

Full Length Research Paper

Effect of dietary protein, lipid and carbohydrate contents on the nutrient and energy utilization and digestibility of *Cyprinus carpio communis* fingerlings

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Fingerlings having average weight 1.64 ± 0.13 g and length 5.26 ± 0.10 cm were fed on four different formulated feeds and a control feed (each in a triplicate set), 6% of their body weight, three times a day, during 90 days. Feeds were formulated using ground nut oil cake, mustard oil cake, rice bran, wheat bran, fish meal and soybean meal in order to suffice the balanced need of protein and energy of the common carp. Nutrient and energy utilization and digestibility were measured. At the end of the study, the nutrient and energy utilization and digestibility of fingerlings were affected significantly ($P < 0.05$) with protein, lipid and carbohydrate contents in the feeds. Highest nutrient and energy utilization and digestibility were observed in fingerlings fed with feed B containing $40 \pm 0.21\%$ protein, $9.31 \pm 0.25\%$ lipid and $10.08 \pm 0.10\%$ carbohydrate. The fingerlings fed with feed C containing $25.98 \pm 0.19\%$ protein, $5.49 \pm 0.18\%$ lipid and $34.63 \pm 0.19\%$ carbohydrate showed least nutrient and energy utilization and digestibility. This work concluded that feed B containing 40% protein, 9.31% lipid and 10.08% carbohydrate is the best one for a more profitable and successful culture of the common carp.

Key words: *Cyprinus carpio communis*, fingerlings, nutrient utilization, energy utilization, digestibility.

INTRODUCTION

Proteins are the major organic materials in most fish tissue, and form an important component of the diet. One of the major requirements of fish culture is the efficient transformation of dietary protein into tissue protein (Webster and Lim, 2002). However, protein is essential for normal tissue function, for the maintenance and renewal of fish body protein and for growth. Due to the cost of the protein, the feed will be more cost effective if all the protein is used for tissue repair and growth and little catabolized for energy (Gauquelina et al., 2007). From a practical point of view, the ideal situation should tend to maximize the use of dietary protein for growth, minimizing the use of proteins for functional protein synthesis, gluconeogenesis, lipogenesis and energy (Jamabo and Alfred, 2008). If adequate protein is not provided in the diet, there is a rapid reduction or cessation of growth and a

loss of weight due to withdrawal of protein from less vital tissues to maintain the functions of more vital tissues. On the other hand, if too much protein is supplied in the diet, only part of it will be used to make new proteins and the remainder will be catabolized to produce energy (Alatise et al., 2006). Although the utilization of proteins for basal energy metabolism is a well-established phenomenon, conventional "energy-yielding" nutrients like fats and carbohydrates can reduce the oxidation of protein to satisfy the energy needs of fish and thus improve the utilization of dietary protein (Kim et al., 2004).

Lipids are an extremely diverse group of compounds, many of which function as important sources of metabolic energy. Among the various types of lipid, it is the simple, glycerol based, fats and oils that are of most interest in terms of general nutrition (Du et al., 2008). Lipids normally occur in foodstuffs and in the fat deposits of most animals in the form of triglycerides, which are esters of fatty acids and glycerol (Kieśliling et al., 2001). Thus, dietary lipids provide a source of indispensable nutrients, the essential fatty acids. In addition, they also act as

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carriers of certain non - fat nutrients, notably the fat-soluble vitamins A, D, E and K and they are also an important source of energy (Storebakken, 2002). Lipids contain more energy per unit weight than any other biological compound e.g., one gram of lipid contains almost twice as much total energy as either one gram of carbohydrate or one gram of protein (Gullaine, 2001). Dietary lipids, mainly in the form of triglycerides, are hydrolyzed to free fatty acids and glycerol by pancreatic lipase, aided by the saponifying and emulsifying action of bile acids in the digestive tract. Absorption generally occurs primarily in the anterior ileum including the caecum (Subhadra et al., 2006). Lipids are transported in the bloodstream either as lipoprotein complexes called very low-density lipoproteins (VLDLs) or as very small droplets called chylomicrons. The triglycerol components of VLDL and chylomicrons are hydrolyzed to free fatty acids and glycerol in the target tissues (generally adipose tissue and skeletal muscle) outside of the cell by an enzyme called lipoprotein lipase (LPL). The other source of long chain fatty acid is synthesized (lipogenesis) from acetyl-CoA derived from carbohydrate (glucose), mainly in adipose tissue and the liver (Mourente et al., 2005).

Unlike protein and fat, carbohydrate as a nutrient was not considered essential to fish because of their ability to synthesize carbohydrate metabolites (glucose/glycogen etc.) from excess dietary protein and fat. Compared to the farmed terrestrial animals, the utilization of dietary carbohydrates in fish is limited, but the inclusion of carbohydrate in fish feeds has certain beneficial effects (Shiau and Lin, 2001). The utilization of carbohydrate in fish varies depending on its complexity, source, level in the diet, pre-treatment and degree of gelatinization. The ability of fish to utilize carbohydrate also differs greatly between species and life stage as a consequence of the marked variations in the anatomy of the digestive tract and in the food habits (Mustafizur et al., 2008). It is also thought that herbivorous and omnivorous fish species utilize carbohydrate better than carnivorous fishes (Hemre et al., 2002). The inability of fish to utilize dietary carbohydrate has been illustrated by glucose tolerance tests. Oral administration of glucose to different fish species led to linear increase of blood glucose concentration, with a poor response of plasma insulin levels. This implies that glucose levels in blood are poorly regulated by fish, their response being frequently similar to diabetic mammals (Stone, 2003; Amoah et al., 2008; Tian et al., 2010). Other carbohydrates such as fibres, hemicellulose, lignin and pentosans which generally form indigestible fractions in the feed, often act as pellet binders. Some fish species can tolerate up to 8% of dietary fibre and depressed growth may occur when the fibre content reaches 20% (NRC, 1993; Amoah et al., 2008; Jesu et al., 2008).

