CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

Faculty of Tropical AgriSciences

Department of Animal Sciences and Food Processing

Influence of selected amino acids on the performance of cervids

Ph.D. Dissertation thesis

Prague 2023

Author: Ing. Veit NY

Supervisor

doc. Francisco Ceacero Herrador, Ph.D.

Consultants

Ing. Luděk Bartoň, Ph.D.

doc. Tersia Needham, Ph.D.

DECLARATION

I, Veit NY, hereby declare that I have written the enclosed PhD thesis entitled "Influence of selected amino acids on the performance of cervids" independently and in collaboration with co-authors in the respective scientific articles related to this work. All the texts in this thesis are original, and all the sources have been quoted and acknowledged by means of complete references and according to the Citation rules of the FTA. I state that the work has not been submitted for any other degree to this or any other university within and outside the Czech Republic.

In Prague,……

Ing. Veit NY

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to my family support both biological family and the adopted one and the same to the SOS orphanage in Cambodia. I would not be able to go to school to reach my position these days without support from this organization, in the first place. Their contribution to my education is priceless. I wouldn't be who I am today without motivation and pushes from my adoptive mother, **Pok Srey Rein**, who sacrificed her time and effort taking care of all orphans including me among other twenty children. Her work is beyond described.

My warmest acknowledges to my supervisor, **doc. Francisco Ceacero Herrador, Ph.D.**, who has helped me during my whole study with his intellectual supervision at all kinds, let alone the success of my master with him as well. He is the main guidance from the conceptualization of the research to my success with all scientific publication, especially his big contribution with statistics nightmare of my data analysis. I would like to also thanks the co-supervisor, **doc. Tersia Needham, Ph.D.,** for all her guidance both in research and as friend's support. It has been a big motivation for me. I would like to warmly thanks the other co-supervisor as well as my boss, **Ing. Luděk Bartoň, Ph.D.,** from the Institute of Animal Science for the motivation and advice and connection to some financial support for me working as research assistant there. From the same institution, I am grateful for my colleague, **Ing. Daniel Bureš, Ph.D.** who has guided and help with my research especially related to meat science and statistic parts. My equal thanks also to **Ing. Radim Kotrba, Ph.D.,** who has helped with technical communication with deer farmers and issuing ethical permits necessary for the experiments. My research cannot carry out without this help. The same reason for me to thanks to **Mr. Pavel Friedberger** and his family and staffs for cooperating and assisting at the deer farm which he allowed us to have access to the animals and using facility. My special appreciation to the Food Research Institute Prague, especially to **Ing. Milan Houška, CSc.,** for having me as a research assistant for my financial support since the beginning of my study up to date. Once again, my equal appreciation to the Institute of Animal Science.

For the research financial support, I would like to express my gratitude to the continuous support from the internal grant agency of the Faculty of Tropical AgriSciences and the Ministry of Agriculture of the Czech Republic. My appreciation also for the sponsor of amino acid additives for my experiments from the **VVS Vermerovice s.r.o.** feed company.

I would like to thanks to all PhD colleagues and friends who helped assisting my data collection and some parts of laboratory work such as **Abubakar Sadiq Musa, Jericó C.**

Mituda, Nicole Lebedová, Thoniso Chitambala, Hajra Munir, Stipan Čupić, Kamila Pokorná, Jana Marešová, and Jiři Turek.

Last but not least, to all the given opportunities and supports, I hope my work and achievements make them proud, especially with contribution back to those organizations mentioned above.

Photos by the author of some field work and laboratory work with research team.

ABSTRACT

Venison, velvet, trophies, or antlers are important products of deer farming, especially from male cervids. Like other ruminant livestock, improving nutrition and welfare not only improves animal well-being, but typically also animal product yield and quality. One of the key nutrients for animals is amino acid (AA), the targeted supplementation of which has proved to be beneficial in improving growth and production performance (meat, milk, hair, etc.) in cattle, sheep, and goats. Optimising AA nutrition in deer is still a novel topic. Only a few studies, especially regarding Lysine (Lys) and Methionine (Met), have shown the potential uses of AA supplementation in improving nutrient digestibility, growth performance, antler production, carcass traits, blood biochemical markers, and gut health. All of these effects can contribute to supporting the production of cervids products, from venison to velvet or trophy antlers, as well as the general performance and well-being of captive-bred cervids. Improved welfare management in male livestock by immunocastration (IC) also adds value to animal well-being and product quality, especially by reducing aggressiveness, easing handling, reducing production losses (carcass bruising from fighting), reducing objectionable meat odours linked to androgens etc. Because IC supresses androgen production, and has consequences on muscle and fat metabolism, it should influence the AA requirements.

The effects of ruminally protected AA (RPAA) supplements, Lys and Met, were examined at an inclusion rate of 3:1 (3 Lys: 1 Met) and based on metabolic body weight, from several experimental settings in real deer farming practices, utilising a large number of yearling male fallow deer (≈ 10 - 15 deer per group). IC was also incorporated into one of the experiments to see the effect of AAs under the anabolic suppressive influence of IC. The studied parameters include growth performance such as body weight, body condition, average daily gain (ADG), antler and bone growth, and carcass performance. On an important note, there were challenges and limitations in this study as the same as common Central Europe deer farming practices for controlling feeding behaviour, feed intake, pasture availability and quality.

However, from the series of experiments performed we found that the supplementation of RP-Lys and RP-Met improved antler weight and burr perimeter, especially under a low-protein diet regime. The AA supplementation during winter increased internal fat storage, evidenced also in increased plasma fat biochemical markers. The supplementation of RP-Lys and RP-Met also increased carcass performance (carcass dressing percentage).

Immunocastration, which suppresses testosterone production that plays an important role in antler and bone development, decreased antler weight, with a thinner cortical bone layer, and less mineralisation compared to entire (non-castrated) bucks. Therefore, AA supplementation can improve deer production, especially for growing animals and when low protein diets are utilised. Care needs to be taken to avoid high levels of supplementation during winter, as it will be used as fat storage. Potential effects of RPAA on gestation, milk production, and long-term antler growth still need to be explored. Furthermore, the doses of RPAA and vaccination schedules of IC on different hormones involved in regulating the antler cycle should be further studied. To some extent, aggressivity and sex behaviour observation should also be monitored in combination with androgen hormone changes by these treatments.

Keywords: Antler; Blood biomarkers; Body condition; Fat deposition; Immunocastration; Lysine; Methionine; Venison

List of tables

Table 1 Summary of chapters, experimental settings, and feeding treatments utilised within the studies conducted for the thesis.

Table 2 Proven effects of supplementary amino acids on cattle, sheep and goat, and its potential applications in cervid nutrition.

Table 3.1 Nutritional composition of the silage, barley and free choice mineral supplement used during the study.

Table 3.2 MANOVAs showing lack of significant differences of diet supplementation with ruminally protected Lys (mean value), on antler the characteristics of fallow deer.

Table 3.3 Multivariate General Linear Models showing the effect of body mass at the beginning of the experiment and treatment (different diet supplementation regimes; see text) on the antler characteristics of fallow deer yearlings in the first experiment described (2015).

Table 3.4 MANOVAs showing significant differences of diet supplementation (mean value), on the antler characteristics of fallow deer yearlings.

