Full Length Research Paper

Evaluation of herring (*clupea spp*) fish eggs as a potential source of dietary protein in animal model

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The protein composition of four different fish eggs was determined. The crude protein (%) in the eggs of Herring fish (Clupea spp) (48.50%) was higher than that of Clarias (27.70%), Heterobranchus (26.60%) and Tilapia (25.60%). The fish eggs with highest crude protein (Herring) was used as protein source to formulate the feed fed to experimental animals while soya beans were used as a protein source for the control. The fish eggs and the soya beans made up 30% of the test feed formula and control respectively. The weights and growth rate of experimental and Swiss albino rats were monitored for three weeks. The animals fed on the diet formulated with herring egg gained more weight than the control which received diet formulated with soya beans. The growth and development indices (body length measurements in cm) of Swiss albino rats fed with fish egg formula gave higher values for the experimental animals over the control. The nose to tail length was 29.3±1.26 cm for the experimental and 27.5 ±1.32 cm for the control. The respective lengths of tail and head circumference were 12.7 ± 1.26 and 2.30±0.2cm for the experimental while 12.5±0.76 and 2.20±0.2cm for the control, respectively. The net protein utilization (NPU) was 0.70mg and 0.40mg for animals fed with fish eggs and soya beans diet, respectively. The toxicological effects of the fish eggs formula determined from the hematological and serum biochemical parameters showed that Hb (%) for control (8.73 ± 0.81) and experimental (8.97±0.65) animals was not significantly p>0.05) different. The Packed Cell Volume (PCV) (%) was 26.30 ± 2.52 and 27.00 ± 2.0 for control and experimental animals respectively. The white blood cell count for experimental animals (3200 ± 608.28) was higher than that of control (3066.67 ± 608.28). The Serum total protein (STP) was 65.70 ± 31.70 g/l for Control and 119.00 ± 42.7g/l for the experimental animals. The serum total albumin (STA) was 25.80 ± 0.67g/l for control and 57.40 ± 16.50g/l for experimental animals.

Keywords: Protein, growth, fish, egg, formula.

INTRODUCTION

Nutrition plays a major role in growth and development of an individual. Dietary proteins are primary nutrient for growth and maintenance of the body's structural parts including muscles in animals. In human, breast milk is the main food recommended to sustain new born babies to at least six month old. In the first year of life, well fed children under favorable environmental conditions grow and develop rapidly and are expected to double in weight after six months and triple by the end of the first year, (Hart *et al.*, 1974). Rapid growth occurs in the first few years of life in humans and requires a high and regular supply of protein, this is because protein cannot be stored in the body. A deficiency or excess of one or more nutrient may cause nutritional disorder. The tragic consequence of mal-nutrition includes death, disability and stunted mental and physical growth. Research reports show that 49% of the 10 million deaths occurred among children below five years in the developing countries are associated with malnutrition including protein deficiency (WHO, 2000).

Protein and energy malnutrition disorders among young children are of the greatest challenge to many developing countries, Nigeria inclusive. This is so because of many factors as; poor nutritional education,

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decline in households income and to a large extent the ever increasing cost of commercial formula (Akpo et al, 1985; Miriam, 2005). Weaning or supplementation stage is an important stage in baby development, for nutrients are needed from complementary food after six months of exclusive breast feeding (Piccaino, 2000). The weaning food supplement is expected to supply adequate but not excessive quantities of the basic nutrients (protein, carbohydrate, fats, vitamins, minerals, dietary fiber and water). However, natural proteins are mainly sourced from plants (sova beans, nuts and wheat), milk, eggs, beef and fish (Owolabi et al., 1996; Foote and Marriot, 2003). Foods from which proteins are derived are relatively more expensive than the other classes of food nutrients. This had resulted in babies and infants taking foods that are poor in protein content resulting in nutritional disorders. In addition, apart from the breast milk, no single natural food substance is known to contain all the required food nutrients in the right proportion. This calls for the formulation or compounding of a balanced food formula for the weaning infants. However, most of the food formulas available commercially are imported. which are quite expensive and mostly available to the urban dwellers while the rural populace, have little or no access to them, majorly because of cost and poor nutrition education. Also, the other classes of nutrients required for formulation are reasonably available, affordable and easier to preserve while the protein components are usually of a major challenge. Hence the need for the continuous search for cheaper and more appropriate protein sources. Traditional weaning foods are usually based on cereal and legume, a typical example 'ogi' is made from corn gruel (Miriam et al., 2005). The indigenous sources of proteins for infant formulas from animal and plant stuffs includes, eggs, fish, crayfish, African oil bean seed, pumpkin seed, melon seed and maize (Achinwewhu, 1987).

