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<b>DRAFT FOR COMMENTS ONLY</b>	<b>Doc: No. FAD 12 (19906) C</b>
<p><i>Draft Indian Standard</i></p> <p><b>मत्स्य आहार — विशिष्टि, भाग 2 मागुर और सिंघी आहार</b> (आई एस 16150, भाग 2 का पहला पुनरीक्षण)</p> <p><b>Fish Feed — Specification, Part 2 <i>Magur</i> and <i>Singhi</i> Feed</b> [First revision of IS 16150 (Part 2)] ICS No 65.120, 67.120.30</p>	
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Fish, Fisheries and Aquaculture Sectional Committee, FAD 12

#### FOREWORD

(Formal clauses to be added later)

Aquaculture is making a rapid progress within the country. Many aquaculture farms have been established where aquaculture has been undertaken on scientific lines. It is important that for producing good quality fish at minimum cost, the fish are properly fed to meet their nutritional requirements. To keep pace with the development of aquaculture, the manufacture of fish feeds and their marketing has also commenced in the country.

This standard was first published in 2014 with a view to enable the manufacturers to prepare fish feeds of known quality. This first revision is being brought about to address changes in feed manufacturing practices and regulatory requirements. In this revision, the following major changes have been incorporated:

- a) The title and scope have been modified to make the standard species specific;
- b) Nutrient composition and aflatoxin B1 limits have been revised based on national and global best practices;
- c) Usage of antibiotics in feed has been prohibited altogether;
- d) New ingredients have been included for use in air-breathing catfish feed formulations; and
- e) Methods of test are updated as per latest available standardized methods and alternate methods are also prescribed where needed.

This is one among the series of Indian Standards formulated to ensure the availability of feeds of suitable quality for fish. Other standards in this series are as follows:

(Part 1) Carp feed

(Part 3) Marine shrimp feed

(Part 4) Freshwater prawn feed

(Part 5) *Pangasius* feed  
Part 6) Marine Carnivorous Fish Feed  
(Part 7) *Tilapia* Feed

In revising this standard considerable assistance has been provided by the Central Institute of Freshwater Aquaculture, Bhubaneswar.

For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test or analysis, shall be rounded off in accordance with IS 2: 2022 'Rules for rounding off numerical values (*second revision*)'. The number of significant places retained in the rounded-off value should be the same as that of the specified value in this standard.

## **1 SCOPE**

This standard prescribes the requirements and the methods of sampling and testing for air-breathing catfish like *Magur (Clarias magur)* and *Singhi (Heteropneustes fossilis)* feeds.

## **2 REFERENCES**

The standards listed in Annex A contain provisions, which constitute provisions of this standard through reference in this text. At the time of publication, the editions indicated were valid. All standards are subject to revision and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent editions of the standards listed in Annex A.

## **3 TYPES**

Air-breathing Catfish feed shall be of the following four types:

- a) *Air-breathing Catfish Larval Feed (ABCLF)* – feed to be fed to catfish larvae up to 20<sup>th</sup> day of hatching/20mm length.
- b) *Air-breathing Catfish Fry Feed (ABCFF)* – feed to be fed to catfish from 21 - 90 days/21 – 70mm length.
- c) *Air-breathing Catfish Grow-out Feed (ABCGF)* – feed to be fed to catfish from 91 days to harvesting.
- d) *Air-breathing Catfish Brood Feed (ABCBF)* – feed to be fed to brood catfish before 4 months of the onset of breeding.

## **4 REQUIREMENTS**

### **4.1 Description**

The air-breathing catfish feed shall be free from rancidity, adulterants, moulds and insect infestation.

### 4.1.1 *Ingredients*

The ingredients listed in Annex B shall only be used for manufacturing air-breathing catfish feed.

## 4.2 **Physical Characteristics**

### 4.2.1 *Feed Form and Size*

**4.2.1.1** Air-breathing Catfish Larval Feed shall be in the form of powder or granules of size less than or equal to 100 µm.

