



## Review Article

# Potential benefits of amino acid supplementation for cervid performance and nutritional ecology, with special focus on lysine and methionine: A review

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## ABSTRACT

Deer farming is a thriving industry for venison, velvet antlers, trophy hunting, and other by-products. Feeding and nutrition are important factors for improving production performance, especially dietary protein and amino acids (AAs), as they are the main components of all tissues. Only a few studies on AA supplementation (Lys, Met, Arg) have been performed on cervids, which show positive effects on weight gain, ADG, feed:gain ratio, plasma AAs, carcass weight, dressing percentage, yield of high-quality muscles, storage of internal fat during winter, DM and CP digestibility, plasma protein- and fat-related metabolite concentrations, antler burr perimeter, weight, length and mineralisation, velvet antler yield, rumen volatile fatty acids, and microbiome composition. All these effects are relevant for supporting the production of cervids products, from venison to velvet or trophy antlers, as well as their general performance and well-being of captive-bred cervids. The current available information suggests that AA supplementation can be especially interesting for animals fed low protein rations, and growing animals, but should be avoided in high rations and during winter, since it may promote the accumulation of internal fat. Potential effects on milk production and the concentrations of different hormones involved in the regulation of the antler cycle should be further explored.

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## 1. Overview and importance of the captive breeding of cervids

Cervids and humans share a long history (Fletcher, 2014) since their products have been always greatly appreciated, not just their meat, skin or tendons as with many other species, but also their antlers for producing tools or just as trophy, and the velvet for medicinal, cultural and religious purposes (McCullough et al., 2009;

Kuba et al., 2015). Early attempts of domestication of certain species, like the European elk (*Alces alces*) and reindeer (*Rangifer tarandus*), lead to the exploitation of other products like milk or even their utilization for riding and ploughing. Modern deer farming started in the 1970s (Blaxter, 1974; Kuba et al., 2015), and nowadays cervids are kept both in extensive and intensive systems worldwide, with the main goals being the production of breeding stock, meat (venison), antlers for trophies or for velvet, and ecotourism (Fletcher, 2019), with global deer farming involving approximately 12 million animals (Serrano et al., 2019). The most commonly farmed species nowadays are red deer (*Cervus elaphus*), fallow deer (*Dama dama*), reindeer, wapiti (*Cervus canadensis*), sika deer (*Cervus nippon*), axis deer (*Axis axis*), rusa deer (*Rusa timorensis*), white-tailed deer (*Odocoileus virginianus*), and sambar deer (*Rusa unicorn*) (Vos, 1982).

**Venison** – Among all deer products, venison is the most in demand, with an estimated market value of over 1.5 billion US dollars. Approximately 75% of the revenue of deer farming in New

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Zealand (the most developed deer industry worldwide) comes from venison. The USA is the primary importer of venison, while it is gradually becoming a “must-have” for supermarkets in Europe, and has a promising market in China (Spencer, 2020). Venison is low in cholesterol and high in polyunsaturated fatty acids, protein, and iron (Wiklund et al., 2014; Kudrnáčová et al., 2018), and has favourable sensory qualities (flavour, aroma, texture) when compared to beef (Bureš et al., 2015). Nowadays, many common livestock farming practices are being implemented in the deer farming industry to improve meat yield and quality, mainly focusing on nutrition and feeding (Volpelli et al., 2002; Phillip et al., 2007; Hutchison et al., 2012).

**Velvet** – Velvet antlers are pre-calcified, soft, and vascularized antlers (Goss, 1983) that are harvested, cut to slices, dried, and prepared as nutraceuticals or functional foods, in the form of liquid extracts, tablets, capsules, or powders (Wu et al., 2013). As cartilaginous tissue, the mid-portion of velvet antlers is rich in collagen, protein, and amino acids (AAs). The basal portion mainly contains macro and micro minerals, while the tip portion mainly contains growth factors, such as insulin-like growth factor 1 (IGF-1) and fibroblast growth factors (Suttie et al., 1985; Suttie and Fennessy, 1992). Farming for velvet requires as much care and management as for venison production. Nutrition also plays an essential role in velvet production, especially dietary minerals, protein, and AA contents (Dryden, 2016).

**Trophy hunting** – Recreational trophy hunting of cervids is currently a lucrative industry (Feldhamer and McShea, 2012), annually generating at least 3 billion US dollars in North America and €16 B in Europe. Trophies are scored based on their antler weight, size, and shape (Goss, 1983; Kuba et al., 2015). Therefore, the higher the score of the antlers (larger trophies), the more expensive the animal is for hunting. To achieve these results, improved nutrition is essential. Not just in terms of minerals but also regarding protein and key AAs for producing the necessary collagen scaffolding (Shin et al., 2000; Dryden, 2016). Casted hard antlers are also widely used as ornamental tools and crafts.

**By-products** – All parts of the carcass are utilized, especially in the Asian markets, where by-products like hearts, penises, testicles, blood, tendons, sinew, etc., are highly appreciated, although this market is still not well developed. On the contrary, skin has a well-established market for producing high-quality leather garments and footwear (Vos, 1982; Feldhamer and McShea, 2012), generating around 25 million US dollars in New Zealand alone (Fletcher, 2014).

**Ecotourism** – Another economic activity of captive cervid breeding is ecotourism and its related activities. England has a long tradition of maintaining numerous deer parks for tourist attraction (Fletcher, 2019). Moreover, both deer-tourism and especially trophy hunting also generate high incomes for locals and businessmen through other various services, accommodation, and hunting gear. Just in the USA, at least 25 billion US dollars is generated from these related services every year (Feldhamer and McShea, 2012), which is, indeed, more than what is generated by the deer products themselves altogether.