Digestibility is the quantification of the digestive processes. Digestibility gives relative measures of the extent to which ingested food and its nutrient components are digested and absorbed by the animal. Part of the food

consumed by fish will pass through the gastrointestinal tract without being digested and absorbed i.e., part of the ingested food will be lost as faeces. If digestibility is high then faecal losses will be low and vice versa (Burel et al., 2000). Proteins in most feedstuffs that are properly processed are highly digestible to fish. The digestion coefficients for protein in protein rich feedstuffs are usually in the range of 75 to 95%. Protein digestibility tends to be depressed as the concentration of dietary carbohydrate increases (Hemre et al., 2002). Digestibility of lipids ranges from 85 to 95% in most fish species (NRC, 1993; Menoyo et al., 2003). Long chain fatty acids exhibit a higher digestibility than short chain ones. Poly-unsaturated fatty acids such as 20:5 or 22:6 are up to 100% digested by rainbow trout (Kaushik, 2004) and, in general, the essential PUFA show a very high digestibility in this fish species (Kaushik, 2004). Source or type, dietary level, and heat treatment affect the digestibility of carbohydrates in fish (Krogdahl et al., 2005). Considerable differences in carbohydrate digestibility between the various fish species can be expected as a consequence of the marked variations in the anatomy of the digestive tract and in the native diet (Gumus and Ikiz, 2009). A factor, which has a major effect on carbohydrate digestibility in fish, is the degree of polymerization. The monosaccharides are well absorbed by fish, while dextrin is moderately digestible and crude starches have comparatively low digestibilities (Krogdahl et al., 2005).

The aim of the present study was to carry out orderly nutritional research with common carp by using different dietary protein, lipid and carbohydrate contents for determination of a feed formulation with optimum protein to energy ratio (P/E ratio) which would result in better nutrient and energy utilization and digestibility so as to make production of Common carp economical.

MATERIALS AND METHODS

Four feeds (feeds A, B, C and D) were formulated using the ingredients like ground nut oil cake, mustard oil cake, rice bran, wheat bran, fish meal and soybean meal. The ingredients were selected so as to suffice the balanced need of protein and energy of the common carp. Feeds were formulated using "Pearson-Square method" with different protein, carbohydrate and lipid contents. Control feed consisted of 50% mustard oil cake and 50% rice bran. Vegetable oil (1.5 ml per 100 g of feed) and cod liver oil (1.5 ml per 100 g of feed) were incorporated in each formulated feed to ensure adequate supply of fatty acids of both n - 6 and n - 3 series, which is assumed to be essential for common carp. Vitamin - mineral mix-ture (2 g per 100 g of feed) was added to each formulated feed for the maintenance of fish health. Sodium alginate (5 g per 100 g of feed) was used as binder and oxytetracycline (500 mg per 100 g of feed) as antibiotic for control and formulated feeds. 1% chromic oxide (BDH 277572Q) was included in control and formulated feeds, as inert indicator for digestibility studies.

A pelleting machine (Hobart, model, A 200) was used to pellet the feeds. An appropriate die was used to form pellets of desired sizes (1.0 to 3.0 mm). Pellets were oven dried and fed to the fishes at 6% of the body weight, three times a day at 10 A.M., 2.0 and 5.0 P.M. every day.

Cyprinus carpio communis fingerlings having average weight

1.64 ± 0.13 g and length 5.26 ± 0.10 cm were used for the experiment. Prior to the initiation of the feeding trail, fingerlings were acclimatized for one week. During this period, traditional mixture of mustard oil cake and rice bran (1:1) was fed to the fingerlings. Each formulated feed and control feed was fed to triplicate group of fingerlings for 90 days. Fifty fingerlings were reared in each fiber glass tank.

Water analysis of the experimental tanks was done regularly to monitor any unusual changes. The tanks were aerated throughout the experiments with aquarium air pumps (RS-180, Zhongshan Risheng Co. Ltd., China). Fingerlings were weighed on an Elite electronic balance.

Faecal matter was collected once a day at about 08.30 A.M. before the feeding commenced for digestibility studies. Faeces of each dietary treatment were pooled together. Biochemical analysis (dry matter, moisture, crude protein, crude lipid, carbohydrate and ash of feed ingredients, feeds, carcass and faecal matter) was determined by using standard procedures (AOAC, 1995). The energy content of feed ingredients, feeds, carcass and faecal matter were calculated calorimetrically. The biochemical analysis of carcass was carried out for the determination of nutrient and energy utilization. The biochemical analysis of faecal matter was carried out for the digestibility determination.

Nutrient and energy utilization

The following parameters were recorded to assess the nutrient and energy utilization of fingerlings fed on control and formulated feeds.

Protein efficiency ratio (PER)

PER = Live weight gain (g) / protein consumed (g)

Apparent net protein utilization (ANPU)

ANPU (%) = [(P2 - P1) / total protein consumed (g)] × 100

Where, P1 is the protein in fish carcass (g) at the beginning of the study and P2 is the protein in fish carcass (g) at the end of the study.