**4.2.1.2** Air-breathing Catfish Fry Feed shall be in the form of granules or crumbles of size 0.25 mm to 1 mm.

**4.2.1.3** Air-breathing Catfish Grow-out Feed shall be in the form of crumbles or pellets of size 1 mm to 2 mm.

**4.2.1.4** Air-breathing Catfish Brood Feed shall be in the form of pellets of size 2 mm and above.

### 4.2.2 *Water Stability of Pellets*

The feed pellets shall be stable without disintegration in water for 2 h minimum. The water stability shall be as follows when tested as per Annex C.

<i>Type of Catfish Feed</i>	<i>Water Stability</i>
Air-breathing Catfish Larval Feed	Not less than 90 per cent after 30 min
Air-breathing Catfish Fry Feed	Not less than 90 per cent after 45 min
Air-breathing Catfish Grow-out Feed	Not less than 90 per cent after 45 min
Air-breathing Catfish Brood Feed	Not less than 90 per cent after 60 min

**4.3** The air-breathing catfish feed shall also conform to the requirements given in Table 1.

**Table 1 Requirements for air-breathing catfish feed**  
(*Clause 4.3*)

SI No.	Characteristic	Requirements for				Method of Test, Ref to
		Air-breathing Catfish Larval Feed	Air-breathing Catfish Fry Feed	Air-breathing Catfish Grow-out Feed	Air-breathing Catfish Brood Feed	
(1)	(2)	(3)	(4)	(5)	(6)	(7)
i)	Moisture, per cent by mass, <i>Max</i>	12	12	12	12	IS/ISO 6496* or 4 of IS 7874 (Part 1)

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ii)	Crude Protein (N x 6.25), per cent by mass, <i>Min</i>	40	35	35	30	IS/ISO 5983: Part 1* or IS/ISO 5983: Part 2 or 5 of IS 7874 (Part 1)
iii)	Crude fat, per cent by mass, <i>Min</i>	8	8	6	6	IS/ISO 6492 or 7 of IS 7874 (Part 1)*
iv)	Crude fibre, percent by mass, <i>Max</i>	4	6	6	6	IS/ISO 6865 <sup>#</sup>
v)	Acid insoluble ash, per cent by mass, <i>Max</i>	2.5	3	3	3	IS 14826* or 10 of IS 7874 (Part 1)
vi)	Gross energy, kcal/kg, <i>Min</i>	3300	3300	3200	3100	Annex D

NOTES –

- 1) The values for requirements specified at sl. no. (ii) to (vi) are on a moisture-free basis.
- 2) For routine analysis, the characteristics at sl. No. (ii) to (v) may be tested by a near infrared-analyzer.
- 3) In case of dispute, the methods indicated by ‘\*’ shall be the referee method.
- 4) <sup>#</sup>This standard includes both manual and semi-automatic procedures. In case of any dispute, manual method of IS/ISO 6865 shall be the referee method.

#### 4.4 Antibiotics

No antibiotics or pharmacologically active substances shall be incorporated into air-breathing catfish feed.

4.5 Aflatoxin B1 contamination in the air-breathing catfish feed shall not exceed 0.02 mg/kg at the time of manufacture. Aflatoxin B1 shall be tested by the manufacturer in accordance with the test method prescribed in IS 14718 and declared on the label.

## 5 PACKING AND MARKING

### 5.1 Packing

The material shall be packed in clean, dry, polythene-lined bags/ jute bags/ laminated paper bags/ HDPE bags/ PP bags/ BOPP bags. Each bag's mouth shall be machine stitched or rolled over and hand stitched.

### 5.2 Marking

5.2. 1 Each bag shall be suitably marked so as to give the following information legibly:

- a) Name of the material and brand name, if any;
- b) Type of the fish feed;
- c) Name and address of the manufacturer;
- d) Net weight;
- e) Batch or Code number;
- f) Date of manufacture (MM/YY);
- g) Best before (MM/YY); and

h) Any other markings required under the *Legal Metrology (Packaged Commodities) Rules, 2011*.

