



WHITEPAPER

AQUA CULTURE

Haemoglobin | Actipro 95 PHS/BHS
**A high quality protein source
for fish & shrimp**

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Haemoglobin vs blood meal in rainbow trout &

THE BENEFITS OF **SPRAY DRIED HAEMOGLOBIN IN FISH FEED**

One of the main benefits of using haemoglobin as a protein source in a fish diet, is the fact that it contains 92% protein of high biological value. This high biological value can be explained by several aspects. Firstly, the proteins remain highly digestible thanks to the spray drying process. Furthermore, haemoglobin does not contain any protease inhibitors, which are known to block proteolytic cleavage of protein precursors. Being an animal protein, haemoglobin is highly palatable and has a rich source of dietary essential amino acids which are complementary to the amino acid-profile of vegetal ingredients.

Compared to blood meal, fish meal, or other Processed Animal Proteins (PAP), haemoglobin has very few biogenic amines. These biogenic amines are metabolic products produced by bacteria and are toxins. By the fact that the blood is hygienically collected and immediately cooled, no bacteria can grow and amino acids stay intact.

For fish feed, haemoglobin is also being used as a pelleting process aid and as a coloring agent.

THE DIFFERENCE BETWEEN SPRAY DRIED HAEMOGLOBIN AND CONTACT DRIED BLOOD MEAL

Thanks to the spray drying method, the proteins in haemoglobin remain highly digestible. 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.

	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 1: spray drying method versus contact drying method

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. By having intact amino acids, animals can use the essential amino acids as building blocks. 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. Those biogenic amines are signal communes by which cells communicate with one another. However, when present in large quantities, biogenic amines are toxic. With the formation of biogenic amines, amino acids are lost and no longer available for digestion.

HOW TO DIFFERENTIATE HAEMOGLOBIN AND BLOOD MEAL?

The industry uses in vitro tests to monitor the quality of their formulated ingredients. For these tests, the Pepsin-HCl solubility of protein is measured, but often it is translated into 'in vitro digestibility'. The in vitro method is a cheap and quick way to create routine analyses which show an indication of the digestibility of a protein source or single ingredient. The method works in presence of digestive enzymes at a low pH level, in which the stomach environment is more or less simulated. However, in vitro tests use much lower pH levels compared to normal pH levels in the stomach of, for example, the rainbow trout. In reality, the residence time in the digestive tract of the fish is much shorter than in the lab conditions. Also other lab conditions such as temperature may be different than in reality. This leads to analysis results that are mostly good indications, but make it impossible to compare these 2 ingredients in a profound way.

HAEMOGLOBIN VS BLOOD MEAL IN RAINBOW TROUT

In order to get a clear picture of the real digestibility of blood meal and haemoglobin in rainbow trout, a study was conducted by DISAFA, Italy.

Preliminary Trial

To evaluate whether the ingredients are suitable for an in vivo trial, which can lead to statistically correct results, a preliminary trial was performed in rainbow trout. The main objective was to evaluate if the high inclusion rate is well supported by the fish.

Setup

During a period of 30 days, the two diets were evaluated. The first diet, hereafter named the control diet, contained 40% fish meal. Table 2 shows the complete ingredient list of the control diet. The second diet, hereafter named the Hb (haemoglobin) diet, was composed of 70% of the control diet and 30% haemoglobin.

The diets were cold pressed into pellets of 3mm and dried at 50°C for 48h. Thereafter, the pellets were distributed twice a day, 5 days per week, in two outdoor fiberglass tanks (0,50m³). Each tank contained 30 fish.

Ingredients	% in Control diet
Fish meal	40
Corn gluten meal	20
Soybean Meal	11
Wheat Meal	12
Binder	7
Fish oil / Vegetal oil	8
Vitamin & mineral premix	2

Table 2: ingredient list of the control diet

Results

Results show no mortality and no health problems of the fish. It also showed good palatability of both diets. Moreover, both groups showed a similar specific growth rate (SGR*), with 0,50% per day in the control group, and 0,51% per day in the Hb group. These results indicate that both diets are suitable for the fish.

