Aminocarp™ – Progress in Modelling Amino Acid Recommendations for Carp

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Abstract

Experiments have shown that cyprinid fish species respond to supplemental free amino acids allowing for dose-response studies to determine optimal concentrations. While dose-response experiments have limitations, the factorial approach considering requirements for performance and maintenance offers an alternative way to define amino acid recommendations. Evonik's AMINOLab® database provides comprehensive information on the amino acid composition of common carp body protein. In addition, various experiments provide data on utilisation of dietary amino acids for deposition in body protein. Research also shows that free amino acids are utilised as effective as amino acids from intact feed proteins. This information can be used for modelling amino acid recommendations. AMINOCarp™ is a software which provides amino acid recommendations for common carp feed. These recommendations can be adjusted to growth rate of carp, feeding rate of carp, average body weight at start of feeding phase, feeding frequency and proportion of natural feed to overall feed intake.

Keywords: aminocarpTm, amino acid, carpfeed.

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Introduction

Among animal production cultured fish and shrimp play an increasingly important role. According to most recent FAO statistics (2010) total aquaculture production increased from 50,225,371 t in 2003 to 68,348,943 t in 2008 which was an increase of about 36 % during this period. From the total aquaculture production in 2008 about 30 % (20,439,469 t) can be assigned to *cyprinidae* representing the largest species group of which common carp makes up about 15 % (2,987,433 t). Thus, common carp belong to the top three species with respect to production volume after silver carp and grass carp – two further cyprinids. Almost 79 % of common carp was produced in China but common carp are also widely spread in Indonesia (8.1 %), Vietnam (2.5 %), Russian Federation (1.6 %), Bangladesh (1.2 %), Brazil (1.2%), Iran (0.7 %), Myanmar (0.6 %), Czech Republic (0.6 %), Poland (0.6 %) and Egypt (0.4 %). It can therefore be concluded that common carp is an important species in aquaculture which is found in many parts in the world including Asia as well as Europe and Africa whereas little commercial production has been reported for countries in Latin America.

Various production systems are described among which we find polyculture or monoculture systems and within those extensive production with low harvest yields or intensive production with comparably high harvest yields. Compound feed is increasingly used in carp production where the importance of nutrient content and balance in compound feed increases with production intensity in order to meet the requirements of the fish and to keep or improve profitability. In this context dietary protein is a main contributor to diet costs which recently increased due to increasing prices of protein sources such as soybean meal and fish meal. Also, nitrogen pollution via ammonia and its further transformation to nitrate as well as the impact of organic excretions on oxygen management of pond systems can be optimised with nutritionally optimised feed.

Similar to other farm animals, carp do not have a requirement for crude protein (nitrogen x 6.25) but require individual essential amino acids. By means of supplemental amino acids

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such as DL-Methionine, L-Lysine, and L-Threonine the dietary amino acid profile can be much better balanced while sparing expensive raw materials as well as dietary protein. It has been demonstrated recently that those supplemental amino acids can effectively be applied in carp nutrition (Lemme, 2010; Schwarz, 1998).

However, questions on amino acid requirements and their optimal dietary concentrations remain. This shall be addressed in the following article in which methodologies for requirement determination are introduced. In addition, a concept is introduced allowing for dynamic and flexible recommendations for all essential amino acids under changing production conditions.

Methods to determine amino acid requirements

Although several methodologies for determining amino acid requirements are described in literature, two approaches are most established: **dose response studies** and the **factorial approach**. Whilst both methods certainly have advantages and disadvantages the factorial approach currently appears to be the most effective and flexible method for determining amino acid recommendations for carp. However, both methods yielded valuable information contributing to defining recommendations which were applied in the development of AMINOCarp™ but in the following the factorial approach will be discussed in detail.