**5.2.2** In addition to the information listed in **5.2.1**, each bag shall have a label or tag attached to it or contain a leaflet giving the following information:

- a) List of ingredients in decreasing order of proportion;
- b) Name and quantity of the additives added, if any;
- c) Moisture content;
- d) Crude protein content;
- e) Crude fibre content;
- f) Crude fat content;
- g) Aflatoxin B<sub>1</sub> content;
- h) Gross energy; and
- i) Direction for use.

### **5.3 BIS Certification Marking**

The product(s) conforming to the requirements of this standard may be certified as per the conformity assessment schemes under the provisions of the *Bureau of Indian Standards Act, 2016*, and the Rules and Regulations framed thereunder, and the products may be marked with the Standard Mark.

## **6 SAMPLING**

Representative samples of the material shall be drawn according to the method prescribed in IS 1374.

## **7 TESTS**

**7.1** Tests shall be carried out as prescribed in **4.2.2**, **4.4** and Table 1.

## **8 QUALITY OF REAGENTS**

Unless specified otherwise, pure chemicals and distilled water (*see* IS 1070) shall be employed in tests.

NOTE — "Pure chemicals" shall mean chemicals that do not contain impurities which affect the result of analysis.

### **ANNEX A** (*Clause 2*)

#### **LIST OF REFERRED INDIAN STANDARDS**

<i>IS No.</i>	<i>Title</i>
IS 920: 1972	Specification for common salt and cattle licks for animal consumption ( <i>first revision</i> )
IS 1070: 2023	Reagent grade water — Specification ( <i>fourth revision</i> )

IS 1162: 2021	Cane molasses — Specification ( <i>first revision</i> )
IS 1374 : 2007	Poultry feeds - Specification ( <i>fifth revision</i> )
IS 1712 : 2022	Cottonseed oilcake as livestock feed ingredient — Specification ( <i>third revision</i> )
IS 1713 : 2022	Decorticated groundnut oilcake as livestock feed ingredient — Specification ( <i>third revision</i> )
IS 1932 : 2022	Mustard and rapeseed oilcake as livestock feed ingredient — Specification ( <i>third revision</i> )
IS 1934 : 2016	Sesamum oilcake as livestock feed ingredient — Specification ( <i>second revision</i> )
IS 1942 : 1968	Specification for bone-meal as livestock feed supplement ( <i>first revision</i> )
IS 2151 : 1985	Specification for maize germ oilcake as livestock feed ingredient ( <i>first revision</i> )
IS 2152 : 2013	Maize gluten as livestock feed ingredient — Specification ( <i>second revision</i> )
IS 2154 : 2014	Coconut oilcake as livestock feed ingredient — Specification ( <i>third revision</i> )
IS 2239 : 2022	Wheat Bran as Livestock Feed Ingredient — Specification ( <i>second revision</i> )
IS 3441 : 2022	Solvent extracted groundnut oilcake (meal) as livestock feed ingredient — Specification ( <i>second revision</i> )
IS 3592: 1985	Specification for solvent extracted decorticated cottonseed oilcake (meal) as livestock feed ingredient ( <i>second revision</i> )
IS 3593 : 2022	Solvent extracted rice bran (de-oiled rice bran) as livestock feed — Specification ( <i>third revision</i> )
IS 3648 : 1975	Specification for rice bran as livestock feed ( <i>first revision</i> )
IS 4193 : 2022	Guar Meal as Livestock Feed Ingredient -Specification ( <i>second revision</i> )
IS 4307 : 1983	Specification for fishmeal as livestock feed ingredient ( <i>second revision</i> )
IS 5065 : 1986	Specification for meat meal as livestock feed ingredient ( <i>first revision</i> )
IS 5470 : 2002	Dicalcium phosphate, animal feed grade - Specification ( <i>first revision</i> )
IS 5862 : 2022	Solvent extracted niger seed oilcake (meal) as livestock feed — Specification ( <i>first revision</i> )
IS/ISO 5983 : Part 1 : 2005	Animal feeding stuffs — Determination of nitrogen content and calculation of crude protein content Part 1 Kjeldahl method
IS 5983 (Part 2) : 2021 / ISO 5983-2 : 2009	Animal feeding stuffs — Determination of nitrogen content and calculation of crude protein content, Part 2 Block digestion steam distillation method ( <i>first revision</i> )
IS 6242 : 1985	Specification for solvent extracted undecorticated safflower oilcake as livestock feed ingredient ( <i>first revision</i> )
IS/ISO 6492 : 1999	Animal feeding stuffs — Determination of fat content
IS/ISO 6496 : 1999	Animal feeding stuffs — Determination of moisture and other volatile matter content
IS/ISO 6865 : 2000	Animal feeding stuffs — Determination of crude fibre content — Method with intermediate filtration
IS 7060 : 1973	Specification for blood meal as livestock feed
IS 7874	Methods of tests for animal feeds and feeding stuffs

