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Digestibility of cod liver oil in the diet of *Labeo rohita* fingerlings

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ABSTRACT

The effect of inclusion level and adaptation time on the apparent digestibility of cod liver oil (CLO) in *Labeo rohita* fingerlings was determined. Cod liver oil was included into a casein based reference diet at 10, 12.5 and 15% inclusion levels with chromic oxide (1%) as an external indicator. Digestibility analyses was done on faeces pooled over 3 sets of 5 successive days (1-5 day; 10 -15 day; 21-25 day respectively) and unpooled daily faecal samples. The apparent digestibility coefficient (ADC) decreased significantly from 85 to 57% as the CLO was increased from 10 to 15% in the diet. The digestibility of gross energy of CLO decreased significantly from 78.4 to 36% as the inclusion level was increased from 10 to 15%. But the protein digestibility of fish was not affected by CLO inclusion in the diet. The analyses on the digestibility of pooled and unpooled samples of faeces revealed that *L.rohita* require more time to adapt to maximum digestibility at higher inclusion levels of CLO in the diet. Results indicated the necessity to determine the ADC of CLO over the range of inclusion levels to be used and also the importance to ensure complete adaptation of the feed to obtain maximum digestibility results.

Introduction

Fish require dietary lipid to provide fatty acids for energy production, hormone synthesis and essential components of cell membrane. In view of the increasing demand and predicted shortage of fish meal (Wijkstrom and New, 1989), it will be useful to determine the potentiality of lipids in fish diets. Assessment of digestibility of ingredients is essential to formulate cost effective feeds because its accurate determination gives greater idea about the bioavailability of the components in fish feed. Studies on protein digestibility of fishes are available but information on other nutrients especially lipids are scarce (Appleford and Anderson, 1997). Majority of lipid digestibility studies provide limited information on energy provision and nutritive value of the types of lipids used in the fish diet. The lipid digestibility of fish greatly varies and depends on many factors such as water temperature (Steffens, 1989), animal size (Storebakken, 1985), other dietary components (Spyridakis *et al.*, 1989; Nandeesha *et al.*,

1991; Storebakken and Austreng, 1987), feeding rates (Steffens, 1989), melting point, (Takeuchi *et al.*, 1979; Austreng *et al.*, 1980), inclusion level and time (Appleford and Anderson, 1997). The present work was undertaken to quantify the digestibility of cod liver oil at three inclusion levels for rohu fingerlings and the effect of adaptation time on digestibility.

Materials and methods

Labeo rohita fingerlings (15±1.5g) were purchased from Fish Farmers Development Agency (FFDA) Manimuthar, Tirunelveli district and acclimatized at the Centre for Aquaculture Research and Extension, (CARE), Palayankottai. Animals were stocked at 15 fish per 50 l rectangular cement tanks. Each tank received a continuous flow of fresh water (4 l/h) with aeration. Feeding trials were carried out at an average water temperature of 27 ± 1°C, pH averaging 7 ± 0.1 and 12 : 12 light and dark cycles. The test feed contained commercially available cod liver oil (99.9%) with 0.27% non-lipid components and a gross energy content of 43.8 μj k^{-1}. Casein based reference diet was used for this experiment (Table 1). Preliminary studies showed that above 5% CLO in the reference diet gave reliable digestibility values and more than 18% inclusion decreased the diet stability. Hence three inclusion levels of 10, 12.5 and 15% of CLO which provided pellet stability were considered to be appropriate to determine the digestibility of purified lipid source. The total dietary lipid ranged from 5.83% in the reference diet to 19.31% in the 15% inclusion diet (Table 1). The diets were prepared using a pelletizer with die hole diameter of 0.2 mm. The pellets were air dried at 50°C for 12 hrs and stored

TABLE 1. *Formulation and (% dry matter) and proximate composition as determined by the analysis (% dry matter) of experimental diets*

Ingredients	Reference diet	Inclusion levels of cod liver oil (%)		
		10	12.5	15
Casein	35	34	34	34
Fish meal	15	12	11	10
Wheat flour	10	10	9	9.0
α- cellulose	20	18	17	14.5
Corn flour	10	9	9	9
Cod liver oil	5	12	15	18.5
*Vitamin and mineral mix	4	4	4	4
Chromic oxide	1	1	1	1
Proximate composition				
Crude protein (%)	47.95	43.71	40.68	38.8
Crude fat (%)	5.83	13.52	16.73	19.31
Ash (%)	4.12	3.93	3.86	3.69
Gross energy (μJ kg^{-1})	17.67	19.03	18.87	18.17

* As 'vitaminets forte', Roche pharmaceuticals, Mumbai, India.