The factorial approach offers flexibility

In the factorial approach requirements are split up into maintenance requirements and requirements for performance. In growing animals performance means amino acid deposition (retention). The factorial approach can be expressed by a mathematical formula:

$$Req_{Total} = Req_{Maintenance} + Req_{Retention}$$

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Maintenance requirement is usually defined as that amount of amino acid which allows for maintaining body weight which means neither gaining nor losing weight. The requirement for retention includes those amino acids which are deposited in form of protein (net retention). However, amino acids absorbed from the digestive tract are not completely utilised for accretion but partly degraded or metabolised into other compounds or utilised as energy source. Therefore, also knowledge about utilisation of absorbed amino acids is required. For example, methionine is an important methyl group donator for the energy metabolism in the cells and a certain portion of the methionine pool is used for this purpose and does consequently not appear in body protein. Finally, as digestibility of individual amino acid may differ between ingredients and fish diets are usually still formulated on basis of total amino acids also digestibility of dietary amino acids need to be considered in this model approach. Therefore, the above formula is extended:

$$Req_{Total} = (Req_{Maintenance} + (Req_{Net\text{-}Retention} \text{ x } Utilisation_{Amino\ acid})) \text{ x } digestibility_{Amino\ acid}$$

Due to the historically grown expertise and the high annual number of amino acid analyses of various materials, Evonik Industries has access to a comparably large data base including amino acid compositions of whole body homogenates of various fish species. More than 100 whole body homogenates of common carp were analysed for crude protein and amino acids in AMINOLab® providing us with a reliable profile of the amino acid composition of common carp. Interestingly, average coefficient of variation for the amino acid concentrations within protein (% of protein) was well below 5 % and none of the individual values exceeded 6.5 %. Table 1 shows the amino acid profile of carp protein composition (Lys = 100) and suggests that the protein contains a rather low level of cysteine and that the concentration of none of the essential amino acids exceeded that of lysine. Moreover, comparisons to the profiles of other fish species analysed in AMINOLab® revealed differences while Kaushik and Seiliez (2010) proposed one set for all species. So, once the amino acid composition of carp whole body protein as well as the protein deposition rate for a defined period of time is known, net retention can be calculated. Knowledge of protein deposition rates which can be determined by comparative slaughter technique are

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needed for calculating the deposition of amino acids at different body weight ranges. The charming aspect of this part of the model is that all essential amino acids can be considered at a time because amino acid analysis via AMINOLab® releases the complete profile.

Table 1. Average amino acid profile of body protein obtained from 110 whole body samples of common carp and finfish in general

Amino acid	**Lys**	**Met**	**Met+Cys**	**Thr**	**Trp**	**Arg**	**Ile**	**Leu**	**Val**	**His**	**Phe**
Common carp*	100	34	46	53		76	52	92	59	38	51
Finfish**	100	37	50	59	14	83	58	98	64	33	56

* Tryptophan was not analysed ** adopted from literature survey by Kaushik and Seiliez (2010)

Utilisation of amino acids for retention can be distinguished between overall and marginal utilisation

With respect to the model component $\text{Utilisation}_{\text{Amino acid}}$ available information is scarce. Basically, overall utilisation of dietary amino acids for retention can easily be determined if - as described above - amino acid retention has been determined by comparative slaughter technique and amino acid intake which is the product of feed intake and dietary amino acid content is known as well. By definition, the proportion of retained to ingested amino acids indicates the utilisation for retention. Whether amino acids which are not deposited in body protein are excreted undigested, utilised for certain metabolic functions, transformed into other molecules or simply degraded as energy source cannot be distinguished by this approach. In the example shown in Figure 4 (middle) the Met+Cys intake increased with dietary Met+Cys level which was actually increased by supplemental DL-Methionine (Lemme *et al.*, 2011). When Met+Cys retention was related to intake, overall utilisation increased up to 54.5 % at a dietary level of 0.98 % Met+Cys and decreased thereafter. Such a Met+Cys utilisation pattern is in line with findings in broilers. The overall utilisation only