(Part 1) : 1975	General methods
(Part 2) : 1975	Minerals and trace elements
IS 13398 : 1992	Alga spirulina, feed grade — Specification
IS/ISO 14718 : 1998	Animal feeding stuffs – Determination of aflatoxin B <sub>1</sub> content of mixed feeding stuffs – Method using high performance liquid chromatography
IS 14826 : 2021 / ISO 5985 : 2002	Animal feeding stuff — Determination of ash insoluble in hydrochloric acid ( <i>first revision</i> )

**ANNEX B**  
(*Clause 4.1.1*)

**INGREDIENTS FOR AIR-BREATHING CATFISH FEED**

**B-1** In the compounding of air-breathing catfish feed a variety of ingredients are used. This annexure gives a list of such ingredients.

**B-1.1 Grain and Seeds**

- a) Maize;
- b) Barley;
- c) Bajra;
- d) Wheat;
- e) Jowar;
- f) Oats;
- g) Ragi; and
- h) Rice / Broken rice

**B-1.2 Grain By-products**

- a) Rice bran or solvent-extracted rice bran and polishing's (*see* IS 3648 and IS 3593);
- b) Wheat bran (*see* IS 2239);
- c) Maize gluten and maize gluten feed (*see* IS 2152);
- d) Grain sievings; and
- e) Rice gluten.

**B-1.3 Oil Cakes and Meals**

- a) Copra cake, coconut oilcake (expeller-pressed or solvent extracted) (*see* IS 2154);
- b) Cottonseed oilcake (decorticated) (expeller-pressed or solvent extracted) (*see* 1712 and IS 3592);
- c) Groundnut oilcake (expeller-pressed or solvent extracted) (*see* 1713 and IS 3441);
- d) Maize germ oilcake (*see* 2151);
- e) Mustard and rapeseed oilcake (*see* IS 1932);
- f) Neem seed kernel cake;

- g) Sal seed meal, solvent extracted;
- k) Soybean meal (solvent extracted);
- h) Safflower (*Canthamus tinctorius*) oilcake (expeller- pressed or solvent extracted) (*see* IS 6242);
- i) Sesame (*Sesamum indicum orientale*) oilcake (expeller- pressed or solvent extracted) (*see* IS 1934);
- j) Sunflower oilcake (decorticated or undecorticated);
- k) Niger seed oilcake (*see* IS 5862);
- l) Guar meal (*see* IS 4193);
- m) Dried distillery grains with solubles (DDGS); and
- n) Seaweed meal.

#### **B-1.4 Tubers and Roots**

- a) Tapioca flour.

