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Improvised protocol for quantitative determination of crude fat in fish feeds

Abstract

The efficacy of formulated feeds in successful aquariculture depends on its proper proximate components which include proteins (amino acids), lipids (fats, oils, fatty acids), carbohydrates (sugars, starch), vitamins, minerals, water and other supplementary additive such as probiotics. Estimation of the oil content is essential for assessing the quality of the formulated feeds meeting their nutritional values. Presently employed methods for quantifying crude fat in feedstuffs require solvent extraction, acid hydrolysis and inconvenient for the farmer community. So, the present investigation deals with designing a basic protocol to quantify the total fat in fish feed using Diethyl ether as a solvent which can be employed at wide range of temperatures however the moisture content of the feed should be around 12%.

Keywords: ornamental fish, formulated feed, crude fat, diethyl ether

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Introduction

In recent decades aquariculture has emerged as a globally growing million dollar industry comprising cultivation of various freshwater and marine species of finfish as well as shellfish.[1] Expansion of the ornamental fish culture industry has enabled Indian producers to market locally and internationally. One of the key drivers for rapid popularity of the hobby of ornamental fish keeping over the past 50 years is the development of manufactured or formulated feed.[2] Correct formulation of feed is the key component for improving nutrient digestibility as well as supplementing the metabolic needs, thereby reducing the maintenance cost and also combat water pollution. For normal growth and survival of ornamental fish the lipids are important sources of energy and fatty acids.[3] Improved growth performance was found in swordtail with increase in dietary lipid from 8% to 16% with same protein level. Additionally the source of lipid in the ovary depends on the muscle lipid content which is a vital indicator of the reproductive performance. To support cell membrane flexibility at lower water temperature fatty acids with low melting points are required.[2] Dietary levels of dietary docosahexaenoic acid significantly affected egg hatchability in the freshwater common carp, *Cyprinus carpio*[4] one of the food fishes having significant commercial value.

Cost effective diets can be formulated by partly sparing the protein increasing the percentage of dietary lipids. Despite of it, elevated levels of fat deposition in the liver can cause detrimental effect on fish health and market quality. Presence of too much fat in the body has profound negative influence in the reproductive potentialities. So, optimum dietary inclusion of fats in fish feed is a vital factor. Assessment of total oil in fish feed content is basically done by organic solvents employing the Soxlet[5] or by cold extraction methods.[6,7] Following extraction the fatty acid composition is estimated by transmethylation using the gas-liquid chromatography.

Commonly used solvent extraction tend be slow, inconvenient, costly and requires skilled personnel. Moreover, hazardous chemicals are used during the process which is unacceptable by many international environmental standards. Additionally these methods cannot be employed by traditional fish culturist. Taking in view, an improvised method is calibrated using Diethyl ether which is economical, simple and fast.

Materials and methods

The experiments were conducted in the Department of Life Sciences, Dibrugarh University.

Feed formulation

Feed was prepared using locally available ingredients and natural carotenoid sources using the trial and error method following Hardy[8] with little modification.

a. **Selection of feed ingredients:** The locally available ingredients such as fish meal, Soybean meal, Groundnut cake, Rice bran and Wheat flour were used for dough preparation.

b. **Feed formulation:** Feed was formulated using the following ingredients using the trial and error method following Hardy[8] with little modification.

c. **Grinding:** The selected ingredients were grinded and mixed in a definite proportions as depicted in Table 1

d. **Dough Preparation:** Lukewarm water was added to the mixed ingredients to prepare the dough.

e. **Cooking:** The prepared dough was steamed cooked in hot water bath at 70^0C for 30 minutes.

f. **Mixing of vitamin-mineral premix and additives (carotenoids, binders etc):** The cooked dough was allowed to cool and the ingredients such as starch powder, vitamin-mineral premix, carotenoids, vitamin C and soybean oil were mixed thoroughly.

g. **Pelleting:** The above mixture was pelleted using a hand pelletizer to form pellets of uniform size.

h. **Pellet Drying:** The pellets were dried in hot air oven at 60^0C for 25-30 minutes until moisture level reach less than 12%.

i. **Storage:** The dried pellets were stored in zipper bags of 8cm length and 6cm breadth and fed to the cultured fish. (Figure 1&2)

Table 1 Effect of temperature and moisture percentage on estimation of Crude fat

Feed type	Temperature	Crude fat %	Moisture %	Crude fat %
1	24	8	6	5
2	25	8	7	6.5
3	26	7.9	8	6.8
4	27	7.9	9	7.1
5	28	7.8	10	7
6	29	7.8	11	7
7	30	7.8	12	7.2
8	31	7.5	13	7.1
9	32	7.4	14	7.2
10	33	7.4	15	7.2

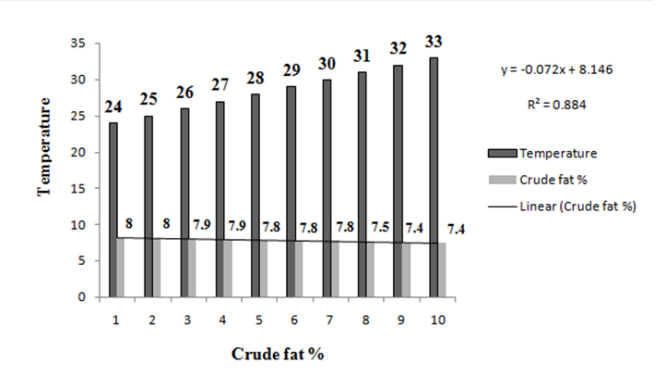

Figure 1 Effect of temperature on fat content.