Apparent net lipid utilization (ANLU)

ANLU (%) = [(L2 - L1) / total lipid consumed (g)] × 100

Where, L1 is the lipid in fish carcass (g) at the beginning of the study and L2 is the lipid in fish carcass (g) at the end of the study.

Apparent net energy utilization (ANEU)

ANEU (%) = [(E2 - E1) / (Total feed consumed (g) × Dietary energy in feed (Kcal))] × 100

Where, E1 is the energy in fish carcass (Kcal) at the beginning of the study and E2 is the energy in fish carcass (Kcal) at the end of the study.

Digestibility determination

The inert indicator chromic oxide was used in feeds for determining digestibility. It passes unaffected by digestion through the alimentary tract of fish. This provides a convenient method of measuring digestibility without the need of quantitative collection of faeces.

Chromic oxide was measured in feed and faeces using acid digestion method of Furukawa and Tsukahara (1966). The digestibility of dry matter, protein, lipid, carbohydrate and energy were calculated using the formula of Maynard and Loosli (1969).

Digestibility (%) =

100-[100 (% Cr₂O₃ in feed / % Cr₂O₃ in faeces) (% nutrient in faeces / % nutrient in feed)]

RESULTS

Biochemical composition of fish feed ingredients

Biochemical composition of fish feed ingredients (% in dry weight basis) used for the present research work is given in Table 1. The dry matter content of fish feed ingredients was the highest (95.37 ± 0.17%) in mustard oil cake and the least (91.55 ± 0.28%) in rice bran. The moisture content of fish feed ingredients was the highest (8.45 ± 0.21%) in rice bran and the least (4.63 ± 0.13%) in mustard oil cake. The crude protein of fish feed ingredients was the highest (53.60 ± 0.21%) in fish meal and the least (13.45 ± 0.13%) in rice bran. The crude lipid of fish feed ingredients was the highest (9.73 ± 0.19%) in mustard oil cake and the least (3.37 ± 0.17%) in rice bran. The carbohydrate content of fish feed ingredients was the highest (19.61 ± 0.17%) in rice bran and the least (4.33 ± 0.14%) in fish meal. The ash content of fish feed ingredients was the highest (12.50 ± 0.16%) in rice bran and the least (4.12 ± 0.17%) in mustard oil cake. The energy content of fish feed ingredients was the highest (4.92 ± 0.21 Kcal/g) in mustard oil cake and the least (1.86 ± 0.22 Kcal/g) in rice bran. Out of six ingredients, ground nut oil cake and mustard oil cake were used as the source of lipid to provide energy of 4.74

± 0.13 and 4.92 ± 0.21 Kcal/g, respectively. Fish meal and soybean meal were used as protein source, providing 53.60 ± 0.21 and 50.12 ± 0.17% crude protein, respectively. Rice bran and wheat bran were used as the source of carbohydrate to provide instant energy of 1.86 ± 0.22 and 1.99 ± 0.26 Kcal/g, respectively. There was no significant difference (P>0.05) in the biochemical composition of ground nut oil cake and mustard oil cake; rice bran and wheat bran; fish meal and soybean meal.

Composition of control and formulated feeds experimented

Four feeds (feeds A, B, C and D) were formulated using the ingredients like ground nut oil cake, mustard oil cake, rice bran, wheat bran, fish meal and soybean meal. The ingredients were selected so as to suffice the balanced need of protein and energy of the common carp. Feeds were formulated using "Pearson-Square method" with different protein, carbohydrate and lipid contents in order to ascertain their effect on growth parameters. Control feed consisted of 50% mustard oil cake and 50% rice bran. Feed A consisted of ground nut oil cake (15%),

Table 1. Biochemical composition of fish feed ingredients (% in dry weight basis)

S/N	Ingredient	Dry matter	Moisture	Crude protein	Crude lipid	Carbohydrate	Ash	Energy (Kcal/g)
1	Ground nut oil cake	95.09 ^C ± 0.21	4.91 ^a ± 0.18	42.21 ^b ± 0.17	9.05 ^C ± 0.28	8.62 ^b ± 0.13	4.62 ^a ± 0.21	4.74 ^b ± 0.13
2	Mustard oil cake	95.37 ^C ± 0.17	4.63 ^a ± 0.13	39.56 ^b ± 0.18	9.73 ^C ± 0.19	7.32 ^b ± 0.12	4.12 ^a ± 0.17	4.92 ^b ± 0.21
3	Rice bran	91.55 ^a ± 0.28	8.45 ^C ± 0.21	13.45 ^a ± 0.13	3.37 ^a ± 0.17	19.61 ^C ± 0.17	12.50 ^C ± 0.16	1.86 ^a ± 0.22
4	Wheat bran	91.84 ^a ± 0.23	8.16 ^C ± 0.26	16.10 ^a ± 0.12	4.58 ^a ± 0.13	16.26 ^C ± 0.19	11.92 ^C ± 0.21	1.99 ^a ± 0.26
5	Fish meal	93.82 ^b ± 0.19	6.18 ^b ± 0.16	53.60 ^C ± 0.21	7.78 ^b ± 0.26	4.33 ^a ± 0.14	10.60 ^b ± 0.20	3.92 ^C ± 0.23
6	Soybean meal	93.63 ^b ± 0.12	6.37 ^b ± 0.15	50.12 ^C ± 0.17	7.56 ^b ± 0.24	4.72 ^a ± 0.10	10.05 ^b ± 0.18	3.63 ^C ± 0.13

Values are means ± SD. Means in the same column having different superscripts are significantly different (P<0.05) and means in the same column with same superscript are not significantly different (P > 0.05).