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for methionine revealed 76.3 % in the basal diet which also agrees well with poultry data and indicates that the utilisation of cysteine is very low. The latter can be explained by cysteine being required for metabolic functions rather than as building block for protein synthesis. However, methionine can be converted to cysteine in order to cover the requirement as well. These data were obtained from an experiment with common carp (*Cyprinus carpio*) with an average initial weight of 57 g being fed over 58 days. In contrast to overall utilisation, marginal utilisation provides information how much of the last unit of ingested amino acid has been retained in protein. This may also provide information on the utilisation of DL-Methionine as increases in dietary Met+Cys were achieved by supplementation. The center graph in Figure 4 shows the daily Met+Cys retention regressed against the daily Met+Cys intake. Accordingly, the first and second inclusion level of supplemental DL-Methionine (0.05, 0.15 %) resulted in improved retention - as already shown in the left graph of Figure 1 -. However, when both retention and intake are given in amounts (mg) the slope of the curve represents the marginal utilisation as also demonstrated by Rodehutscord *et al.* (1995). Mathematically, the slope in each point of a quadratic equation can be described by the first derivation which will be a linear regression equation. This regression or in other words the utilisation of the dietary Met+Cys decrease with increasing intake from about 100 % to negative (Figure 1, right hand graph). This indicates that at deficiency the utilisation for Met+Cys retention was complete suggesting a complete transfer of supplemental DL-Methionine into body protein. With increasing Met+Cys intake utilisation decreases and after exceeding maximum performance and thus maximum protein deposition utilisation turns even negative. This finding confirms results reported for rainbow trout (Rodehutscord *et al.*, 1995) and broilers (Fatufe and Rodehutscord, 2005). Thus, utilisation of amino acids depend on supply status and not on the amino acid source.

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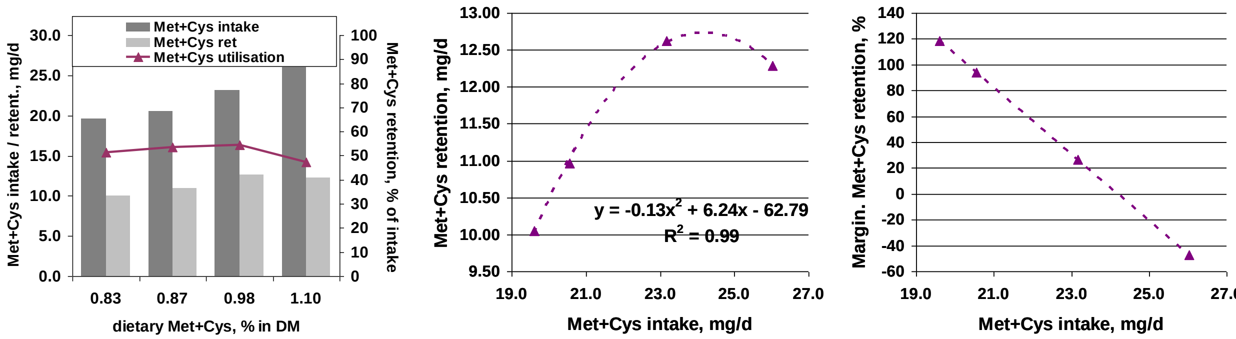

Figure 1. Overall Met+Cys intake, retention and utilisation in common carp with increasing dietary Met+Cys (left), Met+Cys retention regressed against ingested dietary Met+Cys (middle) and marginal Met+Cys retention indicating the utilisation of supplemented DL-Methionine using the first derivation of the quadratic equation (y=-0.26x +6.42; data from Lemme *et al.*, 2011)

Coming back to the overall amino acid utilisation, all essential amino acids would equally limit performance in an ideal protein and thus utilisation of the dietary protein will be maximised - a major objective for protein sparing nutrition. Despite this, overall utilisation coefficients are not equal among amino acids. For example the maximum utilisation of lysine will be higher than that for methionine because lysine is almost exclusively used as building block for protein synthesis whereas substantial amounts of methionine are used as methyl group donators and used for other metabolic purposes and are therefore not recovered in body protein.

Prior to the experiment reported above another trial was conducted at IMARES, Netherlands, with common carp with average initial body weights of about 60 g. In both trials fish were fed either a methionine deficient diet or this diet supplemented with 0.05 %, 0.15 %, or 0.25 % DL-Methionine for 56 days (Evonik, 2011). The difference between experiments was that in the first trial protein content of the diets was about 40 % in DM while in the second trial it was about 34 % in DM. However, in both trials diets were based

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on soybean protein concentrate, pea protein concentrate, wheat and cornstarch and only proportions of those raw materials differed. Fish were fed continuously by automatic feeders at a daily feeding rate of 2.5 % feed dry matter of body weight. Feed amounts were adjusted daily according to an assumed growth curve. Also in the IMARES trial a clear response to incremental DL-Methionine was observed in terms of weight gain and overall Met and Met+Cys retention. However, utilisation of Met or Met+Cys was highest in the basal diet and decreased gradually from 81 % to 61 % or 57 to 51 %.