#### **B-1.5 Animal Products**

- a) Blood meal (*see* IS 7060);
- b) Fish meal (*see* IS 4307);
- c) Liver residue;
- d) Meat meal (*see* IS 5065) and meat scrap;
- e) Meat-cum-bone meal;
- f) Fish trimmings /Fish by product meal
- g) Chicken Trimmings / Poultry by-product meal
- h) Silkworm pupae meal;
- j) Shrimp and shrimp head meal;
- k) Mollusc meat meal;
- m) Bone meal (*see* IS 1942);
- n) Insect meal (the insects used a protein source have to be bred on the plant origin only);
- o) Fish protein hydrolysate;
- p) Squid liver paste;
- q) Squid meal;
- r) Krill meal;
- s) Crab meal;
- t) Crab shell meal; and
- r) Other invertebrate meal.

#### **B-1.6 Oils and Fats**

- a) Fish oil/ Squid liver oil;
- b) Edible vegetable oils;
- c) Tallow;
- d) Insect oil;
- e) Krill oil;
- f) Single cell oil; and
- g) Algal oil.



**B-1.7 Minerals, Vitamins, Amino acids and other Additives**

- a) Dicalcium phosphate (*see* IS 5470), Mono Calcium Phosphate (*see* IS 5470), Mono sodium phosphate, Mono potassium phosphate, Mono ammonium phosphate;
- b) Common salt (*see* IS 920);
- c) Vitamins (mineral stabilized);
- d) Mineral premix;
- e) Vitamin C;
- f) Crystalline amino acids (animal grade);
- g) Feed binders;
- h) Toxin binders;
- i) Immunostimulants;
- j) Carotenoids;
- k) Antioxidants;
- l) Exoenzymes; and
- m) Nucleotides.

**B-1.8 Industrial By-products**

- a) Brewers' grain;
- b) Dried yeast and yeast sludge;
- c) Molasses (*see* IS 1162);
- d) Dried distillery grains with solubles (DDGS); and
- e) Bio-diesel industry by-products.

**B- 1.9 Other Ingredients**

- a) Dunaliella;
- b) Antifungals;
- c) Peptidoglycans;
- d)  $\beta$ -glucans;
- e) Fuceoidan;
- f) Organic acids;
- h) Spirulina meal (*see* IS 13398);
- i) Other single-cell proteins (bacterial, yeast and microalgal meal);
- j) Protein concentrate/isolate from non-edible seed; and
- k) Herbal immunostimulants.

**ANNEX C**

(*Clause 4.2.2*)

**DETERMINATION OF WATER STABILITY OF AIR-BREATHING pCATFISH  
FEED PELLETS**

**C-1 PRINCIPLE**

The water stability of dry catfish feed pellets is determined by the loss in mass of pellets kept in water for a specified time interval. The loss in mass of pellets indicates the stability; higher the loss, the poorer the stability.

**C-2 APPARATUS**

**C-2.1 Oven**

**C-2.2 Nylon Mesh**

**C-2.3 Sieve (2.4 mm)**

**C-2.4 Balance**

**C-2.5 Glass Beaker (1 L)**

**C-2.6 Stop Watch**

**C-3 PROCEDURE**

Wash cone-shaped pouches made of nylon mesh (1 mm mesh size) thoroughly and dry at 70°C to constant mass in an oven. Take about 2 g of feed pellets in each pouch and record the exact initial mass. Take 5-6 such pouches for each sample. Place the pouches with feed pellets at the bottom of a 1 litre beaker containing 1 litre distilled water. Record water temperature. After the prescribed time, slowly take pouches with pellets out of the water. Examine the pellets for their physical shape. Dry the pouches with pellets at 70 °C to constant mass. The difference in the initial mass and final mass of the pellets gives the loss in mass at 70 °C.