**Captive breeding for conservation** – Compared to other mammals, cervids are not well-understood by society regarding their endangered status due to the wide distribution of the best-known species (Blouch et al., 1998). However, just 16 of the 55 living species are considered as “not endangered” (IUCN, 2021). Captive breeding already saved one species from extinction, the Père David’s deer (*Elaphurus davidianus*), and is an important tool for the management of a few others. Studies aiming at designing adequate feeding programs are also essential for these species (Azad et al., 2005; Müller et al., 2010).

## 2. Cervids’ protein nutrition

In ruminants, the absorbed AAs are mostly from microbial protein synthesis, and from dietary AAs that escaped ruminal degradation (Kung and Rode, 1996). Many factors affect the final AA profile available for absorption by ruminants, including degradation and use by rumen microbiota. Therefore, AA supplementation has become a growing topic for improving the nutrition of ruminants. Likewise, for cervids, the protein quality, determined by a balanced AA content, is considered more important than the quantity itself (Brown, 1999; Dryden, 2016). However, protein nutrition and AA requirements are not well-established for cervid species, and like other animals, it depends on the species, sex, age, season, and physiological state of cervids (Brown, 1999; Dryden, 2011). Cervids require at least 6% to 7% crude protein (CP; on a DM basis for the rest of the document) to support rumen functioning (Richardson, 2000), 4% to 9% for maintenance (lowest requirement in adult white-tailed deer; Brown, 1999), and around 16% to 22% for production (pregnancy, lactation, and antler growth; Dryden, 2011). As the microbes in the rumen have an impact on the final absorption of AA, feeding only a high protein diet, or unprotected AA, to cervids are not recommended (Kung and Rode, 1996). Thus, supplementation of rumen-protected AAs (RPAAs) remains the optimal solution for improving the protein nutrition of ruminants.

## 3. Role of amino acids in improving ruminant performance

Amino acid supplementation has already shown to improve nutrient utilization, growth performance and survival of the young animal, reproductive performance, wool and hair production, and especially increase milk and meat production in other ruminant livestock such as cattle, sheep, and goats. The most common studies are on Lys, Met, His, and Arg supplementation (D’Mello, 2003). Methionine and Lys generally have no effects on the growth performance (body weight [BW]; body condition score [BCS]) and feed intake of dairy cow fed various diets (Blum et al., 1999; Lara et al., 2006; Watanabe et al., 2006; Lee et al., 2015). However, these parameters increased when other AAs, such as RP-His, RP-Thr, RP-Leu, RP-Ile, were co-supplemented (Lee et al., 2012; Giallongo et al., 2016; Zhao et al., 2019).

RP-Lys and/or RP-Met and/or RP-His increased milk yield, milk composition (protein, fat, lactose, AAs) (Socha et al., 2005; Watanabe et al., 2006; Lee et al., 2012; Giallongo et al., 2016; Zhao et al., 2019) in dairy cows, especially when fed a lower protein diet. However, the effects of RPAAs on milk production in sheep and goats are inconsistent. For instance, Urbaniak et al. (2001) found no increased milk yield or influence on milk components in goats supplemented RP-Met. Flores et al. (2009) and Titi, 2017 found increased milk production in lactating goats supplemented with RP-Met, and in sheep supplemented with RP-Lys plus RP-Met (Goulas et al., 2003; Tsiplakou et al., 2018, 2020). Other AAs, such as RP-choline (RP-Chol), RP-betaine (RP-Bet), and N-carbamylglutamate (NCG) also increased milk yield and affected milk composition in goats (Fernández et al., 2009; Baldi et al., 2011; Pinotti, 2012; Pinotti et al., 2008, 2020).

RPAAs support maternal health and improve the growth and development of young ruminants. RP-Met supplementation increased feed intake, BW, and body conditions in calves (Alharthi et al., 2018), and NCG or RP-Arg increased BW and improved the organ development of goat kids and lambs, and improved maternal health profiles (Souri et al., 1998; Zhang et al., 2016; Sun et al., 2018; Wang et al., 2019). However, some studies did not find any

improvements in those parameters (Flores et al., 2009; Al-Qaisi and Titi, 2014; Titi, 2017; da Silva et al., 2018).

Feeding RP-Lys and/or RP-Met also increases BW, carcass weight, and meat quality in beef cattle (Klemesrud et al., 2000a, b; Teixeira et al., 2019). RP-Bet, RP-Lys and/or RP-Met increased feed intake, BW, nutrient digestion, and improved meat quality (tenderness and colour) and composition (fatty acid profile) in lambs (Rodehutsord et al., 1999; Araújo et al., 2019; Dong et al., 2020). For wool and hair production, feeding sulphur-containing AAs, such as RP-Met, RP-Bet, or RP-Try increased hair production (growth rate, cashmere length, mohair fibre) in goats (Souri et al., 1998; Ma et al., 2010, 2011), and wool production (growth rate, yield, staple length) in sheep (Mata et al., 1995; Nezamidoust et al., 2014). Moreover, reproductive improvement (fertility and sperm quality) can be achieved by supplementing RP-Arg to rams and ewes (De Chávez et al., 2015; Kaya et al., 2019).

Overall, supplementation of AAs, especially the essential or limiting ones, have various positive effects on the productivity and health of ruminants, improving nutrient utilization, physiology, maintenance, reproduction, health, and production in both large and small ruminants. All of these effects are summarised in Table 1.

