

## **STUDIES-BASED ASSESSMENT ON DAMINOC ® (2020)**

### **PREAMBLE**

Although every organism has its own nutrient profile and needs different amounts of nutrients, we all need the same micro- and macro-nutrients: proteins, fats, carbohydrates and vital substances (vitamins, minerals, trace elements, secondary plant substances) (Johansson, 2012). Protein is of great importance here, as it is the largest building block in the human and animal body, along with water. The skeletal muscles account for 60% of the total body protein (Ballard and Thomas, 1983). The protein metabolism in skeletal muscles, which accounts for more than 25% of the total body's protein turnover (Young and Torun, 1981), has long been an important area of research.

The changed division ratio of carbohydrates, proteins and fats is seen as a cause of modern malnutrition. When comparing the current distribution ratio with previous ones, a clear shift in the nutrient proportions can be seen (Neumann and Hottenrott, 2008).

The available data on the nutrition of our ancestors in the Paleolithic (Stone Age) indicate that Stone Age people consumed fewer and high-fiber carbohydrates (wild plants) to secure their energy needs. Compared to today's diet, they consumed twice as much protein (wild animals, fish) and only half as much carbohydrates with a low glycemic index (Cordian, 2003).

However, the genetic potential of humans for the ingestion of animal and vegetable products has not changed.

Today, the early harvest and long transport and storage times of our food increasingly reduce its value as living food. Heating and boiling or even microwave radiation further destroy valuable nutrients. Even the water-based vast amounts of meat from industrial mass animal production no longer provide the protein nutritional value that can be found in species-appropriate, biological small animal husbandry. Today animals are fattened quickly and kept under sedentary circumstances.

We must also gradually ask ourselves what effects such food has on our consciousness. Most people prefer what tastes good thanks to artificial flavors and what is cheap. The price pressure triggered by the will of the consumer induces the production of inferior food among manufacturers and ultimately culminates in increasingly frequent rotten meat scandals. Industrially produced food, rich in auxiliary substances and E-numbers, is usually rich in hidden sugars and fats, but poor in high-quality animal proteins and vital substances.

Our modern, industrial food management may appear practical, but despite advertising statements to the contrary, it delivers largely nutrient-free food. We eat more and more of less and less until we finally eat too much of nothing (Johansson, 2012).

Looking at the constantly changing diet recommendations for body weight loss, it can be observed that increased attention is currently being paid to increased protein intake when making diet proposals. Proteins cause longer saturation, increase thermogenesis and are a substrate for the formation of new sugar (gluconeogenesis) in the liver.

In performance training, an additional intake of special amino acids with suitable galenics makes sense.

The quality of protein uptake is determined by the specific composition of essential amino acids (Neumann and Hottenrott, 2008).

Proteins trigger chemical reactions that are at the origin of life. They produce vital enzymes, form hormones including sex hormones and neurotransmitters. They form an integral part of the immune system by locating and eliminating harmful invaders.

As hemoglobin, they bind the oxygen in the red blood cells and regulate the transport of oxygen. Muscle formation also depends on proteins. Proteins are associated with all life forms, an observation that dates back to Mulder's original identification of protein as a class in 1838.

The proteins in living matter act as organic catalysts (enzymes), as structural features of the cell, as messenger substances (peptide hormones) and as antibodies.

## PROTEIN SYNTHESIS

The importance of protein in food is mainly to act as a source of amino acids, some of which are essential (indispensable) components of food as their carbon frameworks are not synthesized in the human or animal body. Only the non-essential amino acids can be synthesized in the body.

Often the sequence of the synthesis corresponds to the reverse degradation. The synthesis steps of the essential amino acids are too complex, and the enzymes required for them have probably been lost in the course of evolution (AMBOSS Medical Science).

Depending on the source of the protein, a distinction is made between animal protein and vegetable protein. Both types are made up of amino acids. Metabolism is responsible for converting energy in the body from one form to another. Without amino acids, no metabolic reactions would take place. When a protein is digested or enzymatically hydrolyzed during digestion, the protein releases its constituent amino acids, which after absorption in the small intestine can follow one of the following two metabolic pathways:

a) the catabolic pathway by which their amino acids are deaminated, which means that their amino groups (NH<sub>2</sub>) are separated from their carboxyl groups (COOH) which provide energy and produce energy and nitrogen catabolites (secondary function of amino acids)

b) the anabolic way, in which the amino acids act as precursors of body protein synthesis (primary function of amino acids) without providing energy or nitrogen catabolites.

Catabolism (degradation metabolism) releases potential

chemical energy of complex organic molecules for work performance and for heat generation through oxidation. It breaks down carbohydrates into simple sugars, fats into fatty acids and proteins by breaking them down into amino acids to make sure they can be used. In addition, it breaks down used and toxic material in the cells, a certain proportion of which can be used for reconstruction and the rest is excreted. On the other hand, anabolism (building metabolism) mainly synthesizes organic molecules.

Some of the amino acids therefore take the anabolic metabolic pathway, on which they are reassembled into new proteins as required and used for assembly processes. The anabolic metabolic pathway is responsible for everything that has to be assembled inside the body. Ideally, assembly and dismantling are in balance. Even when the body is at rest, the metabolism continues to run continuously, for example to maintain the body temperature or for individual cell functions (Klinke, 2003).

The liver and other tissues of the body are able to assemble resorbed amino acids and di- or tri-peptides into "new" proteins. Amino acids from food are transported from the liver to other tissues via serum albumin or as free amino acids. The whereabouts of the amino acids after absorption can be categorized into three forms. Amino acids are especially used in muscles and the liver for (1) the synthesis of tissue proteins, (2) for the synthesis of enzymes, albumins, hormones and other nitrogenous substances, or (3) they are used as energy sources using the remaining carbon structure.

High protein synthesis rates exist primarily along with the production of red and white blood cells in the epithelial

cells of the skin and in the cells along the gastrointestinal tract, such as in the intestinal mucosa during the production of exocrine secretion products (digestive enzymes, mucus) (Hand et al., 2003 ).

In general, amino acids in the skeletal muscle tissue pool have three options for entering this pool: (a) breakdown of muscle proteins, (b) conversion of other amino acids and intermediates, and (c) uptake from extracellular fluid, where (b) applies to non essential amino acids.

At the same time, amino acids within the pool have three possibilities to leave this pool: (a) release into the extracellular fluid; (b) reconstruction of proteins and (c) metabolism in muscles (Henriksson 1991).

Essentially, proteins consist of a series of amino acids. Each of these amino acids contains at least one nitrogen atom. If proteins are broken down from the muscles, for example, individual amino acids are created. When they are metabolized, nitrogen is produced, which has to be excreted via the kidneys. Firstly, it is converted to urea in the liver. The nitrogen balance is understood to mean the difference between the amount of nitrogen absorbed and excreted. If more proteins are broken down by the body than built up in the balance, especially in the muscles, which is the case, for example, in times of hunger (catabolic metabolism), the nitrogen balance is negative. The body loses nitrogen compounds through the breakdown of proteins and amino acids. If, on the other hand, a person takes in 10 grams of nitrogen, for example, but only releases 5 grams, there is a positive nitrogen balance. This can be the case, for example, during

growth or regeneration times in strength athletes. More nitrogen is taken up than released, and thus more protein is built up than broken down (Hager et al., 1995).

This also reflects whether the body is undergoing a metabolic crisis (e.g. due to long fasting or illness). When such a phase ends, the body normalizes again and, under the right conditions (surviving an illness, returning to a balanced diet), switches back to the anabolic (anabolic) metabolism, in which proteins are built up again.