**C-4 CALCULATION**

Water stability is calculated using the following formula:

$$\text{Percent Water Stability} = \frac{\text{Final mass (g)} \times \text{Percent dry matter}}{\text{Initial mass (g)} \times \text{Percent dry matter}} \times 100$$

**ANNEX D**

[Table 1, SI No. (vi)]

**GROSS ENERGY**

**D-0** Gross energy in raw materials and finished catfish feeds can be either directly estimated by bomb calorimeter (Method D-1) or calculated by Physiological Fuel Values (Method D-2) and the detailed procedures are given below

**D-I DETERMINATION OF GROSS ENERGY**

The bomb calorimeter provides a means of assessing the amount of energy (gross) made available during the catalytic degradation of combustible solids, liquids and gases in a pressurized oxygen atmosphere. Gross energy is the amount of heat liberated when a substance is completely burnt to carbon dioxide and water. It is also known as the heat of combustion.

**D-1.1 Preparation of Sample Material**

It is essential that the test sample is truly representative of the sample material. In general, the sample material needs to be dried before combustion, and here the sample characteristics will determine the method of drying to be used, that is, whether oven drying or vacuum drying at low temperature should be done before or after selecting a working sample. The drying process should not volatilize or destroy any of the combustible material. If complete dryness cannot be achieved easily without loss, preliminary tests should be made to determine the maximum water content at which this sample material can be ignited and completely burnt in the bomb. All materials with low bulk density and high surface area must be compacted.

## **D-2 PRINCIPLE**

A known quantity of a sample is ignited electrically and burnt in excess of oxygen in the bomb. The maximum temperature rise is measured with the thermometers in a controlled system. By comparing this rise with that obtained when a sample of a known calorific value is burnt, the calorific value of the sample material can be determined.

## **D-3 APPARATUS**

### **D-3.1 Adiabatic Bomb Calorimeter**

### **D-3.2 Pellet Press**

### **D-3.3 Metallic Crucible**

### **D-3.4 Hot Air Oven**

### **D-3.5 Balance**

### **D-3.6 Fuse Wire**

### **D-3.7 Cotton Thread**

### **D-3.8 Beaker**

### **D-3.9 Burette**

### **D-3.10 Pipette**

### **D-3.11 Whatman Filter Paper No. 1**

## **D-4 REAGENTS**

### **D-4.1 Benzoic Acid (Calorimeter Grade, Gross Energy Content 6 318 cal/g)**

### **D-4.2 Distilled Water**

### **D-4.3 Oxygen Gas**

### **D-4.4 Barium Hydroxide**

### **D-4.5 Sodium Carbonate**

### **D-4.6 Hydrochloric Acid**

### **D-4.7 Methyl Red Indicator**

### **D-4.8 Phenolphthalein Indicator**

## **D-5 PROCEDURE**

### **D-5.1 Determination of Bomb Equivalent**

- a) Take about 0.35 g of benzoic acid and make a pellet with the help of a pellet press.
- b) Place the pellet in a pre-weighed metallic crucible. Weigh the pellet and crucible accurately.
- c) Put the bomb top on the stand. Thread a piece of fuse wire through the electrodes and tie a single strand of cotton to it. Keep the lengths of fuse wire and cotton thread constant in order to facilitate the calculation of caloric value.
- d) Swing the crucible into position, clamp the ring and arrange the ends of the cotton thread so that they are in contact with the sample.
- e) Pipette 1 ml of distilled water into the bomb.
- f) Place the electrode assembly into the bomb body, ensuring it fits correctly.
- g) Tighten the bomb closure ring by hand only.
- h) Fill the bomb to 25 atmospheric pressure with oxygen (oxygen must be free from hydrogen).

- j) Fill water into the calorimetric vessel to submerge the bomb completely. The vessel and water should give a total mass of 3 kg. The quantity of water used is not critical, but it must be constant for all tests to an accuracy of  $\pm 0.5$  kg.
- k) Place the bomb on three supports in the calorimeter vessel and check for gas leakage so that the bomb does not show any sign of gas leakage.
- m) Gently slide the top of the calorimeter console onto the bomb. Switch on the main and press down the bomb firing plug to contact the bomb.
- n) Adjust the initial temperature and press the fire switch.
- p) After 8 min, read the temperature on the main thermometer. Note the final temperature when it stabilizes.

