Acta Agraria Kaposváriensis (2017) Vol 21 No 1, 1-14.
Kaposvári Egyetem, Agrár- és Környezettudományi Kar, Kaposvár

Measuring fish metabolism – science and practice of development in fish feeding: A review

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ABSTRACT

Since costs related to feeding comprise determining part of production costs in aquaculture, as in any other branch of animal production, innumerable studies aimed to give information about the feed utilization were done also for fish. The great majority of literature discusses only the simplest indicators as feed conversion ratio (FCR), feed efficiency ratio (FER), protein efficiency ratio (PER) and productive protein value (PPV). One of the key research areas however that made possible the impressive growth of aquaculture in the last decades certainly was the extensive development of feeds based on sophisticated knowledge of nutrient requirements of more and more fish species. The main goal of this literature review was to focus on digestibility of nutrients, its measurement methods and to survey the main directions of contemporary research activity in this field.

In conclusion, determination of apparent digestibility coefficients (ADC) *became an everyday practice of experimental methodology in fish nutrition studies, although there are no standardized methods, neither in marker use nor in feces collection, just to mention two from the most crucial questions. Testing and evaluating new alternative protein and energy sources to minimalize the use of fishmeal (FM) and fish oil (FO) needed to develop the requirement at ration level (RRL) method to determine adequate daily ration and also the diet replacement method (DRM) and ingredient replacement method (IRM) for ingredient inclusion in studies on digestibility. Metabolomics and nutrigenomics offer new ways of approximation in areas of primary importance in the future development of aquaculture.*

(Keywords: fish, metabolism, digestibility, omics)

INTRODUCTION

Since costs related to feeding comprise determining part of production costs in aquaculture, as in any other branch of animal production, innumerable studies aimed to give information about the feed utilization were done also for fish. The great majority of this literature discusses only the simplest indicators as feed conversion ratio (FCR) or its inverse, feed efficiency ratio (FER) which are calculated as the simple ratio of input and output or vice versa, where the feed is the input and the output is the weight gain. Protein efficiency ratio (PER) is also very popular because it is simply calculated as weight gain/protein intake. Productive protein value (PPV) also can be easily calculated: (gain in nitrogen/nitrogen intake) x100, which used also to be termed as NPU (net protein utilization) (*Weatherly and Gill*, 1989). One of the key research areas however that made possible the impressive growth of aquaculture in the last decades certainly was the extensive development of feeds based on more sophisticated knowledge of nutrient requirements of more and more fish species (*Webster and Lim*, 2002). In parallel, more and more detailed and accurate theoretical models of fish metabolism could be elaborated (*Braaten*, 1978; *Smith*, 1980; *Kaushik and de Olivia-Teles*, 1985; *Tytler and Calow*, 1985; *Kaushik*, 1986; *Johnston and Dunn*, 1987; *Clarke and*

Johnston, 1999; *Bureau et al.*, 2002; *Dietz et al., 2013; Grisdale-Helland et al.*, 2013; *Stadtlander et al., 2013*).

There is a huge literature on fish metabolism and apparent digestibility, therefore the purpose of this review is to examine the recent literature dealing with the digestibility of nutrients, its measurement methods and also to survey the main directions of contemporary research activity in this field.

Fish metabolism

Conveniently, investigators couched metabolic problems in terms of energy as it is very well summarized in the book of *Weatherly and Gill* (1989) using the basic biological terms of anabolism and catabolism. They cite, among others the work of *Cho et al*. (1982) who note that any study on bioenergetics of an animal can be defined as investigating the balance between energy supply in food and the energy expenditure of physiological processes of the body. Although the main categories and terms describing fish metabolism are very similar to higher vertebrates partitioning of food energy in fish were discussed by numerous authors from which the energy equation given by *Brett and Groves* (1979) in Weatherly and Gill (1989).

$$I = M + G + E$$

where: I: all ingested energy, M: metabolism, E: excretion, has to be mentioned first. It could be and really was long disputed, but proved to be very useful for practical estimation until now, with their proposed conversion factors for body constituents as 17.15, 39.54 and 20.08 kJ g^{-1} (also given in NRC (1993) for carbohydrate, lipid and protein, respectively, values that are somewhat different from mammalian values (*Kleiber*, 1961) in Weatherly and Gill (1989). To understand similarities and differences the flow of the dietary energy in fish depicted in *Figure 1* can help.