Protein catabolism is of particular importance to the human body. It cannot create glucose from stored fat (although possible the other way around), and as such always needs glucose for the high-energy breakdown of fat, otherwise the energy balance deteriorates. The brain and red blood cells also depend on glucose.

Hypoglycaemia can even lead to over-acidification of the blood. However, liver cells are able to carry out gluconeogenesis, i.e. to produce glucose. In particular, the protein components, i.e. amino acids, are converted into glucose.

In detail, the liver cells split off the amino (nitrogen) ends of the simple amino acids. The remaining carbon structure then develops intermediates of the citric acid cycle and "feeders" of gluconeogenesis and fatty acid synthesis. The above-mentioned nitrogen is produced in the form of ammonia, which is packed in urea and excreted via the kidneys (Müller-Esterl, 2018).

## **AMINO ACIDS**

Of all the amino acids in our genome, twenty are able to form proteins and are therefore referred to as "proteinogenic". As protein building blocks and in the metabolism of the cell, they are indispensable, as they play a central role in numerous vital functions, the structure and regeneration of the body. A balanced and sufficient supply of these amino acids is essential for maintaining health. Each of the proteinogenic amino acids has a characteristic side chain that varies in size, structure and charge and is therefore responsible for its individual chemical properties.

Eight of those twenty proteinogenic amino acids have so far been considered to be essential for humans: isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine, the amino acids arginine and histidine as semi-essential, i.e. conditionally essential because they are only essential under certain circumstances (Biesalski, 2004).

New research results, however, question whether the classic division into essential and non-essential amino acids is still valid in clinical nutrition therapy, since the enzymes required for the synthesis of the essential amino acids are no longer sufficiently available due to evolution. As the following explanations show, some of the non-essential amino acids must actually be classified as indispensable or conditionally indispensable in certain symptoms and therefore administered exogenously.

Histidine was originally regarded as a non-essential amino acid in adults, as it can be released from the sufficiently available endogenous stores (hemoglobin and carnosine) if it

is insufficiently supplied. However, more recent studies on healthy people show that the human organism cannot synthesize histidine to a sufficient extent, which means that a long-term histidine-free diet leads to deficiency symptoms. A prolonged, deficient diet leads to a drop in histidine concentration in the plasma. Thus, histidine is essential for life and must be supplied in sufficient quantities through food. After the administration of histidine, the level in the plasma rises again to the normal value. Histidine deficiency restricts hemoglobin synthesis and promotes a catabolic metabolism. Today it is also undisputed that histidine is essential both in pediatrics and in chronic kidney failure (Biesalski, Hans-Konrad et al., 2004).

In any case, histidine is essential for infants. The actually non-essential amino acid arginine is also essential in infants and toddlers, but even in adults, stress and illnesses (high blood pressure, vascular diseases) can have a higher requirement than the amount produced by the body (Niels Schulz-Ruhtenberg, 2012).

Fundamental findings led to the new concept of functional amino acids, which are defined as those amino acids that are involved in and regulate important metabolic pathways in order to improve health, survival, growth, development, lactation and reproduction of organisms. Functional amino acids are promising in the prevention and treatment of metabolic diseases (e.g. obesity, diabetes and cardiovascular disorders), intrauterine growth restriction, infertility, intestinal and neurological dysfunction and infectious diseases (including viral infections).

Traditionally, amino acids were classified as nutritionally "essential" or "not essential" based on the growth or nitrogen

balance of mammals (Wu, 2009). Studies show that amino acids whose carbon frameworks are not synthesized de novo by cells must be ingested with food in order to sustain life. They are therefore to be classified as essential (Wu, 2013).

However, the nitrogen balance is not a reliable indicator of an optimal amino acid requirement in the diet (Wu 2013). For example, adult men on an arginine-free diet can maintain a nitrogen balance for 9 days, but both the number and vitality of their sperm are reduced by 90% (Wu et al., 2009).

In addition, a deficiency in arginine in the mother diet impairs the survival and growth of the embryo / fetus in pregnant pigs, although there is no negative nitrogen balance (Wu et al., 2010).

According to the tables (Amino Acids in Animal and Human Nutrition for Mammals, Wu, 2009), arginine and histidine can be classified as essential, functional amino acids for all mammals.

Branched-Chain Amino Acids (BCAA) form a special group of essential amino acids. These include leucine, isoleucine, and valine. Due to their structure, they are quickly transported into the muscle. They enter the blood directly from the intestine and, like other amino acids, do not have to be transported via the liver. Branched-chain amino acids are used more and more frequently in the form of supplements in seriously ill patients because of their advantages mentioned in the human medical literature. Use of BCAA as anti-carcinogenic amino acids seems possible (Danner & Priest, 1983; Blomgren et al., 1986; Saito et al., 2001).

Clinical studies on humans have shown longer survival times, more favorable nitrogen balances and an improved quality of life if the food consumed is supplemented with up to 12 grams of BCAAs per day (Ventrucci et al., 2001; Hiroshige et al.,

2001; Inui, 2002; Gomes-Marcondes et al., 2003).

The supply of essential amino acids is necessary for the long-term maintenance of the nitrogen balance and protein synthesis, as the body cannot produce them itself and they must therefore be supplied from outside or through food. The body can basically produce the ten non-essential amino acids alanine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, proline, serine and tyrosine itself.

The constituent essential and semi-essential amino acids, together with the non-essential amino acids that the body produces itself on the basis of the essential ones with the help of transaminase, form the structural basis of the body and all molecules that support life. This process is known as body protein synthesis. If the cleavage processes are insufficient, the half-digested proteins are pushed on to the large intestine, where they are broken down by bacteria and put a strain on the organism.

## **CAUSES AND CONSEQUENCES OF A LACK IN PROTEIN**

A deficiency in protein or individual amino acids can lead to serious health disorders that can affect almost all areas of diseases.

Vegetarians and vegans in particular are at risk of insufficient coverage. The danger is that they are very often undersupplied with nitrogen, as the maximum protein nutritional value of plants is only 18% (Johansson, 2012).

The protein requirements of growing children and adolescents are at least equal to that of their adult mother (Johansson, 2012).

This means that the protein supply is particularly important in this phase in order to avoid growth damage due to protein deficiency in particular and malnutrition in general. Protein metabolism plays an important role in all growth processes because human nutrient requirements are always controlled by a protein hierarchy. What applies to children also applies to the older generation. Up to now, most diseases that were associated with the aging process were considered to be a natural accompaniment to the aging process. Today we know, however, that most of these diseases are caused by malnutrition and are therefore due to an insufficient supply of nutrients (Johansson, 2012).

The consequences of a protein deficiency are diverse and range from fatigue, difficulty in concentrating, reduced serotonin release, weakening of the immune system, muscle breakdown, physical weakness, poor regeneration phases after illness, metabolic, digestive and detoxification disorders, functional disorders of the organs, iron deficiency, mood swings, hair loss, stronger wrinkling and brittle nails up to unwanted weight loss, osteoporosis, obesity, poor sleep, erectile

dysfunction and menstrual disorders, water retention, niacin deficiency, neurological problems and more severe menopausal symptoms.

The causes of a protein deficiency are too little or insufficient quality of protein intake (not containing all essential amino acids or in a less than optimal ratio), one-sided diet, constant stress, decreasing absorption along with ageing, too little stomach acid, poor digestion, protein utilization disorders in the intestine, strong blood sugar fluctuations and due to a higher need along with sporting activities, illnesses and pregnancy (Hamann, 2018).

