

DIGESTIBILITY DETERMINATION IN NILE CATFISH FINGERLINGS USING INTERNAL AND EXTERNAL MARKERS

TALAAAT MAHMOUD SHAHAT

National Institute of Oceanography and Fisheries, Egypt, Cairo, 101 Kasr El-Aney street.

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SUMMARY

Digestibility trials were conducted to compare the use of one external dietary marker (Chromic oxide) and two natural internal dietary markers (Crude fiber and acid insoluble ash) for the estimation of apparent protein and energy digestibility in catfish *Clarias lazera*. The faecal samples were collected daily by filtering the water and from the stomach and from the rectum at the end of each trial.

A 5-day, 6 treatments received essentially single-ingredient diets (Yellow cron, wheat bran, soybean meal, cottonseed meal, fish meal and meat meal). The data showed that the highest digestion values for protein and energy were obtained by using (Cr_2O_3) followed by (A-I-A), but the lowest values were obtained when using (CF). Also the data showed that there were no significant differences ($P \leq 0.05$) in digestibility coefficients of protein and energy when using different faeces collection methods, except for cottonseed meal which was high by estimates depending on faecal samples obtained from the rectum. This may be due to their high of fiber content.

A 10-day digestibility trial was conducted with diets differed in dietary protein levels (20, 25 and 30%). The diets were formulated from the same six ingredients used before. The data showed that there were no significant differences in protein digestibility when using the three markers. The higher digestibility occurred when faecal samples were obtained from the water and rectum might indicate that the absorption of protein occurred far backwards in the rectum. The values of energy

digestibility were high by using (Cr_2O_3), also, were high when the faecal samples were obtained from rectum and from water.

A 10-digestibility trial was conducted with diets differed in gross energy content 4300 and 4700 Kcal/Kg diet. The present data showed no significant differences ($P \leq 0.05$) in protein digestibility by using the three markers. The higher values of protein digestibility were obtained when, the faecal samples collection was from the water and rectum. Also, there are no significant differences ($P \leq 0.05$) in the energy digestibility by using different faeces collection methods.

In conclusion, chromic oxide (0.5%) can be considered as the most suitable "foreign" dietary marker, while, acid-insoluble ash was found to be suitable good internal marker particularly under practical farming conditions.

INTRODUCTION

The formulation of successful practical fish rations is based on understanding not only of the chemical and physical characteristics of individual feedstuffs but also of their relative digestibility in fish.

Due to the difficulties encountered with the quantitative collection of faeces with an aquatic environment, the most widely used method employed by nutritionists for estimation of nutrient digestibility has been an indirect approach involving the use of a dietary inert

marker to follow the progress, of digestion (Cho et al., 1982; NRC, 1983). Chromic oxide (Cr_2O_3) is the most commonly used added indicator to diets for the estimation of nutrient digestibility in terrestrial animals (McDonald et al., 1977) and fish (Furukawa and Tsukahara, 1966; Austreng, 1978).

The indicator method, using chromic oxide, was judged most suitable, but the best method of sampling faeces was uncertain (Austreng, 1978). Although the apparent variability in excretion pattern can be minimised by collecting faeces continuously over a period of days (Dansky and Hill, 1952), there is a need to identify other dietary markers which may be suitable under practical farming conditions where it is not always possible to introduce a "foreign" dietary marker. For example, preliminary studies with fish have indicated that cellulose (Buddington, 1979), hydrolysis resistant organic matter (Buddington, 1980; De-silva and Perera, 1983), crude fiber (Tacon et al., 1983b; De-Silva, and Perera, 1983) and hydrolysis resistant ash (Bowen, 1981; De-Silva and Perera, 1983) may offer particular promise as natural markers for estimating nutrient digestibility. Chromic oxide and crude fiber are reliable external and internal dietary markers for use with rainbow trout. However, considerable further work is required on the suitability of acid-insoluble ash and polyethylene dietary markers for use with fish digestibility trials (Tacon and Rodrigues, 1984).