Table 2. Composition of control and formulated feeds experimented (% in dry weight basis)

Ingredient	Control	Feed A	Feed B	Feed C	Feed D
Ground nut oil cake	Nil	15	18	8	16.66
Mustard oil cake	50	15	60	12	16.66
Rice bran	50	10	2	40	16.66
Wheat bran	Nil	10	8	30	16.66
Fish meal	Nil	25	4	6	16.66
Soybean meal	Nil	25	8	4	16.66
Sodium alginate (g)	5	5	5	5	5
Vitamin ¹ mineral mixture (g)	Nil	2	2	2	2
Vegetable oil (ml)	Nil	1.5	1.5	1.5	1.5
Cod liver oil ² (ml)	Nil	1.5	1.5	1.5	1.5
Oxytetracycline (mg)	500	500	500	500	500
Chromic oxide (%)	1.00	1.00	1.00	1.00	1.00

¹Supplevite – M (Sarabhai Chemicals India) ²Cod liver oil (Sea cod, M/S Universal Medicare Ltd. Mumbai).

mustard oil cake (15%), rice bran (10%), wheat bran (10%), fish meal (25%) and soybean meal (25%). The combination aimed at the supply of maximum protein component than the energy. Feed B consisted of ground nut oil cake (18%), mustard oil cake (60%), rice bran (2%), wheat bran (8%), fish meal (4%) and soybean meal (8%). This combination, instead of having fish meal as a source of protein had mustard oil cake. Feed C consisted of ground nut oil cake (8%), mustard oil

cake (12%), rice bran (40%), wheat bran (30%), fish meal (6%) and soybean meal (4%). This combination aimed at the use of carbohydrate rich diet for the growth. Feed D consisted of the mixture of equal quantity (16.66%) of all the ingredients. Vegetable oil (1.5 ml per 100 g of feed) and cod liver oil (1.5 ml per 100 g of feed) were incorporated in each formulated feed to ensure adequate supply of fatty acids of both n - 6 and n - 3 series, assumed to be essential for common carp. Vitamin

- mineral mixture (2 per 100 g of feed) was added to each formulated feed for the maintenance of fish health. Sodium alginate (5 per 100 g of feed) was used as binder and oxytetra-cycline (500 mg per 100 g of feed) as antibiotic for control and formulated feeds. 1% chromic oxide (BDH 277572Q) was included in control and formulated feeds, as inert indicator for digestibility studies. Composition of control and formulated feeds (% in dry weight basis) experimented is given in Table 2.

Table 3. Biochemical composition of control and formulated feeds experimented (% in dry weight basis).

Biochemical composition	Control	Feed A	Feed B	Feed C	Feed D
Dry Matter	92.89 ^a ± 0.17	93.77 ^b ± 0.21	94.01 ^b ± 0.19	92.73 ^a ± 0.28	93.44 ^b ± 0.16
Moisture	7.11 ^d ± 0.21	6.23 ^a ± 0.16	5.99 ^a ± 0.17	7.27 ^d ± 0.23	6.56 ^a ± 0.19
Crude Protein	26.50 ^a ± 0.31	42.00 ^c ± 0.26	40.00 ^b ± 0.21	25.98 ^a ± 0.19	34.75 ^{ab} ± 0.17
Crude Lipid	5.80 ^a ± 0.26	8.94 ^d ± 0.19	9.31 ^d ± 0.25	5.49 ^a ± 0.18	8.22 ^d ± 0.16
Carbohydrate	32.95 ^b ± 0.18	12.92 ^a ± 0.16	10.08 ^a ± 0.10	34.63 ^b ± 0.19	15.07 ^a ± 0.22
Ash	8.68 ^a ± 0.21	9.39 ^d ± 0.19	9.45 ^d ± 0.16	8.59 ^a ± 0.26	9.15 ^d ± 0.15
Energy (Kcal/g)	3.66 ^a ± 0.15	4.44 ^d ± 0.11	4.65 ^d ± 0.13	3.48 ^a ± 0.16	4.26 ^d ± 0.19
P/E (mg protein/Kj)	17.33 ^a ± 0.22	22.64 ^c ± 0.36	20.54 ^d ± 0.21	17.18 ^a ± 0.19	19.53 ^{ab} ± 0.15

Values are means ± SD. Means in the same row having different superscripts are significantly different ($P < 0.05$) and means in the same row with same superscript are not significantly different ($P > 0.05$).

Biochemical composition of control and formulated feeds experimented

Biochemical composition of control and formulated feeds experimented (% in dry weight basis) is given in Table 3. The highest dry matter content ($94.01 \pm 0.19\%$) was recorded in feed B and the least ($92.73 \pm 0.28\%$) in feed C. The highest moisture content ($7.27 \pm 0.23\%$) was recorded in feed C and the least ($5.99 \pm 0.17\%$) in feed B. The highest crude protein ($42 \pm 0.26\%$) was recorded in feed A and the least ($25.98 \pm 0.19\%$) in feed C. The highest crude lipid ($9.31 \pm 0.25\%$) was recorded in feed B and the least ($5.49 \pm 0.18\%$) in feed C. The highest carbohydrate content ($34.63 \pm 0.19\%$) was recorded in feed C and the least ($10.08 \pm 0.10\%$) in feed B. The highest ash content ($9.45 \pm 0.16\%$) was recorded in feed B and the least ($8.59 \pm 0.26\%$) in feed C. The highest energy content (4.65 ± 0.13 Kcal/g) was recorded in feed B and the least (3.48 ± 0.16 Kcal/g) in feed C. The highest P/E ratio (22.64 ± 0.36 mg protein/Kg) was recorded in feed A and the least (17.18 ± 0.19 mg protein/Kg) in feed C.