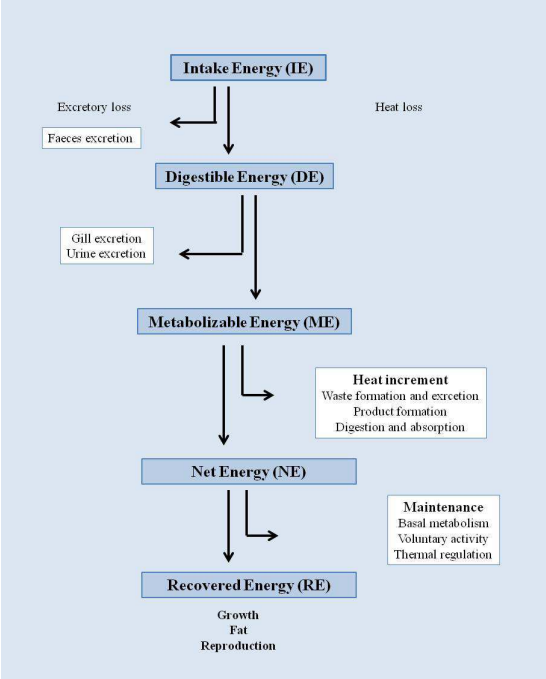

Figure 1.

Fate of dietary energy for fish

(*Adapted from National Research Council. 1993. Nutrient Requirements of Domestic Animals. National Academy Press. Washington, D.C.*)

Discussing peculiarities of fish metabolism from excretion to basal metabolism and some aspects of difficulties caused by the water as the environment are beyond the scope of this review likewise a profound overview of literature on bioenergetics of fish discussed profoundly among others by *Braaten*, 1979; *Smith*, 1980; *Johnston and Dunn*, 1987; *Clarke and Johnston*, 1999; *Bureau et al*., 2002; *Davis, 2015*

However, some basic principles, although known for a long time, have to be mentioned here again. First of all, energy need of ingestion and digestion is small compared to metabolic work (*Brody*, 1945). It has an important consequence, namely that determining metabolizable energy (ME) gives a little advantage over measuring digestible energy (DE) in the evaluation of useful energy of feedstuffs for fish (*NRC*, 1993). The same was already stressed by *Lovell* (1989) demonstrating it by a table showing data of DE/IE and ME/DE calculated for rainbow trout. (Difficulties of exact measurement of ME will be mentioned later.) *Allameh et al*., (2007) citing *Willoughby* (1999) give a simple solution for this problem, calculating ME simply by subtracting 11% from DE as nitrogenous excretion (NE).

Modern fish feeds are developed considering the optimal protein/energy ratio come with lots of information including gross energy (GE), DE and ME values. Whereas the big feed producing companies have their own experimental facilities and/or carry out feed development in cooperation with prominent research institutions, all the DE and ME values for all age groups of all farmed fish species given by feed producers cannot come from accurate experiments. Some good and reliable practice must exist to derive these values from GE content of the feed, about which no information could be found. However, the determination of DE is becoming a standard part of methodology in feeding experiments as it will be demonstrated later.

Gross energy can directly be determined by bomb calorimetry but its calculation from chemical composition using the values given above is a widely applied method (*NRC, 1993*). Then – based on experimental results - an equation to estimate ME, as follows:

$$\text{ME (MJ/kg dry matter)} = -3.064 + 34.82\, x_1 + 17.21\, x_2 + x_3\,(18.52 - 31.2\, x_4),$$

where: x_1 = crude protein, x_2 = crude fat, x_3 = N-free extract, x_4 = crude fibre (all calculated in g/kg units), could be developed, exactly as it was done by *Härtel (*1977) for poultry. This regression equation was used for poultry until 1990 but proved to be applicable even for fish, because it was considered giving less error than laboratory measurements. This situation might have been changing since then but no more similar attempt of estimation could be found in the literature on fish metabolism.

An important energy sparing feature in fish is related to the excretion of a large amount of ammonia as the main product of protein catabolism in place of synthesizing urea lowers heat increment significantly and makes possible to use a bigger part of energy intake for maintenance and growth (*NRC*, 1993). Another peculiarity of fish metabolism comes from the lack of thermoregulation since fish are poikilothermic ectotherms. Moreover, buoyancy made possible by swimming bladder in most species means that fish need much less energy to maintain posture in water compared to terrestrial animals, also an item lessening maintenance energy. *Kaushik and Médale* (1994) compared average maintenance energy needs, expressed as basal metabolic rates (MJ/ $kg^{0.75}$/day) of terrestrial animals and fish giving the corresponding values as 0.70 vs. 0.01- 0.07, respectively. *Jobling* (2017) gives an excellent and simple summary of the differences between poikilothermic and homeothermic animals'metabolism (*Table* 1).