Egg proteins are used as the standard by which all other proteins are measured because of their very high ratio of essential amino acids (Brain and Ilan 1982; Maff, 1991). Eggs contain high quality protein content, with about thirteen essential vitamins and minerals and are second to breast milk. The fish egg however has rarely been given any consideration as a protein source for infant food formula. At present, there are no previous studies to ascertain that fish eggs are good component of weaning food.

The more patronized fish in Nigeria for low income earners is the herring fish commonly called "shawa' and are available locally as fresh or frozen. This is because this species of fish is cheap and very available all the year round.

This study is therefore designed to evaluate the nutritional composition especially the protein content of four different fish eggs, in order to ascertain the suitability of the fish eggs as a available source of cheap protein for infant weaning formula. This will possibly meet the need for a nutritious, cheap and affordable weaning infant formula using simple but adequate technology.

MATERIALS AND METHODS

Fish Eggs Collection

The Herring fish (*Clupea spp*) eggs used for this study were collected from the Federal College of Fresh Water Technology, New Bussa in Niger State, Nigeria. The fish eggs were spread out and oven dried at 55 °C for 24 hours in the College laboratory and stored in air tight containers. The containers were transported in plastic cages to Biochemistry Laboratory, Federal Polytechnic Bida, Niger State for analysis.

Animals

Three week old weaning Swiss albino rats were purchased and acclimatized in the animal house of the Science Laboratory Technology Department, Federal Polytechnic, Bida, Niger State, Nigeria, for one week. The rats were randomly distributed into two groups of five rats each in triplicates. The weight gained was measured daily for each animal for the three weeks of experimental period. At the onset and at the end of the third week (21 days), the head to tail length, designated as Tail length (TL), Girth (G), Head circumference (HC) were measured.

Proximate Analysis

The protein content was determined by the microkjeldhal method as established by the AOAC (AOAC, 1970). Moisture, crude fat, crude fiber, ash and carbohydrate contents were each determined as described by AOAC, (ACOA, 1980).

Feed Formulation

The fish eggs with the highest quantity of crude protein obtained after the proximate analysis were used to formulate weaning feed. The fish eggs served as a source of protein and constitute 30% of the formula containing maize, rice bran, bone meal, common salt, vitamin premix and D.L Methionine according to the method described by Adevemo and Eawim. (Adeyemo and Egwin, 2000), while soya beans served as a source of protein for the control. The animals were placed in animal cage and the total feed intake and weight gained were measured

Component (%)	Clarias	Heterobranchus	Talipia	Herring
Crude protein	27.69	26.6	25.63	48.52
Crude fat	12.82	11.16	16.83	24.48
Ash content	3.67	4.0	2.58	4.09
Carbohydrate	6.0	5.4	4.71	5.54
Moisture Content	49.61	52.82	50.25	17.33

Table 1. Proximate Composition of Fish Eggs

 Table 2. Growth and development indices of rats fed fish eggs formula after 21 days

Growth indices	Control (cm)	Experiment (cm)
Length from nose to tail	27.5 <u>+</u> 1.32	29.3 <u>+</u> 1.26
Length of Tail	12.5 <u>+</u> 0.76	12.7 <u>+</u> 1.26
Girth	3.0 <u>+</u> 0.0	3.0 <u>+</u> 0.0
Circumference of the head	2.2 <u>+</u> 0.2	2.3 <u>+</u> 0.2

Data represents Mean ± SD of five replicates.

daily throughout the duration of the experiment.

RESULTS

Hematological Indices and Biochemical Parameters

(PVC), hemoglobin Packed cell volume (Hb) and the white blood cell count (WBC) were determined as described by Daice and Lewis, (1975). Total serum protein (TSP) and total serum albumin (TSA) determined were according to the method described by Henry et al., (1974) and lawal et al., (2005).

Net protein utilization (NPU)

The NPU was determined from the total protein intake and the urinary urea values (Fashakin and Ogunshola, 1982).

Statistical Analysis

The results represent the mean \pm S.D of five replicates. The student's t-test was used to compare the mean values using the SPSS statistical software package, version 16.0. The proximate composition of the four different fish eggs are presented in Table 1. The result showed that crude protein ranged from 25.63-48.52%, while crude fat and ash ranged from 11.16 - 24.48% and 2.58 - 4.09%, respectively.

The growth and development indices (body length measurements) presented in Table 2 compares the level of growths in the experimental animals fed diet containing fish egg and control animals fed diet formulated with soya beans protein because soya bean has an acceptable protein supplement. The length from nose to tail of the experimental animals (29.3 ± 1.26 cm) was longer than that of control (27.5 ± 1.32 cm). There is no significant difference between the tail length of the experimental animals (12.5 ± 1.27 cm) and that of the control (12.5 ± 0.76 cm). There was also, no difference in the girth of both the control and the experimental animals. The head circumference of the experimental animals (2.3 ± 0.2 cm) was also not significantly (p>0.05) higher than that of the control (2.2 ± 0.2 cm).