Nutrient and energy utilization

The nutrient and energy utilization of fingerlings fed on control and a formulated feed for 90 days is given in Table 4.

Protein efficiency ratio (PER)

After 90 days, the highest PER (2.66 ± 0.01) was recorded in the fingerlings fed on feed B and the least (2.06 ± 0.02) in the fingerlings fed on feed C. There was no significant difference ($P > 0.05$) in the PER of the fingerlings fed on control feed and feed C. The PER of the fingerlings fed on Feed A was significantly lower ($P < 0.05$) as compared to the PER of the fingerlings fed on Feed B.

Apparent net protein utilization (ANPU)

After 90 days, the highest ANPU ($36.67 \pm 0.19\%$) was

recorded in the fingerlings fed on feed B and the least ($26.24 \pm 0.24\%$) in the fingerlings fed on feed C. There was no significant difference ($P > 0.05$) in the ANPU of the fingerlings fed on control feed and feed C. The ANPU of the fingerlings fed on feed A was significantly lower ($P < 0.05$) as compared to the ANPU of the fingerlings fed on feed B.

Apparent net lipid utilization (ANLU)

After 90 days, the highest ANLU ($32.43 \pm 0.12\%$) was recorded in the fingerlings fed on feed B and the least ($25.15 \pm 0.21\%$) in the fingerlings fed on feed C. There was no significant difference ($P > 0.05$) in the ANLU of the fingerlings fed on control feed and feeds C, A, B and D.

Apparent net energy utilization (ANEU)

After 90 days, the highest ANEU ($28.65 \pm 0.27\%$) was recorded in the fingerlings fed on feed B and the least ($21.53 \pm 0.21\%$) in the fingerlings fed on feed C. There was no significant difference ($P > 0.05$) in the ANEU of the fingerlings fed on control feed and feed C, A, B and D.

Digestibility

The nutrient and energy digestibility shown by the fingerlings fed on control and a formulated feed for 90 days is given in Table 4.

Dry matter digestibility

The highest dry matter digestibility (58.87%) was recorded in the fingerlings fed on feed B and the least (48.14%) in the fingerlings fed on feed C.

Protein digestibility

The highest protein digestibility (75.02%) was recorded in

Table 4. Nutrient and energy utilization and digestibility of fingerlings fed on control and formulated feeds for 90 days.

Parameter	Feed					± SEM
	Control	Feed A	Feed B	Feed C	Feed D	
Protein efficiency ratio (PER)	2.09 ± 0.03 ^a	2.48 ± 0.04 ^b	2.66 ± 0.01 ^c	2.06 ± 0.02 ^a	2.26 ± 0.02 ^{ab}	0.02
Apparent net protein utilization (ANPU %)	26.88 ± 0.18 ^a	35.48 ± 0.28 ^b	36.67 ± 0.19 ^c	26.24 ± 0.24 ^a	32.62 ± 0.17 ^{ab}	0.21
Apparent net lipid utilization (ANLU %)	25.61 ± 0.17 ^a	31.63 ± 0.26 ^b	32.43 ± 0.12 ^b	25.15 ± 0.21 ^a	29.54 ± 0.14 ^b	0.16
Apparent net energy utilization (ANEU %)	21.78 ± 0.19 ^a	26.32 ± 0.31 ^b	28.65 ± 0.27 ^b	21.53 ± 0.21 ^a	25.18 ± 0.19 ^b	0.23
Dry matter digestibility (%)*	48.64	57.21	58.87	48.14	56.13	
Protein digestibility (%)*	59.50	73.10	75.02	59	71.65	--
Lipid digestibility (%)*	66.16	87.10	88.14	65.21	86.14	--
Carbohydrate digestibility (%)*	49.64	55.53	55.95	48.82	54.97	--
Energy digestibility (%)*	55.18	61.05	62.15	54.60	60.12	--

Values are means ± SD of three replications (d.f. 5, 17). Means in the same row having different superscripts are significantly different ($P < 0.05$) and means in the same row with same superscript are not significantly different ($P > 0.05$). *No statistical analysis was possible as determinations were performed on pooled samples.

the fingerlings fed on feed B and the least (59%) in the fingerlings fed on feed C.

Lipid digestibility

The highest lipid digestibility (88.14%) was recorded in the fingerlings fed on feed B and the least (65.21%) in the fingerlings fed on feed C.

Carbohydrate digestibility

The highest carbohydrate digestibility (55.95%) was recorded in the fingerlings fed on feed B and the least (48.82%) in the fingerlings fed on feed C.

Energy digestibility

The highest energy digestibility (62.15%) was recorded in the fingerlings fed on feed B and the least (54.60%) in the fingerlings fed on feed C.