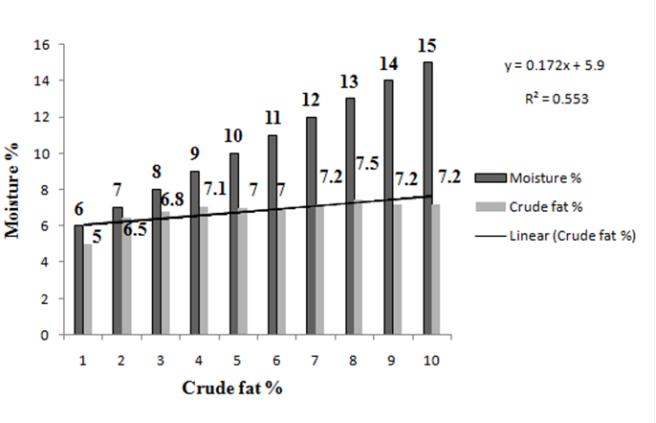

Figure 2 Effect of moisture on fat content.

Characteristics of the formulated feed

a. **Texture:** Uniform in appearance with smooth surface. Light tan to brown in colour having a diameter of 2mm and variation of length from 2.5mm to 5mm.

b. **Floating features:** Freely floating surface extruded (floating or buoyant) feeds.

c. **Results**

d. **Protocol for estimation of crude fat in the formulated feed:** Dry and finely grounded feed when mixed with diethyl ether dissolves fats and fat soluble substances at a temperature of 20^0C as the boiling point of Diethyl ether is 30^0C. Subsequently evaporation of ether from the fat solution leaves the resulting residue referred to as crude fat. Approximately 2grams of feed is weight and grinded to fine powder. In a conical flask finely grounded feed is mixed with 10ml of Diethyl Ether and boiled at temperature of 20^0C on a heating mantle for 20 minutes. Prior to extraction a petri dish is weight and after heating the supernatant is poured on the pre weighted petri dish and weight is taken. The difference in weight of the petri dish indicates the quantity of fats. For confirmation of fats in the sample Sudan III test [9–11] was done which gives a positive result.

Calculations

$$Fat\ content,\ percent = \frac{(B - C)\ X\ 100}{A}$$

Where A=Sample weight

B=Weight of the glass plate after extraction

C=Weight of the glass plate prior to extraction

Discussion

In this protocol significant correlation (0.553) between moisture and crude fat estimation was found however no significant effect of temperature have been observed on fat content. The protocol reveals that for its efficient utility the feed as well as the Diethyl ether should be devoid of moisture to inhibit the co extraction of components in the feed such as carbohydrates, urea, lactic acid etc. which are water soluble. To prevent oxidation of fat, the evaporation of ether and removal of residual moisture was carried out at low temperature. Considering the wide application of this one-step method for quantization of crude fat in fish feed the procedure is appropriate for commercial use as well as research purpose.

Conclusion

Lipids (fats) being high-energy nutrients, can be harnessed to partially spare protein in formulated aquaculture feeds. Next to protein, lipids play a significant role for optimum utilization of dietary protein for growth and reproductive performance. Traditionally applied solvent extraction procedures yield lower crude fat values than anticipation, so a simple and efficient method for quantification of fat in formulated will be great significance. Optimum care and attention should be taken while feeding fish in confined and stagnant water to synchronize the natural conditions. Improvement of feed meal based formulated feed with optimum nutritional attributes facilitate enhancement of growth, reproductive performance as well as quality and quantity of fish production. Crude fat being one of key ingredients of the balanced formulated feed so its optimum inclusion in the feed should be estimated. In this context a stable feed considering adequate requirement of fish using supplementary of feed ingredients will ensure successful fish culturing.

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Conflicts of interest

The author declares that there are no conflicts of interest.

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References

1. Das AP, Biswas SP. Carotenoids and Pigmentation in Ornamental Fish. *Journal of Aquaculture & Marine Biology*. 2016;4(4):00093.
2. Earle KE. The nutritional requirements of ornamental fish. *Veterinary Quarterly.* 1995;17:53–55.
3. Sales J, Janssens GPJ. Nutrient requirements of ornamental fish. *Aquatic Living Resources*. 2003:16(6):533–540.
4. Shimma Y. et al. On the lipids of adult carp raised on fish meal and SCP feeds, and hatchabilities of their eggs. *Bulletin of Freshwater Fisheries Research Laboratory*. 1977;27:35–48.
5. Association of Official Analytical Chemists. *Official Methods of Analysis*. 13th edn. AOAC: Arlington VA, Official Method 7.058. 1980.
6. Bligh EG, Dyer WJ. A Rapid Method of Total Lipid Extraction and Purification. *Can J Biochem Physiol*. 1959;37(8):911–917.
7. Folch J. Lees M, Sloane G. A Simple Method for the Isolation and Purification of Total Lipids from Animal Tissues. *J Biol Chem*. 1957;226(1):497–509.
8. Hardy R. Fish feed formulation. In: ADCP. *Fish feed Technology, Agriculture development coordination programme*. FAO/ADCP/Rep. 1980;80(1–1):233–239.
9. Larsen JC. Legal and illegal colors. *Trends in Food Science & Technology*. 2008;19 (Suppl. 1):S64–S69.
10. Browse J, McCourt PJ, Somerville CR. Fatty Acid Composition of Leaf Lipids Determined after Combined Digestion and Fatty Acid Methyl Ester Formation from Fresh Tissue. *Analytical Biochemistry*. 1986;152(1):141–145.
11. Chong A, Hashim RA Ab. Assessment of soybean meal in diets for discus (*Symphysodon aequifasciata* Heckel) farming through a fishmeal replacement study. *Aquaculture Research*. 2003;34(11):913–922.

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