In a further trial performed at Gesellschaft für Marine Aquakultur by Heinitz and Schulz (2011) in which increasing dietary methionine levels were fed to juvenile common carp with average initial weights of 7 g, weight gain response was not as strong as in the former trials and inclusion levels of 0.03 % and 0.07 % improved body weight gain only slightly. In this trial whole body homogenates of fish were obtained at the start and end of the experimental phase. Those were analysed for amino acid content and formed the basis for subsequent calculation of retention efficiency. Compared to a utilisation of 74 % in the basal diet, it increased to 76 % with 0.03 % supplemental DL-Met and decreased thereafter to 65 % at 0.11 % additional methionine. Other methionine dose-response trials confirm a utilisation of 70 % and higher.

To summarise, overall utilisation amino acids can be determined by means of a dose-response growth study combined with comparative slaughter technique which allows for amino acid retention. Taking methionine as an example utilisation of total amino acids seems to be 70 % or higher in carp. Met+Cys utilisation is 50 % or higher, respectively.

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Amino acid intake can also be varied with daily feeding rate

Another approach to vary amino acid intake is not by varying the dietary amino acid content but by varying feeding rates. Again, only that amino acid which is first limiting performance will maximised its utilisation. Helland *et al.* (2010) recently reported such an approach with smolted salmons. In the experiment by Helland *et al.* (2010) utilisation of digestible amino acids for retention ranged between 45 % (tryptophan) and 62 % (lysine) but, however, diets have not been formulated to be deficient in any of the amino acids and thus it is questionable whether maximum utilisation has been achieved. In addition, this regression method allows estimating the maintenance requirement by calculating required amino acid intake at zero retention. This is only suitable in case of a treatment close to zero amino acid retention and extrapolation of the regression line would bear a big risk for error as small changes in the slope would have a significant impact on calculated maintenance requirement.

A trial with common carp with an average initial body weight of 57 g was conducted at the facility of feedtest, Germany, in order to determine utilisation of methionine and to determine the maintenance requirement of methionine. Four replicate tanks with 10 carp each per treatment were fed two different diets at varying feed intakes for 56 days. One diet was deficient in methionine (0.48 % in DM) whereas the methionine level of the other was increased by supplemental DL-Methionine (0.59 % in DM). While there was a negative control treatment in which fish did not receive any feed, fish were fed the diets at quantities of 3.2, 6.4, and 12.8 g dry matter / d / kg body weight$^{0.8}$. Feed was supplied in 5 meals per day whereas in an 8th extra treatment the deficient diet was given in one meal per day at 3.2 g / d / kg body weight$^{0.8}$. Methionine intake and retention per kg body weight per day was determined using amino acid analysis of feed and whole body homogenates. Body weight development and regression of methionine retention against intake are illustrated in Figure 2. Specific growth rates increased linearly with increasing feed intake but performance was higher with increased dietary methionine resulting in a significant difference at highest feed intake. Interestingly, when feed was given in one meal, weight gain was significantly lower

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than when the same feed quantity was given in 5 meals. However, when methionine retention was regressed against intake both lines had the same slope according to the slope ratio regression equation: Y = -2.01 + 0.798 Met-def + 0.780 Met-sup. Thus, dietary methionine was utilised by 79.8 and 78.0 % in these two diets being in line with above reported numbers. Moreover, confidence intervals suggest that slopes were not different (CI= 87.3 – 108.2 %) which suggest that supplementation of 0.1 % DL-Methionine representing about 17 % of total dietary methionine had no negative impact on overall utilisation or – in other words – the supplemented DL-Methionine was utilised to the same degree for retention as the remaining methionine from protein sources. This finding is in contrast to the belief that free amino acids are not utilisable.