#### 4. Amino acid supplementation and performance of cervids

In the cervid family, as in other ruminants, proteins and AAs are important for body growth, reproductive health, maintenance, production, and lactation, but also for antler growth (Richardson, 2000; Dryden, 2011; Dryden, 2016). Based on the review by Dryden (2011), protein nutrition and AA requirements in cervids cannot simply be predicted using cattle or sheep data. Furthermore, their digestive tract physiology differs to that of other ruminants, with a smaller rumen and shorter tract length (Brown, 1999). The digestive tract of each ruminant is also adapted to the different morphological feeding types of the species, according to if it is a browser, grazer, or mixed feeder (Hofmann, 1989). Nonetheless, available data about the supplementation of specific RPAA in other ruminants, based on metabolic body weight (MBW), may aid in predicting their effects on the growth and production performance of cervids. However, knowledge on protein and AA nutrition in cervids is still very limited compared to other ruminant livestock.

In this review, the level of AA used will be described based on the basal MBW =  $BW^{0.75}$ , adapted from Savage et al. (2004) and Tedeschi et al. (2015), based on the quarter-power scaling in mammalian biology and models of AA requirements in cattle.

##### 4.1. General performance and venison production

Amino acids are important for meat protein synthesis, and thus for venison production. While adequate balanced protein is necessary for cervids, as it is for other ruminants, cervids are different not only in their production purpose (which includes antlers and velvet), but also that their reproductive cycle has a large effect on body condition. Providing adequate AA may help to reduce weight loss in cervids, especially during the rut (stags lose about one fifth of BW) and winter periods, when there is a lack of feed resources (Dryden, 2011; Fletcher, 2014).

Few studies have evaluated the effects of AA supplementation on the feed intake, nutrient digestion, weight gain, growth, and venison quality, particularly essential amino acids (EAAs), such as Lys, Met, and Arg. Mendoza-Nazar et al. (2012) supplemented RP-Met to adult male red deer (0.08, 0.12, or 0.15 g/kg MBW per d) together with a 11.9% CP diet, resulting in a quadratic increase in total weight gain and average daily gains (ADG) at a supplementation level of 0.15 g/kg MBW per d. There were also no increases in protein and fat serum metabolites in this study, at all RP-Met

supplementation levels used. Similarly, Huang et al. (2015a) supplemented RP-Met to sika deer calves (0.08 or 0.15 g/kg MBW per d), and reported a linear increase in ADG during the 35-d supplementation period. However, later (until 70 d of supplementation) there were no differences in BW between the study animals. Even though the effects on BW were not greatly improved, RP-Met supplementation increased the apparent digestibility of Met and other AAs (Val, Ile, Leu, Phe, Asp, Gly, Cys). Moreover, it also increased plasma AAs concentrations (Ser, Leu, Ala, Tyr, Gly, Pro, Ile, His, and Lys) which are important for absorption and protein metabolism. Huang et al. (2015b) continued with almost the same research design, feeding a high protein diet (16.63% CP), compared to a low protein diet (13.77% CP) with RP-Lys (0.23 g/kg MBW per d), or combined with RP-Met (0.08 or 0.16 g/kg MBW per d). A tendency for improving ADG and nutrient digestibility (DM, OM, CP) was observed when adding RP-Met and RP-Lys to the low protein diet, showing the same result as feeding a high dietary CP. However, the overall weight gain and feed intake were not improved. Changes in the serum glutamic pyruvate transaminase and its effects on the mobilisation of AAs for glucose synthesis in muscles have been suggested as the mechanism modulating the effect of RP-Met supplementation on ADG (Mendoza-Nazar et al., 2012).

Moreover, feeding RP-Lys, and in combination with RP-Met 2 g (0.16 g/kg MBW per d), improved protein digestibility and serum protein biochemistry marker levels [total protein, albumin, globulin, blood urea nitrogen, alanine aminotransferase (ALT), aspartate aminotransferase (ATS)] to the same extent as the group fed high dietary protein, compared to the CP-deficient group. This elucidates the potential benefits of RP-Lys and RP-Met supplementation for improving the growth of young animals, instead of feeding a high protein diet, especially at an early age (Huang et al., 2015b). Another study on RP-Lys and RP-Met (with a higher number of animals) using yearling fallow deer raised on pasture and fed low-protein barley (10.75% CP) or supplemented with RP-Lys (0.55 g/kg MBW per d), or RP-Lys (0.55 g/kg MBW per d) plus RP-Met (0.18 g/kg MBW per d) (Ceacero et al., 2020), showed no effects of RPAA on ADG, BW, or carcass weight. However, the RP-Lys-fed group, and the Lys–Met combination group, improved their carcass dressing percentage, internal fat storage, BCS, and some plasma metabolites (creatinine and triglycerides). The key notes from these findings are the important roles of RPAA for nutrient metabolism, particularly the increased lipid metabolites and body condition. This is related to the well-known decrease of feed intake in cervids during winter, mediated by the reduced photoperiod (Scott et al., 2013), which apparently leads to using most of the resources for fat storage for winter survival during a nutrient-scarcity period. On the other hand, a study by Kudrnáčová et al. (2019) supplementing only RP-Lys at 0.39 g/kg MBW per d to yearling fallow deer bucks found no improvements regarding weight gain, carcass weight, or dressing percentage, compared to bucks fed only barley grains (11.27% CP), but less internal fat deposition (kidney, rumen, and scrotal fat) during the summer fattening period. However, these parameters were still better in the RP-Lys group compared to the group which were fed only pasture. Moreover, RP-Lys increased the proportion of the high-priced meat from shoulder, and the weight of the *longissimus thoracis et lumborum* (LTL) muscle. Limited dietary effects were found on the physical meat quality characteristics of the LTL and *semitendinosus* muscles (pH, colour, Warner–Bratzler shear force). It is important to note that this study was done during a drought-summer, which affected the growth of the pasture. Thus, concentrate and/or RP-Lys supplementation improves the muscle development of pasture-raised cervids for meat production, which has good potential for commercial deer farming, especially under poor pasture conditions. Bureš et al. (2020) deeply examined the

**Table 1**  
Proven effects of supplementary amino acids (AAs) on cattle, sheep and goat, and its potential applications in cervid nutrition.