Table 1.
Metabolic characteristics of poikilothermic ectotherm (fish) and endothermic homeotherms (mammals)
(*Jobling*, 2017)

	Fish	***Mammals***
Metabolic rate	Low	High
Starvation resistance	High	Low
Maintenance food requirement	Low	High
Food use for growth	High efficiency	Low efficiency

Before going into details of the determination of digestible part of the energy and the different nutrients, it has to be stressed that there is an other alternative terminology applied for the same physiological processes used for example by *Jobling* (2017) when discussing nutritional requirements of farmed fish. According to this terminology bioavailability of a nutrient in a diet called absorption efficiency (A) defined as follows:

$$A = 100\ (N - F)/N$$

where N is the nutrient content of the food and F represents fecal losses. This absorption efficiency also is known as digestive efficiency, shortly digestibility or more exactly apparent digestibility, as it will be mentioned afterward. "True" absorption efficiency is given by:

$$\text{“true”}A = 100\ [N – (F – F')]/N$$

where F' is the non-food component of the feces derived from cellular and bacterial sources. Determination of "true" digestibility is extremely important when low protein content diet is fed since in this case digestibility of the protein source may be considerably underestimated while in most cases this error is only about 2-3% (*Jobling*, 1998). In spite of its high importance "true" digestibility is almost impossible to determine in fish.

Difficulties of developing a standard method to investigate fish metabolism by adapting methodology elaborated for land animals is summarized by *Smith* (1971) with the main point as follows:

- The small size of individuals makes difficult to obtain an adequate amount of waste product while using large groups of fish gives an alternative but arises other problems.
- Waste products should be separated from the water and also from the uneaten parts of food and measured quantitatively.
- Fish excrete major part (70-80%) of non-fecal waste nitrogen through the gills.
- The metabolic rate of fish as poikilothermic animals depends on water temperature.

Part of these difficulties can be overcome using sophisticated appliances, as it was first demonstrated by *Smith* (1971) who constructed a special metabolic chamber where fish were confined and force-fed. Not surprisingly this pioneering methodology was not directly developed further and would be unimaginable, besides some methodological difficulties, considering today's rules of animal welfare. Fish respirometers used nowadays are similar to that was designed by *Cho et al.* (1982) where, of course, metabolic rate can also be measured by oxygen consumption (*Figure 2*). Nowadays in state-of-the art experiments on fish nutrition

(*Helland et al.*, 1996; *Grisdale-Helland et al*., 2013) fish keeping tanks are equipped with semi-open, semi-closed respirometry (*Helland et al.*, 1996) that makes possible to include determination of heat increment during fasting and growing phase into the evaluation of ration levels when studying energy, protein and amino acid requirement of fish.

