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Evaluation of amino acid availability in feed proteins and mixed feed for monogastrics - possibilities, limitations and outlook

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SUMMARY - The paper deals with a discussion of different definitions and methods for determination of amino acid availability for monogastrics in relation to consequences for diet formulation. Main directions of methodical development are characterized and advantages as well as disadvantages are discussed from a critical perspective for the purpose of an introduction into the practice of diet formulation. One of the most important problems is that based on tabulated data we have only limited possibilities to reliably characterize the amino acid availability of actual feed batches. Endogenous secretions of amino acids and the difficulty for quantitative estimations under different dietary conditions is a further point of significance. From this point of view more standardized methods and a better knowledge about variation factors within different batches of one feed protein, including feed treatments, are prerequisites for further successful developments.

Key words: Monogastrics, protein utilization, amino acids, amino acid availability, methods for estimation.

RESUME - *"Evaluation de la disponibilité en acides aminés dans les protéines alimentaires et les aliments composés pour monogastriques - possibilités, limitations et perspectives". Cet article présente une discussion des différentes définitions et méthodes pour la détermination de la disponibilité en acides aminés pour les monogastriques en relation avec les conséquences pour la formulation des régimes. Les voies principales du développement de méthodes sont caractérisées et les avantages ainsi que les inconvénients sont discutés de façon critique comme introduction dans la pratique de la formulation des régimes. L'un des grands problèmes est le fait qu'en se basant sur les données saisies nous n'ayons que des possibilités limitées de caractériser la disponibilité en acides aminés des apports actuels d'aliments avec suffisamment de fiabilité. Les sécrétions endogènes d'acides aminés et la difficulté d'effectuer des estimations quantitatives sous différentes conditions de régime sont encore un élément important. De ce point de vue, des méthodes plus standardisées et une meilleure connaissance des facteurs de variation d'une protéine alimentaire dans les différents lots, y compris les traitements des aliments, sont des pré-requis pour la réussite des développement ultérieurs.*

Mots-clés : *Monogastriques, utilisation des protéines, disponibilité en acides aminés, méthodes d'estimation.*

Feed protein evaluation and methodical developments dealing with this topic are nearly as old as scientific animal nutrition in general. The first steps in this direction were such classical parameters like "Protein efficiency ratio" (PER), "Biological value" (BV), "Net protein utilization" (NPU) or "Productive protein value" (PPV). These different parameters generally calculate a ratio between performance and intake of feed proteins. The result of such a calculation could be of interest for a complex diet evaluation from viewpoint of protein utilization resp. protein quality determination. But it is well known and an important limitation, that beside protein quality also protein intake has a strong influence on the result of such a calculation. On the other hand there is no possibility for estimation of a mixed feed protein quality by anyway of calculation from complex informations about protein quality of single mixed feed components. Mean time the physiological background for this limitation is well known – only for the limiting amino acid (LAA) exists a significant correlation between intake and performance. In conclusion to this fact nutritionists were more and more looking for single amino acids (AA) mainly under two aspects: (i) quantification of AA in feed proteins; and (ii) evaluation of nutritional value of most limiting AA.

Dealing with the second aspect of protein research work a clear definition should be found to answer the question: "What does it mean to quantify the nutritional value of a single feed AA?"

Following ARC (1981) the nutritional value of feed AA is described as *AA-Availability* (AAA) and defined as:

"The proportion of the total AA that is not combined with compounds which interfere with its digestion, absorption or utilization by the animal, e.g., AA that can be digested, absorbed and utilized by the animal consuming it, for the purpose of maintenance or growth of new tissue".

Mean time it is a classical definition, but in general an actual and precise explanation of the existing scientific problem. Three processes are connected and have to be quantified to describe the physiological value of a feed AA: (i) protein hydrolysis by digestive enzymes; (ii) absorption process for uptake of AA into the circulation; and (iii) postabsorptive utilization of AA in the tissues.

Different terminology and a lot of different methods as well have been developed in the last decades of this century to estimate the level of nutritional efficiency of feed amino acids and sometimes the important connection between declaration of results and methodical background has been lost. Based on such a beginning of confusion Godber (1990) proposed the following hierarchy of terms to define nutrient bioavailability:

- Abundance	The amount of nutrient present in the feed
- Digestibility	The amount of nutrient, which is present, that is broken down by the digestive process into a usable form
- Absorbability	The amount of nutrient, which was broken down to a usable form, that is actually absorbed into the body
- Availability	The amount of nutrient, which was absorbed, that was actually used in a biologically significant function
- Bioavaiability	The unifying term that includes the consideration of all factors that may impact the utilization of nutrients

Actually we have the problem to estimate different parameters of AA-quality on different levels of such a hierarchy. The aim in general is an evaluation of AA-bioavaiability, but methods with different suitability for this purpose were developed up to now. For the evaluation of methodical backgrounds it is essential to get informations about the methods used.

