RAINBOW TROUT

Protein digestibility of spray dried haemoglobin and contact dried blood meal in rainbow trout

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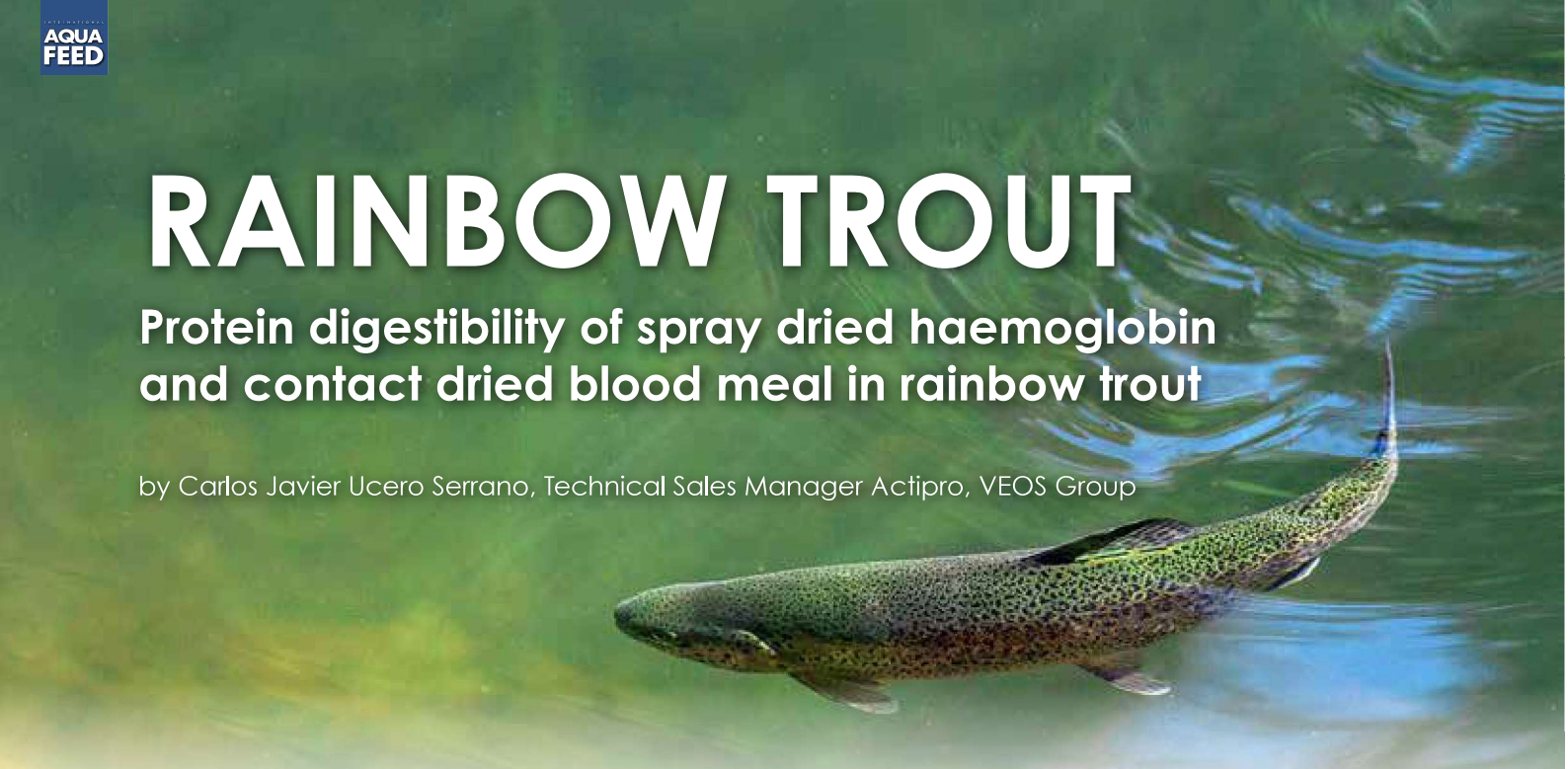


Defined by The Food and Agriculture Organization (FAO) of the United Nations as being a "product obtained by drying and grinding or otherwise treating fish or fish waste to which no other matter has been added," fish meal is considered as an essential ingredient in the diet of carnivorous farmed fish.