	Control	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 3: Specific growth rate

* SGR = $[\ln BW_{fin} (g) - \ln BW_{initial} (g)] \times 100 / \text{time (d)}$

In vivo digestibility trial (Disafa, Italy)

The success of the preliminary trial allowed us to proceed with the in vivo digestibility trial. With this trial, we want to determine the in vivo digestibility of spray dried haemoglobin and contact dried blood meal in fish feed.

Setup

To compare the digestibility of spray dried haemoglobin with the digestibility of blood meal, three diets were compared:

- | | | |
|---|------------------|---|
| 1 | Reference diet | contains 40% high quality fish meal |
| 2 | Haemoglobin diet | consists of 70% of the reference diet and 30% haemoglobin |
| 3 | Blood meal diet | consists of 70% of the reference diet and 30% blood meal |

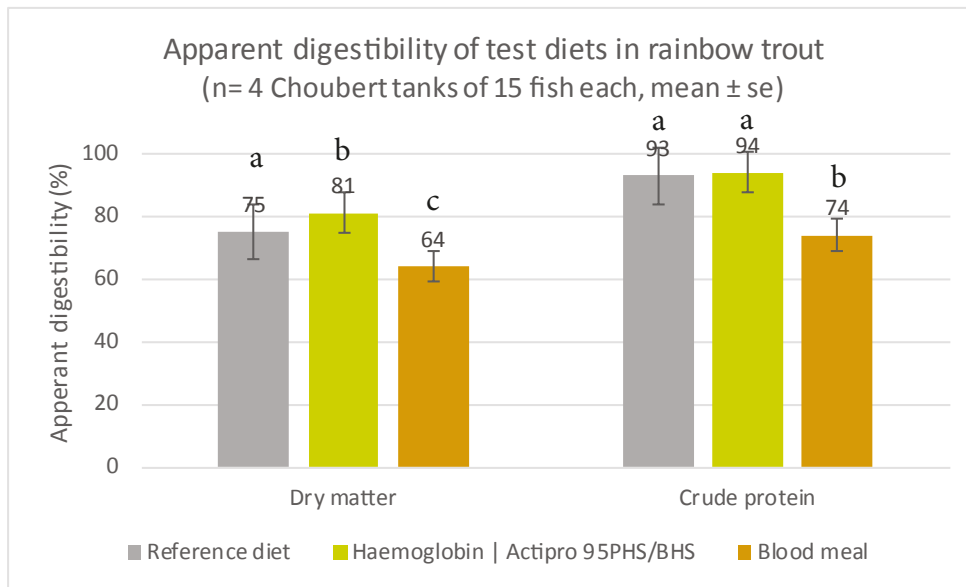
In the trial, 12 Choubert tanks (250L) containing 15 fish each were used. A Choubert tank is a water tank in which the feces are continuously collected. This prevents nutrient leakage and is crucial as nutrient leakage would result in an overestimation of the digestibility.

The trial lasted 4 weeks with first 2 weeks as an adaptation period. The fish were fed two times a day, five days per week.

