

OPINION LEADER

Dietary Amino Acids and Pig Meat Quality

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Abstract

Strategies to improve sensory and technological meat quality receive increasing interest in the mainstream meat production chain, as long as they do not negatively affect growth, feed efficiency and carcass leanness. It is well established that an optimal dietary amino acid supply and protein-to-energy ratio are paramount for realizing the animals' genetic capacity for muscle growth. While feeding below amino acid requirements increases carcass fatness and some aspects of meat quality, this comes evidently at the cost of worse animal performances. Yet, there is emerging information on the role that several amino acids may play in the regulation of key metabolic pathways, thereby potentially also affecting post-mortem meat quality. The literature was reviewed for reports on the effects of dietary supplementation with specific amino acids on defined meat quality traits. Beneficial effects on meat quality of the addition of methionine, leucine, arginine, tryptophan, glutamic acid, β -alanine and histidine have been reported or hypothesized; either by increasing the intramuscular fat content, improving the oxidative stability or reducing animals' stress. It has to be noted that the number of studies is mostly limited and that outcomes are not consistent. More research is warranted on the effects of dietary supplementation with specific amino acids on defined meat quality traits.

Background

Meat quality is a broad concept encompassing both intrinsic product quality traits (i.e. sensory and technological quality, nutritional value, chemical and microbiological safety) and extrinsic quality traits related to the characteristics of the production system (e.g. animal welfare, environmental impact) (Prache et al., 2022; Lebret & Candek-Potocar, 2022a). In most consumer surveys on quality perception and preference for fresh meat, sensory traits are ranked very high if not highest with an emphasis on tenderness and flavour, denoting the importance of these traits. Pig carcasses are also processed for a large part of a variety of processed meats. Hence, technological quality traits such as water-holding capacity and textural properties of the raw materials, e.g. fresh hams for transforming to cooked ham or dry-cured ham, are equally very important (Lebret & Candek-Potocar, 2022b).

The sensory and technological quality of meat is determined by the muscle characteristics and the post-mortem metabolic conversion of muscle to meat. Several animal genetic, feeding and management factors influence the carcass and muscle composition at slaughter and the subsequent conversion to meat, whereby the latter is also strongly affected by the slaughter process and by the stress the animals may experience prior to and during slaughter (Lebret & Candek-Potocar, 2022a). Apart from specific brands and production practices focusing on eating quality traits (e.g. heavy pigs in Italy and Iberian pigs in Spain for high-quality dry-cured ham, local breeds and brands in many countries), the mainstream production of pigs worldwide is oriented toward the efficient conversion of feed into lean muscle mass. Particularly in most European production systems, there has been a continuous trend towards higher growth rate, feed efficiency and carcass leanness through genetic selection and optimized feeding, with less interest in meat quality (Prache et al., 2022). However, there are increasing requests from retailers, processors and consumers for improving the eating quality of fresh pork and its suitability for further processing. Flavour,

tenderness or texture and water-holding capacity are thereby the key traits. E.g. The intramuscular fat (IMF) content of many pork joints has dropped below threshold levels for optimal flavour. It is indeed well established that the degree of marbling is almost linearly related to flavour intensity. Excessive drip loss remains also a concern in the pork processing industry, as well as textural defects when transforming to non-comminuted high-quality products. Colour properties and oxidative stability are also traits of general interest (Lebret & Candek-Potocar, 2022a, 2022b).

The bio-physicochemical background of each of the mentioned traits is specific and complex, meaning also that there is no simple strategy for optimizing meat quality. Including meat quality traits in breeding programs receives increasing interest but remains challenging. On the other hand, diet composition and feeding programs warrant research as a faster approach to customizing meat quality for specific markets. An optimal dietary amino acid (AA) supply and protein-to-energy ratio come into play here as major determinants for realizing the animals' genetic capacity for muscle growth, which may then indirectly influence fat deposition and meat quality.

In addition, there is emerging information on the specific effects of dietary supplementation with individual AA above requirements on pig meat quality traits. Indeed, besides the role of α -AA as building blocks in proteins, several α -AA and non- α -AAs regulate key metabolic pathways (Wu, 2009). The specific aroma of free AA and their contribution to the formation of meat aroma compounds, e.g. through the involvement in Maillard reactions, is also well documented (Khan et al., 2015; Ma et al., 2020). Genotype differences in muscle free AA content and concomitant differences in sensory perception have also been described (Straadt et al., 2014; Ma et al., 2020). A key question is then to what extent the muscle-free AA profile can be modified by adjustments in the dietary AA supply.