#### D-5.1.1 Calculation

$$\text{Bomb equivalent} = \frac{(6\,318 \times M) + A}{T}$$

where

$M$  = mass of benzoic acid (g);

$A$  = correction factor for wire and thread [heat of combustion of thread and wire may be taken as 3 962 cal/g and 1 400 cal/g (or 2.3 cal/cm) respectively]; and

$T$  = rise in temperature ( $^{\circ}\text{C}$ ).

#### D-5.2 Gross Energy Estimation of Feed

Weight 0.5-1 g of finely ground representative sample and make a pellet with the help of a pellet press. All the materials which have low bulk density and the high surface area must be compacted to reduce their rate of combustion, or otherwise, it will lead to a false result due to loss of sample from the crucible, even more serious is the possibility that the combustion will be so rapid that it resembles an explosion. Weigh samples for dry matter determination at the time of pelleting. Put the pellet in a pre-weighed crucible and weigh it again. Follow the steps (c) to (p) as described in **D- 5.1**. Switch off the main switch. Remove the bomb from the vessel. Release the pressure of the bomb using a pressure release cap. Open the bomb and wash the electrodes and the inside top and body of the bomb with distilled water. Collect these washing in a beaker for corrections for nitrogen and sulphur contents.

#### D-5.2.1 Calculation

$$\text{Gross energy (cal/g)} = \frac{(\text{Bomb equivalent} \times T) - A}{\text{Dry mass of sample (g)}}$$

where

$T$  = rise in temperature; and

$A$  = correction factors for wire, thread, nitrogen and sulphur.

#### D-5.3 Nitrogen and Sulphur Corrections

- a) Boil the washings (*see* D-5.2) collected in the beaker for 5 min.
- b) Cool and titrate against N/10 Ba(OH)<sub>2</sub> solution using phenolphthalein indicator.
- c) Add 20 ml of N/10 Na<sub>2</sub>CO<sub>3</sub> solution and boil again.

- d) Cool the contents, filter through Whatman filter paper No. 1, and give 2-3 washings with hot distilled water.
- e) Titrate the washings against N/10 HCl using a methyl orange indicator.
- f) Heat liberated due to the production of  $\text{H}_2\text{SO}_4$  and  $\text{HNO}_3$  can be calculated by using the following factors:

1 ml of N/10  $\text{Ba}(\text{OH})_2$  solution = 3.60 cal

1 ml of N/10  $\text{Na}_2\text{CO}_3$  solution = 1.43 cal

#### **D-5.3.1 Calculations**

Nitric acid correction (cal) = 1.43 ( $B - C$ )

Sulphuric acid correction (cal) = 3.60 [ $A - (B - C)$ ]

where

$A$  = amount of N/10  $\text{Ba}(\text{OH})_2$  solution used (ml);

$B$  = amount of N/10  $\text{Na}_2\text{CO}_3$  solution added (ml); and

$C$  = amount of N/10 HCl used (ml).

#### **D-II: CALCULATION OF GROSS ENERGY**

Moisture, crude protein, crude fat, crude fibre, and total ash in the catfish feed may be estimated per the test methods in table 1. Nitrogen-free extract (NFE), which represents the available carbohydrate portion of the feed material, shall be calculated as below:

Nitrogen free extractives =  $100 - (\% \text{ Crude Protein} + \% \text{ Crude Fat} + \% \text{ Crude Fibre} + \% \text{ Total Ash})$ .

Based on the protein, fat and carbohydrate, the heat of combustion can be calculated, and the gross energy equivalent of the test sample can be estimated. The Physiological Fuel Value (gross energy equivalent) for Crude Protein, Crude Fat and Nitrogen free extractives shall be 5.65, 9.45 and 4.2 kcal/g, respectively and the Gross Energy of the feed in kcal/kg shall be calculated as given below:

Gross Energy of the feed (kcal/kg) = (crude protein (g/kg)  $\times$  5.65) + (crude fat (g/kg)  $\times$  9.45) + (NFE (g/kg)  $\times$  4.2)