RPAA	Measured parameters	Studied in cervids	Effects <sup>1</sup>	Studies species	Comments	References
RP-Met	Weight gain, ADG.	Yes	(+)	Red deer bucks, 2.8 years old	Limited effects on weight gain (only quadratic level). Only slightly improved beam length.	Mendoza-Nazar et al. (2012)
	Serum GLU, CHOL, Urea, CREA, SGPT, SGOT.	Yes	(0)			
	Antler (beam length, brow tine length, points).	Yes	(0)			
	ADG, feed-to-gain ratio.	Yes	(+)	Sika deer bucks, 5 months old	Increased gain only during early 35 d.	Huang et al. (2015a)
	Plasma Gly, Pro, Ile, Ser, Leu, His, Lys, Arg, NH <sub>3</sub> .	Yes	(+)			
	Apparent AA (Val, Leu, Ile, Phe, Asn, Gly, Cys).	Yes	(+)	Lactating cows	Improved protein digestion & AA absorption for growth of young.	Blum et al. (1999); Lara et al. (2006)
	BW, BCS, DMI.	No	(0)			
	BW, ADG, feed intake.	No	(0)			
	Milk yield, milk protein & fat yield, milk urea N.	No	(+)	Dairy cows, lactating goats	No effects on gain and intake, similar to lactating cows and sheep.	Flores et al. (2009); Al-Qaisi and Titi (2014); Titi, 2017
		Milk yield, milk fat, protein, lactose contents.	No			
	Milk SFAs.	No	(+)	Gestating and lactating ewes	Potential for improving milk production and quality of does for supporting early growth of fawns.	Urbaniak et al. (2001); Kudrna et al. (2009); Flores et al. (2009); Titi, 2017; Zhao et al. (2019)
		Milk SFAs.	No			
	DMI, BW, hip height, wither height.	No	(+)	Gestating cow & calves	Potential for improving maternal, postnatal health, and growth of young fawns.	Alharthi et al. (2018)
	BW, feed-to-gain ratio, N retention.	Yes	(0)	Cashmere goats	No effect on gain and intake, similar to cattle and sheep.	Souri et al. (1998)
	Weight of cashmere hair, diameter of mohair fibre.	No	(+)	Wether sheep	Potential for improving growth of pelage/coat during autumn and velvet hair.	Mata et al. (1995); Rodehutschord et al. (1999)
BW, N retention, OM digestion.	No	(+)				
Wool sulphur content, clean wool yield, wool growth rate & fibre diameter.	No	(+)				
Plasma sulphate, Met.	No	(+)	Fallow deer buck, 11 months old	Important for supply of sulphur for coat and velvet hair growth in cervids.	Kudrnáčová et al. (2019)	
Glutathione in blood, liver, skin.	No	(+)				
Weight gain, carcass weight, dressing percentage, weight of LTL muscle.	Yes	(+)	Fallow deer buck, 11 months old	Increased carcass and muscle yield. Increased fat storage for winter period.	Bureš et al. (2020)	
Internal fat (kidney, rumen, scrotal fat).	Yes	(+)				
LTL and ST muscle pH, colour, shear force, tenderness.	Yes	(0)	Fallow deer buck, 11 months old	No effects on some physical meat quality depends on muscle types, similar to beef.	Lancaster et al. (2016)	
IMF, LTL AA (His, Leu, Ala, Glx, Gly).	Yes	(+)				
Intake, gain, hot carcass weight, carcass composition, LTL muscle area, marbling.	Yes	(0)	Finishing feedlot cattle	Increased nutritional quality of venison and marbling quality.	Teixeira et al. (2019)	
N utilization, gain:feed, LTL (muscle area, moisture).	Yes	(+)	Finishing feedlot cattle	No effects on carcass and physical meat quality, similar to cervid.		
				Gain only at early 87 d, similar to cervids.		
				Potential effect for increasing venison yield and quality, especially at early supply.		