Table 3.5 Multivariate General Linear Models showing the effect of body mass at the beginning of the experiment and treatment (different diet supplementation regimes; see text) on the antler characteristics of fallow deer yearlings in the second experiment described (2016).

Table 4.1 Nutrient composition of feedstuffs used during the experiment.

Table 4.2 Mean (SD) values and significant differences (one-way ANOVA) in growth performance of fallow deer (*Dama dama*) supplemented with Lys and Met during the fattening period, and culled on different seasons: LA (n = 18) and LW (n = 27).

Table 4.3 Generalized linear mixed models explaining growth parameters of fallow deer (*Dama dama*) supplemented with Lys and Met during the fattening period, and culled on different seasons.

Table 4.4 Generalized linear mixed models explaining body condition fallow deer (*Dama dama*) supplemented with Lys and Met during the fattening period, and culled on different seasons.

Table 4.5 Generalized linear mixed models explaining blood plasma metabolites concentration in fallow deer (*Dama dama*) supplemented with Lys and Met during the fattening period, and culled on different seasons.

Table 5.1 Mean chemical compositions of the pasture and concentrate supplemented to fattening fallow deer bucks.

Table 5.2 General Linear Model showing the combined effects of immunocastration, supplementary feeding and their interaction on growth patterns of fattening fallow deer bucks measured at slaughter. Statistical differences of the pairwise comparison for the effect of diet within the control (Co) and immunocastrated (IC) groups are also shown.

Table 5.3 Generalized Linear Mixed Models showing the effects of immunocastration, supplementary feeding and their interaction on blood biochemistry markers of fattening fallow deer bucks measured along the experiment (1- start, 2- week 3 and at 3- culling). Statistical differences of the pairwise comparison for the effect of diet within the control (Co) and immunocastrated (IC) groups are also shown.

Table 6.1 Chemical compositions of the pasture and concentrate supplemented to fattening fallow deer bucks.

Table 6.2 Pearson's correlation coefficients (*r*) among the characteristics of the base of the antler.

Table 6.3 Pearson's correlation coefficients (*r*) among the characteristics of the top of the antler.

Table 6.4 Pearson's correlation coefficients (r) between the characteristics of the metatarsus.

Table 6.5 Average values and standard error (s.e.) recorded for each group for the studied variables from the antler base (AB), antler top (AT) and metatarsus (Met).

Table 6.6 Multivariate GLMs showing the effects of the supplementation of amino acids, immunocastration and their interaction on antler and metatarsus characteristics in fallow deer bucks.

List of figures

Figure 4.1 Box-plot of the studied body condition parameters (BCS, KFI and internal fat) after the fattening period of fallow deer (*Dama dama*) culled on different season: LA (*n= 18*) and LW (*n= 27*). Independent samples Student's t tests showed differences for BCS (*P*= 0.006) and KFI (*P*= 0.012). Mann–Whitney *U* test found no differences in internal fat depots (*P*= 0.729). BCS = body condition score; KFI = kidney fat index; LA = late autumn; LW = late winter.

Figure 5.1 The immunocastration vaccination schedule, blood sampling, feed supplementation period, and data collection points from the study onset (week 1) until the slaughter (week 17/18).

Figure 5.2 Interaction of castration and diet on blood ALB concentrations of immunocastrated and control (non-castrated) fattening fallow deer bucks (n=28). High- High level of supplementary feeding; Low- Low level of supplementary feeding. Error bars indicate the standard deviation of the mean values.

Figure 5.3 Interaction of castration and diet on blood GLOB concentrations of immunocastrated and control (non-castrated) fattening fallow deer bucks (n=28). High- High level of supplementary feeding; Low- Low level of supplementary feeding. Error bars indicate the standard deviation of the mean values.

Figure 6.1 Plots showing the variations in bone density across the transversal axis. Note the sharp transition between bone and marrow in the metatarsus sample (top graph) but the smooth transition between the cortical and trabecular bone in the antler top (middle graph) and base (bottom graph)

List of abbreviations

AA	amino acid (s)
ADG	average daily gain
ALB	albumin
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
Bet	betaine
BCS	body condition score
BW	body weight
BHBA	β-hydroxybutyric acid
BUN	blood urea nitrogen
CHOL	cholesterol
Chol	choline
CP	crude protein
CREA	creatinine
DM	dry matter
DMI	dry matter intake
EAA	essential amino acid (s)
ECM	energy corrected milk
FCM	fat corrected milk
GLOB	globulin
GLU	glucose
HDL-CHOL	high-density lipoprotein cholesterol
IGF-1	insulin-like growth factor
IMF	intramuscular fat
LDL-CHOL	low-density lipoprotein cholesterol
LTL	longissimus thoracis et lumborum
MBW	metabolic body weight
NCG	n-carbamoylglutamate
PTH	parathyroid hormone
PUFA	polyunsaturated fatty acid (s)
RPAA	ruminally protected amino acid

(s)

ST	semitendinosus
SFA	saturated fatty acid
SGOT	serum glutamic oxaloacetic transaminase
SGPT	glutamic pyruvate transaminase
TP	total protein
TRIG	triglycerides
UFA	unsaturated fatty acid (s)
VFA	volatile fatty acid (s)
WBSF	Warner-Bratzler shear force

Table of contents

CHAPTER 1

General introduction……..1

1.1 Introduction……1

1.2 The aims of the thesis……3

1.3 Chapter overview……....5

CHAPTER 2

Literature Review…….....9

2.1 Potential benefits of amino acid supplementation for cervid performance and nutritional ecology……11

2.1.1 Overview and importance of the captive breeding of cervids…….....13

2.1.2 Cervids' protein nutrition……15

2.1.3 Role of amino acids in improving ruminant performance……...15

2.1.4 Amino acid supplementation and performance of cervids……..16

2.1.5 Conclusions and recommendations…….....29

2.1.6 References…..…….....29

2.2 Immunocastration and consequences on AA requirements: a novel management tool for male cervids……37

2.2.1 Castration and welfare management……37

2.2.2 Immunocastration and welfare management……...37

2.2.3 Immunocastration and livestock production……38

2.2.4 Application of immunocastration in cervids……39

CHAPTER 3

Effects of Lysine and Methionine supplementation on first antler growth in fallow deer (*Dama dama*)…….41

3.1 Introduction……...45

3.2 Materials and methods……..47

3.2.1 Experimental design……47

3.2.2 Samples and data collection…….50

3.2.3 Antler analyses…….50

3.2.4 Statistical analysis……51

3.3 Results……...53
3.4 Discussion…….53
3.5 Conclusions……...57
3.6 References…….58

CHAPTER 4

Differential effects of ruminally protected amino acids on fattening of fallow deer in two culling periods……...61
4.1 Introductions……..65
4.2 Materials and methods……..67
4.2.1 Study area and experimental animals……..67
4.2.2 Diet and treatments……..67
4.2.3 Data collection…….68
4.2.4 Statistical analyses……...71
4.3 Results……...71
4.4 Discussion…….77
4.5 References…….80