DISCUSSION

Nutrient and energy utilization

The PER was recorded the highest (2.66 ± 0.01) in the fingerlings fed on feed B and the least (2.06 ± 0.02) in the fingerlings fed on feed C. There was no significant difference ($P > 0.05$) in the PER of the fingerlings fed on control feed and feed C. The PER of the fingerlings fed on feed A was significantly lower ($P < 0.05$) as compared to the PER of the fingerlings fed on feed B. The highest PER in feed B having 40% dietary protein level gets support from the work of Babalola and Adebayo (2006) who reported the highest PER 3.98 in catfish fed with higher crude protein (50%). Similar values of higher PER attained at higher dietary protein levels has been reported by Gul et al. (2007), Diyaware et al. (2009), Adewolu and Adoti (2010) and Sotolu (2010) in several fish species. The ANPU was recorded the highest ($36.67 \pm 0.19\%$) in the fingerlings fed on feed B and the least ($26.24 \pm 0.24\%$) in the fingerlings

fed on feed C. There was no significant difference ($P > 0.05$) in the ANPU of the fingerlings fed on control feed and feed C. The ANPU of the fingerlings fed on feed A was significantly lower ($P < 0.05$) as compared to the ANPU of the fingerlings fed on feed B. Diler et al. (2007), while replacing fish meal with *Ulva rigida* having 11.50% crude protein reported the lowest ANPU (23.41%) in common carp, in contrast to Samsons and Fasakin (2008) who reported 45.62% ANPU in African catfish, after inclusion of animal protein meals with crude protein > 50 to 55%, but reported decrease in ANPU and PER after the crude protein content was enhanced to 60%. These findings lend support to the present work showing the highest ANPU in feed B having 40% dietary protein level and the least ANPU in feed C having 25.98% dietary protein level, barring the protein requirements of the test fish species. The protein utilization in terms of PER and ANPU exhibited positive correlation with the dietary protein level up to 40% and above 40% dietary protein level, both the parameters decreased significantly

($P < 0.05$). The PER and ANPU also exhibited positive correlation with the lipid level of feed. Lupatsch et al. (2001) and Bright et al. (2005) reported increase in protein utilization with the increase in dietary protein and lipid level in catfish, these observations support present finding related to the increase in protein utilization with the increase in dietary protein and lipid levels.

The ANLU was found the highest ($32.43 \pm 0.12\%$) in the fingerlings fed on feed B and the least ($25.15 \pm 0.21\%$) in the fingerlings fed on feed C. The ANEU was found the highest ($28.65 \pm 0.27\%$) in the fingerlings fed on feed B and the least ($21.53 \pm 0.21\%$) in the fingerlings fed on feed C. There was no significant difference ($P > 0.05$) in the ANLU and ANEU of the fingerlings fed on control feed and feeds C, A, B and D. Morais et al. (2001) reported ANLU 36.25% in Atlantic cod at 40% dietary protein level, which strongly supports the present observation showing the highest ANLU in feed B having 40% dietary protein level. Kalita et al. (2008) and Alessio et al. (2010) reported higher ANLU and ANEU values in *Catla catla*, *Cirrhinus mrigala* and gilthead sea bream, *Sparus aurata*, respectively, after increasing the dietary crude protein levels from 30 to 40%, which lend support to the present observation of the highest ANLU and ANEU in feed B having 40% dietary protein level. Both ANLU and ANEU exhibited positive correlation with the dietary lipid level, which is in accordance with the findings of Morais et al. (2001) and Lee et al. (2002), who reported the increase in lipid utilization and energy utilization with the increase in dietary lipid levels.

Digestibility

The highest dry matter digestibility (58.87%) was recorded in the fingerlings fed on feed B. The least dry matter digestibility (48.14%) was recorded in the fingerlings fed on feed C. The highest dry matter digestibility in feed B having 40% dietary protein conforms with the work of Cheng et al. (2003) and Gul et al. (2007) who reported better dry matter digestibility in fish fed higher dietary protein levels. The highest protein digestibility (75.02%) was recorded in the fingerlings fed on feed B. The least protein digestibility (59%) was recorded in the fingerlings fed on feed C. The protein digestibility increased with the increase in dietary protein level up to 40% and above 40% of the dietary protein level, decrease in protein digestibility was observed. Gul et al. (2007), Portz and Cyrino (2004) and Tidwell et al. (2005) reported the increase in protein digestibility with the increase in dietary protein level.

The highest lipid digestibility (88.14%) was recorded in the fingerlings fed on feed B. The least lipid digestibility (65.21%) was recorded in the fingerlings fed on feed C. The highest carbohydrate digestibility (55.95%) was recorded in the fingerlings fed on feed B. The least carbohydrate digestibility (48.82%) was recorded in the fingerlings fed on feed C. The highest energy digestibility

(62.15%) was recorded in the fingerlings fed on feed B. The least energy digestibility (54.60%) was recorded in the fingerlings fed on feed C. Similar findings have been reported in literature by Steven and Delbert (2000), Maina et al. (2002) and Perla et al. (2004) who reported higher lipid, carbohydrate and energy digestibility in fishes fed on diets having higher crude protein and low carbohydrate contents. Perla et al. (2004) reported higher carbohydrate and energy digestibility values of 60.5 and 65.3% respectively at dietary crude protein > 45% in juvenile grouper, which lend support to present finding of highest carbohydrate and energy digestibility in feed B having 40% dietary protein level.

The dry matter, protein, lipid, energy and carbohydrate digestibilities decreased with the increase in dietary carbohydrate level. This observation is in conformation to those of Stone (2003), Jesu et al. (2008), Gumus and Ikiz (2009) and Tian et al. (2010).