## **AMINO ACID PATTERN**

Each species, regardless whether human or animal, has its own specific amino acid pattern, a composition that is only valid for this species. Based on this specific amino acid pattern, the proteins are built, which enable the physical substance and life of this species. To ensure optimal protein synthesis, all essential amino acids must be available in sufficient and precisely defined quantities. If only one of the required amino acids occurs in insufficient quantity, the optimal amino acid ratio is disturbed and protein synthesis is consequently limited.

What is optimal for humans can only approximately fit for any other species. For the qualitative assessment of food proteins, their nutritional-physiological value is determined. The so-called protein quality provides information on the extent to which absorbed protein can replace broken down body protein. The two decisive criteria for this are the respective amino acid pattern and the digestibility of the protein (availability of amino acids). In order to be able to estimate the protein quality as precisely as possible, the digestibility of the individual amino acids in the food must be taken into account.

Due to the different amino acid compositions, the organism is only able to synthesize as much body protein from food proteins as their concentration of the most deficient amino acid allows for. This is called the limiting amino acid and determines the value of a dietary protein. The lack of an essential amino acid leads to an inhibition of the formation of new protein and thus limits the value of the total protein contained (Kirchgeßner et al., 2011).

## **DOGS**

Essential amino acids for dogs are isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophane and valine (CASE et al., 1997). Arginine and histidine can only be synthesized in insufficient quantities and are therefore among the semi-essential amino acids. Essential amino acids cannot be produced by the body itself. They must therefore be made available in the required quantity and in the correct ratio via food (Grandjean, 2006).

In 1979 MILNER published two studies on the essentiality of amino acids using young beagles (Milner, 1979a & Milner, 1979b). Pure L-amino acid diets, which lacked the amino acids histidine, isoleucine, methionine, threonine and tryptophane, were used in the experiments. After some time, the beagles showed not only reduced food consumption but also a significant weight loss, a clearly negative nitrogen balance and a significantly increased urea content in the blood and urine. The removal of arginine, leucine, lysine, phenylalanine or valine from the diet also resulted in a clearly negative nitrogen balance and an increased urea content in the blood and urine. The supply of essential amino acids is therefore necessary for the long-term maintenance of the nitrogen balance and protein synthesis (Meier 2004).

A protein deficiency manifests itself in bad fur, skin infections, infectious diseases, diarrhea, food intolerance, allergies and parasite infestation. Young pets, such as puppies, appear sluggish and show little temperament; in addition, their growth is usually reduced. Older dogs, for example, show a significant decrease in performance, they appear sluggish to apathetic, lactating dogs usually have too little milk.

## **CATS**

The total requirement for essential amino acids for cats is about 18% higher than that for dogs (Burger and Smith, 1987). The amino acid pattern in the feed can show imbalances, which can lead to poorer feed intake and poorer growth. Some amino acids are even toxic at higher doses. The cat reacts very sensitively to a leucine content of more than 1.25% in the dry food substance or to a methionine content of more than 1.5% in the TS (Hargrove et al. 1984; Fau et al., 1987). The amino acids arginine, lysine, histidine, isoleucine, leucine, methionine, phenylalanine, threonine, tryptophane and valine are essential for growing cats. Rogers and Morris (1979) found no change in weight gain in the absence of alanine, asparagine, proline, or tyrosine. But if the content of an essential amino acid in the feed was too low, this resulted in a decrease in feed consumption of 30 - 50%. There was also weight loss and a decrease in the plasma level of the corresponding amino acid (Rogers and Morris, 1979).

## **HORSES**

In the course of studies on the growth performance of warm-blooded stallions in rearing, with special consideration of the protein and amino acid supply, feeding tests were used to determine how the growth of riding horse stallions of the DSP breed during rearing between 11 and 27 months of age, depending on the energy and protein supply. The animals were kept in two groups of six animals each in loose pens with daily exercise. The upgrading of the protein supply in the sense of the ideal protein concept took place in the supplement group via a pelleted supplementary feed in which high-quality protein carriers and crystalline amino acids were used.

The dry matter intake was identical in all rations in both groups. According to the aim of the experiment, the protein and amino acid supply was the only different experimental factor, the energy intake of both groups was balanced. Effects of the optimized supply were recorded and evaluated by comparing the groups in regards to growth, tissue and metabolic parameters over a period of 72 weeks.

The increases in live weight and height at the withers were higher in the supplement group over the entire course of the experiment. When considering shorter periods of time, significant, dosage-dependent effects were detectable in these, but also by means of other growth parameters. The increase in muscle size was significantly increased in the corresponding observation period, the other tissue parameters BIA and X-ray provided results corresponding to the age of the animals. In the parameter of urea nitrogen in the serum, a significantly lower value was determined in the mean of the animals of the supplement group at one point in time.

At other times, and also with the indicator substance 1-methyl-histidine, no differences between the groups were detectable. A higher absorption of the essential amino acids from the supplementary feed in the supplement group was demonstrated by analyzing the free amino acids in the blood in several test sections. The improved protein quality in the dosage of the supplement group had a positive effect on the growth of the animals. The use of high-quality protein carriers and crystalline amino acids according to the ideal protein concept could be recommended in supplementary feed for the rearing of horses and for periods of increased performance requirements (Koslowski, 2014).

All tissue proteins of an animal are subject to permanent build-up and breakdown processes with specific half-lives in the respective tissue. This means that protein synthesis and proteolysis constantly work in parallel and ensure constant renewal of the proteins in the organism. When amino acids are broken down, a limited part of them can be used for further

syntheses, but are largely lost through trans- or desamination for new syntheses. These losses arise regardless of the performance of the animals and must also be covered by constant food consumption in the conservation status or in the need for conservation.

Another basic requirement for amino acids is for regulatory functions (Eder, 2010).

The so-called ideal protein concept for monogastric livestock was developed to ensure efficient use of the feed protein and to achieve an optimal composition of the feed protein. This means that protein synthesis is most efficient when the ideal combination of essential amino acids for the respective performance fulfillment is available through the feed (TUITOEK et al., 1997; BOISEN et al., 2000). If the protein quality is improved in the sense of the ideal protein concept via the essential amino acids, constant or increased performance in the animal can be expected even with a reduced absolute protein intake (WANG and FULLER, 1989; STANIAR, 1998; STANIAR et al., 2001).

The amino acid lysine is the key amino acid in the horse's diet (OTT et al., 1981). The derivation of the ideal protein, the creation of relationships between the lysine and the other indispensable amino acids, was carried out via amino acid analyzes in the whole body composition of animals or the examination of partial tissues or products. The approach was based on the assumption that the need for protein approach in growth arises mainly from an increase in muscle mass. The essential amino acids required must therefore be available in appropriate proportions, synchronously with the synthesis, from the suckling period of the foal (GRAHAM-THIERS and KRONFELD, 2005).

A deficiency in amino acids can be counteracted in a targeted manner by changing protein carriers in the feed or by direct crystalline supplementation. Lysine deficits and deficits in other essential amino acids prevent the use of other high-quality protein components and amino acids (BREUER, 1971; WESSELING, 2003).

As described, lysine is regarded as the first limiting amino acid in horses (HINTZ, 1994; BREGA, 2005).

Threonine was identified by the authors as the second limiting amino acid in horses (GRAHAM et al., 1994; BREGA, 2005).

In feeding experiments with horses in which the addition of lysine or lysine and threonine were examined, the increases rose and the feed conversion improved. The levels of urea nitrogen in the blood were negatively correlated with an optimization of the lysine and threonine supply. As a result, in the experiments mentioned, a higher efficiency of feeding and a relief of the metabolism by using the ideal protein concept were achieved. This relationship, proven by indicators of protein metabolism, ammonia and urea discharge, was investigated and proven in several experiments by the working group (GRAHAM et al., 1994; GRAHAM-THIERS and KRONFELD, 2005). GRAHAM-THIERS et al. (2010) concluded after their extensive series of experiments regarding the amino acid supply of horses that one amino acid supply from the feed offered in practice was often not sufficient to satisfy the possible, very high growth rates of young horses of modern genetics. Furthermore, recommendations for digestible protein and digestible amino acids were difficult to meet, even according to the ideal protein concept. Muscle hypertrophy, as it occurred in adult animals through training, had to result in a higher or changed requirement for amino acids and possibly even a changed ratio of amino acids.

