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EVALUATION OF APPARENT DIGESTIBILITY COEFFICIENTS OF DIFFERENT DIETARY PROTEIN LEVELS WITH AND WITHOUT FISH MEAL FOR *LABEO ROHITA*

Y. GUL, M. SALIM AND B. RABBANI

Department of Zoology and Fisheries, University of Agriculture, Faisalabad, Pakistan

ABSTRACT

The apparent digestibility coefficients of three protein levels of reference diets (without fish meal) and test diets (with fish meal) were estimated for *Labeo rohita*. Three test diets (test diet-I, test diet-II and test diet-III) and three reference diets (reference diet-I, reference diet-II and reference diet-III) having 28, 30 and 32% protein levels were prepared. Chromic oxide was used as an internal marker in the experimental diets for the evaluation of digestibility of protein levels. The differences in apparent digestibility for dry matter, crude protein, crude fat and gross energy of reference and test diets were highly significant (P<0.01). The apparent digestibility coefficients (ADCs) of dry matter were 32.10 ± 0.30 and 35.30 ± 0.30% for reference and test diets -III. The ADCs of crude protein and crude fat were 77.92 ± 0.10, 69.23 ± 0.06 and 75.77 ± 0.05, 70.40 ± 0.22% for reference and test diets-III, respectively. The values of ADCs of gross energy were maximum for reference diet-III (52.28 ± 0.25%) and test diet-III (48.65 ± 0.78%). The ADCs of dry matter were 19.80 ± 1.40 and 18.15 ± 0.05% for reference and test diet-I. The ADCs of crude protein and crude fat were 73.93 ± 0.05, 68.43 ± 1.58 and 59.49 ± 1.13, 57.02 ± 2.42% for reference and test diet-I respectively. The ADCs values of gross energy were for reference diet-I (42.43 ± 0.36%) and test diet-I (39.09 ± 1.35%). The better ADCs for dry matter, crude protein, crude fat and gross energy were at 32% protein level.

Key words: Apparent digestibility, fish meal, protein levels, *Labeo rohita.*

INTRODUCTION

Fish nutrition has improved dramatically in recent years with the development of balanced commercial diets. Nutrition involves the ingestion, digestion, absorption and transport of various nutrients throughout the body where the nutrients in food are converted into body tissues and utilized for various activities.

The rapid expansion of aquaculture, along with improvements in fish culture techniques, has increased the demand for fish feeds, which mainly depend on fish meal and fish oil as the major dietary components due to their ideal nutritional quality. Fish meal provides essential amino acids such as lysine and methionine which are deficient in plant sources. Fish oil is a good source of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are not found in plant oils (Watanabe, 2002). However, due to increasing demand, high cost and uncertain availability, investigations have been carried out to partially or completely replace the fish meal in fish diets by plant proteins (Nengas *et al*., 1995; Webster *et al*., 1995; Boonyaratpalin *et al*., 1998). Several experiments conducted with channel catfish in ponds have revealed that diets containing 28 to 32% crude proteins primarily from soybean meal provide growth equivalent to diets containing some animal protein, such as fish meal, meat meal and bone meal (Robinson *et al*., 2000) but the information regarding the effect of dietary protein levels with and without fish meal on the digestibility of *Labeo rohita* is lacking. The apparent digestibility coefficients of different dietary protein levels of reference diets without fish meal and test diets with fish meal for *Laboe rohita* have been described in the present paper.

MATERIALS AND METHODS

The present study was carried out for the evaluation of apparent digestibility coefficients (ADCs) of different dietary protein levels of reference and test diets for *Labeo rohita*. The experiment was conducted in Fish Nutrition Laboratory, Department of Zoology and Fisheries, University of Agriculture, Faisalabad, Pakistan during March-April 2006. The experiment was run for eight weeks.