As demands outstrip the availability of fish meal produced from bycatch and fish trimming, wild fish is specifically caught to meet this need. Within fish meal, there is a great variability of products such as great nutritional differences, whilst the origins of some of the ingredients is also often unknown.

The only way to standardise production is to raise fish to feed other fish, which has no environmental advantage or sustainability over time. The use of fish meal, in addition to being unsustainable, seems to be less nutritious (Johnson, John Alan, 1997) and has less lysine and iron content, among other factors.

All of the above can lead to rising feed costs and growing concerns about the environmental sustainability of farmed fish, prompting the industry to seek for cost-effective and sustainable alternative protein sources. For this purpose, low-ash animal by-products may be suitable ingredients for partial replacement of fish meal in aqua feeds.

The purpose of our study was to evaluate the exchangeability of fish meal for blood by-products in trout and, at the same time, the effect of the processing method on protein digestibility: spray drying versus contact drying.

Absence of anti-nutritional factors typically found in many plant-based protein sources, along with the extremely high protein content and lysine-rich amino acid profile of blood by-products, make these ingredients very promising for partial replacement of fish meal in aqua feed.

Intensive processing of the raw material, however, may damage the nutrients, decreasing both digestibility and bioavailability. Among the blood by-products, haemoglobin (HB) is a highly interesting product as either a substitute for or a complement to fish meal in diets.

One of the main benefits of using HB as a protein source in a fish diet is the fact that it contains 92 percent protein of high biological value. This high biological value can be explained by several aspects. Firstly, the proteins remain highly digestible thanks to the gentle spray drying process, as explained in Table 1.

Furthermore, HB does not contain any protease inhibitors, which are known to block proteolytic cleavage of protein precursors. Being an animal protein, HB is highly palatable and has a rich source of dietary essential amino acids, which are complementary to the amino acid profile of vegetal ingredients (Graph 1).

Compared to fish meal, blood meal (BM) and Processed Animal

Table 1: Main differences between spray and contact drying

	Spray drying	Contact Drying
Cost of drying	Expensive	Cheap
Raw material	Requires high quality blood (low bacterial load)	Necessary for low quality blood (high bacterial load)
Drying Temperature	Moderate and short - low protein damage - high protein digestibility	High and prolonged - high protein damage - reduced protein digestibility
Dried product	Good microbiology Very low biogenic amines	Good microbiology Usually high in biogenic amines

Table 2: In vitro digestibility (%)

	Crude protein	Digestibility coeficient	Digestibility protein
Spray dried Haemoglobin	92.9	100	92
Contact dried blood meal	91.3	97.3	88.9

Table 3: Diets

	CD	Hb-Diet	Bm-Diet
Added (%)		30	30
Fish meal (%)	40	28	28
Corn gluten ml (%)	20	14	14
Soybean meal (%)	11	8	8
Wheat meal (%)	12	8	8
Binder (%)	7	5	5
Fishoil/vegetal oil (%)	8	6	6
Vit & Min premix (%)	2	1	1
Celite® (AIA) (%)	1	<1	<1

Proteins (PAP), HB has very few heat stable biogenic amines. These biogenic amines are metabolic molecules produced by bacterial decarboxylation of corresponding amino acids, being toxic when present in large quantities. Likewise, decarboxylated amino acids are lost and no longer available for digestion.

By the fact that the blood is hygienically collected and immediately cooled, no bacteria can grow and amino acids stay intact.