## **AMINO ACID REQUIREMENTS**

For humans, WHO and FAO have defined an amino acid requirement pattern (mg / g crude protein) by which the amino acid content (mg / g) of a dietary protein can be compared.

The Amino Acid Score (AAS) calculated in this way is then multiplied by the true protein digestibility ( $WV = N \text{ uptake} - [N \text{ faeces} - N \text{ faeces endogenous}] / N \text{ uptake}$ ). This should actually be determined experimentally, but is mostly taken from tables.

According to a publication by the German Nutrition Society from October 2017, the "estimated values" for an appropriate intake of essential amino acids for infants aged 0 to under 4 months are based on the amino acid content in human milk. The values published by the World Health Organization (WHO) 2007 (1) for the requirement for essential amino acids are "adopted" for infants from 6 months of age as well as for children, adolescents and adults. For adults aged 19 and over, these values are "derived" from data from nitrogen balances. Since the protein requirements for adults aged 65 and over cannot be determined with the desired accuracy from the studies available to date, an "estimated value" is given for this age group. According to the WHO, the accuracy of the values is generally not satisfactory. For this reason and because amino acids are usually supplied in the form of proteins, the independent derivation of recommended intake values for the individual essential amino acids is dispensed with. Since 2007, the WHO has therefore not issued any recommendations for an adequate intake of essential amino acids.

For dogs alike these values are often taken from the literature, as the exact determination of the actual protein digestibility seems extremely complex.

(N Faeces endogenous = nitrogen excretion with the faeces with protein-free feeding.)

This is how the "Protein Digestibility Corrected Amino Acid Score" (PDCAAS) is obtained. With this method, protein evaluation is relatively simple to carry out. However, it does not coincide with the methods carried out by the "biological value" (BV) of food proteins. The BV can be determined from the net protein utilization (NPU = Net Protein Utilization) and the digestibility of food proteins:  $BV = \frac{N \text{ uptake} - (N \text{ faeces} - N \text{ faeces endogenous}) - (N \text{ urine} - N \text{ urine endogenous})}{\text{uptake of digestible nitrogen (N)}}$ . NPU and BV both provide information about the proportion of the protein (nitrogen) taken in with food that is used to build body proteins.

While the NPU refers to the total intake of protein (nitrogen uptake), this applies exclusively to the digestible portion (amount of nitrogen absorbed) for the biological value. However, the PDCAAS method has the advantage that, in addition to the protein assessment, it is also possible to determine the currently limiting amino acid (s) and thus information about the supplementary value of a dietary protein can be given. This is particularly important for protein combinations. Proteins of animal origin, such as those found in fish, meat, eggs, milk etc., contribute to the supply of all essential amino acids and are therefore referred to as "complete proteins". Vegetable proteins, on the other hand, which are deficient in one or more essential amino acids, are accordingly called "incomplete proteins" (Gaßmann, 2006).

Since our body cells are constantly renewing themselves, they need a regular supply of proteins. The decisive factor is not just the quantity, but the quality.

Unfortunately, the quality of the protein requirement is very often underestimated, because it is crucial that the essential amino acids are all available in the body at the same time, i.e. synchronously with the synthesis, in the proportion corresponding to the respective species. According to Block and Mitchell, all amino acids generally follow the catabolic (degrading) metabolic pathway, which inevitably leads to an increase in urea levels if not all amino acids are administered at the same time.

The biological value of a dietary protein is therefore directly dependent on its amino acid components. If not all essential amino acids are available at the time of protein synthesis, the intracellular deficit limits the body's protein synthesis, even if only one amino acid is not available in sufficient quantity (Block and Mitchell, 1946). This is called the limiting amino acid because it stops protein build-up. The limiting amino acid sets the limit for the amount of essential amino acids that can be used for protein synthesis. If only 60% of one amino acid is present in relation to the others, the utilization of all amino acids is limited to 60% (Liebig's minimum principle). There are currently many and varied sources of protein used. However, the prior art does not provide any information about the concept of providing an optimal protein supply, the aim of which is essentially a complete absorption and utilization of the amino acids contained therein which are administered to an individual.

Up to now, it has been customary to determine the suitable composition of food supplements for the human and veterinary field on the basis of experimental values and to adapt them from time to time. Analogous recommendations are usually issued by the World Health Organization (WHO) for humans or the National Research Center (NRC) for dogs, cats and horses or in accordance with the guidelines for complete and complementary feed for cats and dogs (FEDIAF).

Corresponding values with regard to the pure, essential amino acid requirement can be found in the following table (1):

Table 1

	Human		Dog		Cat		Horse	
	WHO (2007)		FEDIAF (2019)		FEDIAF (2019)		Meyer & Coenen (2014)	
	<i>daily requirement</i>		<i>daily requirement</i>		<i>daily requirement</i>		<i>daily requirement</i>	
	<i>g/kg BW</i>	<i>70kg BW</i>	<i>g/kg BW<sup>.75</sup></i>	<i>20kg BW</i>	<i>g/kg BW<sup>.67</sup></i>	<i>4kg BW</i>	<i>g/kg BW<sup>.75</sup></i>	<i>500kg BW</i>
<b>Isoleucine</b>	0.02	1.40	0.13	1.23	0.12	0.30	0.22	23.55
<b>Leucine</b>	0.04	2.73	0.23	2.18	0.29	0.73	0.44	46.23
<b>Lysine</b>	0.03	2.10	0.12	1.13	0.09	0.23	0.27	28.78
<b>Methionine</b>	0.01	0.70	0.11	1.04	0.04	0.10	0.18	19.19
<b>Phenylalanine</b>	0.03	1.75	0.15	1.42	0.12	0.30	0.29	30.53
<b>Threonine</b>	0.02	1.05	0.14	1.32	0.15	0.38	0.45	47.10
<b>Tryptophane</b>	0.00	0.28	0.05	0.47	0.04	0.10	0.07	6.98
<b>Valine</b>	0.03	1.82	0.16	1.51	0.15	0.38	0.30	31.40
<b>Arginine</b>	n.a.	n.a.	0.14	1.32	0.25	0.63	0.51	54.08
<b>Histidine</b>	0.01	0.70	0.06	0.57	0.08	0.20	0.11	11.34
<b>Total</b>	0.18	12.53	1.29	12.20	1.33	3.37	2.83	299.16

Conventional, well-known food supplements for humans and animals (dogs, cats and horses) are therefore mostly compiled on the basis of these recommendations.

According to the recommendation, the need for protein in adults is between 0.8 g and 0.93 g per kg of body weight, depending on their constitution and living conditions, in infants even between 0.9 g and 2.7 g per kg of body weight,

whereby the daily amount should be distributed over meals in order to keep the protein level as constant as possible.

Studies show that a higher intake of protein (amino acids) promotes protein synthesis in muscles (D. Paddon-Jones, 2004) (G. Biolo et al., 1997) (T.B. Symons, 2007).

A protein intake of less than 1 g / kg body mass led to a negative nitrogen balance in competitive athletes (Friedman & Lemon, 1989).

Researchers found that 0.8 g / kg body weight is not enough to maintain muscle mass in older people (RDA, Resommended Daily Allowance) (W.W Campbell et al., 2001).