Results

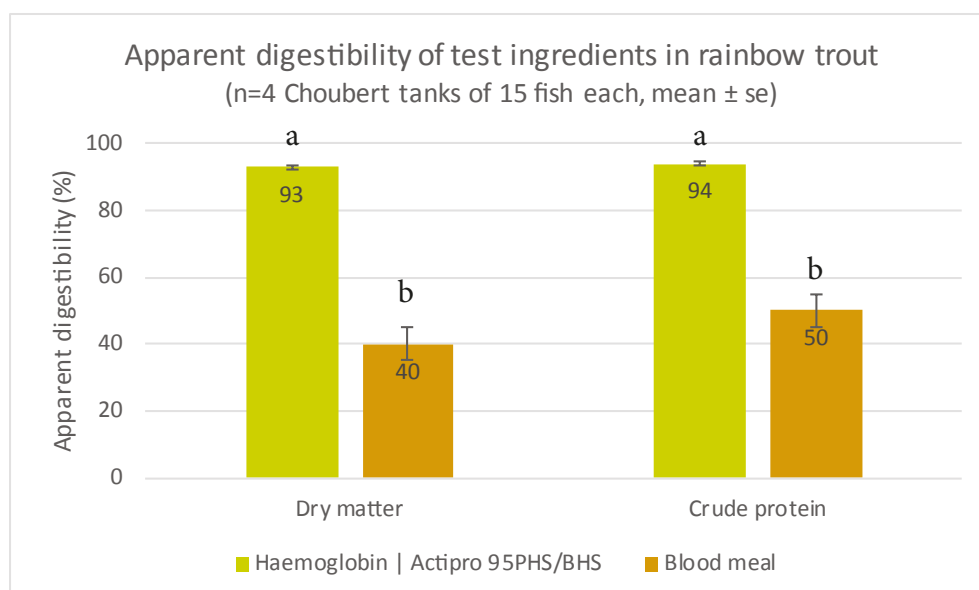
An external marker was used to determine and calculate the apparent digestibility of the feed. The digestibility of a feed determines the amount of nutrients that is actually absorbed by an animal and therefore available for growth, reproduction, etc. Apparent digestibility is calculated by subtracting nutrients contained in the feces from nutrients contained in the dietary intake.

The following figure shows the results of the apparent digestibility of the reference diet, haemoglobin diet and blood meal diet in rainbow trout.



Graph 1: Apparent digestibility of test diets

The crude protein apparent digestibility in the haemoglobin diet is similar to the reference diet (94% vs 93%), but significantly higher than the blood meal diet (74%). This means that the crude protein apparent digestibility of haemoglobin is similar to fish meal, but significantly better than blood meal. Further, we noticed a higher dry matter apparent digestibility of the haemoglobin diet compared with the reference diet. This may indicate that the digestion of other nutrients are also improved by adding haemoglobin to the diet (Graph 1).



Graph 2: Apparent digestibility of test ingredients

The dry matter apparent digestibility and the crude protein apparent digestibility of haemoglobin is almost the double as of blood meal (93% vs 40% and 94% vs 50%). This means that the haemoglobin is two times more digestible than blood meal (Graph 2).

In vitro digestibility

An in vitro Pepsin-HCl digestibility test (Method 72/199//EEC) was done on the diets.

Table 4 shows the results of our trial.

		Crude protein	Digestibility coefficient	Digestibility protein
Spray dried haemoglobin	(%)	92,9	100	92
Contact dried blood meal	(%)	91,3	97,3	88,9

Table 4: Pepsin-HCl solubility of protein

It is very clear that the crude protein content of both ingredients is more or less the same (92,9% and 91,3%). The in vitro digestibility coefficients are also close to one another. The same applies for the digestibility of protein; 92% versus 88,9%. However, when we look at the actual in vivo digestibility in rainbow trout, results show 87,3% for haemoglobin, and only 45,2% for blood meal (table 5). That means that high in vitro digestibility does not indicate high in vivo digestibility. Therefore, in vitro trials does not reflect the real value of ingredients.

		Crude protein	Digestibility coefficient	Digestibility protein
Spray dried haemoglobin	(%)	92,9	94	87,3
Contact dried blood meal	(%)	91,3	49,5	45,2

Table 5: In Vivo digestibility of rainbow trout

HAEMOGLOBIN VS BLOOD MEAL IN SHRIMP

Apart from trout, shrimp is one of the main species cultivated within aquaculture, so it is also interesting to clarify the digestibility of haemoglobin in such specie.

Setup

We have done a study at IMAQUA where the protein digestibility of spray dried haemoglobin powder, ring dried blood meal and disc dried blood meal was measured in shrimp.