in a refrigerator for further use. Triplicates were maintained for each test diets. The fish were fed two times with equal food allocation (9 am and 4 pm) at a feeding rate of 5% body weight day^{-1} to ensure that all feed was ingested. The faeces were collected daily morning by siphoning. The left over food was removed prior to faeces collection. Analyses were done for daily samples and pooled samples over 10 days of the experimental period. The test diets and daily faeces were dried to constant weight at 50°C and packed for biochemical analyses. Lipid was determined by methanol chloroform extraction (Bligh and Dyer, 1959), energy by semi micro bomb calorimeter and protein by kjeldahl nitrogen (N x 6.25) by using the method of Lowry *et al.* (1951). Chromic oxide was determined using the method of Furukawa and Thsukahara (1966). Apparent digestibility co-efficient for reference and test diets was calculated by using the following formula:

$$\text{ADC}(\%) = 100 - \frac{\%\ Cr_2O_3 \text{ in diet}}{\%\ Cr_2O_3 \text{ in faeces}} \times \frac{\%\ \text{nutrient in faeces}}{\%\ \text{nutrient in diet}} \times 100$$

The test ingredients were included in the ratio of 85 : 15; 87.5 : 12.5 and 90 : 10 of reference diet mixture and test ingredients.

$$\text{ADC}(\%) = \frac{100}{x} \times \text{test} - \frac{(100 - x)}{100} \times \text{ref}$$

Where 'X' is the inclusion of (10 or 12.5 or 15%) test ingredients into test diet, 'test' is the ADC of nutrient in the test diet and 'ref' is the ADC of the same nutrient in the reference diet.

Data were analysed using ANOVA and Tukey's test to find out the difference among means (Snedecor and Cochran, 1968).

Results and discussion

The inclusion of CLO significantly affected ($P < 0.05$) the dry matter digestibility of the experimental diets. Inclusion of 15% CLO in the diet caused a significant decrease ($P < 0.05$) in whole diet digestibility (73.3%). The digestibility of dry matter of CLO at 10% inclusion obtained from pooled samples (84.6%) observed in the present study coincides with the previous values found for lipid source for *C.carpio* (Kurzinger *et al.*, 1986; Chu *et al.*, 1991; Appleford and Anderson, 1997). However, the digestibility of CLO at 15% inclusion (56.9%) was lower than the previous reports and revealed that the addition of CLO in the diet has surpassed the optimal lipid digestion rate of the fish. This result suggests the need to determine the digestibility of dietary ingredients at levels in which they are to be included.

The digestibility of CLO was significantly affected ($P < 0.05$) by the level of CLO incorporation which was higher at 10% inclusion level (84.6%) than 12.5 or 15% inclusion levels (70.8 or 56.9%) respectively. A significant decrease ($P < 0.05$) in ADC of gross energy in the experimental diets was reflected by the incorporation of CLO from 10 to 15%. The ADC of reference diet and 10% inclusion level in the test diet was more or less the same (81.4 and 80.56%), while inclusion level of 12.5 and 15% CLO resulted in a decrease in gross energy digestibility (76.8 and 71.8%). The digestibility of gross energy from CLO was found to be significantly higher ($P < 0.05$) at 10% (78.4%) than at 12.5% inclusion (53.7%) and also at 15% inclusion (35.9%) (Table 2). During the initial periods of experimental trials, the digestibility of CLO at 10% was more than that of 15% inclusion and by the end of the trial, the ADC of CLO for the fish seemed to narrow

TABLE 2. *Apparent digestibility coefficients (%) of the experimental diets and test ingredient as analysed by pooled faeces. Values are mean ± standard error (n = 5)*

Diet	Diet-apparent digestibility coefficients (%)				Cod liver oil-Apparent digestibility coefficients (%)	
	Dry matter	Protein	Lipid	Gross energy	Dry matter	Gross energy
Reference diet	78.9^{a} ± 0.82	96.2 ± 0.13	63.7^{a} ± 2.4	81.4^{a} ± 0.66	-	-
10%	79.6^{a} ± 0.97	96.6 ± 0.14	73.81^{b} ± 1.4	80.56^{a} ± 2.1	84.6^{a} ± 2.4	78.4^{a} ± 4.1
12.5%	76.4^{b} ± 0.27	96.2 ± 0.11	69.88^{b} ± 1.8	76.81^{b} ± 0.6	70.8^{b} ± 2.8	53.7^{b} ± 2.8
15%	73.3^{c} ± 0.81	95.8 ± 1.7	66.43^{ab} ± 1.7	71.8^{c} ± 0.3	56.9^{c} ± 1.7	35.9^{c} ± 3.3

(Values with same superscripts in the same column are not statistically significant (P<0.05).

down which appears that the diet containing higher levels of dietary lipid required a longer period of time for complete digestion. The ADC calculated for daily and pooled samples did not show any significant difference indicating the fact that there was a true effect of pooling faeces produced during the adaptation period of the fish. The result also indicates that digestibility determination should be done after ensuring that the experimental animals adapted to experimental diets. The present experiment also suggests that higher levels of CLO could be incorporated either in experimental or in commercial diets only when the diets are to be used over extended period of time.