So it should be declared more detailed what we do in the field of methodical developments (Table 1).

Carpenter and Ellinger (1955) started to use the reaction of FDNB with free epsilon-amino group of lysine for evaluation of blockage of lysine. But generally chemical methods have reached only a limited importance because of their limited information about the utilization process *in vivo*. Biological methods *in vitro* have the same limitation. Additional, the growth of test microbes depends on supply of amino acids in agreement with AA-requirement of microbes. Required protein composition for microbes differs markedly from mammals and poultry, furthermore species differ in ability for synthesis of single AA (essentiality). Based on this situation *in vivo* measurements seem to be unavoidable.

Biological methods *in vivo* can be divided in *two main directions*: (i) estimation of the extent of digestion and absorption of dietary AA; and (ii) estimation of the extent of total utilization of dietary AA (including digestion and absorption).

In a first step of experimental development digestion and absorption process for dietary amino acids was quantified based on analyses of faecal protein. But 25 years ago the influence of microbial fermentation on quantity and composition of faecal protein could be stated out. Actually different methods exist (Table 2) for partly compensation of these microbial processes by evaluation of digestion and absorption of dietary AA up to the end of small intestine (ileal resp. praecaecal level).

Different methods (Table 2) have different advantages and disadvantages from physiological and ethical point of view. It is not the place for discussion here, but in general we can only get informations about digestion and absorption. Consequently it has to be postulated that postabsorptive losses of AA are neglectable. This assumption is in discrepancy with reality. Furthermore we have only very limited possibilities to consider endogenous losses in the gastrointestinal tract (Table 3), because: (i)

determination is expensive and methods for determination are still under discussion; (ii) quantitative and also qualitative variation is high and cannot be estimated reliable for feed evaluation; and (iii) variations of the extent of reutilization of AA from endogenous secretions under normal feeding conditions are only inaccurately known.

Table 1. Methodical developments for estimation of AA-bioavailability

A - Chemical methods

Level of lysine blockage
- FDNB-method
- TNBS-method
- Further coloured indicators
- Alternative reagent (Methylacrylate/Aethylvinylsulfon/Methylisourea)

Level of methionine oxidation
- DMSO-method
- Jodoplatinate-Titantrichloride-method
- GC-method for intact Met

B - Biological methods in vitro
- Liberation of AA by enzymes
- Growth of test microbes
- Growth of test microbes after liberation of AA by enzymes

C - Biological methods in vivo
- AA-balance of GIT
- AA-balance of small intestine
- Mobile NBT
- Postabsorptive "AA area under curve"
- Growth test (Slope ratio assay)
- N-balance (N-utilization model)

Table 2. Some experimental techniques for AA-balance of small intestine

- T-cannula
- Reentrant cannula
- Ileorectal shunt (anastomosis)
- Postvalvular T-cannula caecum
- Direct sampling (Ileal dissection technique)
- Caecectomy (poultry)
- Mobile bag technique -combined T- and PVTC-cannula after a gastric (or *in vitro*) predigestion

Remarks:
- Various function of the GIT!
- Use of indicator substances is in some cases essential!
- No perfect indicator substance available!

Table 3. Some factors affecting quantity and/or quality (composition) of endogenous losses

- Protein intake
- Protein AA-balance
- protein structure
- ANFs
- Fibre quantity + quality
- GIT conditions (p.e., motility/passage/viscosity

Remarks:

- Methods for quantification are still under development
- Extent of reutilization?
- Limitations for estimations of endogenous AA for feed evaluation systems

Otherwise endogenous secretions are of quantitative importance in the utilization process (Table 4). Normally 40 ... 60 percent of the total N-intake of pigs is contributed from endogenous secretions.

Table 4. Proportion of endogenous N secretion sources in pigs (Auclair, 1986)

	Total N (g/24 h)	N-intake (%)
Salivary + gastric secretion	2.0 ... 3.3	5 ... 8
Pancreatic secretion	2.5 ... 6.7	4 ... 15.6
Bile secretion	1.8 ... 3.0	4.5 ... 6.5
Small intestinal secretion	14.4	22 ... 26.5
Sloughed cells	1.4 ... 2.0	2.5 ... 3.5
Total endogenous secretion	22.1 ... 29.4	38 ... 60.1

Another methodical direction is, to quantify the result of utilization process by parameters of performance (Growth, N-balance, N-deposition). Slope ratio assays based on short time growth trial and N-balance trials in connection with physiological based N-utilization models are the most important ways for quantification of total utilization in dependence on bioavailability of limiting AA (Table 5).

Table 5. Some experimental techniques for estimation of total utilization of limiting AA

Slope ratio assay

Based on different performance parameters like:
- Body weight gain
- Protein deposition
- Carcass gain
- Feed conversion rate

N-balance trial

Based on physiological description of protein deposition by an exponential function in relation to:

- Age, sex and genotype
- Protein quality of the diet
- Bioavailability of limiting AA

It has to be noticed however that correlations between AA-intake and performance parameters only exist for the limiting amino acid under study.