Four diets were prepared, being one of them the control diet.

Raw materials (%)	CTRL	PC-H	PL-BM	PC-BM
Wheat flour	35.63	24.94	24.94	24.94
Soybean meal	22.00	15.40	15.40	15.40
Fish meal	25.00	17.50	17.50	17.50
Sepioliet	3.10	2.05	2.05	2.05
Fish and tuna oil	2.90	2.03	2.03	2.03
Squidmeal	5.00	3.50	3.50	3.50
Soy lecithin	1.56	1.09	1.09	1.09
Limestone	0.75	0.53	0.53	0.53
Vitamin premix	0.50	0.35	0.35	0.35
Monocalcium phosphate	1.00	0.70	0.70	0.70
Cholesterol, feed grade	0.10	0.07	0.07	0.07
Vit C35%	0.06	0.04	0.04	0.04
Gelatin	2.00	1.40	1.40	1.40
Titanium Dioxide	0.4	0.4	0.4	0.4
Spray dried haemoglobin	0.0	30.0	0.0	0.0
Ring dried blood meal	0.0	0.0	30.0	0.0
Disc dried blood meal	0.0	0.0	0.0	30.0
Total	100	100	100	100

Table 6: Dietary treatments

The mixtures were homogenized and pelletized to be able to be supplied to the shrimp, never exceeding the temperature of 50°C during the process.

To allow digestibility analysis, the TiO marker was included at a concentration of 0.4%.

Experimental animals were *Penaeus Vannamei*, imported in the post-larval state, being certified as specific pathogen free: WSSV, YHV/GAV/LOV, TSV, IMNV, PNV, CMNV, BP, MBV, AHPND/EMS, EHP, NHP-B and IHNV. A total of 600 shrimp, with a starting average body weight of approximately 8.5g, was used to randomly compose 12 groups of 50 individuals. Each group was housed in one feeding unit. The animals were subjected to an adaptation process in saline water (20g / L) and fed an artificial diet to replace live food. They were then fed a pelleted diet. The feed was given automatically 6 times a day and the amount of feed was checked and adjusted daily according to the size of the animals.

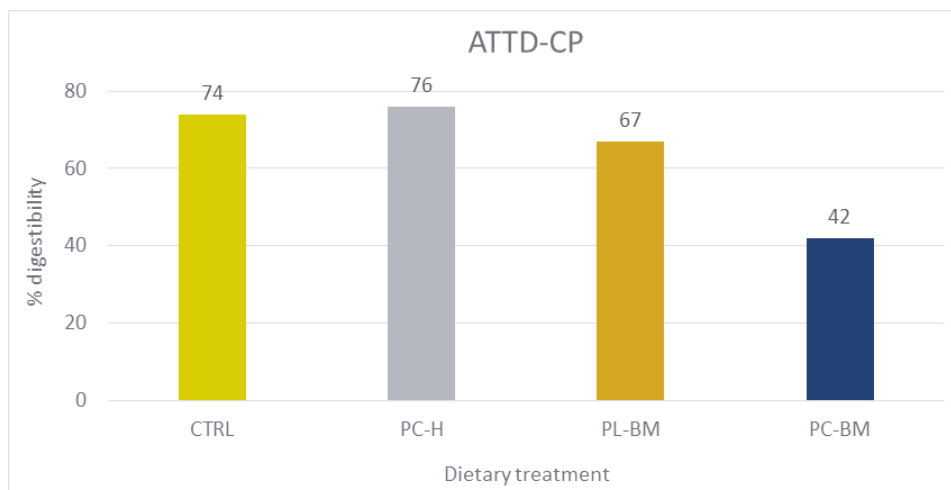
The water temperature was 27°C ± 1, kept constant throughout the study. Nitrite and ammonia levels were kept constant (less than or equal to 0.8 and less than 0.05 respectively) during the study, through the use of mechanical and biological filters and regular water changes. Light / dark cycles will last 12 hours.