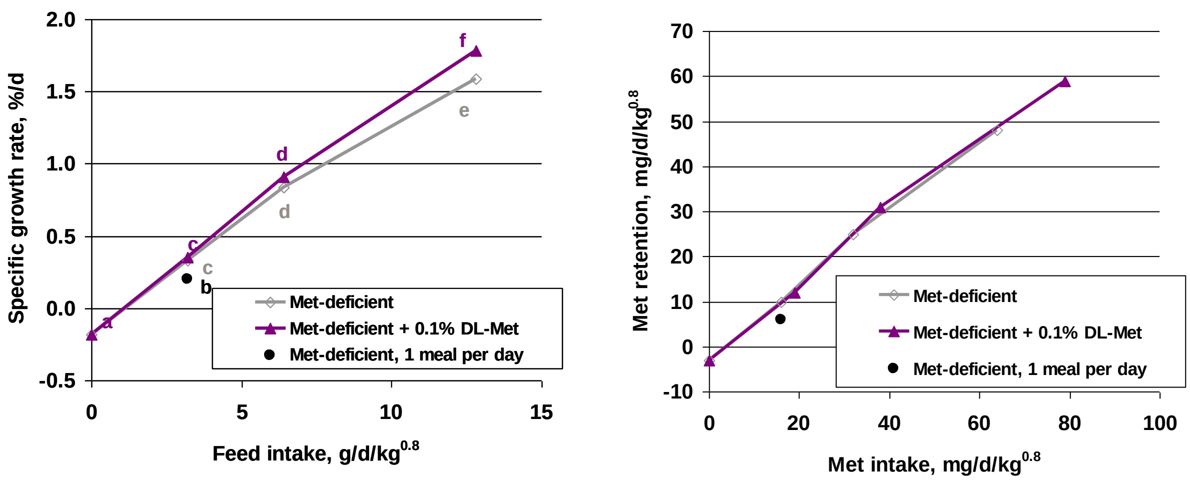

Figure 2. Specific growth rate (%/d, left) and retention (mg/d/kg BW$^{0.8}$, right) of dietary methionine at changing feed and thus methionine intakes in common carp fed either a methionine deficient diet (rhombs, grey line) or a diet supplemented with methionine (triangles, purple line) (Elwert, 2011)

Using the equations given in Figure 2 also maintenance requirement for methionine can be calculated. Accordingly, between 0.37 and 0.41 mg/d/kg BW$^{0.8}$ would be needed. Helland *et al.* (2010) estimated a maintenance requirement of 3.4 mg methionine/kg BW/d for salmon in the above mentioned study which appears low in contrast to 16.1 mg methionine/kg BW/d estimated for rainbow trout (Rodehutscord *et al.*, 1997). This

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substantial difference might be due to the fact that the experiment of Helland *et al.* (2010) was not conducted under the condition of suboptimal methionine supply.

Amino acid requirements of carp were estimated by factorial approach

According to the factorial approach, we have developed AMINOCarp™, a simple model for estimating optimal levels of all essential amino acids in carp diets. Body weight development of carp is defined by the input variables initial average body weight, specific growth rate and feeding period. Background information on changing body protein composition with increasing size enables calculating protein and thus amino acid retention. Defining the feeding rate, a further input variable of the tool, will determine the required dietary concentration needed to meet the requirement.

For example, a scenario in which 60 g carp will be fed over 8 weeks at a feeding rate of 2.5 % of body weight per day (in 3 meals) and performing at a specific growth rate of 2.1 % per day would result in an optimal lysine, methionine and methionine+cysteine level of 2.33 %, 1.29 % and 1.87 %, respectively, in the diet. These amino acids are just taken as an example and the model displays recommendations also for Thr, Arg, Ile, Leu, Val, His and Phe.