Experimental fish

Two hundred *Labeo rohita* fingerlings were purchased from the Government Fish Seed Hatchery, Faisalabad, Pakistan. The fingerlings were acclimatized for one week in glass aquaria (37 x 29 x 45 cm). Before the start of experiment, fish were treated with 0.5% sodium chloride to prevent ectoparasites or fungal infection (Rowland and Ingram, 1991). During this period, fish were fed once daily on the reference diet used in subsequent digestibility study (Allan and Rowland, 1992).

Feed ingredients and diets preparation

Each test diet was composed of 70 percent reference diet and 30 percent test ingredient (fish meal) on dry weight basis. Three test diets (with fish meal) and reference diets (without fish meal) having three protein levels (28, 30 and 32%) were prepared by applying Win feed formulation Package, ver. 2.6 (software program). Chromic oxide was used as an inert marker and incorporated into experimental diets at 1.0 percent inclusion level.

The ingredients used in reference and test diets were ground, sieved and mixed in mixer for 30 minutes, where after, fish oil was gradually added, while mixing constantly. Then 85 ml of water per 100g of feed was slowly blended into the mixture, resulting in a suitably dough texture for fish food (Lovell, 1989). Drying was carried out in a convection oven at 35°C for 48 hours. The dry product was cut into pellets of 2.5 mm diameter. The composition of ingredients of reference and test diets is shown in Table 1.

Experimental protocol

An eight week digestibility experiment was conducted by using faecal collection tanks in which a settling column was used to separate the faecal material of fish from effluent water. Water temperature remained 30-32°C during the study period. Air pumps were used to maintain the level of dissolved oxygen (5-5.5 mg/l). For each treatment, two replicates were used and in each replicate 10 fingerlings were stocked (average body weight 16 gm). Fish were fed at the rate of 2 percent of their live wet body weight twice daily (morning and afternoon) in the feeding chamber. After a feeding session of 2-3 hours, fingerlings were shifted to faecal collection tanks for faecal collection.

Analytical procedure

Samples of six experimental diets and their respective faeces were homogenized using a mortar pestle and analyzed by standard AOAC (1995) procedures: dry matter (DM) by oven drying at 105°C for 16 hours, crude protein (CP) by micro-kjeldhal analysis and gross-energy by oxygen bomb calorimetry. Crude fat was determined following petroleum ether extraction method (Bligh and Dyer, 1959) through 10454 soxtec system HTz. Chromic oxide was estimated by using acid digestion method (Divakaran *et al*., 2002), through UV/VIS 2001 spectrophotometer. Apparent digestibility coefficients of nutrients for each diet were calculated by using the standard method of Maynard and Loosli (1969).

Finally, data were subjected to one-way analysis of variance (Steel *et al.*, 1996). Differences between means were evaluated by Tukey's Honesty Significant Difference Test (Snedecor and Cochran, 1991).

RESULTS

The result of proximate analysis of reference and test diets, having three dietary protein levels and estimation of chromic oxide in diets and faeces are shown in Tables 2 and 3. The effects of diets on apparent digestibility for dry matter, crude protein, crude fat and gross energy of all the experimental diets were highly significant (P<0.01). The apparent digestibility of dry matter was maximum for reference and test diets-III (32.10 ± 0.30 and 35.30 ± 0.30%) at 32% protein level. The difference in apparent digestibility of dry matter between reference diet-I and test diet-I was non-significant, whereas the means of remaining reference and test diets were significantly different from each other (Table 4).