The study lasted 53 days with triplicate groups of animals, focusing on weight gain and protein digestibility, analyzing faeces collected with a precipitation system. The analysis was performed for protein with a method based on ISO 5983-2: 2009 (Kjeldahl) and an own method for TiO₂ based on UV-spectrophotometry. Crude protein digestibility calculation (ATTD-CP) was done according to Lemos et al. (2009).

Dietary treatments	Feeding trials		Analysis of digestibility at the end of the trial	
	Number of shrinks (replicate tanks)	Feeding period and collection of faeces	CP and TiO ₂ in feed	CP and TiO ₂ in faeces
CTRL	150 (50x3)	53 days	1	3 (1x3)
PC-HM	151 (50x3)		1	4 (1x3)
PL-BM	152 (50x3)		1	5 (1x3)
PC-BM	153 (50x3)		1	6 (1x3)
Total	600		4	12

Results

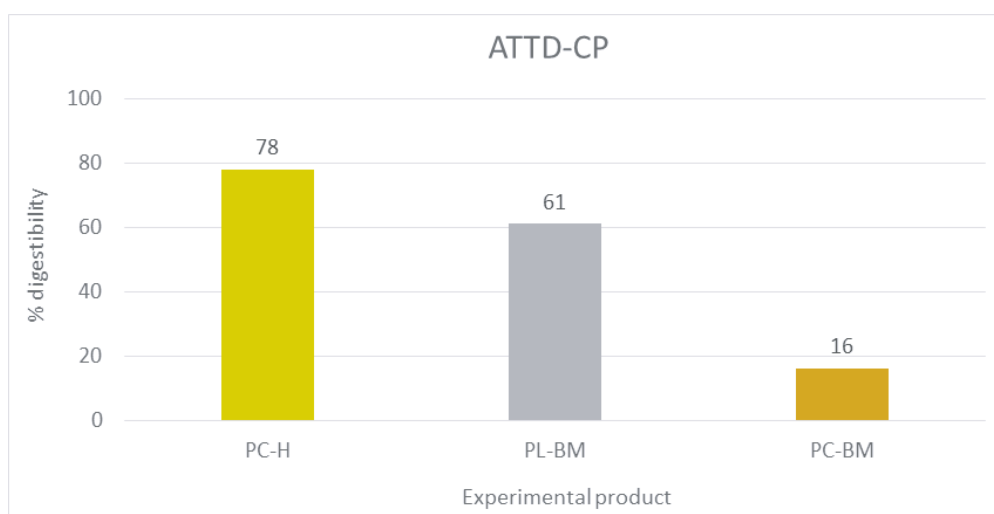
Apparent total tract digestibility of the different diets results as:



Graph 3: ATTD-CP

Bars sharing the same label are not significantly different from each other ($p > 0.05$). Bars that have no label in common are significantly different from each other ($p < 0.05$).

The apparent digestibility of the three ingredients studied resulted in:



Graph 4: ATTD-CP

Conclusion

The main conclusions that we can draw are:

With an apparent total tract digestibility for crude protein (ATTD-CP) of 78% within shrimp, spray dried haemoglobin powder has the potential to be used as a fish meal replacement as fish meal typically scores around 80% in shrimp.

The way of processing (drying) blood has a big influence on protein digestibility in shrimp. Haemoglobin powder produced by the gentle spray drying technology has a higher protein digestibility (78%) in shrimp than ring dried (61%) or disc dried (16%) blood meal.