Based on nutrient and energy utilization and digestibility this work concludes that feed B containing 40% protein, 9.31% lipid, 10.08% carbohydrate and having P/E ratio 20.54 mg protein/Kg is the best one for a more profitable and successful culture of the common carp.

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REFERENCES

- Adewolu MA, Adoti AJ (2010). Effect of mixed feeding of varying dietary protein levels on the growth and feed utilization of *Clarias gariepinus* fingerlings. J. Anim. Vet. Adv. 9(10): 1415-1419.
- Alatise PS, Ogundele O, Eyo AA, Oludunjoye F (2006). Evaluation of different soybean-based diets on growth and nutrient utilization of *Heterobranchus longifilis* in aquaria tanks. FISON conference proceeding. pp. 255-262.
- Alessio B, Gloria I, Ramon F, Luca P, Ester G, Pier PG (2010). Growth and feed utilization of gilthead sea bream, *Sparus aurata* fed increasing dietary energy levels. J. Aquacult. 282(2): 309-314.
- Amoah A, Shawn DC, Carl DW, Robert MD, Leigh AB, James HD (2008). Effect of graded levels of carbohydrates on growth and survival of largemouth bass, *Micropterus salmoides*. J. World Aquacult. Soc. 39(3): 397-405.
- AOAC (1995). Official methods of analysis of the Association of Official Analytical Chemist. 16th edition, Association of Official Analytical Chemist, Washington, DC, USA. pp. 1234-1565.
- Babalola F, Adebayo P (2006). Effect of dietary protein on the growth of catfish. J. Aquacult. Int. 39(4): 233-237.
- Bright LA, Coyle SD, Tidwell JH (2005). Effect of dietary lipid level and protein energy ratio on growth and body composition of largemouth bass, *Micropterus salmoides*. J. World Aquacult. Soci. 36(1): 129-134.