The average weight presented in figure 1 shows that the weight of control animals was significantly lower than that of the experimental ones (P<0.05). This implies that weight gain may have been enhanced by the protein from the herring eggs used in the formulation of the

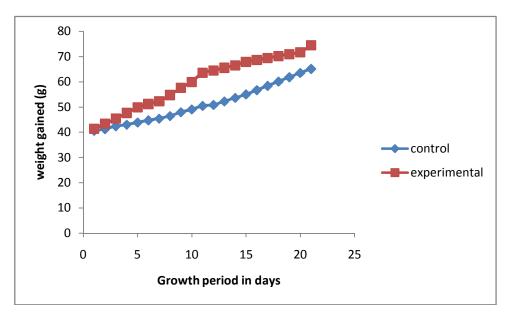


Figure 1. Growth profile of rats fed control and experimental diets after 21 days

Table 3.	Net protein	Utilization*
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	Control	Experiment
Weight (g)	68.33 ^b	733.33 ^a
Protein intake (g)	3.35	4.5
Urine nitrogen (g/l)	9.5	7.5
NPU (mg)	0.4 ^b	0.7 ^a

*Values with different letter superscripts on the row are statistically different (p<0.05)

experimental feed compared to the control which were fed with soya beans.

The net protein utilization (Table 3) in the experimental animals (0.7 mg) was significantly higher than that of the control (0.4mg). Also the urine nitrogen was lower in the experimental animals (7.5g/l) than in the control (9.5g/l),

The amino acid profile of Herrings fish eggs is shown in Table 4. The result reveals that the fish eggs contain all the essential amino acids and their quantities were above the recommended values.

The results of the hematological and biochemical parameters are presented in Table 5. The Haemoglobin level in the control $(8.73 \pm 0.81\%)$ is significantly (p<0.05) lower than that of the experimental animal (8.97 \pm 0.65%). The packed cell volume (PCV) in the experimental animal (27.0 \pm 2.0%) is higher than that of the control (26.33 \pm 2.2.52%). The serum total protein (STP) and serum total albumin (STP) were significantly (p<0.05) higher in the experimental animals compared to that of the controls.

DISCUSSION

From the result, herring eggs have higher nutrient values than other fish eggs evaluated. On the basis of this, the herring eggs were used to formulate a weaning diet. From this study, it became clear that the nutrients in the herring egg compares favorably with that of previous research finding for complimentary infant weaning foods. For instance, (Miriam, 2005) reported a range of 2.50 - 5.15 % for moisture, 2.05 - 2.60 % for ash, 15.0 - 35.60 for crude protein, 9 - 38.1 % for crude fat and 2.95 - 9.07 % for crude fiber in cereal-legume based complimentary infant feed.

The protein content of herring egg (48.52 %) is higher than the recommended protein nutrient intake (10 - 20%) (WHO, 2005) and also higher than that obtained from crayfish (35.60%) and soya beans (30 - 40 %) that have been recommended for infant feeding due to their positive contribution to protein nutrition levels (Akpo et al., 1985; Piccaino, 2000; Owolabi et al., 1996). Previous

Amino Acid	FAO Ref. Value	Concentration g/100g
Lysine	4.2	7.24
Histidine		2.80
Arginine	*	5.22
Aspartic acid		9.82
Threonine	2.8	5.22
Serine		3.32
Glutamic acid		15.14
Proline		5.47
Glycine		6.00
Alanine		5.12
Cystine	2.0	0.79
Valine	4.2	6.34
Methionine	2.2	3.48
Isoluecine	4.2	5.02
Leucine	4.2	10.01
Tyrosine	2.8	4.08
Phenylalanine	2.8	5.05

 Table 4. The amino acid profile of herrings eggs

Table 5. Mean and Standard Deviation of Hematological index and Serum biochemical Parameters

	Control	Experiment
Hb	8.73 <u>+</u> 0.81%	8.97 <u>+</u> 0.65%
PCV	26.33 <u>+</u> 2.52%	27 <u>+</u> 2.0%
WBC	3066.67 <u>+</u> 737.11cm ³	3200 <u>+</u> 608.28cm ³
S.T.P	65.70 <u>+</u> 31.72g/1	119.33 <u>+</u> 42.71g/1
S.T.A	25.83 <u>+</u> 0.67g/1	57.37 <u>+</u> 16.45g/1

studies also reported that the protein content found in imported commercial weaning foods ranged from 15-20 % which is lower than that obtained from herring eggs (48.52 %) (Owolabi *et al.*, 1996). It therefore suggests that herring eggs may be a good and cheaper source of protein for infant formula for the poor rural communities.