Faeces have been collected by several methods (Nose, 1960), faecal stripping (Inaba et al., 1962), suction (Windell et al., 1978), collection in chambers with false bottoms (Cho. et al., 1974) in overnight collection tubes supplied with drainage systems (Cho et al., 1976) and collection of faeces directly from the rectum (Lovell, 1977).

In view of the lack of information regarding the performance of internal (indigenous) markers, present within the two natural internal markers (acid-insoluble ash, crude fiber) and one external marker (chromic oxide) a 15-day digestibility trial was conducted with Nile catfish fingerlings *Clarias lazera* to determine:-

a) the protein and energy digestion coefficients for

grains and grains by-products (yellow corn and wheat bran), oilseed meals (soybean and cottonseed meal and animal products (fish meal and meat meal).

b) the effect of different dietary levels of protein and energy on their digestibility.

All values of digestibility estimates obtained from faeces samples collected by water filtering and by dissection from both stomach and rectum.

MATERIALS AND METHODS

Animals:

Nile catfish fingerlings *Clarias lazera* weighing 36.4 ± 5.7 were obtained from common populations of Barrage Fish Farm, Qadisiya, Egypt which belongs to National Institute of Oceanography and Fisheries. The fishes were randomly stocked in 12 lots of 10 individuals each, in 50-L glass aquaria provided with artificial aeration devices.

Diets:

Twelve glass aquaria received dry, pelleted fish as essentially single-ingredient (yellow corn wheat bran; soy bean meal, cottonseed meal, fish meal and meat meal). Were used Cottonseed meal was added to every ingredient at a rate of 3%. Vitamins and minerals premix was used at rate 1%. Rice starch was used as a binder at a rate 0.5%. Chromic oxide (Cr_2O_3) was added as external indicator at a rate of 0.5%. The fish were fed every ingredient at a rate of 3% of wet body weight per day for 5 days Table (1).

Table (1) Chemical Composition of tested feedstuffs.

	Dry matter %	Gross energy (kcal / kg)	Crude fiber %	Crude Protein %	Acid insoluble ash %
Corn, Yellow	89	4220	8.12	2.2	0.012
Wheat bran	90	4426	8.81	11.8	0.012
Soybean meal	89	4565	44.25	4.4	0.012
D. Cotton seed meal	93	4559	41.50	18.55	0.012
Fish meal	92	4662	61.26	1.25	0.012
Meat meal	92	4318	54.18	8.5	0.012
Cotton seed oil	100	8820	—	—	—
Starch	93	4384.2	1.25	—	0.012

Six glass aquaria received three diets differed dietary protein level 20, 25 and 30%. Another

Digestibility determination

Table (2) Composition of the practical diets.

Ingredients	Diets different in dietary protein level			Diets differed in gross energy level		
	20	25 %	30	4300	4500 Kcal / Kg	4700
Corn. Yellow	35	29	24	42	29	42
Wheat bran	30	28	20	18	28	8
Soy bean meal	12	15	15	15	15	17
D. Cotton seed meal	10	7	15	7	7	7
Dish meal	5	10	15	10	10	10
Meat meal	3	6	6	6	6	6
Cotton seed oil	3	3	3	----	3	8
Vitamines and Minerals premix	1	1	1	1	1	1
Strach "as a binder"	0.5	0.5	0.5	0.5	0.5	0.5
Chromic oxide (Cr ₂ O ₃)	0.5	0.5	0.5	0.5	0.5	0.5
Crude protein %	20.82	24.85	29.81	24.63	24.85	24.40
Gross energy Kcal / Kg	4432.74	4450.84	4480.58	4292.24	4450.84	4646.54
P/E ratio	46.96	55.81	66.50	57.33	55.81	52.46
Crude fiber %	6.21	6.05	5.97	5.24	6.05	4.22
Acid-Insoluble ash %	0.0647	0.0905	0.1135	0.0862	0.0905	0.0809
Chromic oxide (Cr₂ O₃)	0.5	0.5	0.5	0.5	0.5	0.5

Table (3) Chemical composition of catfish faeces fed selected feedstuffs.