The following table shows the difference between two drying methods; spray drying and contact drying. In order to achieve a high protein digestibility, the choice of drying method and the quality of the raw material are very important (Table 1).

Spray drying is a gentler heating process than contact drying. The process is less aggressive against bacteria, but also has a less negative impact on proteins. As a result, the proteins are less damaged which make the amino acids available for digestion. The gentle heating process of spray drying implies the need for a high quality blood with low bacterial load as a raw material.

The high temperature, and the prolonged process time of contact drying, is ideal to decontaminate materials that have a high bacteriological load. As bacteria are sufficiently heat-labile, this process eliminates bacteria with its high temperature. However, the large amount of biogenic amines that are present in low quality blood, are not eliminated by the contact drying process, as they are heat-stable.

The real protein digestibility of spray dried haemoglobin powder

In order to get a clear picture of the real protein digestibly of spray dried haemoglobin powder and contact dried blood

Graph 1: Haemoglobin amino acid profile g/100g protein

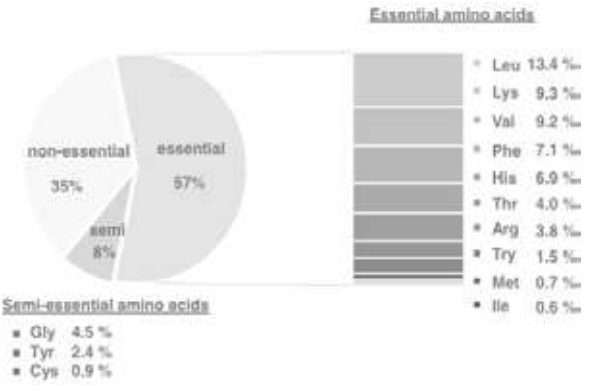
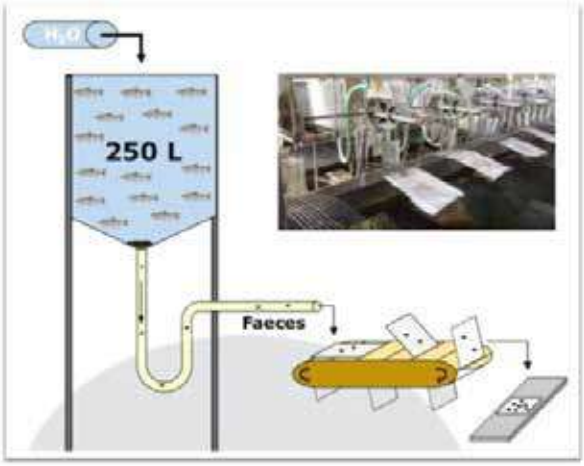


Figure 1: Choubert system



meal in rainbow trout, a study was conducted by DISAFA, Italy, divided in several stages.

Prior to the main study, we conducted an in vitro test, where we faced the two diets to be studied with the in vitro Pepsin-HCl digestibility test (Method 72/199 // EEC). The results, being very similar (Table 2), revealed that the crude protein content of both ingredients is more or less the same (92.9 and 91.3 percent).

In vitro digestibility coefficients are also close to each other, with the same observation also applying to the digestibility of proteins; 92 versus 88.9 percent.

Preliminary trial

To evaluate whether the ingredients are suitable for an in vivo trial, a preliminary trial was performed. The main objective was to evaluate if the high inclusion rate was well supported by rainbow trout. During a period of 30 days, two diets were evaluated. Table 3 shows the complete ingredient list of the Control Diet (CD). The second diet, hereafter named the Hb-diet, was composed of 70 percent of the CD and 30 percent HB (Actipro® PHS/BHS).

At this early stage, the external marker (AIA) was not included and its function will be explained later as the diets were not iso-energetic nor iso-nitrogenous.

The diets were cold pressed into three millimetre pellets and dried at 50°C for 48h. Thereafter, the pellets were distributed twice a day, five days per week, in two outdoor fiberglass tanks (0,50m³), with each tank containing 30 fish.