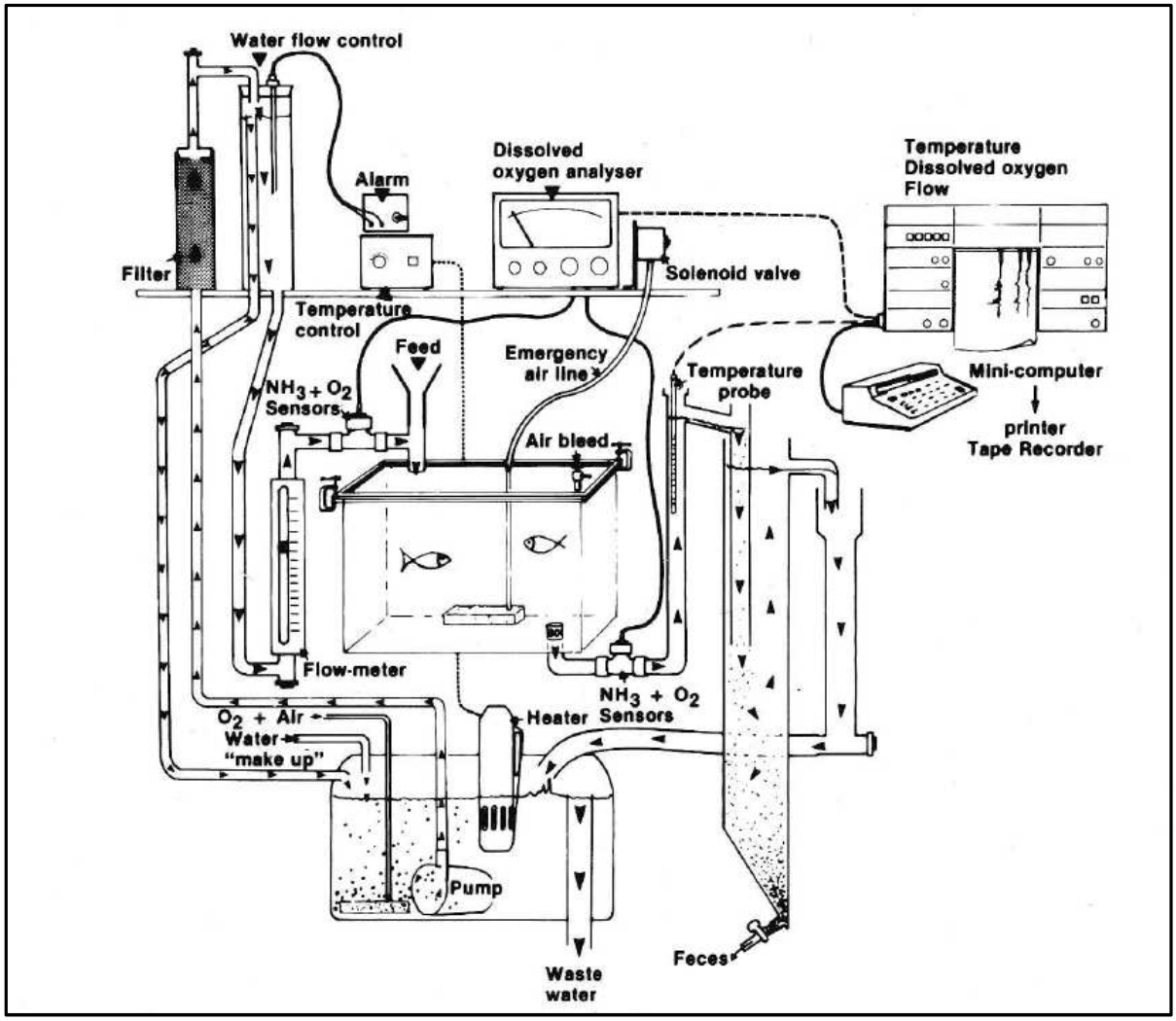

Figure 2.

Fish respirometer system designed and used by *Cho et al.* (1982)

An important part of "heat loss" category, called "basal metabolism" in *Figure 1*, which is also termed in literature as standard metabolic rate (SMR), is so extremely difficult to determine, that *Weatherly and Gill* (1989) considered it as a misnomer and proposed to call this kind of energy loss "standard catabolism". *Chabot et al.* (2016a) discuss extensively the methodology of the determination of SMR by respirometry comparing eight methods of it, so SMR remained the name of the minimal aerobic metabolic rate. There are some other controversies in the terminology of respiratory or bioenergetic costs of fish metabolism. *Weatherly and Gill* (1989), show a figure of fish energy budget where energy losses related to food processing of the organism is called "specific dynamic action" (SDA) a term which was used extensively in a great number of publications for a long time. *Smith* (1980) for example, discussing this question stresses the importance of energy sparing effect of carbohydrates and lipids reckoning this later as a minimally investigated area. Things changed a lot since then, as it could be proved by citing innumerable publications but formulas of modern fish feeds prove

it best. (On the other hand, the above described apparent terminological controversies are quite normal phenomena in science and rarely hinder its development).

Eventually, with the extremely fast growth of aquaculture industry, a plethora of publications are appearing that gives more and more reliable information on different facets of fish metabolism (*Carter and Brafield*, 1991; *Focken* et al., 1994; *Gao et al.*, 2005; *Smith et al*., 1995; *Watanabe and Otha*, 1995; *Otha and Watanabe*, 1996; *Gao et al.*, 2012; *Dietz et al*., 2013; *Saravanan et al*., 2013; *Skov et al.*, 2013; *Jobling*, 2017).