Faeces collection method	Water						Stomach						Rectum					
	DM %	CP %	CE Kcal/kg	CF %	A.I.A %	Cr ₂ O ₃ %	DM %	CP %	CE Kcal/kg	CP %	A.I.A %	Cr ₂ O ₃ %	DM %	CP %	CE Kcal/kg	CF %	A.I.A %	Cr ₂ O ₃ %
Ingredient: Corn and grain by-products																		
Corn. Yellow "raw"	8.63	5.71	3184.7	3.95	0.0226	0.133	9.75	5.95	3218.4	4.1	0.0211	0.135	9.63	5.33	3101.2	3.37	0.0236	0.134
SE _s	0.49	0.20	17.23	0.11	0.00	0.01	0.51	0.21	18.11	0.12	0.00	0.00	0.50	0.19	20.11	0.11	0.00	0.00
Wheat bran	7.81	2.70	1917.9	19.23	0.1093	0.126	7.67	2.81	1993.0	24.3	0.1061	0.129	7.27	2.19	1913.0	21.35	0.1120	0.127
SE _s	0.31	0.17	12.33	0.29	0.01	0.00	0.33	0.19	13.13	0.40	0.01	0.01	0.35	0.17	18.71	0.31	0.00	0.00
Grained meals:																		
Soy bean meal	7.33	15.00	2018.0	11.92	0.1287	0.139	7.81	17.2	2060.0	11.29	0.1231	0.137	7.73	15.81	1986.0	12.7	0.1311	0.132
SE _s	0.35	0.72	22.61	0.17	0.01	0.00	0.33	0.82	21.31	0.20	0.00	0.00	0.32	0.73	19.25	0.18	0.00	0.00
Cotton seed meal	7.85	6.57	2007.8	20.01	0.1417	0.128	7.68	8.31	2036.0	19.31	0.1378	0.122	6.88	0.17	1998.7	20.14	0.1464	0.126
SE _s	0.42	0.27	14.87	0.40	0.00	0.00	0.45	0.33	22.65	0.38	0.00	0.00	0.36	0.25	23.95	0.45	0.00	0.00
Meat products:																		
Pork meal	5.80	13.92	836.0	2.71	0.8793	0.121	6.66	15.27	888.0	2.83	0.8211	0.129	6.08	13.61	762.0	2.67	0.9071	0.125
SE _s	0.29	0.81	10.3	0.11	0.01	0.00	0.31	0.91	9.11	0.09	0.00	0.00	0.25	0.85	9.33	0.10	0.00	0.00
Meat meal	7.57	16.24	787.0	16.22	0.3798	0.131	7.81	17.25	828.0	17.45	0.3576	0.121	8.03	16.08	748.0	16.08	0.3939	0.128
SE _s	0.37	0.83	9.81	0.00	0.00	0.00	0.38	0.84	8.23	0.40	0.00	0.00	0.34	0.88	8.96	0.51	0.00	0.00

Skat

glass aquaria received three diets differed in gross energy content (4300, 4500 and 4700 Kcal/Kg diet) Table (2). All the six diets were formulated from the six ingredients which mentioned above. Also, cottonseed oil, vitamins and minerals premix, starch and chromic oxide were added to diets with the same manner mentioned above for ingredients and at the same percentages respectively.

Faeces collection methods:

The fish were fed at a daily rate of 3% from their live body weight between 11.00h and 16.00h. Deposited faeces were removed every morning at 10.00h using a siphon tube and a fine mesh (20µ) and transferred to petridishes for drying. At the end of the experiment, the faecal samples were collected from stomach and rectum of the fish.

Analytical procedures:

Faecal samples were oven-dried at 70°C for 24 hours and finely ground for subsequent Diets analysis. Diets (Table 2) and faeces (Tables 3,4) analysis were conducted on duplicate or triplicate samples. Total nitrogen (N) was measured using the micro-Kjeldahl technique (AOAC, 1975), and crude protein was calculated as NX 6.25. Gross energy content was determined directly by bomb calorimetry using oxygen bomb calorimeter

according to (Nijkamp, 1966). Insoluble ash was estimated according to (Pearson, 1976). Crude fiber was estimated according to (AOAC, 1975).

The concentration of chromic oxide was determined spectrophotometrically by the method of Furukawa and Tsukahara (1966).