The advantages of these methods are: (i) the direct connection with the performance; (ii) the possibility for determination of AA-requirements in different terms (p.e., bioavailable AA) in dependence on performance level; and (iii) no manipulations of the animals by surgery methods.

The disadvantage is, that we can get only results for the limiting AA.

In summarizing of experimental results from *in vivo* determination of AA-quality parameters in different feed proteins by different methods some critical observations are obvious (Table 6). Many of these practical questions for feed and mixed feed evaluation actually cannot be answered definitely. This is a limiting factor for a too fast introduction of such systems in mixed feed optimization indeed.

Table 6. Critical questions

- Sometimes similar estimations of Lys-availability by different methods (p.e., ileal digestibility ~ slope ratio method)

Remarks:

- Influence of different feed batches?
- Influence of the genotype of test animals?
- Methodical limitations in general?
- Level of compensation of mistakes?
  - How different are different feed batches?
  - How are we able to control different feed batches?
  - How can we quantify improvements in diet formulation by use of available AA? (comparison of methods)
  - Quantification and consideration of feed treatment effects? (AA damage, endogenous losses)
  - Additivity of single feed data in mixed diets? (endogenous losses)

Only two examples from actual research should demonstrate the problem (Table 7 and 8).

Table 7. Evaluation of Lys-availability for pigs in heat treated peas (van Barneveld *et al.*, 1994)

Method	Raw	110°C	135°C	150°C	165°C
Total Lys (g/16 gN)	7.1	6.7	6.9	5.6	4.0
	Coefficients of Lys-availability				
Reactive Lys†/total Lys	1.0	0.99	0.97	0.89	0.70
Ileal digestibility (T-Cannula)	0.75	0.79	0.74	0.74	0.56
Ileal digestibility (IDT††)	0.76	0.83	0.80	0.74	0.67
Faecal digestibility (partial sampling)	0.81	0.83	0.80	0.74	0.46
Faecal digestibility (total collection)	0.82	0.79	0.75	0.70	0.45
Slope ratio method					
Empty body weight gain	1.01	0.74	0.83	0.59	0.16
Gain: feed intake	0.96	0.71	0.77	0.56	0.18
Daily CP-deposition	1.13	0.91	0.97	0.70	0.38
CP-deposition: feed intake	1.10	0.89	0.93	0.68	0.39

†After Roach *et al.* (1967)
††Ileal dissection technique ("direct sampling")

Table 8. Performance and N-balance of pigs fed different diets after different calculation to meet AA-requirement (Buraczewska and Buraczewki, 1997, selected data)

	Supplementations of Lys, Met, Thr bzw. Trp							
	I		II		III		IV	
	A	B	A	B	A	B	A	B
Barley	340		580		190		300	
Wheat	390				150			
Wheat bran							100	
Rye					350		320	
Soybeanmeal	80							
Rapeseed	150		120		120		80	
Peas			260					
Field beans					150		80	
Lupines							80	
Growth(g/d)	756	780	793	818	905	921	875	879
N-balance (g/d)	19.4[a]	20.7[b]	20.9	22.1	21.0	21.0	19.6	19.9
Urine-N (g/d)	16.0	15.1	15.2	14.4	20.5	20.6	29.0	28.7

a: Calculation based on total AA; b: Calculation based on ileal digestible AA (CVB, 1995)

The results in Table 7 demonstrate many differences in evaluation of Lys-availability between the methods used mainly after different steps of heat treatment. On the other hand a central question is the improvement in quality of diet formulation based on actual AA-availability data. Results in Table 8 give an indication about the effects under conditions of a scientific study. Summarizing these data the improvement in diet formulation is rather low. These actual observations should not be generalized but are a reflection of the sum of complicating factors in the field of estimation of generally accepted AA-availability parameters of single feeds which may have important variations between different feed batches. So it seems to be a main conclusion for further research work to clarify this field of variation factors.

Conclusions

In conclusion the following points are from special importance for the further development of systems for evaluation of amino acid quality in monogastric nutrion:

(i) Selection of correct method(s) for determination of available amino acid content of a feed protein.

(ii) Standardization of selected method(s) as good as possible for comparable results about available amino acid content of feed proteins from different laboratories.

(iii) Combination of methods for physiological based determination of amino acid requirements in terms of available amino acids.

(iv) Combination of methods for a more precise determination of different feed treatment effects on amino acid availability.

(v) Development of *in vitro* techniques for quick measurements in actual feed protein batches.

(vi) Higher level of co-ordination of scientific work, mainly in two directions:

- Standardized methods for determination of AA-availability resp. AA-requirement.
- Harmonization of feed evaluation for tabulated data of AA-availability.

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