RP-Lys/RP-Lys + RP-Met	BW, feed intake.	Yes	(0)	Sika deer bucks, 5 months old	No effect on gain and intake, similar to cattle, sheep, and goat.	Huang et al. (2015b)
	Digestibility (DM, OM, CP).	Yes	(+)			
	Blood GLU, GLOB, BUN, TP, ALB, ALT, AST.	Yes	(+)			
	ADG, BW, carcass weight.	Yes	(0)	Fallow deer bucks, 11 months old	No effects on gain and carcass, like in beef cattle.	Ceacero et al. (2020)
	Dressing percentage, internal fat, kidney fat.	Yes	(+)			
	Plasma CREA, BUN, TP, ALB, GLOB, TRIG.	Yes	(+)			
	Antler burr perimeter, antler weight, length.	Yes	(+)	Fallow deer bucks, 11 months old	Improved antler growth for trophy hunting.	Ny et al. (2020)
	Cortical bone diameter & area, density, ash.	Yes	(0)		Only when controlled initial BW.	
	Antler chemical compositions.	Yes	(+)		No effects on the rigidity of antlers.	
	Antler mechanical properties.	Yes	(0)			
	BW, DMI, DM digestibility.	No	(0)	Lactating cows	No effects on gain similar to lactating sheep and goat.	Watanabe et al. (2006); Lee et al. (2015)
	ADG, ADG:DMI ratio.	Yes	(+)	Cross-bred beef calves	Potential effect for improving gain and intake of fawn.	Klemesrud et al. (2000a,b)
Milk yield, milk fat & protein yield, ECM, FCM.	No	(+)	Lactating cows, goats	Potential for improving milk yield and composition for lactating hinds to supply to the young calves.	Madsen et al. (2005); Socha et al. (2005); Watanabe et al. (2006)	
RP-Lys/RP-Lys + RP-Met	Anti-inflammatory gene expression.	No	(+)	Lactating ewes	Potential to prevent mammary gland inflammation, improve immune system, and increase milk production and quality in does.	Tsiplakou et al. (2018, 2020)
	Milk yield, milk fat, protein, and lactose contents.	No	(+)			
	6% FCM, ECM.	No	(+)			
	BHBA, plasma BUN.	No	(-)			
	DMI, BW, hip height, wither height.	No	(0)	Young cow calves	No effect on supply in young calves, but maybe maternal supplementation might have some positive effects.	da Silva et al. (2018)
	DMI, N balance.	Yes	(+)	Lambs	Potential for growth of young.	Araújo et al. (2019)
RP-Lys + RP-Met + RP-His	Faecal score, water intake, ingestive behaviour.	No	(-)			
	Carcass weight.	Yes	(+)	Cross-bred beef calves	Potential for increasing carcass yield in cervids.	Klemesrud et al. (2000b)
	DMI, milk yield, milk protein & fat yield, ECM, ECM feed efficiency.	No	(+)	Lactating cows	Potential for improving intake, milk supply and AA to young fawns during lactation.	Lee et al. (2012); Giallongo et al. (2016)
RP-Met + RP-Thre + RP-Leu + RP-Ile	Plasma (GLU, Lys, His, Met).	No	(+)			
	DMI, milk yield, milk lactose, milk protein yield.	No	(+)	Dairy cows	Important for improving intake, milk supply and AA to young during lactation.	Zhao et al. (2019)
RP-Arg/Arg/NCG	Velvet antler ADG & weight.	Yes	(0/+)	Sika deer bucks, 5-6 years old	No effect for Arg, but increase of velvet weight with RP-Arg.	Si et al. (2021a, b)
	Blood metabolites (TRIG, CHOL, HDL-CHOL, LDL-CHOL, ALB, GLU, ALP, ALT, AST, IGF-1, citrulline, orthonine, phosphoserine).	Yes	(+)			
	Rumen volatile fatty acids.	Yes	(+)		Increase of growth hormones, protein, and fat metabolites.	
	Good rumen microbes	Yes	(+)		Increase good rumen microbes for better rumen health and improve digestibility of carbohydrate and protein.	
	( <i>Fibrobacter</i> spp., <i>Prevotellaceae</i> UCG-003, <i>Bacteroides</i> spp., <i>Rikenellaceae</i> RC9, <i>Treponema</i>				Decrease rumen pathogenic microbes, thus improve gut health.	

(continued on next page)

Table 1 (continued)

RPAAs	Measured parameters	Studied in cervids	Effects <sup>1</sup>	Studies species	Comments	References
RP-Arg/Arg/NCG	2, <i>Turicibacter</i> spp., <i>Romboutsia</i> spp., <i>Alistipes</i> spp., <i>Phascolarctobacterium</i> spp.). Bad rumen microbes	Yes	(–)			
	<i>Clostridium sensu</i> 1, <i>Corynebacterium</i> 1.					
	ADG, milk yield, milk fat & protein yield.	No	(+)	Lactating goat & young	Potential for supporting milk production and compositions for supplying early growth of calves.	Wang et al. (2019)
	Rumen length and thickness of muscularis, density of papillae.	No	(+)			
	Plasma Arg, citrulline, ornithine, insulin.	No	(+)			
	Plasma ammonia, urea.	No	(–)			
	BW of ewes & lambs, foetal organ weight.	No	(+)	Pregnant ewes, foetus & kids	Potential for increasing survival of young fawns at birth and in early growth.	Zhang et al. (2016); Sun et al. (2018)
	Liver enzymatic activity (FA, cholesterol metabolism), hormones and metabolites (IGF-1, insulin, total AA, lactate, thyroxine).	No	(+)			
	Percentage of lamb born dead.	No	(–)	Gestating/lambing ewes	Important for increasing survival rate of young at birth.	Lassala et al. (2011)
	Percentage of lamb born alive, lamb birth weight.	No	(+)			
	Maternal plasma AA, ornithine, Cys, Pro.	No	(+)			
	Ewe intake, BW, plasma AA and metabolites.	No	(0)	Gestating/lambing ewes	Potential for supporting birth survival and postnatal growth of young fawns, especially females.	McCoard et al. (2013)
	Foetal brown fat store, female lamb weight.	No	(+)			
	Foetal BW, body dimension, organ weight.	No	(0)			
	Foetal plasma insulin, IGF-1, GLU, TRIG.	No	(+/0)			
Sperm mass activity, motility, concentration, membrane integrity.	No	(+)	Rams	Important for improving reproduction in bucks for breeding & hunting stags.	Kaya et al. (2019)	
BCS, oestrus presentation, multiple ovulations, prolificacy, fertility of synchronized oestrus.	No	(0)	Ewes in oestrus	Potential for increasing reproduction in does.	De Chávez et al. (2015)	
Fertility to synchronized oestrus.	No	(+)				
RP-Chol	Milk yield, 4% FCM, protein & fat yield, liver function, lipoprotein and fat metabolism in mammary gland.	No	(+)	Lactating Saanen goats	Important for supporting milk production and composition for improving early growth of calves.	Pinotti et al. (2008); Baldi et al. (2011); Pinotti (2012); Pinotti et al. (2020)
RP-Bet	Milk yield, milk fat and short chain FAs.	No	(+)	Lactating goats	Potential for supporting milk production and composition in does.	Fernández et al. (2009)
	ADG, BW, feed intake.	No	(+)	Lambs	Improve feed intake and gain.	Dong et al. (2020)
	LTL shear force, water loss, SFA.	No	(–)		Important for balancing UFAs and SFAs and increase yield and quality of venison.	
	pH <sub>24</sub> , eye muscle area, UFA, UFA/SFA ratio, free AA, Ile, Phe, in LTL muscle.	No	(+)			
	Wool growth rate & yield, staple length.	No	(+)	Lactating ewes	Potential effect for growth and quality of pelage/coat.	Nezamidoust et al. (2014)