- Burel C, Boujard T, Tulli F, Kaushik SJ (2000). Digestibility of extruded peas, extruded lupin, and rapeseed meal in rainbow trout, *Oncorhynchus mykiss* and turbot, *Psetta maxima*. J. Aquacult. 188: 285-298.
- Cheng ZJ, Hardy RW, Idler DR (2003). Assessment of dry matter and protein digestibilities of selected raw ingredients by discus fish, *Symphysodon aequifasciata*. J. Aquacult. Nutri. 9(2): 123-128.
- Diler I, Adem TD, Guroy BK, Soyuturk M (2007). Effects of *Ulva rigida* on the growth, feed intake and body composition of Common carp, *Cyprinus carpio*. J. Bio. Sci. 7(2): 305-309.
- Diyaware MY, Modu BM, Yakubu UP (2009). Effect of dietary protein levels on growth performance and feed utilization of hybrid catfish, *Heterobranchius bidorsalis* x *Clarias anguillaris*. Afr. J. Biotech. 8(16): 3954-3957.
- Du ZY, Clouet P, Huang LM, Degrace P, Zheng WH, Tian LX, Liu YJ (2008). Utilization of different dietary lipid sources at high level in herbivorous grass carp, *Ctenopharyngodon idella* and mechanism related to hepatic fatty acid oxidation. J. Aquacult. Nutr. 14: 77-92.
- Gauquelina F, Cuzona G, Gaxiolab G, Rosasb C, Arenab L, Bureau DP, Cochara JC (2007). Effect of dietary protein level on growth and energy utilization by *Litopenaeus stylirostris* under laboratory conditions. J. Aquacult. 271(1-4): 439-448.
- Gul Y, Salim M, Rabbani B (2007). Evaluation of apparent digestibility coefficients of different dietary protein levels with and without fish meal for *Labeo rohita*. Pak. Vet. J. 27(3): 121-125.
- Gullaine JC, Kaushik S, Bergot P, Metailler R (2001). Nutrition and feeding of fish and crustaceans. Springer-Praxis books edition. pp. 1-396.
- Gumus E, Ikiz R (2009). Effect of dietary levels of lipid and carbohydrate on growth performance, chemical contents and digestibility in rainbow trout, *Oncorhynchus mykiss*. Pak. Vet. J. 29(2): 59-63.
- Hemre GI, Mommsen TP, Krogdahl A (2002). Carbohydrates in fish nutrition: effects of growth, glucose metabolism and hepatic enzymes. Aquacult. Nutr. 8:175-194.
- Jamabo NA, Alfred OJF (2008). Effects of dietary protein levels on the growth performance of *Heterobranchius bidorsalis* fingerlings from Niger delta. Afr. J. Biotechnol. 7(14): 2483-2485.
- Jesu AR, Haniffa MA, Seetharaman S, Appelbaum S (2008). Utilization of various dietary carbohydrate levels by the freshwater Catfish, *Mystus montanus* (Jerdon). Turk. J. Fish. Aquacult. Sci. 8: 31-35.
- Kalita S, Dabrowski J, Haynu S (2008). Effect of supplementation of four non- conventional aquatic weeds to the basal diet on growth, nutrient utilization and body composition of *Catla catla* and *Cirrhinus mrigala* fingerlings. J. World Aquacult. Soc. 39(3): 353-359.
- Kaushik SJ (2004). Fish oil replacement in aquafeeds. Aqua Feeds: Formulation and Beyond. pp. 1: 3-6.
- Kiessling A, Pickova J, Johansson L, Asgard T, Storebakken T, Kiessling KH (2001). Changes in fatty acid composition in muscle and adipose tissue of farmed rainbow trout, *Oncorhynchus mykiss* in relation to ration and age. J. Food Chem. 73: 271-284.
- Kim KW, Wang X, Choi SM, Park GJ, Bai SC (2004). Evaluation of optimum dietary protein to energy ratio in juvenile olive flounder, *Paralichthys olivaceus*. J. Aquacult. Res. 35: 250-255.
- Krogdahl A, Hemre GI, Mommsen TP (2005). Carbohydrates in fish nutrition. Digestion and absorption in postlarval stages. J. Aquacult. Nutri. 11: 103-122.
- Lee SM, Jeon IG, Lee JY (2001). Effects of digestible protein and lipid levels in practical diets on growth, protein utilization and body composition of juvenile rockfish, *Sebastes schlegelii*. J. Aquacult. 211: 227-39.
- Lupatsch I, Kissil GW, Sklan D, Pfeffer, E (2001). Effects of varying dietary protein and energy supply on growth, body composition and protein utilization in gilthead seabream, *Sparus aurata*. J. Aquacult. Nutri. 7: 71-80.
- Maina JG, Beames RM, Higgs D, Iwama G, Kisia SM (2002). Digestibility of some feed ingredients fed to tilapia, *Oreochromis niloticus*. J. Aquacult. Res. 33(2): 853-859.
- Maynard LA, Loosli JK (1969). Animal nutrition. 6th edition, McGraw-Hill, New York, USA. pp. 234-236.
- Menoyo D, Lopez BCJ, Baustita JM, Obach A (2003). Growth, digestibility and fatty acid utilization in large Atlantic salmon, *Salmo salar* fed varying levels of n-3 and saturated fatty acids. J. Aquacult. 225: 295-307.
- Morais S, Bell JG, Robertson DA, Roy WJ, Morris PC (2001). Protein-lipid ratios in extruded diets for Atlantic cod, *Gadus morhua*. Effects on growth, feed utilization, muscle composition and liver histology. J. Aquacult. 203: 101-119.
- Mourente G, Goodand JE, Bell JG (2005). Partial substitution of fish oil with rapeseed, linseed and olive oils in diets for European sea bass, *Dicentrarchus labrax*. Effects on flesh fatty acid composition, plasma prostaglandins E2 and F2a, immune function and effectiveness of a fish oil finishing diet. J. Aquacult. Nutr. 11: 25-40.
- Mustafizur R, Leopold AJ, Nagelkerke MCJ, Verdegem M, Wahab A, Johan AJ (2008). Relationships among water quality, food resources, fish diet and fish growth in polyculture ponds. J. Aquacult. 272(1): 108-115.
- NRC (1993). Nutrient requirements of fish. National Academy Press, Washington, DC, USA. pp. 114-398.
- Perla S, Eusebio R, Coloso M, Roger EP (2004). Apparent digestibility of selected ingredients in diets for juvenile grouper, *Epinephelus coioides*. J. Aquacult. Res. 35(3): 1261-1268.
- Portz L, Cyrino JEP (2004). Digestibility of nutrients and amino acids of different protein sources in practical diets by largemouth bass, *Micropterus salmoides*. J. Aquacult. Res. 35: 312-320.
- Samsons YA, Fasakin AE (2008). Use of animal protein meals as fish meal replacer in the diets of the African catfish, *Clarias gariepinus* juveniles. J. Tropicult. 26(2): 89- 93.
- Shiau SY, Lin YH (2001). Carbohydrate utilization and its protein-sparing effect in diets for grouper, *Epinephelus malabaricus*. J. Anim. Sci. 73: 299-304.
- Sotolu AO (2010). Effect of varying dietary protein levels on the growth performance of *Clarias gariepinus* fry. J. Livestock Res. Rural Dev. 22(4): 312-318.
- Steven D, Delbert M (2000). Nutrient digestibility of common feedstuffs in extruded diets for sunshine bass, *Morone chrysops* x *M. saxatilis*. J. World Aquacult. Soc. 31(4): 570-575.
- Stone DAJ (2003). Dietary carbohydrate utilization by fish. Rev. Fish. Sci. 11: 337-369.
- Storebakken T (2002). Atlantic salmon. Webster, CD and Lim C, editors. Nutrient requirements and feeding of finfish for aquaculture. CABI Publishing, Wallingford, UK. pp. 79-102.
- Subhadra B, Lochmann R, Rawles S, Chen R (2006). Effect of dietary lipid source on the growth, tissue composition, and hematological parameters of largemouth bass, *Micropterus salmoides*. J. Aquacult. 255: 210-222.
- Tian LX, Liu YJ, Hung SSO, Deng DF, Yang HJ, Niu J, Liang GY (2010). Effect of feeding strategy and carbohydrate source on carbohydrate utilization by Grass carp, *Ctenopharyngodon idella*. Am. J. Agric. Bio. Sci. 5(2): 135-142.
- Tidwell JH, Coyle SD, Bright LA, Yasharian D (2005). Evaluation of plant and animal source proteins for replacement of fish meal in practical diets for the largemouth bass, *Micropterus salmoides*. J. World Aquacult. Soc. 36: 454-463.
- Webster CD, Lim L (2002). Introduction to fish nutrition. Webster, CD and Lim C, editors. Nutrient requirements and feeding of finfish for aquaculture. CABI Publishing, Wallingford, UK. pp. 1-27.