According to their recommendation, the elderly should ingest up to 1.2 g / kg body weight. Other studies came to the same conclusion. In the event of illness or chronic stress, the value can be higher. A 2007 study looked at the consequences of administering less protein - only 0.5 g / kg body weight. The result was that muscle formation and energy levels decreased significantly (A.E. Thalacker-Mercer et al., 2007). The main focus here is on how much endogenous protein can actually be formed from the respective dietary protein.

## **PROTEIN QUALITY**

In 1909, Karl Thomas developed a method for evaluating the protein quality in food. Accordingly, the biological value of the proteins is a way to measure the efficiency by which these food proteins can be converted into the body's own proteins, and thus one of the options for determining the value of proteins.

The more similar the dietary proteins are to the body proteins in terms of their amino acid composition, the less effort the body has to put into their conversion. The content of essential amino acids is of particular importance.

The protein quality therefore corresponds to the ability of the amino acids contained in food to meet the requirement for essential amino acids to a sufficient extent.

The need for amino acids varies for specific age groups and according to the physiological needs (Moughan, 2012). In a suitable formulation, the essential amino acids are absorbed after 23 minutes.

The digestibility of a food is primarily measured by the time the organism needs to completely absorb it. High-quality and easily digestible protein can be found in muscle meat, fish, dairy products and offal, for example. These are considered to be animal proteins. Soy also contains large amounts of protein, but this is vegetable protein, which is of poor quality (Table 2). The digestibility of vegetable protein is generally lower than that of animal protein, although the protein quality can be improved by supplementing essential amino acids (Neirinck et al., 1991).

Table 2

Values in Gram per 100 Gram		Pork Meat	Chicken Meat	Whole Egg	Soy Beans	Peas
1.	Isoleucine	1.17	0.90	0.79	0.54	0.30
2.	Leucine	1.62	1.36	1.08	0.85	0.43
3.	Lysine	1.86	1.66	0.74	0.71	0.42
4.	Methionine	0.58	0.49	0.39	0.12	0.07
5.	Phenylalanine	0.83	0.78	0.75	0.55	0.28
6.	Threonine	0.92	0.86	0.66	0.46	0.27
7.	Tryptophane	0.26	0.22	0.18	0.14	0.06
8.	Valine	1.07	0.99	1.05	0.54	0.31
9.	Arginine	1.27	1.15	0.83	0.95	0.66
10.	Histidine	0.56	0.66	0.26	0.31	0.15
<b>Total</b>		<b>10.14</b>	<b>9.06</b>	<b>6.71</b>	<b>5.17</b>	<b>2.95</b>

Source: <https://www.naehrwertrechner.de/naehrwerttabelle/>

Protein digestion begins in the stomach with denaturation and hydrolytic cleavage by pepsin. In the duodenum, further peptidases from the pancreas split the proteins and polypeptides into oligopeptides. The further digestion is carried out by amino and oligopeptidases in the brush border membrane of the enterocytes. The cleavage products are taken up in the enterocytes and the remaining di- and tripeptides are further broken down into individual amino acids by di- and tripeptidases. The amino acids get from the mucosal cells into the blood and via the portal vein to the liver, where a large part of the proteins required by the body is synthesized (Thieme 2013).

Studies on protein synthesis in the human body have shown that synthesis and release into the portal blood begins after 10 minutes after the amino acid composition has been entered into the intestine (Kauffmann 2013).

In contrast to carbohydrates and fats, there is no direct storage facility for proteins. Only 100 to 150 g of the freely available amino acids can be stored in the "amino acid pool". The amino acid pool describes the total amount of all freely available amino acids in the organism (De Marées 2003).

In order to prevent the body's own protein from breaking down, all amino acids that the body needs must therefore be constantly ingested with food. If more proteins are consumed than required, the amino acids released during digestion end up in the amino acid pool. If this is already full, the body uses the excess amino acids for energy supply. Most of the nitrogen bound in the amino acids is excreted in the urine.

#### **MEDIUM CHAIN TRIGLYCERIDES (MCT)**

MCT (MCT = medium chain triglycerides or also medium-chain triglycerides) are fats with fatty acids of medium chain length (6-12 carbon atoms). These saturated fatty acids are found in coconut oil, palm oil and, in much smaller quantities, in butter. Industrially produced since the mid-1950s and mainly used in the fields of cosmetics and pharmaceuticals, MCTs are gaining more and more attention in medical and nutritional research and practical application. Compared to the usual dietary fats with long-chain fatty acids (LCT = long chain triglycerides, > 12 carbon atoms), they are split faster, absorbed and transported directly to the liver via the portal vein. MCT are broken down more quickly in the intestine than LCT and also absorbed by the intestinal epithelial cells without being split. The medium-chain fatty acids are bound to albumin and transported directly to the liver via the blood of the portal vein. Long-chain fatty

acids, on the other hand, are inserted into chylomicrons and reach the blood via the lymphatic system. Medium-chain fatty acids are preferentially oxidized in the liver. In contrast to long-chain fatty acids, they are absorbed into the mitochondria independently of fatty acid-binding proteins, where they are quickly broken down. The resulting acetate serves in the form of acetyl-CoA as a building block for the new synthesis of i.e. ketone bodies, fatty acids, cholesterol or for energy production by means of  $\beta$ -oxidation (Karlson, 1984).

The results of further studies show that MCT, in addition to the lower energy content, induce higher postprandial thermogenesis and thus greater energy consumption than LCT, as Nagao and Yanagita (2010) summarize in their review.

Because of these differences between MCT and LCT, it has been postulated that the replacement of LCT by MCT could be beneficial for weight control or prevention of obesity. Our body cells are dependent on energy. Usually they get this in the form of glucose, but they can also use ketones as an energy source. Ketones are oxygen-containing carbonyl groups found in MCT.

The term "medium-chain triglycerides" used here refers to triglycerides with an almost exclusive content of octanoic acid (caprylic acid; C8: 0) according to the systematic and trivial name in chemical nomenclature. The preferred source of these medium-chain triglycerides is coconut fat. Caprylic acid (C8) has fewer carbons (8 carbons) than capric acid (C10) and lauric acid (C12). This is why it has better ability to produce ketones compared to longer carbon MCTs. Studies have shown that the intake of caprylic acid (C8) increases ketone production (McGarry, J. D., & Foster, D. W., 1971) (Miles, J. M., Haymond, M. W., Nissen, S. L., & Gerich,

J. E., 1983). A slight increase in plasma caprylic acid levels of 1 mmol increases ketone production five-fold. This is important for ketone diets as ketones are the primary energy source here. Caprylic acid (C8) is converted to ketones regardless of whether you are in ketosis, on a ketogenic diet or not.

In addition, other studies show that caprylic acid has strong antibacterial, antiviral and antifungal activities (Nair, MKM, Joy, J., Vasudevan, P., Hinckley, L., Hoagland, TA, & Venkitanarayanan, KS, 2005), and that blood lipid profiles are being improved. Recent research confirms how much more effective caprylic acid (C8) is over capric acid (C10) in its ability to increase ketones. C8 increases ketones by about three times that of C10 (Vandenberghe, C., St-Pierre, V., Pierotti, T., Fortier, M., Castellano, C. and Cunnane, S. C. Vandenberghe, Camille et al., 2019).

Caprylic acid (C8) can supplement meals with a higher content of carbohydrates and / or protein to reduce blood sugar spikes. This happens because after taking caprylic acid, insulin levels rise due to an increase in ketone production. This increases the release of glucose.

Research has shown that MCTs provide a clean, fast-acting source of energy for the body and brain. MCT fats are reabsorbed into the circulation very quickly (similar to glucose) and they need half less oxygen to burn than other fatty acids.