Metabolic rate and its measurement became also important from the point of view of an emerging new discipline: ecophysiology or conservation physiology. Climate change and its ecological consequences inspire the rapid development of this research field which has already produced valuable results presented last year in a special issue of the Journal of Fish Biology. *Chabot et al.* (2016b) presenting this voluminous issue of 442 pages in their editorial ascertain that 13 papers of the total 22 discuss the problem of measuring standard and maximum metabolic rates of fish and clarify definitions and methods. Results achieved in this area certainly will be useful also in research work aiming aquaculture development.

Measurement of digestibility

Digestibility was already defined above as the efficiency ratio of the available energy of the food or a nutrient in it and the difference between the apparent and true digestibility was also discussed. Basically, there are two main methods to measure the digestibility of the food. The direct method measure of the total feed intake and the produced faeces. This method is often used for land animals.However, indirect method often used in fish research: none digestible tracer is mixed in the feed and is totally found again in faeces. Thanks to the dosage results (tracer and nutriments), feed and raw material digestibilities can be calculated. According to *Smith* (1979), Apparent Digestibility Coefficient (ADC) of a nutrient in the feed can be calculated as

ADC = 100 - [(% indicator in feed/% indicator in feces) x (% nutrient in feces x 100/nutrient in feed)]

This formula naturally can be used for ADC of energy (*Lovell, 198*9) in which case the energy content has to be determined directly by bomb calorimetry or calculated from the chemical composition as it was mentioned before.

The most used marker undoubtedly was chromic oxide but *Jobling* (1998) mentions also titanium oxide, rare earth elements, celite (SiO_2), lignin, acid insoluble ash and chromogens, pointing to the fact that experiment aimed to compare the efficiency of these markers gave no unequivocal results. Nowadays yttrium oxide as a marker is also gaining popularity (*Helland et al.*, 2010; *Grisdale-Helland et al*., 2013) as well as titanium oxide (*Heinitz et al*., 2015). In special cases, even crude fiber can be used as a marker (*Krontveit et al*., 2014).

Results of the estimation of absorption efficiency depend greatly on the method of feces collection and treating before analysis. Simplest and cheapest is siphoning or netting but because of the leaching of the remaining part of nutrients from the feces, these methods lead to overestimation of ADC. Citing various authors *Lovell* (1979) states that in the case of estimation from feces collected one hour after defecation this overestimation is around 10%. It worth mentioning that even siphoning from keeping tanks in adequate time can result in reliable results (*Sklan et al.*, 2004). Collection systems developed to minimize the connection with water like Guelph System (*Cho et al*., 1982; *Figure 3*) proved to be effective and are in use until now (*Bureau*, 2013).

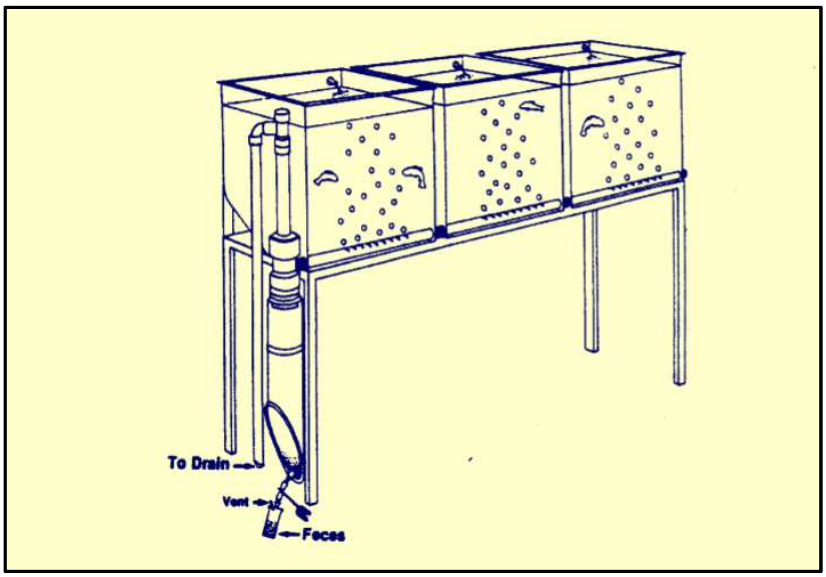

Figure 3.