Feeding frequency strongly influences nutrient utilisation

As indicated earlier, number of meals influences effectiveness of the diet (Figure 2). Recent research indicated that feed conversion ratio and, therefore, nutrient utilisation improved with increasing feeding frequency. In a trial by Schwarz *et al.* (2010) carp with initial body weights of 239 g/fish were fed identical amounts of experimental diets either twice a day or continuously via automatic feeders (Figure 3). Ignoring the different diet compositions, feed conversion ratio was on average 2.11 g/g in case of twice daily feeding until apparent satiation whereas feeding exactly the same amount of feed by automatic feeders resulted in improved growth and thus a significantly lower feed conversion ratio of 1.58 g/g (P<0.05)

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in the 82 day lasting trial. This effect might partly be explained by an improved digestibility as Schwarz *et al.* (2010) also observed a significant increase of protein digestibility (P<0.05) when switching from twice daily feeding to continuous feeding. The latter is confirmed by Zhou *et al.* (2003) who reported a gradual increase in protein digestibility (P<0.05) of juvenile gibel carp at feeding frequencies of 2, 3, 4, and 12 times per day. At the same time these authors observed an increased protein retention rate which clearly suggests better utilisation of digestible protein for retention. The latter effect was much stronger than the impact on digestibility. In another trial presented by Lemme and Elwert (2010) feed conversion ratio improved from 1.50 g/g to 1.31 g/g (P<0.05) when feeding five instead of two meals per day (Figure 3). In this experiment, common carp with initial body weights of 51 g/fish were fed experimental diets for 63 days at a feeding rate of 2.5 % of body weight. Feeding rate was adjusted every day according to a growth curve which in turn was adjusted weekly according to recorded body weights.

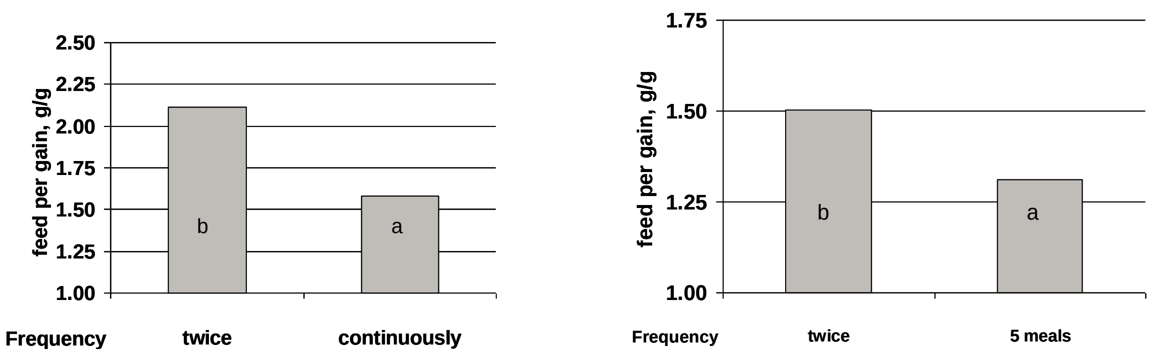

Figure 3. Feed conversion of common carp fed identical amounts of feed either twice dail or by a continuous system (left, Schwarz *et al.*, 2010) or twice daily or in 5 meals per day (right, Lemme and Elwert, 2010)

Also data from more practical production systems are available. Biswas *et al.* (2006) reported results of a pond trial in a polyculture system with Indian major carp species including *Catla catla*, *Labeo rohita*, and *Cirrhinus mrigala*. Accordingly, feed conversion improved from 2.31 to 2.28, and 2.10 at feeding frequencies of 1, 2, and 3 times per day. While in the above experiments exogenous conditions such as oxygen supply were

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optimised, oxygen demand might interact with feeding frequency in terms of nutrient utilisation. As reported by Carter *et al.* (2002) a common response to reduced feeding frequency is an increased meal size. These larger meals will be followed by a higher oxygen consumption compared to smaller meals which can also be observed in experimental units (Elwert, 2010, personal communication).

In the light of these findings the impact of feeding frequency on nutrient conversion has been considered in AMINOCarp™. The user can choose between 2 and 5 meals per day. According to the earlier mentioned scenario created in AMINOCarp™ a switch from 3 times daily feeding to 5 times daily feeding would reduce optimal Lys, Met or Met+Cys levels from 2.33 %, 1.29 % or 1.87 % to 2.12 %, 1.19 % or 1.72 % respectively. Adjustments for the other amino acids are done accordingly. In contrast, reduced feeding frequency to only two meals per day would result in optimal Lys, Met and Met+Cys levels of 2.44 %, 1.35 % and 1.95 % in the diet.