CHAPTER 5

Effects of immunocastration and supplementary feeding level on the performance and blood biochemical markers of farmed yearling fallow deer (*Dama dama*)…….83
5.1 Introduction……...87
5.2 Material and methods……89
5.2.1 Animals, diets and experimental design……..89
5.2.3 Sample collection and analysis……91
5.2.4 Statistical analysis……92
5.3 Results……...93
5.4 Discussion…….99
5.5 Conclusions…….103
5.6 References……...104

CHAPTER 6

Combined effects of supplementation of amino acids and immunocastration in first antler growth of farmed fallow deer (*Dama dama*)…….109
6.1 Introductions……113

6.2 Material and Methods……..115
6.3 Statistical analyses……...119
6.4 Results…….119
6.5 Discussion……...125
6.6 References……...128

CHAPTER 7
General discussion……..133
CHAPTER 8
Overall conclusions……139
CHAPTER 9
General references…….141
CHAPTER 10
Curriculum Vitae……...151

CHAPTER 1

General introduction

1.1 Introduction

Deer farming of male cervids are important, as only male deer produce antlers and typically more males are slaughtered for venison as less are required for breeding replacement animals (Serrano et al. 2019). Improving nutrition and feeding, especially dietary protein (Dryden 2011), to maximise production yields and quality are crucial (Volpelli et al. 2002; Phillip et al. 2007; Hutchison et al. 2012). Deer require high dietary protein levels (16% to 22%) for production purposes, such as pregnancy, lactation, and antler growth (Dryden 2011). Instead of feeding high dietary protein levels, AA supplementation is important, especially with low protein diets, for improving product performance in many ruminant livestock, including cervids (Dryden 2011; Ny et al. 2022). Moreover, unlike monogastric animals, AA supplementation in ruminants is recommended in the rumen-protected (RP) form, and the most commonly known limiting / co-limiting or essential AAs are Lys and Met, and to some extent His and Arg (Kung & Rode 1996; D'Mello 2003). Many studies have found improvements in animal performance when using AA supplementation, especially the effects of RP-Lys and/or RP-Met and/or RP-His on improving milk yield and quality in dairy cattle and the growth and development of young cows (Lee et al. 2012; Giallongo et al. 2016; Alharthi et al. 2018; Zhao et al. 2019). Moreover, AA supplementation also improves meat quality, wool and hair production, and reproductive performance in cattle, sheep and goats (Ma et al. 2010, 2011; Kaya et al. 2019; Teixeira et al. 2019; Dong et al. 2020). In deer nutrition, as reviewed by Ny et al. (2022), the few studies on RPAA supplementation in cervids have shown positive effects on weight gain, 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. Moreover, with regards to the benefits of deer utilization and the potential effects of AA supplementation on improving production performance (proven in other ruminants and to a limited extent in deer), further exploration of the use of these feed additives in nutritional ecology is motivated.

As nutritional responses are affected by the physiological state of animals, which can be also influenced by welfare management, the nutritional responses of deer to AA

supplementation under commercial deer farming conditions needs to be well-considered before routine implementation (Mattiello 2009). In deer farming, especially in intensive systems, welfare issues include injuries during handling, housing, and slaughtering, playing an important role in product performance (Mattiello 2009; Serrano et al. 2020). Keeping deer in high densities, especially male deer for venison production, is problematic regarding agonistic interactions (aggressiveness), social stress, or fighting for resources (Mattiello et al. 1997). It also causes issues for breeding females and their young calves if not separated from aggressive males or if they are not well-habituated to handling (Bartošová et al. 2012; Ceacero et al. 2014). These issues can supress growth performance, and diminish meat quality (carcass bruising after slaughtering, low ultimate pH of muscles, dark firm and dry meat, etc) (Mattiello 2009; Serrano et al. 2019, 2020).

In other livestock, castration has been used to reduce aggressive and agonistic behaviours via reducing androgen hormones, thereby also improving various meat quality issues (Stafford & Mellor 2005). Traditional physical castration negatively effects animal welfare because of pain, stress, and issues with post-operative infection, and thus can be considered as ethically unacceptable (Needham et al. 2017a; Palmer et al. 2018). Immunocastration is a welfare-friendly alternative and is effective in controlling agonistic behaviour whilst improving various meat quality issues in pigs, sheep, goats, cattle, and deer (Claus et al. 2007; Needham et al. 2017a; Ahmed et al. 2022; Zeng et al. 2022; Lincoln et al. 1982; Curtis et al. 2008). In cervids, IC is mainly used to control deer populations (Miller et al. 2000a,b; Curtis et al. 2002; Palmer et al. 2018). However, IC is not well-explored in commercial deer production in terms of its effects on welfare improvement and production performance, despite its potential effects have been well studied in males of other species. Moreover, the use of IC is also interesting for venison production because of its effects on protein and fat metabolism (Claus et al. 2007), altering nutrient responses (particularly regarding dietary protein), and carcass and meat quality (Needham et al. 2016, 2017a,b).

This thesis explores the potential effects of RPAA supplementation on the production performance of yearling fallow deer bucks under commercial deer farming conditions for venison production. The potential effects of RP-Lys and/or RP-Met supplementation (with and without immunocastration) on growth performance (such as weight gain and body condition), physiological responses (including plasma protein and fat biochemical markers) and product performance (first antler, metatarsus bone, carcass performance) were studied.

1.2 The aims of the thesis

The aim of this research was to thoroughly study the effects of RP-Lys and RP-Met supplementation on the production performance and product quality of farmed yearling fallow deer bucks, with or without immunocastration. To achieve this aim, the following objectives were included:

1) To evaluate the effect of RP-Lys and/or RP-Met on the general growth performance (body weight, body condition) and carcass performance (carcass weight, dressing percentage).
2) To analyse the physiological effects of RPAA supplementation through selected blood protein and fat biochemical markers.
3) To determine the effects of RPAA on first antler growth.
4) To evaluate the combined effects of RPAA with IC on the growth performance, protein and fat blood biochemical markers, carcass traits, and antler and bone growth.

1.3 Chapter overview

The thesis consists of five published papers. The research outputs are linked together, and within the scopes of aims and research problem statements, as presented in Chapters 2-6 below. Furthermore, all research settings are summarized in Table 1.

Deer keeping and utilization, either for farming or for other purposes, are described in **Chapter 2**. The review also thoroughly summarises the effects of AA supplementation on other ruminant livestock (cattle, sheep, goats) as well as the limited information available for deer, to highlight the need for deeper exploration into its application in cervid nutrition (especially Lys and Met) (Ny et al. 2022). The nutritional response to dietary protein/AA supplementation as influenced by immunocastration, as a novel management tool for male cervids, is also briefly discussed.

The first experiment conducted within this project focused on the effects of RP-Lys on yearling antler growth in fallow deer bucks due to its importance as a collagen precursor, the results of which are presented in **Chapter 3** (Ny et al. 2020). Based on the limited effect on antler growth, the second experiment utilized an increased RP-Lys level, as well as the combination of RP-Lys and RP-Met, to determine their effects on first antler growth. The RPAAs improved antler growth, especially burr perimeter which is important for further development of antlers. Moreover, the effects of these RPAA supplementation treatments were investigated on other parameters, such as growth, carcass traits, and blood biochemical markers within different culling periods, as presented in **Chapter 4** (Ceacero et al. 2020).