Lipid digestibility was significantly higher (P < 0.05) in 10% inclusion diet (73.8%) than in reference diet (63.7%) while lipid digestibility of 15% inclusion diet (66.43%) was not significantly different (P > 0.05) from that of either reference diet or from 10 and 12.5% inclusion level diets. The incorporation of CLO in the reference diet did not significantly affect the whole diet protein digestibility (P < 0.05). It was also observed that the dietary lipid digestibility of pooled faeces decreased with lipid inclusion from 10 to 15% in the diet. This result is similar to the observation of Medale *et al.* (1991) on sturgeon; Singh (1991) on *C. mrigala* and *C. idella* and Appleford and Anderson (1997) on *C. carpio*. The low digestibility of lipid in the reference diet may be due to decreased lipid secretion of the fish and also high dietary fibre content (11.1%) (Kurzinger *et al.*, 1986; Lie *et al.*, 1988; Berge and Storebakken, 1991). The decreased lipid digestibility at higher inclusion level in the present study is similar to the results of De Silva *et al.* (1990) and Nandeesha *et al.* (1991) who observed that digestibility of lipid from animal and plant sources decreased with higher levels of inclusion in the diet.

The digestibility values of faecal samples revealed that there was marked difference over the duration of digestibility ranging from 74.2% on 6th day to 90.4% on 15th day (mean 82,4 + 2.4%) at 10%

inclusion level and 51.2% (day 7) to 78.4% (day 16) (mean 64.8 ± 4.2%) at 12.5% inclusion level and 72.6% (day 8) to 57.4% (day 18) (mean 44.8 ± 3.3%) at 15% inclusion levels (Fig. 1.) The degree of variation in CLO digestibility did not differ with inclusion of test ingredients, with the mean variation between consecutive days being 11.2 ± 16%, 9.6 ± 1.3% and 6.7 ± 1.1% at 10, 12.5 and 15% inclusion respectively. The digestibility of CLO at 10% inclusion did not appear to increase over the experimental period but a large difference was seen between the digestibility of 10 and 12 .5% and also between 12.5 and 15% inclusion levels in the initial period of trials, then decreased and the values come closer at the final period of the experiment (Fig.1).

Determination of daily faecal samples also revealed a daily variation in the digestibility and the variations are quite similar at all inclusion levels. A similar result was also noticed in *Chanos chanos* (Ferraris *et al.*, 1986), *Etroplus suratensis* (De Silva and Perara, 1984) Nile tilapia

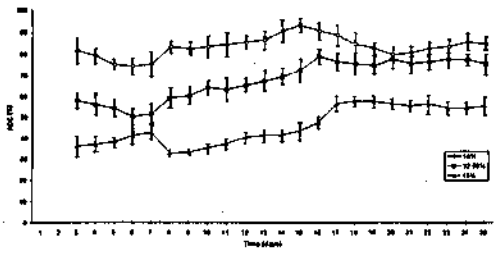

Fig. 1. Daily apparent drymatter digestibility coefficients (%) of cod liver oil inclusion levels at 10, 12.5 and 15% into reference diet with time (days). Values are mean ± standard errors (n=5).

(De Silva *et al.*, 1988) *C. mrigal* and *C. idella* (Smith *et al.*, 1988). But the daily variation in digestibility did not appear to follow any clear pattern. The increase and decrease in the digestibility coefficient after 5 to 6 days showed a rhythm, which lasted for 9 weeks. This rhythmic digestive process clearly indicates the presence of natural rhythmic digestive process of the fish. The same type of rhythmic digestion was previously reported in Nile tilapia (De Silva and Perrera, 1984), Atlantic salmon (Helland *et al.*, 1996) and *C. carpio* (Appleford and Anderson, 1994).

The protein digestibility of the fish was more than 96% in all the trials. This observation is in accordance with the values reported by NRC (1983), Ogino and Chen (1973), Smith *et al.* (1980), Lied *et al.* (1982) and Tacon and Jackson (1985). Protein digestibility was not significantly affected by the inclusion levels of CLO in the diet. This is in agreement with the studies on *C. carpio* (Kitamikado *et al.*, 1964) and *C. idella* (Yong quing *et al.*, 1994) fed with casein based diet. Protein digestibility was not affected by the addition of 30% olive oil or 20% fish liver oil to the diets. Similar studies in rainbow trout (De la Higuera *et al.*, 1977) and channel catfish (Page and Andrews, 1973) also showed no effect on protein digestibility.

The present study has indicated that digestibility of CLO is affected by the inclusion levels into the diet and the diets containing elevated levels of CLO require a given period of time for the fish to adapt to the diet. These results also emphasise the need to analyse ingredients digestibility over a range of levels to be included into the diets to ensure that the fish have completely adapted to the diet for digestibility determinations.

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