Table 1: Composition of reference and test diets (%)

Ingredients	Dietary protein levels					
	28%		30%		32%	
	Reference diet-I	Test diet-I	Reference diet-II	Test diet-II	Reference diet-III	Test diet-III
Rice polishing	29.23	35.81	26.94	42.42	24.66	41.36
Wheat bran	29.37	13.49	27.32	3.55	25.26	0.03
Corn gluten 60%	33.40	11.70	37.74	16.03	42.08	20.46
Fish meal	0.00	30.00	0.00	30.00	0.00	30.00
Fish oil	6.00	7.00	6.00	6.00	6.00	6.15
Vitamin premix	1.00	1.00	1.00	1.00	1.00	1.00
Chromic oxide	1.00	1.00	1.00	1.00	1.00	1.00
Total .	100.00	100.00	100.00	100.00	100.00	100.00

Table 2: Proximate analysis of diets and estimation of chromic oxide

Diets	Protein level (%)	Dry matter (%)	Crude protein (%)	Crude fat (%)	Gross energy (k cal/g)	Chromic oxide (%)
Reference diet-I	28	91.31± .03	27.13±0.05	3.39±0.34	2.66±0.02	0.97±0.01
Test diet-1	28	91.97±0.05	27.58±0.02	3.47±0.03	2.97±0.10	0.95±0.04
Reference diet-II	30	93.43±0.07	29.08±0.02	5.93±0.05	3.03±0.06	0.86±0.01
Test diet-II	30	93.31±0.04	29.37±0.02	5.00±0.00	3.20±0.08	0.90±0.02
Reference diet-III	32	93.27±0.03	31.40±0.09	6.47±0.02	3.19±0.05	0.72±0.01
Test diet-III	32	93.74±0.24	31.63±0.05	6.48±0.02	3.35±0.04	0.68±0.01

Table 3: Proximate analysis of faeces and estimation of chromic oxide

Faeces	Protein levels (%)	Dry matter (%)	Crude protein (%)	Crude fat (%)	Gross energy (k cal/g)	Chromic oxide (%)
Reference diet-I	28	88.50±0.08	8.84±0.012	1.71±0.02	1.90±0.05	1.21±0.02
Test diet-1	28	89.01±0.09	10.09±0.01	1.73±0.02	2.18±0.10	1.16±0.05
Reference diet-II	30	89.93±0.02	9.40±0.13	2.76±0.17	2.22±0.05	1.14±0.02
Test diet-II	30	90.19±0.03	11.45±0.08	2.95±0.06	2.32±0.03	1.16±0.02
Reference diet-III	32	91.11±0.06	10.16±0.13	2.93±0.01	2.35±0.06	1.06±0.01
Test diet-III	32	91.48±0.18	11.87±0.09	2.97±0.04	2.66±0.06	1.05±0.01

Apparent crude protein digestibility coefficients were also highest for reference diet-III (77.92 ± 0.10%) and test diet-III (75.77 ± 0.05%) at 32% protein level. The apparent digestibility of crude protein (Table 4) of reference diet-I was non-significantly different from reference diet-II, test diet-II and test diet-III. Test diet-I was significantly different from reference diet-II, III and test diet-III, but non-significantly different from test diet-II. Reference diet-II was significantly different from test diet-II but non-significantly different from reference and test diets-III. Test diet-II was significantly different from remaining diets except reference diet-I. Reference diet-III and test diet-III were however, non-significantly different from each other.

The apparent crude fat digestibility coefficients were also high for reference diet-III (69.23 ± 0.06%) and test diet-III (70.40 ± 0.22%) at 32% protein level. The difference of means of crude fat digestibility for reference diet-I, test diet-I and II were non-significantly different from each other. The means of reference diet-II was non-significantly different from test diet-II and reference diet-III. The remaining diets were significantly different from each other (Table 4).

The high values of apparent gross energy digestibility coefficients were 52.28 ± 0.25 and 48.65 ± 0.78% for reference-III and test diet-III at 32% protein level. The differences of means of digestibility of gross energy between reference diet-I, II and test diet-II were non-significant. The mean of test diet-I was significantly different from the remaining diets. Reference diet-II and test diet-II were non-significantly different from each other but differed significantly from reference and test diets-III (Table 4).