Immunocastration, which is used to improve welfare management in male livestock and which has potential effects on protein and fat metabolism, was integrated in this research focus. Thus, the effects of different levels of concentrate supplementation on IC or non-IC deer on growth performance and plasma protein and fat biochemical markers is presented in **Chapter 5** (Ny et al. 2023). Moreover, RP-Lys and RP-Met supplementation with or without IC treatment was performed in another similar study. **Chapter 6** presents the effects of the combined use of RPAA and IC on first antler and bone growth (Ceacero et al. 2023). Finally, the overall discussion and conclusions summarize the research findings, their applications, and provide recommendations for future research (**Chapter 7** and **Chapter 8**).

Table 1 Summary of chapters, experimental settings, and feeding treatments utilised within the studies conducted for the thesis.

Chapter	Title	Animals & timeline	Groups	Basic diets ** (g/deer/d)	RPAA (g/deer/d)	Sex
3*	Effects of Lysine and Methionine supplementation on first antler growth in fallow deer (*Dama dama*)	Fallow deer bucks (n = 45) Age ~ 10 months old **Start:** April **End:** October	Control Group 1 Group 2	Pasture only Barley 200 Barley 200	 RP-Lys 5	
4	Differential effects of ruminally protected amino acids on fattening of fallow deer in two culling periods	Fallow deer bucks (n = 45) Age ~ 12 months old **Start:** June **End:** Late Autumn: December Late Winter: February	Control Group 1 Group 2	Barley 500 Barley 500 Barley 500	 RP-Lys 9 RP-Lys+RP-Met (9+3)	
5	Immunocastration and supplementary feeding level on the performance and blood biochemical markers of farmed yearling fallow deer (*Dama dama*)	Fallow deer buck (n= 40) Age ~ 13 months old **Start:** IC in June, July **Start:** Supplementation July **End:** October	Low level Low level*IC High level High level*IC	Pellet 100+Concentrate 300 Pellet 100+Concentrate 300 Pellet 200+Concentrate 600 Pellet 200+Concentrate 600	 	 IC (2x2mL) IC (2x2mL)
6	Combined effects of supplementation of amino acids and immunocastration in first antler growth of farmed fallow deer (*Dama dama*)	Fallow deer buck (n= 40) Age ~ 10 months old **Start:** IC in March, April, July **Start:** Supplementation April **End:** November	Control Group 1 Group 2 Group 3	Concentrate 250 Concentrate 250 Concentrate 250 Concentrate 250	NONE RP-Lys+RP-Met (6.3+2.1) NONE RP-Lys+RP-Met (6.3+2.1)	E E IC (3x2mL) IC (3x2mL)

* This paper also included the experiment from Chapter 4. **Basic diet of barley, concentrate (mixture of 90% oats and 10% wheat grains), commercial pellet for deer feed (soybean meal, rapeseed meal, alfalfa meal, and a premixture of minerals), expressed as g/deer/d.

The RPAA level presented was the AA additive (commercial supplement), not the amount calculated (estimated) amounts of ruminally protected AA.

RPAA: Ruminally Protected AA (Lys+Met); **E**= entire; **NONE**= no RPAA, **IC**= immunocastration with Improvac® in dose 2ml/deer.

CHAPTER 2: Literature Review

2.1 Potential benefits of amino acid supplementation for cervid performance and nutritional ecology

Adapted from: Ny V, Needham T, Ceacero F. 2022. Potential benefits of amino acid supplementation for cervid performance and nutritional ecology, with special focus on lysine and methionine: A review. Animal Nutrition **11**: 391-401. https://doi.org/10.1016/j.aninu.2022.09.001.

Authors' contribution: the first author, **Veit NY** participated in conceptualization, resources, writing - original draft, writing - review & editing. The paper was finally reviewed, commented, and edited for finally published by all authors.

Review Article

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

Veit Ny [a, b, c], Tersia Needham [a], Francisco Ceacero [a]

[a] Department of Animal Science and Food Processing, Faculty of Tropical AgriSciences, Czech University of Life Sciences Prague, Czech Republic

[b] Department of Cattle Breeding, Institute of Animal Science, Prague, Czech Republic

[c] Food Research Institute Prague, Czech Republic

A B S T R A C T

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, antler length in adult deer, 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.

Keywords: Antler; Deer; Feed additive; Protein; Ruminant; Venison

2.1.1 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 unicolor*) (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 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; Kudrnacova et al., 2018), and has favourable sensory qualities (flavour, aroma, texture) when compared to beef (Bures 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 Pere 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).

2.1.2 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 used 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.

2.1.3 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 like, such as RP-choline (RP-Chol), RP-betaine (RP-Bet), and N-carbamylglutamate (NCG) also increased milk yield and affected milk composition in goats (Fernandez 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 (Rodehutscord 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 Chavez 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 2.

2.1.4 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.

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 35d 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 in the AA supplemented group compared to CP deficient diet. 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 Kudrnacova 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. Bures et al. (2020) deeply examined the 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, 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. A summary of the reported and potential effects of RPAAs in venison production, in comparison with the other ruminants, is presented in Table 2.

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 (Baldi et al., 2011; Pinotti, 2012; Pinotti et al., 2008, 2020).

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 amino acids, 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). Lys 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).

Met 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 turns 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 concentrations in the rumen. However, the supplementation of Arg at 0.16 g/kg MBW/d increased serum IGF-1, compared to the 0.08 g/kg MBW/d group and the control group, which is the most important hormone involved in promoting antler growth. The level of ALT and ATS 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 ornithine, 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/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/d. Moreover, RP-Arg supplementation linearly decreased plasma ALT and aspartate aminotransferase 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 ornithine, 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.

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; Putto 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/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/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/d, and a combination treatment of RP-Lys plus RP-Met (0.18 g/kg MBW/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, 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 lysine 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).

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). Met 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/d, RP-Lys (0.23 g/kg MBW/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 2.

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.

Table 2 Proven effects of supplementary amino acids on cattle, sheep and goat, and its potential applications in cervid nutrition.