Calculation of the apparent digestibility

The apparent digestibility coefficient was estimated using a formula suggested by (Lofgren and Loosli (1962):

$$\text{Digestibility coefficient (\%)} = 100 \cdot \left(\frac{I - F}{I} \right)$$

Where:-

I= concentration of chromic oxide, the concentration of nutrient (% DM), i= ingesta, f= faeces.

Statistical analysis:

It was made after Steel and Torrie (1980) using the factorial analysis of variance. Duncan's multiple range test was applied in each experiment when possible to test mean differences (Duncan, 1955).

RESULTS AND DISCUSSION

Table (5) shows digestibility coefficients

Table (4) Chemical composition of catfish faeces fed diets differed in dietary protein and the others fed diets differed in gross energy

Faeces collection method	Water						Stomach						Rectum					
	DM %	CP %	CE Kcal/kg	CF %	A.I.A %	Cr ₂ O ₃ %	DM %	CP %	CE Kcal/kg	CF %	A.I.A %	Cr ₂ O ₃ %	DM %	CP %	CE Kcal/kg	CF %	A.I.A %	Cr ₂ O ₃ %
Diets differed in dietary protein level																		
20 %	8.84	3.2	2175.41	11.87	0.1163	0.138	8.37	15.12	2215.32	11.87	0.1152	0.142	8.76	4.3	2117.8	10.29	0.113	0.133
SE ±	0.24	0.36	14.17	0.28	0.03	0.03	0.14	0.27	4.51	0.31	0.00	0.06	0.24	0.21	22.85	0.104	0.008	0.008
25 %	4.36	2.4	1983.72	11.85	0.1717	0.099	9.50	17.60	2021.50	11.81	0.1981	0.110	9.98	3.5	1971.87	10.94	0.188	0.108
SE ±	0.19	0.30	12.98	0.27	0.01	0.01	0.31	0.20	5.11	0.25	0.00	0.00	0.20	0.18	18.18	0.12	0.008	0.008
30 %	4.56	1.90	1884.35	11.53	0.214	0.122	9.81	21.3	1979.2	11.32	0.2271	0.126	10.01	2.9	1853.8	10.52	0.238	0.128
SE ±	0.28	0.05	16.27	0.23	0.02	0.01	0.21	0.27	4.66	0.41	0.00	0.00	0.20	0.20	17.19	0.11	0.008	0.008
Diets differed in gross energy content																		
4300 Kcal/Kg	2.27	3.87	2119.3	16.39	0.1154	0.096	7.64	15.73	2407.1	16.17	0.1135	0.107	7.83	4.22	2327.9	16.40	0.1173	0.1173
SE ±	0.11	0.12	13.23	0.02	0.00	0.00	0.15	0.23	6.21	0.20	0.00	0.00	0.21	0.20	18.21	0.12	0.008	0.008
4500 Kcal/Kg	5.50	2.66	2472.7	11.79	0.1708	0.083	5.92	17.52	2533.4	11.67	0.1818	0.091	5.82	3.81	2462.5	11.82	0.181	0.101
SE ±	0.13	0.07	12.29	0.20	0.00	0.00	0.19	0.18	4.92	0.21	0.00	0.00	0.11	0.15	17.32	0.13	0.008	0.008
4700 Kcal/Kg	6.30	2.05	2611.5	7.89	0.2189	0.099	5.48	22.09	2676.5	8.7	0.2267	0.100	5.88	2.87	2662.3	8.81	0.2267	0.100
SE ±	0.17	0.08	14.92	0.18	0.00	0.00	0.17	0.20	5.11	0.31	0.00	0.00	0.10	0.11	18.29	0.17	0.008	0.008

Digestibility determination

protein and energy of grains and grain by-products (yellow corn, wheat bran), oilseed meals (soybean meal, cottonseed meal) and animal products (fish meal, and meat meal).