RP-Tryp	ADG, feed efficiency, N retention, cashmere length and growth rate.	No	(+)	Cashmere goats	Important effect for growth and quality of pelage/coat and velvet hair.	Ma et al. (2010, 2011)
L-Leu/L-Phe/L-Leu + L-Phe	Pancreatic cell growth, protein content, plasma insulin, cholecystokinin, trypsin gene expression. Activity of trypsin, lipase, $\alpha$ -amylase, DM digestion.	No	(+)	Holstein calves	Important for improving growth of digestive organ and its enzymatic production in young.	Cao et al. (2018)
		No	(+)	Yearling ewes	Potential effect for protein and carbohydrate digestion in cervid.	Yu et al. (2014)

RPAA = rumen-protected amino acid; GLU = glucose; CHOL = cholesterol; CREA = creatinine; SGPT = glutamic pyruvate transaminase; SGOT = serum glutamic oxaloacetic transaminase; BCS = body condition score; SFA = saturated fatty acid; LTL = longissimus thoracis et lumborum; ST = semitendinosus; IMF = intramuscular fat; GLOB = globulin; BUN = blood urea nitrogen; TP = total protein; ALB = albumin; ALT = alanine aminotransferase; AST = aspartate aminotransferase; TRIG = triglycerides; ECM = energy corrected milk; FCM = fat corrected milk; BHBA =  $\beta$ -hydroxybutyric acid; NCG = N-carbamoylglutamate; HDL-CHOL = high-density lipoprotein cholesterol; LDL-CHOL = low-density lipoprotein cholesterol; IGF-1 = insulin-like growth factor 1; UFA = unsaturated fatty acid.

<sup>1</sup> (-): negative effects; (0): no effect; (+): positive effects.

meat quality of the animals from this same study, analysing the chemical composition (proximate composition, AA, and fatty acids) and sensorial quality of grilled LTL muscles. Intramuscular fat, which contributes to the juiciness and tenderness of meat, was found to be higher in the meat from the barley, and barley plus RP-Lys, supplemented groups, compared to the pasture-fed group. In contrast, the only-pasture-fed group produced meat higher in n-3 polyunsaturated fatty acids. RP-Lys supplementation increased the essential and non-essential AA contents in the LTL muscle compared to the other nutritional treatment groups (His, Leu, Ala, Glu, and Gly). There were few differences in the sensorial attributes of the LTL muscles, but the only-pasture-fed group produced meat with a higher grassy flavour score, which is not considered favourable for some consumers. Still, RP-Bet, RP-Lys and RP-Met have been suggested to improve meat fatty acid profiles in lambs (Araújo et al., 2019; Dong et al., 2020), and should be further studied in cervids considering the links of meat fatty acid composition with consumer health.

Only RP-Lys and/or RP-Met supplementation has been investigated in cervid nutrition, the supplementation of which increased body weight, feed intake, fat storage, carcass and meat quality, especially when supplemented at an early age and under poor nutritional conditions. This data is still limited and is not adequate for formulating AA inclusion levels in cervids based on different production stages and purposes. However, based on the current findings, supplementation of RP-Met at a level of 0.15 to 0.18 g/kg MBW per d, and/or RP-Lys at 0.23 or 0.55 g/kg MBW per d, can be potentially used to improve the growth performance and meat quality of cervids for venison production. A summary of the reported and potential effects of RPAA in venison production, in comparison with the other ruminants, is presented in Table 1.

Further research should also focus on growth effects mediated by milk production, which has not been studied in cervids yet. Nevertheless, RP-Lys, RP-Met and RP-His supplementation have been found to increase milk yield and compositions in dairy cows (Lee et al., 2012), especially under low protein diets, and RP-Chol and RP-Bet increased milk yield and compositions in goats (Baladi et al., 2011; Pinotti, 2012; Pinotti et al., 2008, 2020).

#### 4.2. Velvet

The antler growth period is the most critical time in terms of the dietary protein and AA requirements for cervids, especially for the first antler growth of yearlings. Cervids require high dietary protein levels of up to 16% to 22% during antler growth and approximately 11.5% for initiating pedicle development (Puttoo et al., 1998; Dryden, 2011). Cervids also need to reach a minimum threshold body weight to start pedicle growth (Fennessy and Suttie, 1985). The main reason for this high nutrient demand during velvet growth is because it is formed by a cartilage matrix made up of collagen fibres and covered by a rich blood vessel dermis (Goss, 1983; Price et al., 2005; Jeon et al., 2011). Therefore, the composition of velvet antlers is very high in protein and AAs, especially at the top of the antlers (Sunwoo et al., 1995; Jeon et al., 2011). Thus, AA can both directly and indirectly influence velvet antler growth, supporting the cartilage matrix and affecting the levels of different hormones involved in antler growth (Chapman, 1975). Lysine is a good candidate for promoting velvet antler growth, due to its contribution to collagen formation (hydroxylysine) and its role as a precursor of bone tissue (McDonald et al., 2011). Methionine is also indirectly involved in the development of muscles and onset of maturity in animals, which is associated with the initiation of antler growth driven by sex hormones (Li and Suttie, 2001). Regarding hormonal regulation, the already-described effect of dietary protein and AAs on body weight and BCS affects testosterone levels,

and this in turn affects antler growth (Gaspar-López et al., 2010), specifically the start of the antler cycle and the intensity of mineralization (Ceacero et al., 2019). The production and activity of IGF-1, which is the hormone responsible for promoting cartilage growth, is reduced when dietary protein is not adequately supplied (Suttie et al., 1985; Bonjour et al., 2001). Parathyroid hormone (PTH) is the third most relevant hormone for antler development, through its role in regulating serum calcium concentration. There are no specific studies testing the effect of protein or AAs supplementation on PTH, but Sun et al. (2019) found no effects of protein digestibility on PTH levels.