Natural feed affects optimal amino acid levels in compound feed

Research on nutrient requirement is usually conducted in experimental units using tanks or aquaria. Those systems are well managed and factors which may disturb the experiment are typically excluded. However, under practical conditions the environment differs from that in experimental facilities (Bégout *et al.*, 2002). With respect to nutrition one major factor is the natural feed in form of algae, zooplankton and other sources which contribute to the nutrient intake of fish. According to Bégout *et al.* (2002) the majority of the feed consumed by fish in pond systems is usually from natural sources. Indeed, the proportion of this contribution to overall nutrient intake depends on the production system. The more intensive the system which means the higher the stocking density in the ponds is, the lower the proportion of natural feed in total feed intake will be. In this context two investigations in juvenile seabass by Reymond (1989, cited by Bégout *et al.*, 2002) demonstrated that fish ingested on average between 38 % to 43 % natural feed. Interestingly, the stomach of the larger fishes in these trials contained between 75 % and 100 % supplemental feed while in

Lemme, A. 2011. Aminocarp™ – Progress in Modelling Amino Acid Recommendations for Carp. En: Cruz-Suárez, L.E., Ricque-Marie, D., Tapia-Salazar, M., Nieto-López, M.G., Villarreal-Cavazos, D. A., Gamboa-Delgado, J., Hernández-Hernández, L. (Eds), Avances en Nutrición Acuícola XI - Memorias del Décimo Primer Simposio Internacional de Nutrición Acuícola, 23-25 de Noviembre, San Nicolás de los Garza, N. L., México.
ISBN 978-607-433-775-4. Universidad Autónoma de Nuevo León, Monterrey, México, pp. 284-300.

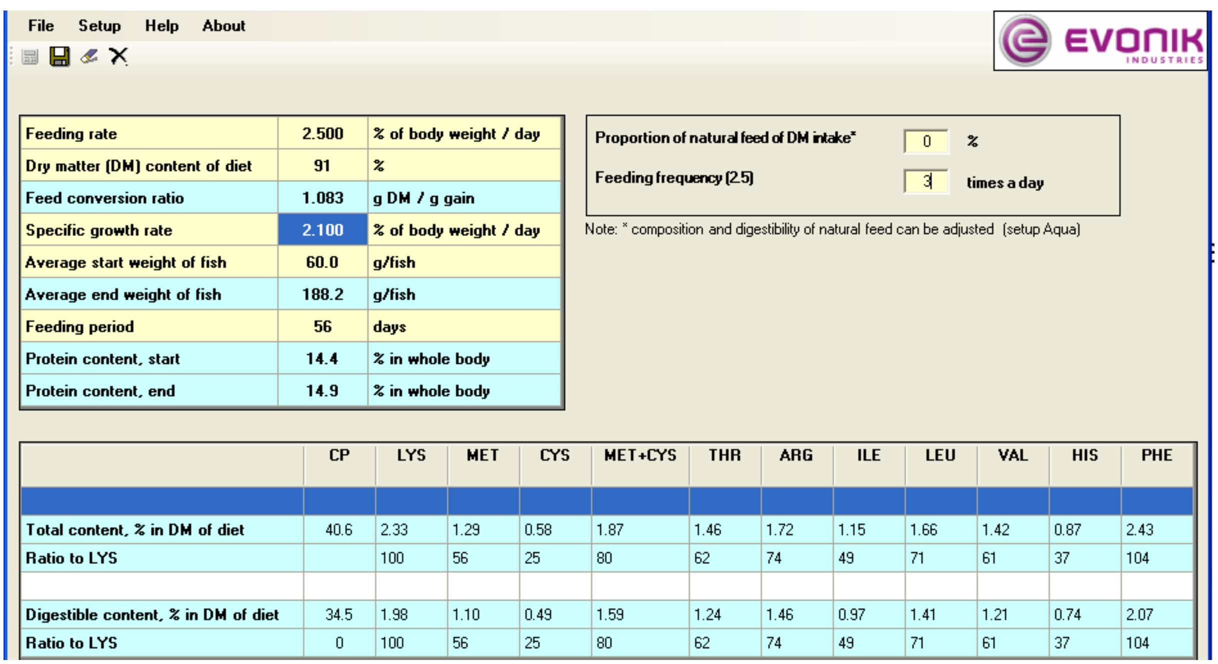
the stomachs of small sized fishes of the same production only 18 % to 37 % supplemental feed was found.