Comparison between estimates, regardless of faeces collection method (Table 6) showed, that, the higher digestion was obtained by using (Cr₂O₃) followed by (A-I.A) for all classes of ingredients. The efficiency of (Cr₂O₃) in digestibility determination, may be due to its passage through the gastro-intestinal tract at a faster rate/relative to digesta (Tacon and Rodrigues, 1984). Many studies reported that

(CF) failed as a dietary marker for estimation of digestibility coefficients for many foodstuffs (Buddington, 1980; De-silva and perera, 1983 and Tacon et al., 1983b). This may have been due to the different crude fiber contents in foodstuffs.

Comparison between estimates from faeces obtained by different methods (Table, 6) showed that there were no significant differences ($P \leq 0.05$) found in protein and energy digestion, except, for cottonseed meal which were high for faeces obtained from the rectum, followed by those obtained from water, while for faeces obtained from stomach they were low, which might be due to the large quantity of fiber content.

Table (5) Digestibility coefficients for the protein and energy of feedstuffs:-

Faeces collection methods	Water			Stomach			Rectum			± SE	
	Nutrien %	CF	A.I.A	Cr ₂ O ₃	CF	A.I.A	Cr ₂ O ₃	CF	A.I.A		Cr ₂ O ₃
Grains and grain by-products:											
		B	B	A	A	A	A	BC	AB	A	
Cor. Yellow	Protein	60.83	62.97	71.18	60.68	69.72	71.17	57.15	66.90	74.95	6.54
	Energy	57.97	60.26	69.07	59.08	56.99	69.98	52.02	62.94	71.95	7.15
Wheat bran	Protein	80.72	82.11	87.70	84.12	80.82	87.20	83.15	83.06	88.15	3.17
	Energy	75.21	77.00	84.18	79.62	75.38	83.56	77.73	77.62	84.34	4.06
Oil seed meals:											
Soy bean meal	Protein	81.80	82.83	86.65	77.96	79.41	85.92	81.99	82.23	87.15	3.58
	Energy	76.26	77.60	82.60	74.42	76.09	83.65	78.08	78.36	84.35	4.08
Cotton seed meal	Protein	91.65	91.50	94.00	89.06	88.94	92.30	92.21	92.27	94.65	2.20
	Energy	76.79	76.36	83.32	75.6	75.34	82.82	77.03	77.21	84.23	4.16
Animal products:											
Fish meal	Protein	89.54	88.53	91.21	89.01	86.53	90.64	89.61	89.13	91.90	1.70
	Energy	91.62	90.82	92.96	91.48	89.56	92.74	92.25	91.89	93.96	2.03
Meat meal	Protein	84.27	84.67	88.88	84.47	82.71	88.10	84.29	85.37	89.31	2.65
	Energy	90.45	90.69	93.25	90.71	89.66	92.89	90.84	91.47	93.77	1.60

Standard error.

A, B, ... etc. means in same row with different superscripts are different ($P < 0.05$)

Table (6) Digestibility coefficients for the protein and energy of feedstuffs. Whereas markers irrespective faeces collection methods and vic versa.

	Markers regardless faeces collection methods				Faeces collection method regardless markers				
	Nutrient %	CF	A.I.A	Cr ₂ O ₃	± SE	Water	Stomach	Rectum	± SE
Grains and grains by products:-									
Corn, Yellow, raw	Protein	C	B	A	9.12	64.99	67.19	66.33	1.65
	Energy	C	B	A	10.07	63.34	62.02	62.30	0.98
Wheat bran	Protein	B	B	A	4.4	83.51	84.05	84.79	0.91
	Energy	B	B	A	5.69	78.80	79.25	79.90	0.79
Oil seed meals:-									
Soy bean meal	Protein	C	B	A	4.56	83.76	81.10	83.79	2.19
	Energy	C	B	A	5.55	78.82	78.05	80.26	1.59
Cotton seed meal	Protein	B	B	A	2.21	92.38	90.10	93.04	2.18
	Energy	B	B	A	5.77	78.82	77.92	97.49	1.11
Animal products:-									
Fish meal	Protein	B	C	A	2.26	89.76	88.73	90.21	1.08
	Energy	B	C	A	1.75	91.8	91.26	92.70	1.03
Meat meal	Protein	B	B	A	3.65	85.94	85.09	86.32	0.89
	Energy	B	B	A	2.18	91.46	91.09	92.03	0.67

Table (7) Digestibility coefficients for the protein and energy of diets differed in dietary protein level and the other differed in gross energy content.