The Guelph System for fish feces collection
(*Cho et al.*, 1982)

The system developed by *Velázquez and Martínez* (2005) also resolves the collection of feed residues (*Figure 4*) which makes possible a more reliable calculation of FCR. *Choubert et al.* (1979) also developed a sophisticated collection system in which the feces is collected continuously, by filtering and stored frozen until analysis. However, after the paper of *De LA Noüe and Choubert* (1986), who compared the direct and indirect method for ADC estimation with rainbow trout, only one paper (*Amirkolaie, et al.*, 2005) mentions this system where it was compared with settling tank.

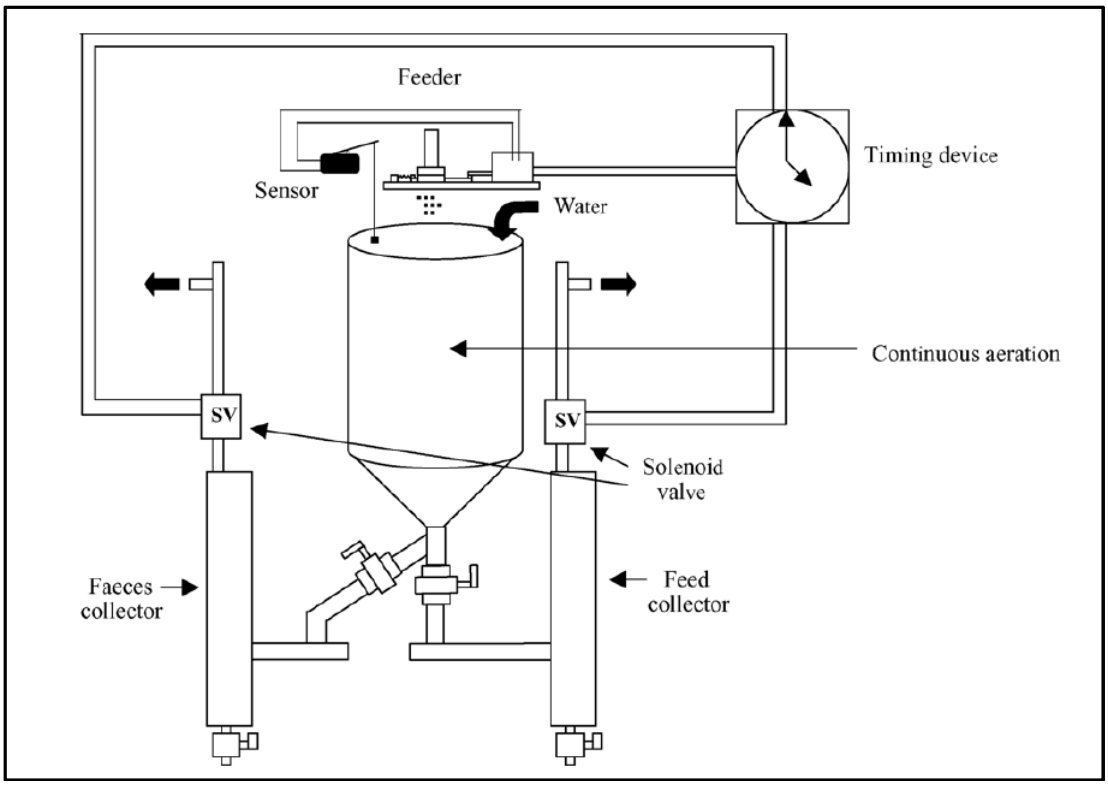

Figure 4.

Modified "Guelph system" developed by *Velázquez and Martínez* (2005)

Feces can be removed directly from the fish by anal suction, stripping or intestinal dissection, methods with which leaching can be avoided but have their drawbacks, too (*Jobling,* 1998). First of all these methods can't be applied to small fish, contamination of the feces with urine and mucus is hardly avoidable and there is a risk of collecting incompletely digested food and all these factors lead to underestimation of ADC. However, direct or active methods also remained a viable alternative firstly for fish with a short and straight intestine like salmon and trout but *Heinitz et al*. (2015) applied stripping also for common carp with very satisfactory results to determine ADC of energy, nutrients and amino acids of common feed ingredients. *Blyth et al.* (2015) also found that stripping resulted in more conservative ADCs, which were also more consistent than those obtained by using the settlement technique. It seems that the most reliable way to obtain adequate and practically useful results is using the same system consequently (*Bureau,* 2013) while comparison results obtained by different methodologies emerges lots of problems (*Rawles et al*., 2010).