The results show that there was no mortality and no health problems, whilst also showing good palatability for both diets.

Moreover, both groups showed a similar specific growth rate (SGR*), with 0.50 percent per day in the control group, and 0.51 percent per day in the Hb-Diet group (as in Table 4). These results indicate that both diets are suitable for the fish.

*SGR= [In BW fin (g) – In BW initial (g)] x 100 / time (d)

Table 4: Body Weight trial

	CD	Hb-Diet
Body Weight initial (g)	140.0 ± 8.2	138.7 ± 8.6
Body Weight final (g)	163.2 ± 14.7	160.1 ± 12.7

Table 5: In vivo results

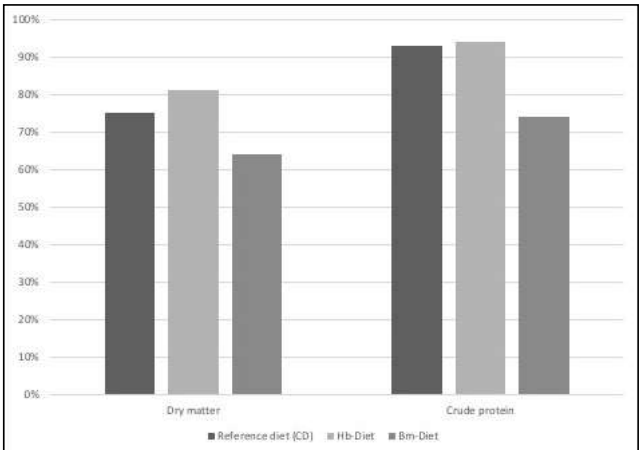
	CD	Hb-Diet	Bm-Diet
Dry matter	75%	81%	64%
Crude protein	93.3%	93.6%	74.4%

Table 6: Digestibility results (%)

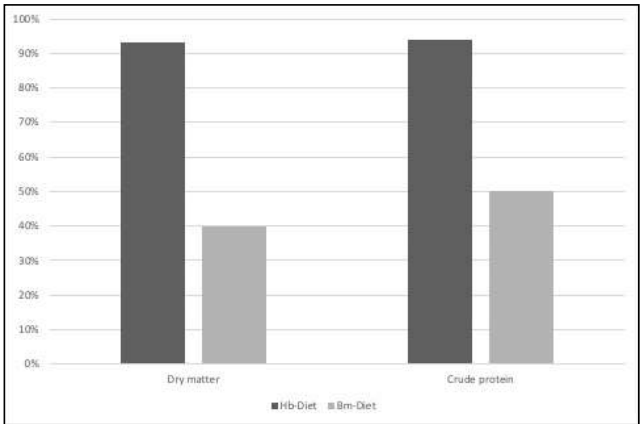
	Crude protein	Digestibility coeficient	Digestibility protein
IN VITRO			
Spray dried Hemoglobin	92.9	100	92
Contact dried blood meal	91.3	97.3	88.9
IN VIVO			
Spray dried Hemoglobin	92.9	94	87.3
Contact dried blood meal	91.3	49.5	45.2

In vivo digestibility trial

The success of the preliminary trial allowed us to proceed with the in vivo digestibility study. With this trial, we want to determine the in vivo digestibility of spray dried HB and contact



Graph 2: Apparent digestibility of test diets in rainbow trout (n=4 Choubert tanks of 15 fish each)



Graph 3: Apparent digestibility of test ingredients in rainbow trout (n=4 Choubert tanks of 15 fish each)

dried BM in fish feed. The trial lasted four weeks with the first two weeks used as an adaptation period. The fish were fed to visual satiety, two times a day and five days per week.

To compare the digestibility of spray-dried HB with the digestibility of BM, three diets were compared: Reference diet (CD), haemoglobin diet (Hb-Diet) and blood meal diet (Bm-Diet) (Table 3). Hb-Diet and Bm-Diet are mainly composed of the same amount of HB as BM, with the rest of their composition remaining identical.