Marker	Nutrient %	Water			Stomach			Rectum			± SE	
		CF	A.I.A	Cr ₂ O ₃	CF	A.I.A	Cr ₂ O ₃	CF	A.I.A	Cr ₂ O ₃		
Protein dietary level %	20	Protein	A	A	A	B	BC	B	A	A	A	12.83
		Energy	BC	BCDE	A	BCD	BCDE	A	B	ACD	A	4.32
	25	Protein	A	A	A	B	B	B	A	A	A	13.16
		Energy	B	BC	B	BC	A	B	B	B	A	0.71
	30	Protein	A	A	A	B	B	B	A	A	A	13.90
		Energy	B	B	A	B	B	A	B	B	A	2.57
Energy level (Kcal/Kg)	4300	Protein	A	A	B	B	B	B	A	A	A	13.27
		Energy	A	B	A	A	A	A	A	B	A	7.15
	4500	Protein	A	A	B	B	B	B	A	A	A	14.11
		Energy	A	B	B	B	BC	BC	A	A	AB	1.11
	4700	Protein	A	A	B	B	B	B	A	A	A	16.19
		Energy	B	B	A	A	A	A	AB	B	A	4.35

SE, standard error.

A, b, ... etc. means in same row with different superscripts are different (P < 0.05).

The high levels of fiber in foodstuffs reduced the passage time and consequently reduce the digestion of nutrients.

The present data showed that the catfish *Clarias lazera* was able to digest the protein and energy of the animal-based feedstuffs more efficiently than those of the plant-based feedstuffs. This data agreed with catfish results obtained by Stickney and Lovell (1977) and with tilapia results cited by Hanley (1987). This was thought to occur because the energy content of the former residues largely in their protein and lipid fractions which are highly digestible by catfish, while, much of the energy content of the plant-based foodstuffs derives from complex carbohydrates, which are either indigestible, or poorly digested by catfish (Stickney and Lovell, 1977).

The data in Table (7) present the comparison between digestibility of diets differed in dietary

protein / level 20, 25 and 30% using the same three markers, also, the faecal samples obtained by the same methods.

Regardless, the faeces collection methods (Table 8) showed that there was no significant differences ($P \leq 0.05$) in protein digestion by using the three markers.

These results are contradictory to studies of De Silve and Perera (1983) with the Asian cichlid *Etilopis suratensis* where hydrolysis resistant ash (as external marker) was found to give consistently higher digestibility coefficients than either (CF) or hydrolysis resistant organic matter as internal markers. While, estimations of energy digestibility showed that the estimates depending on (Cr_2O_3) were higher than that from the other two markers.

Regardless type of markers, (Table 8) the data

Table (8) Digestibility coefficients for the protein and energy of diets differed in protein level and the others differed in energy levels whereas kind of markers irreversible faeces collection methods and vice versa.

	Markers regardless faeces collection methods				Faeces collection method regardless markers				
	CF	A.I.A	Cr ₂ O ₃	±SE	Water	Stomach	Rectum	± SE	
Diets differed in dietary protein level									
20 %	Protein	81.08	79.76	87.15	5.57	A	B	A	21.51
	Energy	74.48	72.78	82.6	7.42	92.64	65.16	90.21	
25 %	Protein	83.86	85.17	82.96	1.60	B	B	A	0.96
	Energy	77.11	77.87	78.29	0.84	76.49	76.02	77.35	
30 %	Protein	84.61	85.2	87.04	1.79	A	B	A	22.74
	Energy	77.83	77.88	83.18	4.35	93.44	65.43	93.12	
Diets differed in gross energy level									
4300 (Kcal / Kg)	Protein	83.44	75.62	86.01	7.65	A	B	A	21.67
	Energy	72.17	59.06	75.48	12.28	90.48	63.99	90.60	
4500 (Kcal / Kg)	Protein	83.37	83.86	82.54	0.94	A	C	B	1.66
	Energy	71.22	71.52	69.56	1.50	94.25	63.34	92.17	
4700 (Kcal / Kg)	Protein	81.8	86.75	83.63	3.54	A	B	A	24.43
	Energy	70.60	63.54	73.74	7.38	70.16	70.40	71.74	
						A	B	A	1.20
						96.05	61.36	94.77	27.82
						68.2	70.37	69.32	1.53

SE, standard error.
A, b, ... etc. means in same row with different superscripts are different ($P < 0.05$).