They are characterized by a combustion energy that is twice as high (8.3 kcal / h) than it is the case with carbohydrates or proteins (4 kcal / g). MCT are completely metabolized by the liver with the release of energy, without the predisposition to be stored in the body in the form of adipose tissue.

Due to their shorter fatty acid chain length, MCTs are relatively soluble in an aqueous environment and can therefore be metabolized without bile acids. Their structure does not need to be split by pancreatic lipase (enzyme of the pancreas). They are transported directly in the blood to the liver, bypassing the lymphatic system, where they are oxidized more favorably than conventional fats and more ketone bodies are formed (Tosiaki, Aoyama et al, 2007).

It is important to supply the body with sufficient energy through fats and carbohydrates, otherwise the protein is not used to build up the cells, but as an energy supplier and is therefore lacking in structure.

Medium-chain fatty acids (MCT) can be transported in the blood solely by coupling to the protein albumin. They are thus quickly available as energy suppliers or as starting material for the triglyceride synthesis in the liver and thus prevent amino acids from being used to meet the energy requirement (DGFF, Lipid-Liga e.V. 1999).

Because MCT fats have a higher ketogenicity than conventional fats, they are also used as part of the ketogenic diet as a partial replacement for LCT fats (P. R. Huttenlocher, A. J. Wilbourn, J. M. Signore, 1971).

Our bodies have low levels of glucose, and if we don't eat for more than a day, there is a "plan B" that prevents us from instantly dying. The brain and most other organs can use certain other sources of energy when glucose is not available. Without this ability, humanity would have long since died. During starvation, we use our fat stores and release fatty acids, some of which are converted into "ketone bodies". They can cross the blood-brain barrier and provide our cells with alternative energy. In order to "produce" these ketone bodies,

we don't necessarily have to fast or starve. Another option is to follow a consistent ketogenic diet.

Another easier option is to eat foods that contain medium-chain fatty acids.

The latter are easily absorbed by the intestines during the digestive process and largely converted into ketone bodies in the liver.

According to a study that was carried out in Florida with 184 people with an average age of 72.5 years who took medium-chain saturated fatty acids in the form of coconut oil and / or MCT oil for therapy, significant improvements such as better speaking skills and easier social behavior were achieved better memory, a resumption of stopped activities and an overall improvement in symptoms.

## **RECOMMENDED INTAKES**

The range of nutritional supplements is extensive and unmanageable.

It ranges from the classic nutrients such as vitamins and minerals to various fatty acids, amino acids, secondary plant substances and pharmacologically effective plant extracts [Hahn A., 2000].

The expansion of the respective substance spectrum is based in many cases on in-vitro studies, rarely on intervention studies (Grossklaus R., 2000) or analytical analysis of the body's own amino acid profile of the respective species. The common, customary intake recommendations with regard to amino acid compositions for dogs, cats and horses are summarized in the following table (3):

Table 3

Quantitative proportions of essential and semi-essential Amino Acids						
	Dog		Cat		Horse	
Isoleucine	10.08 %	0.13 g	9.02 %	0.12 g	7.87 %	0.22 g
Leucine	17.83 %	0.23 g	21.80 %	0.29 g	15.45 %	0.44 g
Lysine	9.30 %	0.12 g	6.77 %	0.09 g	9.62 %	0.27 g
Methionine	8.53 %	0.11 g	3.01 %	0.04 g	6.41 %	0.18 g
Phenylalanine	11.63 %	0.15 g	9.02 %	0.12 g	10.20 %	0.29 g
Threonine	10.85 %	0.14 g	11.28 %	0.15 g	15.74 %	0.45 g
Tryptophane	3.88 %	0.05 g	3.01 %	0.04 g	2.33 %	0.07 g
Valine	12.40 %	0.16 g	11.28 %	0.15 g	10.50 %	0.30 g
Arginine	10.05 %	0.14 g	18.80 %	0.25 g	18.08 %	0.51 g
Histidine	5.03 %	0.06 g	6.02 %	0.08 g	3.79 %	0.11 g
Total	100 %	1.29 g	100 %	1.33 g	100 %	2.83 g

Source: Meyer & Coenen (2008), FEDIAF (2019)

Note: In the absence of WHO information on the recommended intake of the semi-essential amino acid arginine for humans, humans were not included in Table 3

The protein content in animal feed, given in %, is mandatory. However, its informative value is limited, because not all proteins are created equal. Here, too, there are big differences in terms of digestibility and biological value. If the origin of the animal protein source is not declared (e.g. beef, chicken, turkey, etc.), it simply means that the manufacturer leaves a lot of leeway here. According to a 2015 study of British dog and cat foods with "meat and animal derivatives" in the list of ingredients, they looked for DNA from, among others, beef, pork and chicken in these feeds and came to the following findings: A mixture of pig, chicken and bovine DNA was found in 14 of 17 products tested. In all of these foods, only some of the animals used were specified in the list of ingredients, if at all.

For foods with prominently placed flavors on the packaging ("with beef", "with chicken"), this protein source often only made up a small fraction of all the proteins contained and was

ultimately just a misleading advertising gag (Maine, IR, Atterbury, R . & Chang, K., 2015).

Feed declarations, advertising and packaging are often designed to be intransparent and misleading. No wonder that there is no trust in the good intention of the feed company to only want to deliver the best for the dog. It is warned again and again that feathers, horn, skin, hair, beak or hooves end up in the feed in order to increase the analytical protein content of a feed. The amino acids in these keratin-containing components can only be used by the dog in a highly processed form and are therefore fairly inferior proteins. According to a publication by Stiftung Warentest on July 20, 2020, many products for dogs and cats were rated as "poor". Accordingly, many of the products contain inexpensive fillers that can be harmful to pets. The filler most commonly found in dog and cat foods is grain. Some types of feed contain up to 90% grain. This can lead to obesity, diabetes, inflammation of the digestive tract, and behavioral disorders. The proportion of dogs who get problems with gluten in the long term is relatively high.

Imbalances in serum amino acids, nitrogen levels and urea levels, which are frequently observed, are largely based on a nutritionally poorly balanced amino acid composition.

## **PRESENTATION**

The possible side effects associated with the respective current composition and dosage type, such as allergies and intolerances, are well known and not only reserved for human use, but increasingly also an appearance in the veterinary field.

The current state of the art with regard to the supply of protein and / or amino acid products consists predominantly of the following dosage forms

Tablets, which as a rule require additional auxiliaries (tableting aids) in addition to the actual active ingredient. The disadvantages of a dosage form in tablet form can be listed as follows:

-If the tablets are taken incorrectly, without sufficient amount of liquid, the tablets will not reach the stomach, but will "stick" in the esophagus for a while.

-The compliance when taking particularly large tablets is reduced as some people have problems swallowing large or multiple tablets up to several times a day.

-In some people, tablets stick to the throat area while swallowing, which causes uncomfortable foreign body sensations.

Capsules, consisting of a capsule shell and a filling, the shell mostly consisting of gelatin, occasionally cellulose or carrageenan. The disadvantages of a dosage form in capsule form can be listed as follows:

-The main component of the polypeptide gelatin is denatured or hydrolyzed collagen, which is predominantly made from slaughterhouse waste such as the skin and bones of cattle and pigs is obtained and is therefore unsuitable for vegans and vegetarians.

-Capsules are best taken with plenty of liquid standing up to prevent the gelatine from sticking to the esophagus.

-Compliance when taking particularly large capsules is reduced as some people have problems swallowing large or multiple capsules up to several times a day.

Powders, that consist of animal and / or vegetable proteins or proteins.