PTH influences bone remodelling; despite such importance, supplementing AA to cervids for velvet antler development is an almost unexplored research area. The first study of this kind was recently published by Si et al. (2021a), investigating the effect of Arg supplementation on velvet antler growth in sika deer bucks. No effects of Arg were found on velvet weight, average daily body weight gain, or volatile fatty acids (VFAs) concentrations in the rumen. However, the supplementation of Arg at 0.16 g/kg MBW per d increased serum IGF-1, compared to the 0.08 g/kg MBW per d group and the control group, which is the most important hormone involved in promoting antler growth. The level of ALT and AST in blood serum decreased in Arg-supplemented groups, indicating an improvement of liver functionality, and hence, stimulating the production and secretion of IGF-1. This study also shows the efficient use of Arg by the supplemented groups, through increasing serum citrulline and orthenine, which are the intermediate AA used to synthesize Arg in the urea cycle. This leads to the decrease of ammonia concentrations and increased utilization of Arg. Arg also improved rumen gut health and digestion; for instance, Arg improved growth and population of *Fibrobacter* spp., and Prevotellaceae UCG-003 which are important for carbohydrate and protein metabolism and decreasing harmful rumen microbes like *Clostridium sensu 1* and *Corynebacterium 1*. The authors also suggested that Arg supplementation may reduce serum Lys levels, which may explain the lack of effect on velvet antler weight. Through supplementing relatively lower levels of Arg supplemented in the rumen-protected form (RP-Arg at a level 0.03, 0.06, or 0.08 g/kg MBW per d), Si et al. (2021b) found increased final velvet antler weight and velvet ADG compared to the control group, and the highest weight was realized in the group fed RP-Arg at the level of 0.08 g/kg MBW per d. Moreover, RP-Arg supplementation linearly decreased plasma ALT and AST concentrations, while linearly increasing the levels of plasma glucose and triglyceride, which can be due to the improvement of liver functioning regarding carbohydrate metabolism. RP-Arg linearly increased gut microbiota concentrations (*Bacteroides* spp., Rikenellaceae RC9, *Treponema 2*, *Turicibacter* spp., *Romboutsia* spp., *Alistipes* spp., and *Phascolarctobacterium* spp.), which play an important role in carbohydrate and fibre metabolism, particularly in pyruvate, propionate, and butyrate metabolism pathways. However, an important bacterium in carbohydrate and nitrogen metabolism and fibre degradation, *Prevotella* spp., was the most abundant in the control group compared to the RP-Arg groups. RP-Arg groups seem to utilize Arg more efficiently, evidenced by an increase in plasma citrulline and orthenine, which are intermediate AAs that can be used to synthesize Arg in the urea cycle. Moreover, there was a decrease in plasma urea concentration and increased in plasma Arg concentration in the RP-Arg group. This result indicates the potential of RP-Arg in supporting velvet production of adult sika deer. However, further investigation into the aspect of the gut microbial effects and liver functioning in young calves may provide promising results, through the support of early digestive tract and organ development and functioning.

#### 4.3. Trophy hunting

Protein and AA nutrition can also benefit trophy hunting, as it can improve antler quality, body weight and condition, and the reproductive performance of stags. The whole antler growth process is important for the production of hard antlers, especially in young animals, for the initiation of pedicle formation, and during early antler growth (Goss, 1983; Dryden, 2016). Beside breeding management to achieve good quality trophy stags, good nutrition must be considered since the prenatal and early growth stages. Some studies have demonstrated that good nutrition during the early growth phase, especially protein and AA, can advance the initiation of antler growth, and can promote better growth of future antlers (especially the number of antler points) (French et al., 1956; Puttoo et al., 1998). For instance, red deer fawns suckling milk rich in proteins have also shown to have a higher spike weight (Gómez et al., 2006).

As mentioned by Dryden (2016), an adequate AA supply is more crucial than the amount of protein for antler growth in cervids. Only a few studies have focused on AA supplementation for antler growth. Mendoza-Nazar et al. (2012) found a quadratic effect of RP-Met (0.08, 0.12, or 0.15 g/kg MBW per d) on the antler beam length of adult red deer (but this study is limited by the number of animals per group). Thus, Ny et al. (2020) conducted two consecutive studies regarding the supplementation of RPAA to fallow deer. The authors found that the first experiment supplementing 0.39 g/kg MBW per d of RP-Lys did not improve any antler characteristics. However, there was a marginal effect of RP-Lys on the antlers' chemical composition, after including the initial body mass in the statistical analyses. In the second experiment, RP-Lys level was increased to 0.55 g/kg MBW per d, and a combination treatment of RP-Lys plus RP-Met (0.18 g/kg MBW per d) was used. The combination of RPAA improved the external antler characteristics, especially the burr perimeter and antler weight, which is important not only for the first antler growth but also for the future antler cycles (Chapman, 1975). The authors concluded that the use of RPAA shows potential for improving antler growth, particularly when using Lys and Met combined. Stronger effects were found when animals were in a worse general body condition, showing the benefits of RPAA as a complementary feeding source.