Table (9) Growth performance of Nile catfish fingerlings fed single-ingredient and practical diets.

Items	Initial Weight (g.)	Final Weight (g.)	Crain in Weight (g.)	Average daily gain (g./day)	Specific growth (% / day)
<u>Single ingredient which fed to 5 days</u>					
Corn, Yellow	30.15 ± 1.68	31.27 ± 1.80	1.12	0.22	0.73
Wheat bran	35.15 ± 1.60	36.30 ± 1.62	1.15	0.23	0.64
Soy bean meal	40.60 ± 1.55	41.97 ± 1.44	1.37	0.27	0.66
D. cotton seed meal	41.65 ± 2.47	43.32 ± 2.37	1.67	0.33	0.79
Fish meal	35.15 ± 1.84	37.02 ± 2.30	1.87	0.37	1.04
Meat meal	35.7 ± 2.31	37.62 ± 2.30	1.92	0.38	1.05
<u>Diets differed in</u>					
<u>Protein level fed to 10 days</u>					
20 %	28.4 ± 1.81	31.27 ± 1.8	2.87	0.29	0.96
25 %	30.6 ± 1.60	36.30 ± 1.62	5.70	0.57	1.71
30 %	30.77 ± 1.76	37.47 ± 2.01	6.7	0.67	1.13
<u>Diets differed in</u>					
<u>gross energy level fed to 10 days</u>					
4300 Kcal / Kg	33.22 ± 2.25	41.32 ± 2.23	8.10	0.81	2.18
4500 Kcal / Kg	26.9 ± 1.65	34.07 ± 1.82	7.17	0.72	2.36
4700 Kcal / Kg	32.22 ± 2.16	37.37 ± 2.25	5.15	0.51	1.48

indicated that the higher values of protein digestion occur when faecal samples were collected from the water and the rectum, but, those collected from the stomach were found to be low. This data indicated that absorption of protein occurs far backwards in the rectum reported by Austreng (1978).

This supports the conclusion that it is advisable to take faeces samples from as close to the anus as possible.

On the other hand no significant differences ($P \leq 0.05$) were found in energy digestion for diets contained 25 and 30 % CP by using the different faeces collection methods, but, the estimates from rectum were higher than from water and stomach in case of 20 % protein.

However, numerical comparisons showed little increase in protein digestion by increasing dietary protein level from 20 to 30 % (about 4.3, 6.8 and 11 % by using CF, A-I.A and Cr_2O_3 respectively and about 3, 1.3 and 5.7 % by using the faecal samples obtained from the water, stomach and-the rectum respectively.

Also, the data in Table (7) present the comparison between digestibility of diets differed in energy level 4300, 4500 and 4700 Kcal / Kg

Regarding, the faeces collection methods (8), showed no significant differences ($P \leq 0.05$) were found in protein digestion by using the markers. Digestibility coefficients of energy high by using (Cr_2O_3).

For the kind of marker (Table 8), the protein digestion values were obtained from faecal samples which collected from water and rectum, but, there are no significant differences ($P \leq 0.05$) found in energy digestion values.

The comparisons showed little increase or decrease in energy digestion by increasing energy level from 4300 to 4700 kcal / Kg. diet.

In general, the markers evaluated during present investigation often yielded different digestibility coefficients clearly for the diets especially with protein digestion. This may be due to their different physical and chemical characteristics which influence the individual flow patterns through the

gastro-intestinal tract with respect to the digesta. These results agreed with those reported by Tacon and Rodrigues (1984) on rainbow trout.

The digestibility coefficients obtained from different faeces collection methods indicated the effect of absorption. Though the results may be affected by the presence of digestive juices, it seems that absorption occurs in the stomach while protein is absorbed mainly from the small intestine.

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