DISCUSSION

The apparent digestibility of dry matter was much lower than the values of 84-89 and 66-77% reported by Eusebio *et al*. (2004) and Salim *et al.* (2004), respectively. The apparent digestibility coefficient for dry matter may be affected by the type of raw material used (whole fish or its by-products). Fish by-products, such as those generated by the filleting industry (sometime referred to as white fish meal) have higher level of ash and lower level of protein than whole fish meals. High level of ash generally affects digestibility

Table 4: Apparent digestibility (%) of dietary protein levels of reference and test diets

Diets	Protein level	Dry matter	Crude protein	Crude fat	Gross energy
Reference diet- I	28	19.80^{e}±1.40	73.93^{bc}±0.05	59.49^{d}±1.13	42.43^{c}±0.36
Test diet-I	28	18.15^{e}±0.05	68.43^{d} ± 1.58	57.02^{d}± 2.42	39.09^{d}±1.35
Reference diet-II	30	24.50^{c}±0.30	75.64^{ab} ± 0.24	64.85^{bc}±1.64	44.65^{c}±0.30
Test diet-II	30	22.05^{d}±0.05	70.99^{cd} ± 0.21	61.25^{cd}±0.73	43.30^{c}±1.05
Reference diet-III	32	32.10^{b}± 0.30	77.92^{a} ± 0.10	69.23^{b} ±0.06	52.28^{a}±0.25
Test diet-III	32	35.30^{a}± 0.30	75.77^{ab} ±0.05	70.40^{a}± 0.22	48.65^{b}±0.78

Mean values with different superscripts within a row differ significantly (P<0.01).

of dry matter and results in high waste outputs and can also produce mineral imbalance (Cho and Bureau, 1998). The apparent digestibility for crude protein in the present study was less than that reported by Hossain and Jauncey (1989), who observed that apparent digestibility coefficient for crude protein of fish meal in carp was 88.9%. Similar value of fish meal digestibility for *Labeo rohita* was also reported by Salim *et al.* (2004). According to Anonymous (1997), carp can digest up to 95% of proteins in fish meal. However, the value can decrease from 92 to 68%, depending on source and treatment of the meals (Pike *et al.,* 1990). The difference in protein digestibility may be due to differences in chemical composition, origin and processing of various feed ingredients, method of faeces collection and fish species (Koproco *et al.,* 2004). Digestibility of fish meal may be improved by applying low temperature in the drying process (Pike *et al.,* 1990). The high apparent digestibility on 32% protein level might be due to better availability of amino acids than 30 and 28% protein levels. Muzamel *et al.* (2003) observed that the level of essential amino acids in 30% protein diet was comparatively higher than 25 and 20 percent dietary protein levels.

The apparent digestibility of crude fat in the present study was also lower than the values reported by NRC (1993). The values of NRC were in range of 85-95% for fish meal. The values of crude fat digestibility of 81.35 ± 3.64% reported by Jalal *et al.* (2000) were also higher than our findings. However, the fat digestibility of current study was slightly higher than the value (68%) reported by Gaylord and Gatlin (1996). They concluded that differences in lipid digestibility values for red drum (*Sciaenops ocellatus*) compared to other species might be attributable to differences in technique used to extract lipid.

The apparent gross energy digestibility was slightly higher than the finding of Laining *et al*. (2003), who observed apparent gross energy digestibility of 51.1 ± 0.89% for fish meal. The apparent gross energy digestibility in the present study was lower than that reported by other researchers (74%, Windell *et al*., 1978; 91%, Cho *et al.*, 1982; 91.5%, Smith *et al*., 1980) for rain bow trout, (83%; Law, 1986) for grass carp (73.5%, Kirchgessner *et al*., 1986).

Conclusions

The apparent digestibility of dry matter, crude protein and crude fat observed in the present study was comparatively lower than other reported studies. However, the comparison of dietary protein levels revealed that apparent digestibility increased with the increased in dietary protein levels and the digestibility was maximum at 32% dietary protein level.

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