Amino acid deficiency leads to more (intramuscular) fat deposition

Diets low in crude protein or deficient in one or more AA reduce the capacity of muscle deposition, hence the ingested energy that cannot be used for protein synthesis, results in more fat deposition. It can be anticipated that amino acid shortages lead to an increase in fat deposition in all fat depots, including IMF (Essen-Gustavsson et al., 1994; Hyun et al., 2006; Li et al., 2018). Of course, since fat deposition requires more energy than muscle deposition, this comes at

the cost of lower feed efficiency and lower daily gain (Apple, 2004). A consistent effect of feeding below amino acid requirements on IMF content or marbling has been shown in numerous studies (e.g. Goodband et al., 1993; Witte et al., 2000; Apple, 2004; Li et al., 2018), concomitant with improved tenderness or lower shear force values in most but not all studies.

Methionine and cysteine

The sulfur-containing AA methionine and cysteine are essential AA needed for protein synthesis. Methionine also plays other physiological roles, such as its function as a methyl donor. For meat quality, the role of methionine and cysteine as precursors of glutathione, taurine and hypotaurine is especially interesting. These compounds are crucial in the muscle fibre's intracellular antioxidant defence systems (Estevez et al., 2020).

In a study by Lebret et al. (2018), doses of methionine well above the requirement in the last two weeks

before slaughter led to lower TBARS (a measure of lipid oxidation), higher glutathione levels and higher ultimate pH in muscle. In a follow-up metabolomic study of the same group (Gondret et al., 2021), these effects were explained by a modification of the muscle-free AA profile and its redox capacities. In a study by Li and colleagues (2017), methionine supplementation to low-birthweight piglets resulted in lower drip loss and higher ultimate pH after the slaughter at around 95 kg BW.

Leucine

Leucine is an amino acid that plays a crucial role in protein and energy metabolism. It specifically stimulates protein synthesis through the activation of mTOR in muscle (Duan et al., 2016). By stimulating mTOR in the brain, excessive leucine levels may depress feed intake, which can be partially counteracted by adding more valine to the diet (Millet et al., 2015). Its positive effect on muscle deposition and potential inhibiting effect on feed intake logically leads to the assumption that leucine may increase the carcass lean percentage and decrease the carcass fat content.

Still, it has been shown to do the opposite: in pigs with a deficient lysine level, feeding high leucine levels increased the IMF content (Hyun et al., 2007). Similarly, Yu et al. (2007) observed higher IMF levels in diets with supplemental conjugated linoleic acid and leucine. Since Leucine is a ketogenic amino acid, the breakdown of excess leucine in muscle may lead to acetyl-CoA production, which could then be used for fatty acid synthesis. Madeira et al. (2014) did not observe an effect on IMF level or other meat quality parameters, except for a higher juiciness score when leucine was added to a low crude protein diet.

Arginine

Arginine exerts a myriad of functions in the animal body. Apart from its role as a building block in protein synthesis, it is also a precursor for molecules such as nitric oxide, ornithine, polyamines, proline, and creatine. It can be synthesized from glutamine and proline in most mammals, including pigs (Ma et al., 2009). However, it is a conditionally essential amino acid and adding extra arginine has affected the metabolism of pigs in several studies. According to Tan et al. (2011), arginine increases mRNA expression of fatty acid synthase in muscle, while decreasing those for lipoprotein lipase and other enzymes in adipose tissue, therefore favouring lipogenesis in muscle while stimulating lipolysis in adipose tissue. Theoretically, this could be a way to

uncouple fat deposition in muscle and fat tissue. In a study by Tan et al. (2009), arginine supplementation increased lipid deposition in longissimus dorsi, but not in the semitendinosus muscle. However, the marbling score was not affected whereas pH 45 min post-mortem was higher. In a study by Go et al. (2012), pH 45 min post-mortem was lower when additional arginine was fed. In a study by Ma et al. (2010), supplementing with 1% arginine increased the IMF content and decreased drip losses. Similarly, supplementing with arginine and glutamic acid increased the IMF level in a study by Hu et al. (2017). However, in other studies, there was no effect of arginine supplementation on the IMF level (Go et al., 2012; Madeira et al., 2014).

Tryptophan

Tryptophan is the precursor of the monoaminergic neurotransmitter serotonin (5-hydroxytryptamine, 5-HT). Increased dietary tryptophan may lead to an increase in the synthesis of brain 5-HT (25) and this increase may lead to reductions in aggressive tendencies and abnormal behaviour in pigs, although this was not always confirmed in experimental studies (Henry et al., 2021). Similarly, short-term (5 days pre-slaughter) supplementation with tryptophan has been linked with reduced stress levels around slaughter and

therefore reduced occurrence of PSE and improved meat quality (Adeola and Ball, 1992; Paniella-Riera et al., 2009).