The total fat in herring eggs (24.4 %) is higher than that obtained for other fish eggs studied as well as that of soya beans (Kaitaranta and Ackman, 1981). This level of fat is adequate to meet the requirement for the infant body. Fat and oil in food meant for infants and children will not only increase the energy density but also serve as a transport vehicle for fat soluble vitamins. The fat can also provide essential fatty acids needed to ensure proper neural development (Fernadez *et al.*, 2002). Essential fatty acids are required to produce prostaglandins useful in the regulation of blood pressure, blood clotting, immune response, the inflammation response to injury and infections (Wikipedia, 2009). Vegetable oils (corn oil, sunflower, soya beans) are rich in n-6 fatty acids. Fish oil is however, richer in n-3 and n-6 fatty acids that are essential for the normal function and development of the retina and brain. Because omega 3 fatty acids are essential in growth and development throughout the life cycle, they should be included in the diets of all humans. Omega-3 and omega-6 fatty acids are important components of practically all cell membranes.

Omega-3 fatty acids increase bleeding time; decrease platelet aggregation, blood viscosity, and fibrinogen; and increase erythrocyte deformability, thus decreasing the tendency to thrombus formation (simopoulous, 1991). Herring egg can therefore be favorably used to formulate infant weaning diets. The higher protein and fat in the herring eggs may have been responsible for the higher growth and weight gain observed in the animals fed experimental fish egg diet compared to the control which received soya beans as source of protein as shown in Figure 1.

The ash value which is a reflection of the mineral content is higher in the herring eggs (4.09 %) compared to that reported for commercial formula and supplementary diets compounded with soya and crayfish (Owolabi *et al.*, 1996). Other researchers reported poor micronutrient content of plant based diets (Piccaino, 2000). This implies that herring eggs have a great potential as a source of essential minerals when used in diet formulation, therefore, further fortification of weaning formula with minerals may not be necessary.

The experimental animals were well able to utilize the protein from the herring eggs better than the control animals fed soya protein diet. This may be attributed to the fact that animal protein has all the essential amino acids required for protein anabolism (Owolabi et al., 1996). The present result also shows that the fish eggs contain all the essential amino acids (Table 4).

The higher body measurements (tail length and head circumference) of the experimental animals compared to the control indicates that the experimental diet compounded with herring eggs supported more growth compared to soya beans protein. The present observation has further established that the fish eggs may be a good and cheap source of protein for infant weaning formula.

Furthermore, a positive nitrogen balance observed in the experimental animals implies lower urine nitrogen. This may explain why the animals fed experimental diets grew more than the control animals and also showed better growth indices (Table 2). (Kallan, 2009) reported that lower protein in diets will lower body growth and development. Therefore, the better growth performance observed in animals fed with the experimental fish egg diet may be attributed to the high quality protein in the herring eggs. This result ascertains the nutritive adequacy of this locally available protein source as a complementary source of protein for weaning food formulation, especially by the rural and poor urban mothers.

The hematological and biochemical parameters monitored in the present study, showed that the fish may not have any deleterious effect on the animals fed with the experimental diet. The enhanced Hb level observed in the animals receiving the experimental diet will increase the blood oxygen carrying capacity and allow higher availability of oxygen to tissues for metabolism and growth, this observation has further established why herring egg supported more growth than the control.

The packed cell volume in the experimental animals $(27.0 \pm 2.0 \%)$ is higher than that of the control $(26.33 \pm 2.2.52 \%)$. Elevated PCV is associated with increased production of red blood cells (Bobboi et al., 2004). The high quality nutrients in the herring eggs may have enhanced red blood cell proliferation. The serum total protein (STP) and serum total albumin (STA) were much higher in the animals receiving the experimental diet compared to the controls, this is a further indication that the herring eggs have a high potential for enhancing food nutrient supply in infants.

The high levels of essential amino acids present in the herring eggs has finally shown why the animals receiving the experimental diet were able better to utilize the fish eggs protein than their control counterparts which received soya protein (Oyenuga, 1978).

CONCLUSION AND RECOMENDATION

High nutrient density weaning foods is a feasible vehicle for improving nutrient supply to infants. The protein in herring egg is able to meet the recommended nutrient intake in infants. The present study ascertains the nutritive adequacy of this locally available and reasonably affordable protein source as a complement for weaning food formulation, especially for rural and poor urban mothers. Through exclusive breast feeding, babies normally can get their required protein from their mother's milk for the first six months of life, after which there is usually a need to introduce babies to semi-solid or solid foods as the digestive and immune systems have become stronger and more active. Also formulas are required during the periods of weaning, maternal ill health and possibly maternal death and these are critical periods in the life of a child. Weaning food formulas that are nutritious, easily available and affordable are required as this will determine the child's growth and development. Breeding of fish for eggs is an area that fish geneticist should explore to enhance global food/protein adequacies, particularly for the developing countries.

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