Main areas of digestibility studies in fish feed development

Albeit the determination of ADC doesn't have a generally used, standardized method it has many advantages over measuring correctly the metabolizable energy or nutrients of fish foods or industrial feeds (*Lovell,* 1989). Partly due to the rapid development of analytical methods determining ADC became a routine in good quality feeding trials in aquaculture but sometimes studies with very practical objectives apply even also respirometry (*Stadtlander et al.*, 2013).

One important direction of using ADC as an evaluation criterion of efficiency of feeding technology is excellently demonstrated by *Helland et al*., (2010); *Grisdale-Helland et al*., (2013) who tested different macronutrient ratios of salmon feeds combining with different ration levels. The requirement by ration level (RRL) method means that firstly the satiation level is determined (100 %) then decreasing levels (e.g 75, 50, 25 %) are fed and tested. Using the whole methodological weaponry developed till now from respirometry, calorimetry to ADC determination yield very elegant and accurate regression equations describing the DE – energy gain, DE – protein gain or digestive SumAA intake - SumAA gain relationships. This, having, of course, scientific value provides also information of vital importance for fish feed manufacturers and fish producers.

Using the most economically producible feed that satisfies the nutritional requirements of a given age-group of a fish species was the main goal of feed developers since the beginning of the aquaculture industry. However, a new era has started when sustainability became the key question also in fish production. As in many other areas of fish nutrition, this question was most studied in Atlantic salmon a fish requiring high levels of fish meal (FM) and fish oil (FO) in his feed. The concept "fish in fish out" (FIFO) ratio proved to be a very useful tool for the estimation of sustainability and helped to develop a new generation of fish feeds by reducing this ratio significantly. *Tacon and Metian* (2008) gave the figure for salmon as 4.9:1, what means that it takes 4.9 tons of wild fish to produce 1 tonne of salmon while this ratio of modern feeds is around 1.7 (IFFO http://www.ifffo.net/). Countless articles on replacement of FM have appeared until now and systematizing them is quite a difficult task. *Glencross et al*., (2007) reviewed comprehensively the ingredient evaluation strategies for aquaculture feeds up to date. According to them alternative ingredients to FM can be sorted in two groups of plant and terrestrial animal origin. Nowadays the number of alternative protein sources can be increased with meals made from insects and worms (*Magalhães et al.,* 2017 *Pucher et al.,* 2006) and fish protein hydrolysate and other fish processing byproducts (NOAA/USDA (2011); (*Wei et al.*, 2006) in the animal origin group while in the plant origin group soybean

products remain in the first place but alternatives of soybean are also heavily investigated (*Teuling et al.,* 2017; *Hien et al*., 2017).

Glencross et al., (2007) list the key concerns in ingredient assessment as follows: digestibility, palatability, nutrient utilization and functionality. Palatability of feed is a key question which is also discussed in great detail by *Glencross et al*. (2007) stressing that fish must be given the opportunity to refuse feed, therefore feeding beyond apparent satiety is an imperative which was followed by the RRL method (see above). This aspect of studies on feed utilization is especially important when alternatives of FM and FO with possible unfavorable organoleptic and/or antinutritive properties.

Essentially, there are two methods of ingredient inclusion for specific ingredient digestibility assessment (*Glencross et al*., 2007): the diet replacement method (DRM) and the ingredient replacement method (IRM) (*Aksnes et al.,* 1996). In the DRM method, a test ingredient is added to replace a portion of the reference diet to create a test diet but it is highly important that the portion of the reference diet within any test diet must be fully representative of the complete reference diet. The IRM also uses a reference diet but differs in that the reference diet usually has a single reference ingredient at a fixed, moderately high inclusion level (*Aksnes et al.,* 1996). This single ingredient is then replaced with the ingredients wanted to be tested. The assessment of the digestibility of any ingredient is based on the relative diet digestibility with regard to the reference ingredient. With this method, the basis of the digestible value of the test ingredient is largely dependent on the choice of the reference ingredient and its assigned or measured digestibility values (*Morales et al.* 1994). By the choice of a reference ingredient as one of the test ingredients in the DRM method, both methods' strengths can effectively be combined (*Glencross and Hawkins* 2004a; *Glencross et al.* 2004b). Studying nutrient utilization and ingredient use was carried out by a variety of experiment types differing in diet design, ingredient inclusion and feeding strategy as it well demonstrated by (*Glencross et al*. (2007).