However, contrasting results have been reported in the literature, and effects are not surprisingly dependent on genotype and imposed stress level. Several studies concluded that short-term supplementation with tryptophan prior to slaughter had no effect on meat quality (Geesink et al., 2004; Guzik et al., 2006; Paniella-Riera et al., 2008).



β -alanine and histidine

L-histidine and β -alanine are the building blocks of the endogenous muscle dipeptide carnosine and its methylated analogues anserine and ophidine. Whereas methylated analogues are more abundant compared to carnosine in almost all species, carnosine is predominant in bovine and porcine muscles (Boldyrev et al., 2013). Being present at high concentrations in muscle, these dipeptides exert multiple physiological roles *in vivo*, of which the muscle buffering capacity and antioxidant activity are the most relevant ones with regard to meat quality. Greater pH 24 h post-mortem, better water-holding capacity and improved meat color have been found in pigs with high muscle carnosine content (D'Astous-Pagé et al., 2017). In an NMR-based metabolomics study of pork from four different crossbreeds, the muscle carnosine content was significantly correlated to several sensory attributes (Straadt et al., 2014). Although these associations do not necessarily imply direct causal effects, it could nevertheless be hypothesized that dietary β -alanine and/or histidine supplementation may increase the concentra-

tion of these dipeptides in muscle and subsequently affect meat quality.

However, in an early study by Mei et al. (1998), supplementation of swine diets with histidine (0.40%) and/or β -alanine (0.225%) had no and a moderate effect on the concentration of carnosine and anserine in longissimus and vastus intermedius muscle respectively, and did not improve the oxidative stability of pork.

On the other hand, Ma et al. (2010) reported that the direct addition of 100 mg carnosine per kg feed improved the antioxidant capacity and meat quality of pigs by increasing muscle pH at 45 min, 24 h, and 48 h post-mortem, decreasing drip loss, and reducing the concentration of malondialdehyde and carbonyl protein complexes, possibly mediated by altered antioxidant enzyme activities.

Other amino acids and derivates

Glutamic acid is an important flavour-contributing AA in pork. As mentioned above, supplementation of the diet with glutamic acid resulted in higher IMF levels (Hu et al., 2017). In a follow-up study on the same meat samples, Guo et al. (2019) showed that dietary supplementation with arginine, glutamic acid, or arginine plus glutamic acid had little effect on free amino acids, no effect on meat sensory taste traits, but supplementation with arginine plus glutamic acid significantly increased the fatty acid content in muscle, the formation of multiple fatty acid oxidation-derived volatile compounds, and improved the tenderness, juiciness, and overall eating quality of meat. Along the same line, monosodium L-glutamate supplementation increased the IMF content

and altered muscle fibre composition (Kong et al., 2015).

Effects on meat quality of the dietary supplementation with some AA derivates have been described but are not discussed here, e.g. sarcosine (tripeptide synthesized from arginine, glycine and methionine), betaine (an effective methyl donor) or cysteamine. Yet, these effects are not consistent in the literature. We refer to the review of Ma et al. (2020) in this respect.

Table 1. Summary of reported or hypothesized beneficial effects of dietary supplementation with specific amino acids on meat quality traits

	Mechanism(s)	Effect(s) on meat quality
Methionine	Methyl donor, precursor of glutathione and taurine	oxidative stability ↑, drip loss ↓, ultimate pH ↑
Leucine	Ketogenic AA involved in fatty acid synthesis	IMF ↑
Arginine (w or w/o glutamic acid)	Stimulates lipogenesis in muscle and lipolysis in adipose tissue	IMF ↑, drip loss ↓
Tryptophan	Precursor of serotonin, stress reduction	PSE incidence ↓
β-alanine and histidine	Building blocks of carnosine	oxidative stability ↑, pH ↑, drip loss ↓

IMF = intramuscular fat content; PSE = Pale, Soft, Exudative meat

Conclusions

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Amino acids are not only the building blocks of muscle proteins but several AAs are also involved in the regulation of key metabolic pathways such as lipid metabolism and antioxidant defence, with potential post-mortem effects on sensory and technological meat quality. In addition, the content and profile of free AA in meat are related to the specific flavour of meat. Hence, there are good arguments for studying the effect of dietary supplementation with specific AA on defined meat quality traits.

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