In the later stage of growth, stag body weight and antler size (weight) are important factors for trophy production, because the bigger the stag (BW), the larger the antler size (weight) predicted (Huxley, 1931; Dryden, 2016). Moreover, cervids need to recover to a good body condition at the casting stage for antler growth in the next seasons. Therefore, protein intake for replenishing the loss in body growth and weight during the breeding season is very important for stags (Dryden, 2016). The sooner the young animals reach puberty, and the better body condition they have, the better the quality of the trophy achieved. The two studies currently available on young sika calves by Huang et al. (2015a, b) showed that RP-Met and RP-Lys improved weight gain and nutrient utilization, particularly at the early stage of development.

Another relationship of body weight and body condition on trophy production is through reaching maturity, thus affecting steroid hormone and peptide hormone production and antler growth (Suttie et al., 1995; Li and Suttie, 2001; Price et al., 2005). Bartoš et al. (2012) reviewed the crucial roles of testosterone in triggering antler growth, by stimulating and increasing antler bone mass. This can also influence the hierarchy of animals, in terms of getting access to further resources, and thus reaching the necessary body weight for antler initiation. Moreover, as excess protein and AA can be used as an energy source (Ceacero et al., 2020), it can indirectly improve sexual development for male



cervids by reducing their negative energy balance. This is important for reproduction, especially for the breeding selection of stags (Ros-Santaella et al., 2019). However, excess feeding of degradable protein can cause a higher production of ammonia released into the rumen, through the process of deamination by bacterial enzymes. High ammonia concentrations can be diffused from the rumen into peripheral circulation, and can affect endocrine functioning (Kaur and Arora, 1995). Thus, the direct supplementation of only EAA to improve endocrine hormones and reproduction is a better solution. For instance, Lys can improve spermatogenesis, as it is involved in the Lys acetylation during this process (Pang and Rennert, 2013). Arginine is a building block of the nucleoprotein in sperm during spermatogenesis, and improves sperm functionality, vitality, and mobility, which are important for reproductive success (Hegazy et al., 2021). In addition, Arg increased IGF-1, which is an important regulator in antler growth as well (Si et al., 2021a, b). Some sulphur-containing AA, such as Met and Cys, are also important in the methylation process in early spermatogenesis (Menezo et al., 2020).

#### 4.4. Conservation

All the above-mentioned benefits of AA supplementation can also apply for the conservation of endangered cervid species, particularly for the support of pregnant females, improving survival of their young, aiding in breeding management, and supporting general early calf growth. In caribou, it was shown that nitrogen is deposited in the foetus for winter survival, and thus if the maternal reserves of N are insufficient, there may be reduced mass of calves at birth, leading to poor survival rates (Parker, 2003). Methionine is important in milk composition, supporting early growth of the young (Ali et al., 2009). As observed in sheep, RP-Arg or NCG may improve maternal health and support the prenatal growth of foetuses (Zhang et al., 2016; Sun et al., 2018). Therefore, Met can increase the survival rate of those endangered species, especially under limited nutritional resources. Moreover, early growth of the young fawns is also critical, and will affect the future growth and reproduction in cervids. The study by Huang et al. (2015a, b) also proved that the supplementation of RP-Met (0.15 or 0.16 g/kg MBW per d, RP-Lys (0.23 g/kg MBW per d), or combined, improved nutrient digestibility, weight gain, and general growth of young fawns.

Some AA may be involved in reproduction performance, improving the breeding success of endangered cervids. For instance, Arg increases sperm quality and supports reproductive health in both male and female animals (De Chávez et al., 2015; Kaya et al., 2019; Hegazy et al., 2021). Moreover, genetic selection for successful breeding is not a common practice for those endangered species, due to the lack of knowledge of their biology and ecology in general. Hence, complementary AA enrichment is a potential solution to improving reproductive success. Moreover, as excess dietary AA or protein will be used as energy source, it could reduce negative energy balances and indirectly affect the sexual development of males. This is important for reproduction, especially for breeding for conservation programs (Ros-Santaella et al., 2019). All studies of AA in cervid nutrition and their related effects are summarized in Table 1.

Despite the limited data, the studies on fallow deer by Kudrnáčová et al. (2019), Bureš et al. (2020), Ceacero et al. (2020), and Ny et al. (2020) following the inclusion 3:1 (Lys:Met) found interesting improvements in growth performance and production. However, more detailed, and inclusive studies need to be carried out extensively in different species, stages of growth, and especially under typical deer farming conditions.

## 5. Conclusions and recommendations

Amino acid supplementation has shown to be a valuable tool for improving nutrition of farmed cervids through improving weight gain, BCS, ADG, feed-to-gain ratio, plasma AA concentrations, carcass weight, dressing percentage, yield of high-value muscles, storage of internal fat during winter, DM and CP digestibility, plasma protein- and fat-related metabolite concentrations, antler burr perimeter, weight, length and mineralisation, velvet antler yield, rumen VFA concentrations, and microbiome composition. All these effects are relevant for supporting the quality and yield of different cervids products, from venison to velvet or trophy antlers, and the general performance and well-being of cervids for ecotourism or conservation purposes. On the other hand, these positive results are not always clear and thus it is not possible to recommend its generalised use, which would be probably economically unsustainable. The supplementation of different AAs has interesting effects, especially in animals fed low protein rations, and in growing animals with high nutritional demands. However, the supplementation of AAs should be well-adjusted to the MBW, since high inclusion rates may activate fat deposition resulting in negative effects on carcass quality. Similarly, supplementation of AAs during winter should be avoided, since the resource will be used mainly for increasing body fat stores. Further research should focus on promising but unexplored areas, like the effects on milk production and the direct effects on hormonal levels involved in the regulation of the antler cycle.

### Author contributions

**Veit Ny:** Conceptualization, Resources, Writing - Original Draft, Writing - Review & Editing; **Tersia Needham:** Resources, Writing - Review & Editing, Supervision; **Francisco Ceacero:** Conceptualization, Resources, Writing - Review & Editing, Supervision, Project administration, Funding acquisition.

### Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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