-Depending on the protein source used, food intolerances or organic pollution may occur.

-Protein powder also contains carbohydrates, fats and additives and often added flavors and sugar substitutes that dilute the nutritional value of the product. The protein and amino acid content of a protein powder can be very different. While some consist almost entirely of proteins, others only contain them in smaller amounts.

## **WHY WAS DAMINOC® DEVELOPPED?**

The task of DAMINOC® is to provide medium-chain triglyceride and amino acid mixtures with an optimized amino acid composition in the form of a tissue-specific blueprint of the essential and semi-essential amino acid profile of the respective species, which can be used for admixture and production of individual foods and nutritional supplements in the embodiment corresponding to the respective species (humans, dogs, cats and / or horses).

It is no novelty that classy food and nutritional supplements for humans and animals (dogs, cats and / or horses) are incomplete or inadequate with regard to their amino acid profile. Furthermore, according to the WHO, the accuracy of the values for an adequate intake of essential amino acids is generally not satisfactory. The recommendations are largely based on estimates and tables that are adjusted from time to time. This applies to both the human (humans) and the veterinary (dog, cat and / or horse) area.

When it comes to food products in particular, many consumers do not spare any costs, and even pet owners want only the best for their beloved animals. A deficiency in protein or individual amino acids due to malnutrition can, as already shown, lead to serious health disorders that can affect almost all areas of disease.

There is a risk of insufficient coverage not only of vegetarians and vegans, but of all species in all age groups, especially the elderly. Their share is increasing, especially in the industrialized countries, and their deficits in the course of the aging process consist more in a nutritional deficiency or inadequate nutrition than in natural causes.

The biological value of a protein is higher, the more the protein in the diet or in the feed corresponds to the protein of the respective species to which it is administered, since the synthesis is simplified.

Conversely, dog meat would have the highest biological value for dogs, human meat for humans, cat meat for cats and horse meat for horses, which is not feasible in practice from an ethical aspect.

The underlying task was therefore to develop a formula in accordance with the species-specific profile of essential and semi-essential amino acids for the purpose of optimizing the supply of amino acids to humans and animals (dog, cat and / or horse), i.e. which contains a specially tailored composition of essential and semi-essential amino acids to which medium-chain triglycerides have been added as a nutritionally valuable carrier component.

DAMINOC® guarantees that the contained amino acid mixture is exclusively available for protein synthesis and the medium-chain triglycerides solely for energy release. It is provided by means of a suspension.

DAMINOC® contains fermented, free, crystalline and hypoallergenic L-amino acids that are readily bioavailable to the body. The stomach and intestines are not stressed. Since the amino acids do not require to be broken down in the intestine, absorption into the bloodstream begins after 10 minutes without any strain on the stomach and intestines, whereas the digestion time for food protein, which firstly has to be broken down into amino acids, is 3-6 hours. The more similar the dietary proteins are to the body proteins in terms of their amino acid composition, the less effort the body has to put into their implementation. This is especially

true for the content of essential amino acids. DAMINOC® guarantees that all essential and semi-essential amino acids are available to the body in precise proportions at the time of protein synthesis.

The free form of the amino acids facilitates absorption and also supports the formation of non-essential amino acids.

In addition to the hassle-free synthesis, which is due to the fact that the composition of essential and semi-essential amino acids provided corresponds to the body's own composition of essential and semi-essential amino acids, the advantage over conventional products is that food allergies, intolerances, lactose intolerances and excess - or undersupply, which are mostly based on the use of insufficient or unsuitable protein sources, can be avoided.

Even with a meat-free or low-protein diet, DAMINOC® guarantees an optimal supply of amino acids and energy with a low caloric value.

The advantages of a presentation in the form of a suspension over tablets and capsules are that it is easier to take and that it is absorbed more quickly, especially in older patients or children. While older people often have difficulty swallowing, children are often unable to swallow larger volumes, which makes it difficult to take solid medicinal forms such as capsules or tablets.

DAMINOC® can be mixed with food and drinks as well as animal feed. Due to its consistency and dosage form, it does not have to dissolve first, and absorption therefore occurs very quickly.

The determination of the amino acid profile of the respective species was carried out on the basis of examinations (autopsies) of muscle tissue from humans, dogs, cats and horses by means of amino acid analysis.

The composition of amino acids in the target tissues thus served as the basis for deciphering the amount and relationships of essential and semi-essential amino acids.

It was surprisingly found here that the profile of essential and semi-essential amino acids only differs from one another with regard to the relative composition with regard to the respective species (human, dog, cat and / or horse), but not within the respective species.

This means that the dog's muscle tissue always has the same profile of essential and semi-essential amino acids, regardless of breed. The same applies to cats, horses and humans.

The test results can be found in the following table (4):

Table 4

Profile of essential and semi-essential Amino Acids in Muscle Tissue										
Ratio in %	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Threonine	Tryptophane	Valine	Arginine	Histidine
Human	12.40	14.67	19.83	4.55	9.50	7.64	2.27	11.78	12.60	4.75
Dog	9.31	16.56	18.08	6.14	8.70	9.34	2.58	10.04	13.81	5.45
Cat	9.57	16.04	17.97	6.09	8.21	8.99	2.61	10.14	13.14	7.25
Horse	9.40	15.94	18.76	4.44	8.62	9.51	1.41	10.19	13.01	8.73

Source: Amino Acid Composition of Human Tissues (Bocobo, Skellenger, Shaw, Steele)

AGES Institute for Animal Nutrition and Feed

Similarly, DAMINOC® is species-specifically made available in the following versions:

- A-MINO® (for Humans)
- A-DOG® (for Dogs)
- A-KAT® (for Cats)
- A-HORSE® (for Horses)

The dosage of DAMINOC® is significantly simplified because, depending on the body weight and the activity level of the respective species, only the quantitative intake or feeding recommendation in the form of the number of daily, individual portions changes, but not the type of composition.

The composition of DAMINOC® is based exclusively on biologically natural ingredients, e.g. free, crystalline and hypoallergenic L-amino acids, obtained from natural raw materials derived from a bacterial fermentation process, and medium-chain triglycerides with a minimum content of 99%, based on their content of octanoic acid (Caprylic acid; C8: 0) that act as energy and carrier components, and as such serve as unique carrier components not only from a technological, but also from a nutritional and medical point of view. Caprylic acid (C8) extracted from coconut oil is converted to ketones regardless of whether you are in ketosis, on a ketogenic diet or not.

The combination of essential and semi-essential amino acids with medium-chain triglycerides is useful and novel, as medium-chain triglycerides can be used directly to release energy, and the essential and semi-essential amino acids supplied are therefore primarily available for protein synthesis rather than for energy supply.

A slight increase in plasma caprylic acid levels of 1 mmol increases ketone production five-fold. Last but not least, this is important for ketogenic diets, as ketones are the primary energy source here.

Due to its content of medium-chain triglycerides, DAMINOC® is not only suitable for ketogenic applications, but also for vegetarians, vegans, flexitarians, pescetarians and fruitarians due to the compositions based exclusively on plant and natural components for optimized protein supply and protein synthesis.

DAMINOC® is used as a dietary protein substitute or for "upgrading" a regular meal with regard to the content of essential and semi-essential amino acids as an admixture to or component of a food or as an additional supplement for all species (humans, dogs, cats and / or horses). Without mandatorily changing your eating or feeding habits DAMINOC® is a safe and unique way to optimize the body's protein synthesis and thereby improve anthropometric properties and physical and physiological performance.

DAMINOC® is particularly suitable for food and feed intolerances, lactose intolerances, allergies or for use in a reduced-calorie diet to supplement the amino acid requirement while avoiding stress on organs and / or cell metabolism.