RPAA	Measured parameters	Studied in cervids	Studies species	Effects	Comments	References
RP-Met	Weight gain, ADG. Serum GLU, CHOL, Urea, CREA, SGPT, SGOT. Antler (beam length, brow tine length, points).	Yes Yes Yes	Red deer bucks, 2.8 years old	(+) (0) (0)	Limited effects on weight gain (only quadratic level). Only slightly improved beam length.	Mendoza-Nazar et al. (2012)
	ADG, feed:gain ratio. Plasma Gly, Pro, Ile, Ser, Leu, His, Lys, Arg, NH_3. Apparent AA (Val, Leu, Ile, Phe, Asn, Gly, Cys).	Yes Yes Yes	Sika deer bucks, 5 months old	(+) (+) (+)	Increased gain only during early 35 days. Improved protein digestion & AA absorption for growth of young.	Huang et al. (2015a)
	BW, BCS, DMI.	No	Lactating cows	(0)	No effect on gain and intake, similar to lactating sheep and goats.	Blum et al. (1999); Lara et al. (2006)
	BW, ADG, feed intake.	No	Lactating goats & kids	(0)	No effects on gain and intake, similar to lactating cows and sheep.	Flores et al. (2009); Al-Qaisi and Titi (2014); Titi et al. (2017)
	Milk yield, milk protein & fat yield, milk urea N.	No	Dairy cows, lactating goats	(+)	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 et al. (2017); Zhao et al. (2019)
	Milk yield, milk fat, protein, lactose contents. Milk SFAs.	No No	Gestating/ lactating ewes	(+) (−)	Potential for improving milk production and quality of does for supporting early growth of fawns.	Goulas et al. (2003)
	DMI, BW, hip height, wither height.	No	Gestating cow & calves	(+)	Potential for supporting maternal, postnatal health, and growth of young fawns.	Alharthi et al. (2018)
	BW, feed:gain ratio, N retention. Weight of cashmere hair, diameter of mohair fibre.	Yes No	Cashmere goats	(0) (+)	No effect on gain and intake, similar to cattle and sheep. Potential for improving growth of pelage/coat during autumn and velvet hair.	Souri et al. (1998)
	BW, N retention, OM digestion. Wool sulphur content, clean wool yield, wool growth rate & fibre diameter. Plasma sulphate, Met. Glutathione in blood, liver, skin.	No No No No	Wether sheep	(+) (+) (+) (+)	Important for supply of sulphur for coat and velvet hair growth in cervids.	Mata et al. (1995); Rodehutscord et al. (1999)

RP-Lys	Weight gain, carcass weight, dressing percentage, weight of LTL muscle. Internal fat (kidney, rumen, scrotal fat). LTL and ST muscle pH, colour, shear force, tenderness.	Yes Yes Yes	Fallow deer buck, 11 months old	(+) (+) (0)	Increased carcass and muscle yield. Increased fat storage for winter period. No effects on some physical meat quality depends on muscle types, similar to beef.	Kudrnáčová et al. (2019)
	IMF, LTL AA (His, Leu, Ala, Glx, Gly).	Yes	Fallow deer buck, 11 months old	(+)	Increased nutritional quality of venison and marbling quality.	Bureš et al. (2020)
	Intake, gain, hot carcass weight, carcass composition, LTL muscle area, marbling.	Yes	Finishing feedlot cattle	(0)	No effects on carcass and physical meat quality, similar to cervid.	Lancaster et al. (2016)
	N utilization, gain:feed, LTL (muscle area, moisture).	Yes	Finishing feedlot cattle	(+)	Gain only at early 87 days, similar to cervids. Potential effect for increasing venison yield and quality, especially at early supply.	Teixeira et al. (2019)
RP-Lys /RP-Lys + RP-Met	BW, feed intake. Digestibility (DM, OM, CP). Blood GLU, GLOB, BUN, TP, ALB, ALT, AST.	Yes Yes Yes	Sika deer bucks, 5 months old	(0) (+) (+)	No effect on gain and intake, similar to cattle, sheep, and goat. Increase of some plasma protein and fat metabolites (TP, ALB, GLOB).	Huang et al. (2015b)
	BW, DMI, DM digestibility.	No	Lactating cows	(0)	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. Milk yield, milk fat, protein, and lactose contents. 6% FCM, ECM. BHBA, plasma BUN.	No No No 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)
	DMI, BW, hip height, wither height.	No	Young cow calves	(0)	No effect on supply in young calves, but maybe maternal supplementation might have some positive effects.	da Silva et al. (2018)
	DMI, N balance. Faecal score, water intake, ingestive behaviour.	Yes No	Lambs	(+) (−)	Potential for growth of young.	Araújo et al. (2019)
	Carcass weight.	Yes	Cross-bred beef calves	(+)	Potential for increasing carcass yield in cervids.	Klemesrud et al. (2000b)
RP-Lys + RP-Met + RP-His	DMI, milk yield, milk protein & fat yield, ECM, ECM feed efficiency. Plasma (GLU, Lys, His, Met).	No 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	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. Blood metabolites (TRIG, CHOL, HDL-CHOL, LDL-CHOL, ALB, GLU, ALP, ALT, AST, IGF-1, citrulline, orthinine, phosphoserine). Rumen VFAs. Good rumen microbes (*Fibrobacter* spp., *Prevotellaceae* UCG-003, *Bacteroides* spp., *Rikenellaceae* RC9, *Treponema* 2, *Turicibacter* spp., *Romboutsia* spp., *Alistipes* spp., *Phascolarctobacterium* spp.,). Bad rumen microbes *Clostridium sensu* 1, *Corynebacterium 1*.	Yes Yes Yes Yes Yes	Sika deer bucks, 5-6 years old	(0/+) (+) (+) (+) (−)	No effect for Arg, but increase of velvet weight with RP-Arg. Increase of growth hormones, protein, and fat metabolites. Increase good rumen microbes for better rumen health and improve digestibility of carbohydrate and protein. Decrease rumen pathogenic microbes, thus improve gut health.	Si et al. (2021a,b)
	ADG, milk yield, milk fat & protein yield. Rumen length and thickness of muscularis, density of papillae. Plasma Arg, citrulline, ornithine, insulin. Plasma ammonia, urea.	No No No No	Lactating goat & young	(+) (+) (+) (−)	Potential for supporting milk production and compositions for supplying early growth of calves.	Wang et al. (2019)

RP-Arg/ Arg/ NCG	BW of ewes & lambs, foetal organ weight. Liver enzymatic activity (FA, cholesterol metabolism), hormones and metabolites (IGF-1, insulin, total AA, lactate, thyroxine).	No 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)
	Percentage of lamb born dead. Percentage of lamb born alive, lamb birth weight. Maternal plasma AA, ornithine, Cys, Pro.	No No No	Gestating/ lambing ewes	(−) (+) (+)	Important for increasing survival rate of young at birth.	Lassala et al. (2011)
	Ewe intake, BW, plasma AA and metabolites. Foetal brown fat store, female lamb weight. Foetal BW, body dimension, organ weight. Foetal plasma insulin, IGF-1, GLU, TRIG.	No No No No	Gestating/ lambing ewes	(0) (+) (0) (+/0)	Potential for supporting birth survival and postnatal growth of young fawns, especially females.	McCoard et al. (2013)
	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, Fertility to synchronized oestrus.	No No	Ewes in oestrus	(0) (+)	Potential for increasing reproduction in does.	De Chávez et al. (2015)
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. LTL shear force, water loss, SFA. pH_{24}, eye muscle area, UFA, UFA/SFA ratio, free AA, Ile, Phe, in LTL muscle.	No No No	Lambs	(+) (−) (+)	Improve feed intake and gain. Important for balancing UFAs and SFAs and increase yield and quality of venison.	Dong et al. (2020)
	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.	No	Holstein calves	(+)	Important for improving growth of digestive organ and its enzymatic production in young.	Cao et al. (2018)
	Activity of trypsin, lipase, α-amylase, DM digestion.	No	Yearling ewes	(+)	Potential effect for protein and carbohydrate digestion in cervid.	Yu et al. (2014)

(−) Negative effects; (0) No effect; (+) Positive effect

2.1.5 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: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, antler length, 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.