In the trial, 12 Choubert tanks (250L) containing 15 fish each were used. A Choubert tank (Figure 1) is a water tank in which the faeces are continuously collected. This system prevents nutrient leakage which is especially crucial, as nutrient leakage would result in an overestimation of the digestibility.

An external marker, Acid Insoluble Ash (AIA), was used to determine and calculate the apparent digestibility of the feed by subtracting nutrients contained in the faeces from nutrients contained in the dietary intake (Lemos et al. 2009; J. Sales and G.P.J. Janssens 2003).

Crude protein digestibility of HB is similar to fish meal

The results of the in vivo test of the apparent digestibility of the CD, Hb-Diet and Bm-Diet in rainbow trout can be found in Table 5.

The crude protein apparent digestibility in the Hb-Diet is similar to the CD (93.6 ± 0.8 percent vs. 93.3 ± 0.3 percent), and significantly higher than the BM diet (74.4 ± 1.5 percent) ($p<0.001$).

This means that the crude protein apparent digestibility of HB is similar to fish meal but significantly better than BM. Further,

we noticed a higher dry matter apparent digestibility of the haemoglobin diet compared with the CD. This may indicate that the digestion of other nutrients are also improved by adding HB to the diet (Graph 2).

This difference is even more obvious when comparing test ingredients (HB vs BM).. The dry matter and the crude protein apparent digestibility of HB is almost double that of BM (93 vs 40% and 94 vs 50%). This means that the HB is two times more digestible than BM (Graph 3).

From the results it is very clear that the crude protein content of both ingredients is more or less the same (92.9 and 91.3 percent). The in vitro digestibility coefficients are also close to one another, with the same applying to the digestibility of protein; 92 versus 88.9 percent.

However, when we look at the actual in vivo digestibility in rainbow trout, results show 87.3 percent for HB, and only 45.2 percent for BM (Table 6). This means that the high in vitro digestibility does not indicate high in vivo digestibility. Therefore, in vitro trials does not reflect the real value of ingredients.

Moreover, digestible protein content in HB was almost two times higher, compared to BM. Still, pepsin-HCL solubility of protein was comparable between both test ingredients (Table1). The latter is not illogical given the exceptional incubation conditions applied in this routine in vitro assay compared to the intestinal physiology of trout.

The observed higher protein digestibility in HB is likely thanks to the gentle drying process of spray drying, which better preserves the chemical integrity of nutrients. In broiler chickens, for instance, lysine availability has been shown to be much higher in spray dried compared to severely heat-treated animal by-products.

Additional security for the feed formulator

The studies showed that spray dried haemoglobin has almost two times higher apparent digestibility (dry matter and crude protein) compared to contact dried blood meal in rainbow trout.

Spray dried haemoglobin can also be perfectly used for replacing fish meal with increased dry matter apparent digestibility of the diet as result. Similar protein digestibility in reference diet (40%t fish meal) and haemoglobin diet.

The premium quality of haemoglobin is the result from careful collection and processing of the raw materials; starting at the collection, transportation and further processing at the factory, being quality preserved from the start.

As spray dried haemoglobin passes much more quality checks than blood meal, it gives an additional security for the feed formulator to use haemoglobin as an ingredient.

Further, haemoglobin enables the use of other types of ingredients in the feed formulation, even increasing their digestibility, which provides more flexibility.

By using haemoglobin as a better digestible protein source, water pollution will be less, by means of reduced faecal Nitrogen. This creates a more sustainable environment and a better health status of the fish.

The pepsin-HCl solubility test cannot reliably evaluate the suitability of ingredients for use in aqua feed.

How raw ingredients are processed matters. Spray-drying of haemoglobin makes a highly digestible animal protein source for trout suitable for partial replacement of fish meal, contact-dried blood meal is much less digestible.

References available upon request

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