In the course of an investigation of complete feed for dogs, six different products from different manufacturers from the high-price segment were analyzed with regard to their content and profile of essential and semi-essential amino acids, calculated on a feed amount of 100 g. The results were compared with the values stated in Table 4, those found in muscle tissue of dogs (p. 42) and likewise the A-DOG formula

with regard to their composition of essential and semi-essential amino acids (referred to as A-DOG in Table 5).

Table 5

	A-DOG	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
<b>Isoleucine</b>	9.31	0.84	0.89	0.58	0.28	0.49	0.30
<b>Leucine</b>	16.56	1.92	1.57	1.15	0.47	0.90	0.68
<b>Lysine</b>	18.08	1.58	1.46	0.72	0.50	0.86	0.57
<b>Methionine</b>	6.14	0.44	0.51	0.41	0.19	0.24	0.17
<b>Phenylalanine</b>	8.70	1.22	0.91	0.71	0.27	0.51	0.38
<b>Threonine</b>	9.34	0.90	0.92	0.53	0.29	0.49	0.34
<b>Tryptophane</b>	2.58	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
<b>Valine</b>	10.04	1.32	1.11	0.68	0.35	0.62	0.49
<b>Arginine</b>	13.81	2.09	1.53	0.94	0.46	0.87	0.48
<b>Histidine</b>	5.45	0.73	0.50	0.35	0.18	0.37	0.23

According to this, the contents of essential and semi-essential amino acids analyzed in the feed were not only in terms of their grammage but also their quantitative ratios far away from those values that were determined by means of the species-appropriate, A-DOG formulation for dogs (in Table 9 specified as A-DOG).

This fact is mostly due the use of poor quality protein sources.

On the basis of the amino acid (Liebig's principle), which is the limiting factor depending on the individual dog feed, the following results could be calculated (quotient of A-DOG value divided by the amino acid value determined in the respective feed) according to Table 6:

**Table 6**

	A-DOG	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Isoleucine	9.31	11.08	10.46	16.05	33.25	19.00	31.03
Leucine	16.56	8.63	10.55	14.40	35.23	18.40	24.35
Lysine	18.08	11.44	<b><u>12.38</u></b>	<b><u>25.11</u></b>	<b><u>36.16</u></b>	21.02	31.71
Methionine	6.14	<b><u>13.96</u></b>	12.04	14.98	32.33	<b><u>25.59</u></b>	<b><u>36.13</u></b>
Phenylalanine	8.70	7.13	9.55	12.25	32.20	17.05	22.88
Threonine	9.34	10.37	10.15	17.61	32.19	19.05	27.46
Tryptophane	2.58	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Valine	10.04	7.61	9.05	14.76	28.69	16.19	20.49
Arginine	13.81	6.61	9.03	14.69	30.02	15.87	28.77
Histidine	5.45	7.47	10.91	15.58	30.29	14.74	23.71

In sample 1, according to Table 6, methionine is the limiting amino acid, which means that 13.96 times the amount of feed would have to be administered (1.396 kg) to achieve the methionine value of 100 g of the medium-chain triglyceride and amino acid mixture contained in A-DOG.

In sample 2, according to Table 6, lysine is the limiting amino acid, which means that 12.38 times the amount of feed would have to be administered (1.238 kg) in order to reach the lysine value of 100 g of the medium-chain triglyceride and amino acid mixture contained in A-DOG.

In sample 3, according to Table 6, lysine is the limiting amino acid, which means that 25.11 times the amount of feed would have to be administered (2.511 kg) in order to reach the lysine value of 100 g of the medium-chain triglyceride and amino acid mixture contained in A-DOG.

In sample 4, according to Table 6, lysine is the limiting amino acid, which means that 36.16 times the amount of feed would have to be administered (3.616 kg) to achieve the lysine

value of 100 g of the medium-chain triglyceride and amino acid mixture contained in A-DOG.

In sample 5, according to Table 6, methionine is the limiting amino acid, which means that 25.59 times the amount of feed would have to be administered (2.559 kg) to achieve the methionine value of 100 g of the medium-chain triglyceride and amino acid mixture contained in A-DOG.

In sample 6, according to Table 6, methionine is the limiting amino acid, which means that 36.13 times the amount of feed would have to be administered (3.613 kg) in order to achieve the methionine value of 100 g of the medium-chain triglyceride and amino acid mixture contained in A-DOG.

While maintaining the analyzed ratios to the other amino acids, the supply of such increased amounts of feed would again lead to a collision with the values of these amino acids, since the amino acid proportions in the feed do not match the ratios of the species-appropriate amino acid profile.

In addition, in the absence of the appropriate amino acid ratio in the related feed, this would result in completely uncontrolled and counterproductive overfeeding, which in the long term could lead to organ strain or damage, especially in less active, overweight or dogs with medical histories.

Assuming that the body's effort for the synthesis of food proteins is lower, the more they are similar to the body proprietary protein in terms of their amino acid composition, this conversely implies, that the analyzed feed with regard to their amino acid profile is far away from the value of A-DOG formulation for dogs because it cannot adequately cover the requirement for essential and semi-essential amino acids.

In the long term, the possible consequences range, as already explained, from deficiency symptoms and organ stress to a wide variety of clinical issues.

As described at the beginning, similar results are to be expected from analyzes of feed for cats and horses, since the composition of the products is mostly not appropriate to the species.

For example, cat food usually contains too little meat, however it does contain grain, vegetable by-products and sugar, all of which are unsuitable for the cat's appropriate diet.

Cats lack the necessary enzyme to digest vegetable proteins just as well as animal proteins, and they also need a lot more protein. The need for kittens is one and a half times larger than for puppies, adult cats even need two to three times more protein than adult dogs and therefore more amino acids (Deine Tierwelt,2020).

According to various studies, around 50 percent of horses are overweight, and almost 20 percent must be classified as obese. The problem: As with humans, obesity also triggers numerous secondary diseases in horses. This is due, on the one hand, to a completely unsuitable relationship between the supply of food and exercise. On the other hand, it is a false ideal of what a healthy horse should look like in the optimal nutritional state (Sladky, 2018).

The quality of horse feed results on the one hand from the content of nutrients, minerals and vitamins and on the other hand from the microbiological load. Bacteria, fungi and yeasts very often adhere to feed. Up to a certain amount, they are still considered harmless. However, if the levels are too high due to various causes, physical stress occurs, which can

subsequently manifest itself in symptoms such as refusal to feed, cough, colic, faecal water and allergic reactions.

An insufficient supply of essential amino acids, which then also affects the structure and supply of organs, muscles and tissues, is more common than a general protein deficiency. If only one of these essential amino acids is available in insufficient quantities, the body's own protein build-up is impaired. Therefore one should pay attention to their continuous supply (Stögmüller, Sladky, 2018).

In terms of providing the species-specific, optimal amino acid composition in combination with non-stressful, easily digestible MCT oil to ensure a longer aerobic phase of the energy metabolism, DAMINOC® in the appropriate version for the respective species is therefore equally suitable for use in cats and horses feeds.

DAMINOC® is also suitable under the aspect of sustainability to cover the individual protein requirement for avoidance of factory farming and animal slaughter and to avoid the associated intake of hormones, auxiliary substances and medicals.

DAMINOC® is free from preservatives, flavorings and additives. With regard to the administration in the form of a suspension, it was possible to go without fillers, capsule or tableting aids and coatings.

DAMINOC® is supplied or ingested once or several times a day depending on the respective species, their body weight and activity level, whereby it can be administered directly or in combination with a meal, a drink or the feed. It comes in individual single portions packed in sachets.