Acknowledgements

The authors would like to thank the internal grant agency of the Faculty of Tropical AgriSciences-CZU Prague (IGA- 20223107) for the financial support. The authors state that there are no personal conflicts of interest.

2.1.6 References

Alharthi AS, Batistel F, Abdelmegeid MK, Lascano G, Parys C, Helmbrecht A, et al. Maternal supply of methionine during late-pregnancy enhances rate of Holstein calf development in utero and postnatal growth to a greater extent than colostrum source. J Anim Sci Biotechnol 2018;9:1e12.

Ali CS, Sharif M, Nisa M, Javaid A, Hashmi N, Sarwar M. Supplementation of ruminally protected proteins and amino acids: feed consumption, digestion and performance of cattle and sheep. Int J Agric Biol 2009;11:477e82.

Al-Qaisi MA, Titi HH. Effect of rumen-protected methionine on production and composition of early lactating Shami goats milk and growth performance of their kids. Arch Tierz 2014;57:1e11.

Araújo CM, Junior GD, Oliveira KA, Silva AL, Siqueira MT. Levels of protected Lys and methionine in the diet of lambs: nutritional parameters and protein metabolites. Semin-Cienc Agrar 2019;40:709-718.

Azad MA, Hossain MM, Bhuiyan AK. Feeding and management of Spotted deer at Dhaka Zoo. Int J Zool 2005;1:48e52.

Baldi A, Bruckmaier R, D'ambrosio F, Campagnoli A, Pecorini C, Rebucci R, et al. Rumen-protected choline supplementation in periparturient dairy goats: effects on liver and mammary gland. J Agric Sci 2011;149:55e61.

Bartos L, Bubenik GA, Kuzmova E. Endocrine relationships between rank-related behaviour and antler growth in deer. Front Biosci 2012;4:1111e26.

Blaxter KL. Deer farming. Mamm Rev 1974;4:119e22.

Blouch R, McCarthy A, Moore D, Wemmer CM. Deer: status survey and conservation action plan. https://portals.iucn.org/library/node/7479. [Accessed 22 October 2021].

Blum JW, Bruckmaier RM, Jans F. Rumen-protected methionine fed to dairy cows: Bioavailability and effects on plasma amino acid pattern and plasma metabolite and insulin concentrations. J Dairy Sci 1999;82:1991e8.

Bonjour JP, Ammann P, Chevalley T, Rizzoli R. Protein intake and bone growth. Can J Appl Physiol 2001;26:S153e66.

Brown RD. Deer nutrition. In: Non-Native Deer Farming Symposium. Texas Agricultural Experimental Station; 1999. https://oaktrust.library.tamu.edu/ bitstream/handle/1969.1/190210/article774.pdf?sequence¼1 [accessed 14 Mar 2021].

Bureš D, Barton L, Kotrba R, Hakl J. Quality attributes and composition of meat from red deer (*Cervus elaphus*), fallow deer (*Dama dama*) and Aberdeen Angus and Holstein cattle (*Bos taurus*). J Sci Food Agric 2015;95:2299e306.

Bureš D, Barton L, Kudrnacova E, Kotrba R, Hoffman LC. The effect of barley and lysine supplementation on the longissimus lumborum meat quality of pasture raised fallow deer (*Dama dama*). Foods 2020;9:1255.

Cao YC, Yang XJ, Guo L, Zheng C, Wang DD, Cai CJ, et al. Effects of dietary leucine and phenylalanine on pancreas development, enzyme activity, and relative gene expression in milk-fed Holstein dairy calves. J Dairy Sci 2018;101:4235e44.

Ceacero F, Villagran M, Gambín-Pozo P, García AJ, Cappelli J, Ungerfeld R. Better antlers when surrounded by females? The social context influence mineralization in pampas deer (*Ozotozeros bezoarticus*). Ethol Ecol Evol 2019;31: 358e68.

Ceacero F, Clar MA, Ny V, Kotrba R. Differential effects of ruminally protected amino acids on fattening of fallow deer in two culling periods. Anim 2020;14:648e55.

Chapman DI. Antlers, bones of contention. Mamm Rev 1975;5:121e72.

da Silva JT, Manzoni T, Rocha NB, Santos G, Miqueo E, Slanzon GS, et al. Evaluation of milk replacer supplemented with lysine and methionine in combination with glutamate and glutamine in dairy calves. J Appl Anim Res 2018;46:960e6.

De Chavez JA, Guzman A, Zamora-Gutierrez D, Mendoza GD, Melgoza LM, Montes S, et al. Supplementation with rumen-protected L-arginine-HCl increased fertility in sheep with synchronized oestrus. Trop Anim Health Prod 2015;47:1067e73.

D'Mello JPF. Amino acids in animal nutrition. 2nd ed. London: CAB International; 2003.

Dong L, Zhong ZX, Cui HH, Wang SN, Luo Y, Yu LH, et al. Effects of rumen-protected betaine supplementation on meat quality and the composition of fatty and amino acids in growing lambs. Anim 2020;14:435e44.

Dryden GM. Quantitative nutrition of deer: energy, protein, and water. Anim Prod Sci 2011;51:292e302.

Dryden GM. Nutrition of antler growth in deer. Anim Prod Sci 2016;56:962e70.

Feldhamer GA, McShea WJ. Deer: the animal answer guide. Baltimore: JHU Press; 2012.

Fennessy PF, Suttie JM. Antler growth: nutritional and endocrine factors. In: Fenessy PF, Drew KR, editors. Biology of deer production. Wellington: The Royal Society of New Zealand; 1985. p. 242e50.

Fernandez C, Rodríguez-Alcala LM, Abilleira E, de la Fuente JM, Fontecha J. Influence of betaine on milk yield and fatty acid composition in lactating dairy goats. J Appl Anim Res 2009;36:89e92.

Fletcher J. Deer: how deer survive. London: REAKTION Books; 2014.

Fletcher J. Deer farming in the UK. In: Veterinary practice. Improve international; 2019. https://www.veterinary-practice.com/article/deer-farming-in-the-uk [accessed 30 Mar 2020].

Flores A, Mendoza G, Pinos-Rodriguez JM, Plata F, Vega S, Barcena R. Effects of rumen-protected methionine on milk production of dairy goats. Ital J Anim Sci 2009;8:271e5.

French CE, McEwen LC, Magruder ND, Ingram RH, Swift RW. Nutrient requirements for growth and antler development in the white-tailed deer. J Wildl Manage 1956;20:221e32.

Gaspar-Lopez E, Landete-Castillejos T, Estevez JA, Ceacero F, Gallego L, García AJ. Biometrics, testosterone, cortisol and antler growth cycle in Iberian red deer stags (*Cervus elaphus hispanicus*). Reprod Dom Anim 2010;45:243e9.

Giallongo F, Harper MT, Oh J, Lopes JC, Lapierre H, Patton RA, et al. Effects of rumen-protected methionine, lysine, and histidine on lactation performance of dairy cows. J Dairy Sci 2016;99:4437e52.

Gomez JA, Landete-Castillejos T, García AJ, Gallego L. Importance of growth during lactation on body size and antler development in the Iberian red deer (*Cervus elaphus hispanicus*). Livest Sci 2006;105:27e34.

Goss RJ. Deer antlers: regeneration, function, and evolution. New York: Academic Press; 1983.

