\% indicator in feed} \times \text{\% nutrient in faeces}}{\text{\% indicator in faeces} \times \text{\% nutrient in feed}}$$

Statistics

Oneway analysis of variance (ANOVA) was used for statistical evaluation of digestibility, blood glucose and glucose in the urine. Linear regression analyses were carried out to correlate glucose digestion to the levels of glucose in the blood and urine.

RESULTS AND DISCUSSION

The carbohydrate in the fat-extracted soybean meal contained about 50% water-soluble fibre, which according to Krogdahl (1987) inhibits the feed digestibility in fish. Water soluble fibre is known to distend the unstirred water-layer in the intestinal muscosa, resulting in a lowered absorption of several water-soluble nutrients, particularly glucose.

Glucose from gelatinized and extruded wheat and commercially processed potato-starch were digested in both the first and the middle part of the intestine in cod, while glucose from raw wheat was digested only in the first part of the intestine (Table 2). Long starch chains exert a negative feed-back in-

Table 2: Carbohydrate digestibilities (percent) in cod given diets containing soybean meal (I), raw wheat (II), gelatinized wheat (III), extruded wheat (IV) and processed potato starch (V), (see methods section).

Group	I	II	III	IV	V
First part of the intestine	0	48	48	50	39
Middle intestinal section	0	46	53	68	46
Last part of the intestine	0	48	58	69	42
Rectum	0	45	59	62	46

Table 3. Glucose in blood plasma and in urine (mg/L)

Group	I	II	III	IV	V
Plasma	199	308	408	459	425
SD (N = 10)	81	14	17	68	14
Urine	29	42	46	48	46
SD (n = 10)	2	6	7	4	4

hibition on amylase by adsorbing the enzyme. This inhibition is not active when highly processed meals containing limit-dextrins and disaccarides are used. In other fish species carbohydrate is known to be slowly hydrolysed and absorbed all along the intestine (Buddington and Diamond, 1987). However, fat (Lie et al., 1987) and protein in processed feed (Lied and Solbakken, 1984) are fully absorbed in the first part of the intestine in cod.

The carbohydrate digestibility varied according to processing as seen from the results with raw, gelatinized and extruded wheat. A correlation between digestibility and processing may be explained by destruction of amylase inhibitors from the wheat kernel and by shortening of the starch chains (Silano et al., 1975; Hofer and Sturmbauer, 1985). Digestibility of 50–70% for the most processed meal is in accordance with findings for carp (Chiou and Ogino, 1975) and for rainbow trout (Bergot and Breque, 1983).

A difference between meal types was also seen when extruded wheat and highly processed potato-starch, and when raw wheat and fat-extracted soybean meal were compared (Table 2). This may be due to the structural differences of the carbohydrate chains, varying contents of different saccharides and interactions with other constituents in the meals. Differences in carbohydrate digestibilities of different meals have been found in several experiments with rainbow trout (Ufodike and Matty, 1984, Hofer and Sturmbauer, 1985; Degani et al., 1986).

The protein digestibility averaged 80% and did not vary between the fish groups ($p > 0.05$), except for the group given fat-extracted soybean meal in the diet ($p < 0.001$). An apparent protein digestibility of 64% was measured in this group, and may probably be explained by the amount of water-soluble fibre in the feed (Krogdahl, 1987). An average protein digestibility of 80% is fairly good according to earlier experiments with cod (Lied et al., 1982).

Blood glucose and glucose excretion in the urine

Cod given fat-extracted soybean meal had low blood glucose levels. The groups on raw, gelatinized and extruded wheat had blood glucose levels reflecting the amount of glucose absorbed ($0.001 < p < 0.01$) while the fish given a highly processed potato starch showed a somewhat lower blood glucose level (Table 3).

The regulation of blood glucose is described as delayed and diabetic-like for both rainbow trout (Bergot, 1979; Hilton et al., 1987) and coho salmon (*Oncorhynchus kisutch*) (Plisetskaya et al., 1985) and a 24 hour delay before blood glucose is back to normal after a glucose-load must be considered normal. Earlier experiments with cod showed increased blood glucose levels even 24 hours after feeding when the amount of precooked potato starch increased in the feed (Hemre et al., 1989). In the present study mean digestibility values from the middle intestine, last intestine and rectum correlated ($p < 0.01$) with the blood glucose levels measured 24 hours after feeding. These results support the hypothesis that «normal» blood glucose levels in cod depend on the amount of glucose absorbed from the diet (Hemre et al., 1989). However, the fish given fat-extracted soybean meal having no detectable glucose absorbtion, contained about half the level of glucose in the blood compared to the groups on processed wheat. This indicates gluconeogenetic activity. A steady gluconeogenetic activity has been described in salmonids by Walton and Cowey (1982). Despite a poor utilization of dietary glucose, fish presumably require glucose as the preferred fuel for brain and nervous tissue, red blood cells and gonads. Several amino acids have been shown to be metabolized to glucose or glycogen in rainbow trout (Cowey et al., 1977).

The amount of urine in salt-water fishes is known to be limited and urine is claimed to be iso-osmotic to the blood (Bone and Marshall, 1986). An iso-osmotic condition seems also to be the case with respect to glucose in this experiment as the fish from all groups contained glucose in the urine (Table 3) and a significant correlation ($0.001 < p < 0.01$) between blood glucose and glucose in the urine was found. Since the fish without carbohydrate digestion also had glucose in the urine, no relation can be proposed between a state of diabetes and glucose excretion in the urine of cod. Probably a study on gill excretion would show whether a diabetic-like status with surplus glucose excretion from dietary carbohydrate exists in this species.

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