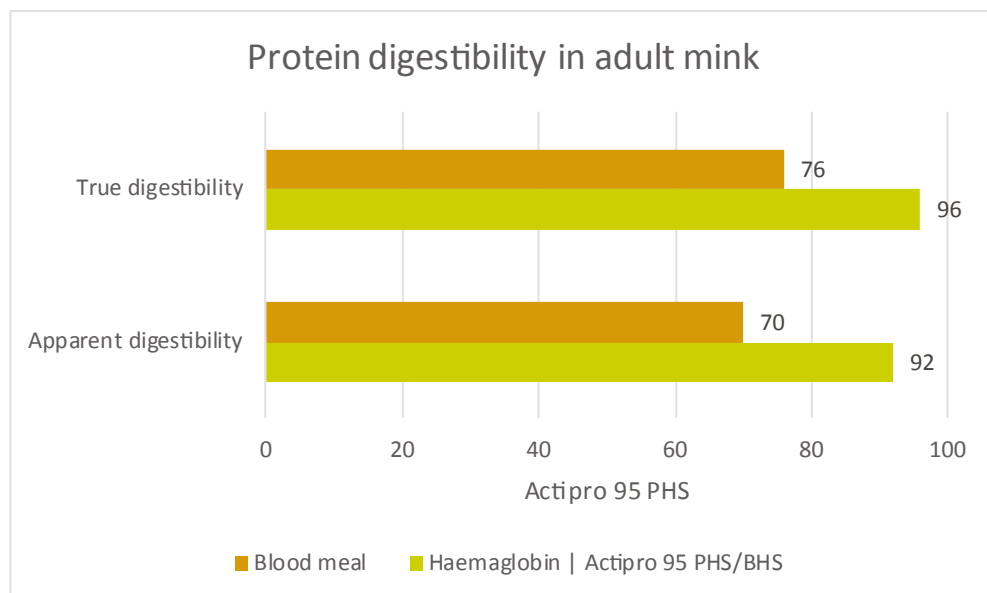
Haemoglobin powder is of higher nutritional value for shrimp and creates less nitrogen pollution in the water thanks to the higher protein digestibility in shrimp.

HAEMOGLOBIN IN OTHER SPECIES

Next, we compared our findings with in vivo digestibility studies in other animal species to verify whether we found similar results.

Adult male mink

In 2003, a study conducted by the Danish fur breeders research center analyzed the digestibility of spray dried haemoglobin and drum dried blood meal in adult male mink. The following graph shows the true and apparent digestibility in mink. Apparent digestibility measures the difference between the nutrients that are fed to the mink and the nutrients that are found in their feces. True digestibility on the other hand takes endogenous losses into account. In daily practice, the apparent digestibility is mostly used.

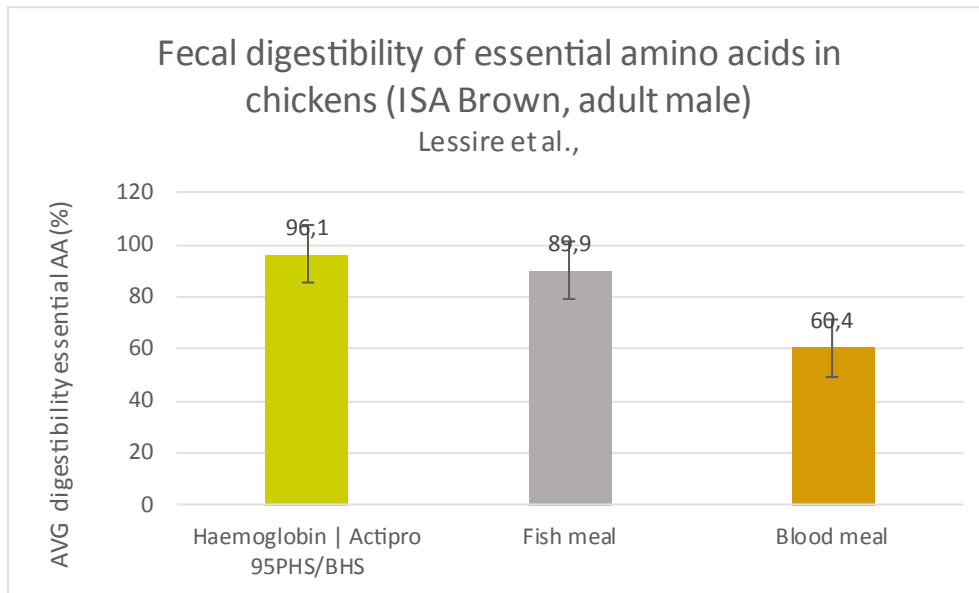


Graph 5: Protein digestibility in adult mink

In adult mink, the average digestibility of proteins in spray dried haemoglobin is much higher than the average digestibility of proteins in drum dried blood meal.