Goulas C, Zervas G, Papadopoulos G. Effect of dietary animal fat and methionine on dairy ewes milk yield and milk composition. Anim Feed Sci Technol 2003;105: 43e54.

Hegazy MM, Sakr AEAM, Abd El-Aziz AH, Swelum AA. Effect of adding different concentrations of L-arginine to Tris-yolk extender on the quality of sub-fertile ejaculates in buffalo. Trop Anim Health Prod 2021;53:1e7.

Hofmann RR. Evolutionary steps of ecophysiological adaptation and diversification of ruminants: a comparative view of their digestive system. Oecologia 1989;78: 443e57.

Huang J, Sun WL, Li CY, Liu HL, Zhang TT, Bao K, et al. Effects of DL-methionine supplement on growth performance and amino acid digestion and plasma concentrations in sika deer calves (*Cervus nippon*). Anim Prod Sci 2015a;56:1002e7.

Huang J, Zhang TT, Kun B, Li GY, Wang KY. Effect of supplementation of lysine and methionine on growth performance, nutrients digestibility and serum biochemical indices for growing sika deer (*Cervus nippon*) fed protein deficient diet. Ital J Anim Sci 2015b;14:61e5.

Hutchison CL, Mulley RC, Wiklund E, Flesch JS. Effect of concentrate feeding on instrumental meat quality and sensory characteristics of fallow deer venison. Meat Sci 2012;90:801e6.

Huxley JS. The relative size of antlers in deer. P Zool Soc Lond 1931;101:819e64.

IUCN. The IUCN Red list of threatened species. 2021. Version 2021-3, https://www. iucnredlist.org. [Accessed 20 April 2022].

Jeon BT, Cheong SH, Kim DH, Park JH, Park PJ, Sung SH, et al. Effect of antler development stage on the chemical composition of velvet antler in elk (*Cervus elaphus canadensis*). Asian-Australas J Anim Sci 2011;24:1303e13.

Kaur H, Arora SP. Dietary effects on ruminant livestock reproduction with particular reference to protein. Nutr Res Rev 1995;8:121e36.

Kaya SO, Gur S, Kaya E. The effect of L-arginine on erection time, sperm quality and the seminal plasma arginase activity in rams. Rev Med Vet 2019;170:73e9.

Klemesrud MJ, Klopfenstein TJ, Lewis AJ. Metabolizable methionine and lysine requirements of growing cattle. J Anim Sci 2000a;78:199e206.

Klemesrud MJ, Klopfenstein TJ, Stock RA, Lewis AJ, Herold DW. Effect of dietary concentration of metabolizable lysine on finishing cattle performance. J Anim Sci 2000b;78:1060e6.

Kuba J, Landete-Castillejos T, Udala J. Red deer farming: breeding practice, trends, and potential in Poland- A Review. Ann Anim Sci 2015;15:591e9.

Kudrna V, Illek J, Marounek M, Nguyen A. Feeding ruminally protected methionine to pre-and postpartum dairy cows: effect on milk performance, milk composition and blood parameters. Czech J Anim Sci 2009;54:395e402.

Kudrnacova E, Barton L, Bures D, Hoffman LC. Carcass and meat characteristics from farm-raised and wild fallow deer (*Dama dam*a) and red deer (*Cervus elaphus*): a review. Meat Sci 2018;141:9e27.

Kudrnacova E, Bures D, Barton L, Kotrba R, Ceacero F, Hoffman LC, et al. The effect of barley and Lysine supplementation of pasture-based diet on growth, carcass composition and physical quality attributes of meat from farmed fallow deer (*Dama dama*). Anim 2019;9:1e33.

Kung L, Rode LM. Amino acid metabolism in ruminants. Anim Feed Sci Technol 1996;59:167e72.

Lancaster NA, Tekippe JA, Claeys MC, Schoonmaker JP. Effect of ruminal bypass lysine on amino acid status, performance, and carcass characteristics of steers fed corn product-based diets. J Anim Sci 2016;94:778.

Lara A, Mendoza GD, Landois L, Barcena R, Sanchez-Torres MT, Rojo R, et al. Milk production in Holstein cows supplemented with different levels of ruminally protected methionine. Livest Sci 2006;105:105e8.

Lassala A, Bazer FW, Cudd TA, Datta S, Keisler DH, Satterfield MC, et al. Parenteral administration of L-arginine enhances foetal survival and growth in sheep carrying multiple foetuses. J Nutr 2011;141:849e55.

Lee C, Hristov AN, Cassidy TW, Heyler KS, Lapierre H, Varga GA, et al. Rumen-protected lysine, methionine, and histidine increase milk protein yield in dairy cows fed a metabolizable protein-deficient diet. J Dairy Sci 2012;95:6042e56.

Lee C, Giallongo F, Hristov AN, Lapierre H, Cassidy TW, Heyler KS, et al. Effect of dietary protein level and rumen-protected amino acid supplementation on amino acid utilization for milk protein in lactating dairy cows. J Dairy Sci 2015;98:1885e902.

Li C, Suttie JM. Deer antlerogenic periosteum: a piece of postnatally retained embryonic tissue? Anat Embryol 2001;204:375e88.

Ma H, Zhang W, Zhu X, Song W, Liu J, Jia Z. Effects of rumen-protected tryptophan on growth performance, fibre characteristics, nutrient utilization and plasma essential amino acids in Cashmere goats during the cashmere slow growth period. Livest Sci 2010;131:227e33.

Ma H, Cheng J, Zhu X, Jia Z. Effects of rumen-protected tryptophan on performance, nutrient utilization and plasma tryptophan in cashmere goats. Afr J Biotechnol 2011;10:5806e11.

Madsen TG, Nielsen L, Nielsen MO. Mammary nutrient uptake in response to dietary supplementation of rumen protected lysine and methionine in late and early lactating dairy goats. Small Rumin Res 2005;56:151e64.

Mata G, Masters DG, Buscall D, Street K, Schlink AC. Responses in wool growth, liveweight, glutathione and amino acids, in Merino wethers fed increasing amounts of methionine protected from degradation in the rumen. Aust J Agric Res 1995;46:1189e204.

McCoard S, Sales F, Wards N, Sciascia Q, Oliver M, Koolaard J, et al. Parenteral administration of twin-bearing ewes with L-arginine enhances the birth weight and brown fat stores in sheep. Springer Plus 2013;2:1e2.

McCullough DR, Takatsuki S, Kaji K, editors. Sika deer: Biology and Management of Native and Introduced Populations. Tokyo: Springer; 2009. p. 61e82.

McDonald P, Edwards RA, Greenhalgh JFD, Morgan CA, Sinclair LA, Wilkinson RG. Animal nutrition. 7th ed. London: PEARSON; 2011.

Mendoza-Nazar P, Mendoza-Martínez GD, Herrera-Haro J, Ruiz-Sesma B, BarcenaGama R, Tarango-Arambula L. Effect of ruminally protected methionine on body weight gain and growth of antlers in red deer (*Cervus elaphus*) in the humid tropics. Trop Anim Health Prod 2012;44:681e4.