The mechanisms become more complicate if seasonal and daily rhythms are considered. At the beginning of the growth season proportion of natural feed is relatively high whereas with progressing season carp biomass is gaining which results in a relative decrease in intake of natural feed (Horváth *et al.*, 2002). With decreasing contribution of natural feed the requirement for supplemental compound feed and particularly the requirement for dietary protein and amino acids from compound feed increases. This is not only to meet the requirement of carp for amino acids but is also very important in terms of preserving the natural algae which are particularly important for oxygen production. While the latter takes place during the day, there is only oxygen consumption during the night. Thus, also feeding regimes needs to be adjusted in order to avoid overstressing the system because digestion and nutrient utilisation of fish will increase oxygen demand (Bégout *et al.*, 2002). In this context, also water temperature plays a crucial role. Feeding carp therefore means also to balance the system for biomass growth of carp, algae and natural feed production, as well as oxygen supply in the pond (Horváth *et al.*, 2002). Certainly, there are more factors such as pond fertilisation or polyculture but by these three the interrelationship of this complex production system can nicely be demonstrated.

Because of the above mentioned interrelationships, AMINOCarp™ includes a feature in which the proportion of natural feed compared to overall feed intake can be chosen. This means that with increasing percentage of natural feed the required amino acid levels in compound feed will decrease. This is particularly important for amino acids such as methionine as the methionine content in algae and zooplankton is comparably low. Thus, the use of the model might help understanding and optimising amino acid nutrition. A difficulty is the composition of natural feed and thus AMINOCarp™ can just provide an approximation. However, in case more detailed information on the amino acid composition of natural feed is available the user of AMINOCarp™ can adjust the respective numbers in the software.

AMINOCarp™ recommends optimal dietary amino acid levels for different production conditions

Using analytical data as well as experimental results allowed for developing a tool which gives amino acid recommendation for common carp at changing production conditions named AMINOCarp™. Taking growth rate and duration of the feeding period as input variables, the model estimates protein and amino acid retention. Once the feeding rate is defined, required concentration of amino acids in the feed can be calculated based on net retention data of amino acids as well as on maintenance requirement estimates and conversion factors. In addition, AMINOCarp™ allows for an adjustment of feeding frequency which strongly affects feed and nutrient utilisation.. Finally, carp in pond systems may have substantial dry matter intake from natural feed such as algae and zooplankton. Depending on the proportion of natural feed optimal amino acid levels in the supplemental feed may need to be adapted and AMINOCarp™ takes this into account . Overall, AMINOCarp™ enables the user to simulate different scenarios and provides an idea as to how dietary amino acids need to be adjusted.



Feeding rate	2.500	% of body weight / day
Dry matter (DM) content of diet	91	%
Feed conversion ratio	1.083	g DM / g gain
Specific growth rate	2.100	% of body weight / day
Average start weight of fish	60.0	g/fish
Average end weight of fish	188.2	g/fish
Feeding period	56	days
Protein content, start	14.4	% in whole body
Protein content, end	14.9	% in whole body

	CP	LYS	MET	CYS	MET+CYS	THR	ARG	ILE	LEU	VAL	HIS	PHE
Total content, % in DM of diet	40.6	2.33	1.29	0.58	1.87	1.46	1.72	1.15	1.66	1.42	0.87	2.43
Ratio to LYS		100	56	25	80	62	74	49	71	61	37	104
Digestible content, % in DM of diet	34.5	1.98	1.10	0.49	1.59	1.24	1.46	0.97	1.41	1.21	0.74	2.07
Ratio to LYS	0	100	56	25	80	62	74	49	71	61	37	104

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