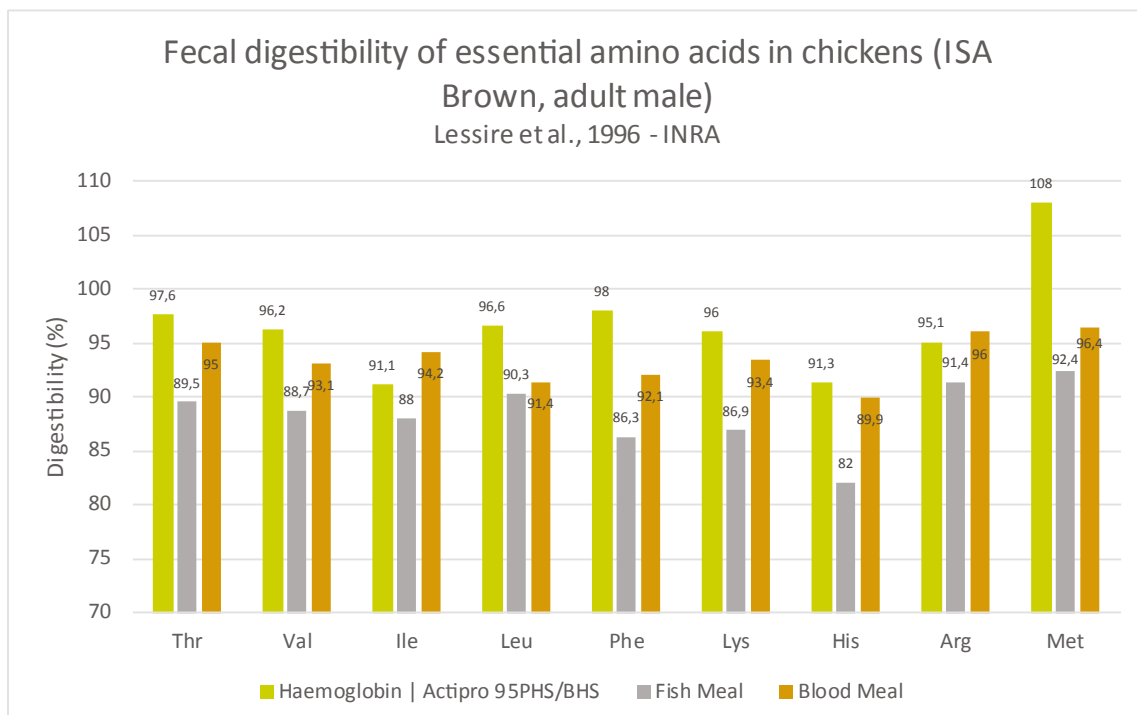
Poultry feed

Another study, conducted in 1996 by Lessire et al., compared the average digestibility of essential amino acids within different protein sources in poultry feed. The different sources that were compared are: haemoglobin, fish meal and blood meal. The graph below shows the results of that study.



Graph 6: Fecal digestibility of AA in chickens

As you can see, blood meal has the lowest digestibility of essential amino acids, whereas haemoglobin has the highest digestibility. Also the individual amino acid digestibility confirms earlier found results.



Graph 7: Fecal digestibility of essential AA in chickens

CONCLUSION

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.

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. The quality is 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 to use other types of ingredients in the feed formulation, which provides more flexibility. By using a better digestible protein source, water pollution will be less, by means of reduced fecal Nitrogen. This creates a more sustainable environment and a better health status of the fish.

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