The functionality of ingredients can be taken into account from point of view of feed industry requirements for pelletization or extrusion (*Thomas and van der Poel*, 2001) or even their effect on fish growth and fillet quality (e.g. producing functional food by Se supplementation (*Pacitti et al*., 2015) of feed or using alternative vegetable oils) (*Monge-Ortiz et al*., 2017).

Using special feed additives is a growing practice in fish feed development. Such additives can be exogenous enzymes (*Hardy*, 2000; *Kazerani and Shahsavani*, 2011) or phytic acid (*Liu et al.,* 2017) which affect digestibility directly. Other kinds of feed additives that influence digestibility indirectly but effectively are the so called synergetics which is the name coined for pre- and probiotics jointly. Use of pre- and probiotics is growing extensively (*Carnevali et al.,* 2017; *Cerezuela et al.,* 2011; *Ganguly et al., 2013*). Application of phytochemicals can also be done by feeding them which can affect fish growth and feed utilization by different ways (*Chakraborty and Hancz*, 2011; *Chakraborty et al*. 2013).

A new branch of biological sciences called "omics" is gaining more and influence also in different areas of today's aquaculture from genetics and immunology to nutrition. First results of metabolomics (*Samuelsson and Larson*, 2008) and nutrigenomics (*Alfaro and Young*, 2016; *Young and Alfaro*, 2016; *Leaver et al.,* 2008; *Sam and Król*, 2017) have already appeared, and surely will be followed by many others opening new perspectives in fish nutrition science and practice as it was foretold by *Jobling* (2017) who also presented the setup of "omics" in a figure (*Figure* 5).

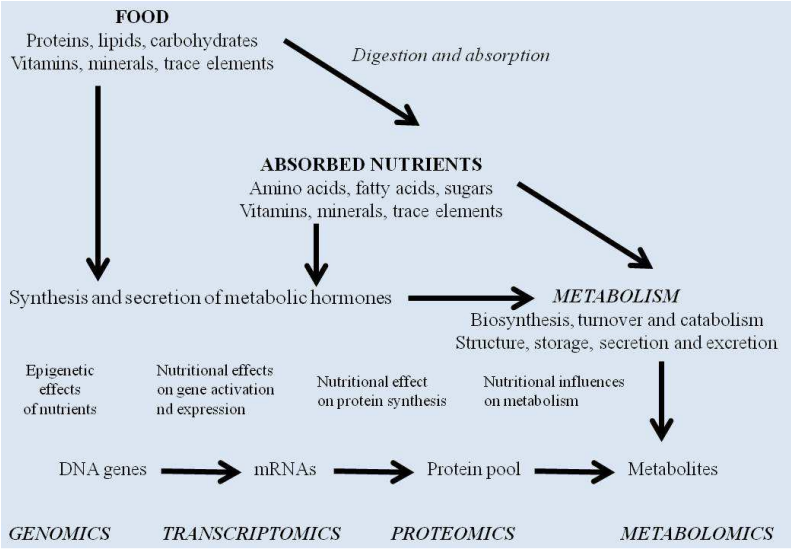

Figure 5.

Interrelationship of "omics" and metabolism
Jobling (2017)

CONCLUSIONS

Specific features of fish metabolism guarantee competitiveness with homeotherms in meat production. However, high protein requirement of fish inspired an intense metabolism research from the beginnings of the intensive aquaculture to make ground for the development of sustainable feed production.

The fast development of ecophysiology is producing valuable results in measuring standard and maximum metabolic rates of fish which certainly will be useful also in research work aiming aquaculture development.

Determination of ADC became an everyday practice of experimental methodology in fish nutrition studies, although there are no standardized methods, neither in marker use nor in feces collection, so to mention two from the most crucial questions.

Testing and evaluating new alternative protein and energy sources to minimalize the use of FM and FO needed to develop the RRL method to determine adequate daily ration and also the DRM and IRM method for ingredient inclusion in studies on digestibility.

Metabolomics and nutrigenomics offer new ways of approximation in areas of primary importance in the future development of aquaculture.

ACKNOWLEDGEMENTS

The work was supported by the European Fisheries Fund Fisheries Operation Programme III. axis "European Fisheries Fund for Renewable Fisheries" provided by the EU and Hungary.

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