Menezo Y, Clement P, Clement A, Elder K. Methylation: an ineluctable biochemical and physiological process essential to the transmission of life. Int J Mol Sci 2020;21:9311.

Müller DW, Lackey LB, Streich WJ, Hatt JM, Clauss M. Relevance of management and feeding regimens on life expectancy in captive deer. Am J Vet Res 2010;71: 275e80.

Nezamidoust M, Alikhani M, Ghorbani GR, Edriss MA. Responses to betaine and inorganic sulphur of sheep in growth performance and fibre growth. J Anim Physiol Anim Nutr 2014;98:1031e8.

Ny V, Kotrba R, Cappelli J, Bures D, Clar MA, García AJ, et al. Effects of Lysine and Methionine supplementation on first antler growth in fallow deer (*Dama dama*). Small Rumin Res 2020;187:106119.

Pang ALY, Rennert OM. Protein acetylation and spermatogenesis. Reprod Syst Sex Disord 2013;5.

Parker KL. Advances in the nutritional ecology of cervids at different scales. Ecoscience 2003;10:395e411.

Phillip LE, Oresanya TF, Jacques JS. Fatty acid profile, carcass traits and growth rate of red deer fed diets varying in the ratio of concentrate: dried and pelleted roughage, and raised for venison production. Small Rumin Res 2007;71:215e21.

Pinotti L, Campagnoli A, D'ambrosio F, Susca F, Innocenti M, Rebucci R, et al. Rumen-protected choline and vitamin E supplementation in periparturient dairy goats: effects on milk production and folate, vitamin B12 and vitamin E status. Anim 2008;2:1019e27.

Pinotti L. Vitamin-like supplementation in dairy ruminants: the case of choline. In: Chaiyabutr N, editor. Milk Production-an up-to-date Overview of Animal Nutrition, Management, and Health. London: IntechOpen Limited; 2012. p. 65e86.

Pinotti L, Manoni M, Fumagalli F, Rovere N, Tretola M, Baldi A. The role of micronutrients in high-yielding dairy ruminants: choline and vitamin E. Ankara Univ Vet Fak Derg 2020;67:209e14.

Price JS, Allen S, Faucheux C, Althnaian T, Mount JG. Deer antlers: a zoological curiosity or the key to understanding organ regeneration in mammals? J Nat 2005;207:603e18.

Puttoo M, Dryden GMcL, McCosker JE. Performance of weaned rusa (*Cervus timorensis*) deer given concentrates of varying protein content with sorghum hay. Aust J Exp Agric 1998;38:33e9.

Richardson C. Factors affecting deer diets and nutrition. 2000. https://hdl.handle. net/1969.1/86928 [accessed 30 Mar 2021].

Rodehutscord M, Young P, Phillips N, White CL. Wool growth in Merino wethers fed lupins untreated or treated with heat or formaldehyde, with and without a supplementation of rumen protected methionine. Anim Feed Sci Technol 1999;82:213e26.

Ros-Santaella JL, Kotrba R, Pintus E. High-energy diet enhances spermatogenic function and increases sperm midpiece length in fallow deer (*Dama dama*) yearlings, vol. 6. Royal Soc Open Sci; 2019, 181972.

Savage VM, Gillooly JF, Woodruff WH, West GB, Allen AP, Enquist BJ, et al. The predominance of quarter-power scaling in biology. Funct Ecol 2004;18:257e82.

Scott IC, Asher GW, Barrell GK, Juan JV. Voluntary food intake of pregnant and nonpregnant red deer hinds. Livestock Sci 2013;158:230e9.

Serrano MP, Maggiolino A, Pateiro M, Landete-Castillejos T, Domínguez R, García A, et al. Carcass characteristics and meat quality of deer. In: Lorenzo JM, Munekata PE, Barba FJ, Toldra F, editors. More than Beef, Pork and Chicken- The production, processing, and quality traits of other sources of meat for human diet. Cham: Springer; 2019. p. 227e68.

Shin HT, Hudson RJ, Gai XH, Suttie JM. Nutritional requirements and management strategies for farmed deer-review. Asian-Austral J Anim Sci 2000;13:561e73.

Si H, Liu H, Nan W, Li G, Li Z, Lou Y. Effects of arginine supplementation on serum metabolites and the rumen bacterial community of sika deer (*Cervus nippon*). Front Vet Sci 2021a;8:630686.

Si H, Han Y, Liu H, Lou Y, Li Z. Effects of rumen-protected arginine supplementation on the plasma amino acids and gut microbiota of sika deer (*Cervus nippon*). Anim Feed Sci Technol 2021b;273:114828.

Socha MT, Putnam DE, Garthwaite BD, Whitehouse NL, Kierstead NA, Schwab CG, et al. Improving intestinal amino acid supply of pre-and postpartum dairy cows with rumen-protected methionine and lysine. J Dairy Sci 2005;88:1113e26.

Souri M, Galbraith H, Scaife JR. Comparisons of the effect of genotype and protected methionine supplementation on growth, digestive characteristics and fibre yield in cashmere-yielding and Angora goats. Anim Sci 1998;66:217e23.

Spencer A. Cervidor to China. Deer industry news southern safari for hawke's bay AP, Issue 99; 2020. https://www.deernz.org/assets/News-and-publications/ Deer-Industry-News-Magazine/DIN-2018/Deer-Industry-News-Issue-99-DecJan-2019.pdf [accessed 20 Mar 2020].

Sun L, Zhang H, Wang Z, Fan Y, Guo Y, Wang F. Dietary rumen-protected arginine and N-carbamylglutamate supplementation enhances fetal growth in underfed ewes. Reprod Fertil Dev 2018;30:1116e27.

Sun W, Zhao H, Bao K, Li C, Li G. Dietary calcium supplementation affects nutrient digestibility and antler-production performance during the antler-velvet growth period of male sika deer. Anim Prod Sci 2019;59:1689e95.

Sunwoo HH, Nakano T, Hudson RJ, SimSutt JS. Chemical composition of antlers from wapiti (*Cervus elaphus*). J Agric Food Chem 1995;43:2846e9.

Suttie JM, Gluckman PD, Butler JH, Fennessy PF, Corson ID, Laa FJ. Insulin-like growth factor 1 (IGF-1) antler-stimulating hormone? Endocrinology 1985;116: 846e8.

Suttie JM, Fennessy PF. Recent advances in the physiological control of velvet antler growth. In: Brown RD, editor. The biology of deer. New York: Springer; 1992. p. 471e86.

Suttie JM, Fennessy PF, Lapwood KR, Corson ID. Role of steroids in antler growth of red deer stags. J Exp Zool 1995;271:120e30.

Tedeschi LO, Fox DG, Fonseca MA, Cavalcanti LF. Models of protein and amino acid requirements for cattle. Rev Bras Zootec 2015;44:109e32.

Teixeira PD, Tekippe JA, Rodrigues LM, Ladeira MM, Pukrop JR, Kim YB, et al. Effect of ruminally protected arginine and lysine supplementation on serum amino acids, performance, and carcass traits of feedlot steers. J Anim Sci 2019;97: 3511e22.

Titi HH. Effect of long-term rumen-protected methionine supplementation on performance of Shami goats and growth performance of their kids. Anim Prod Sci 2017;57:1713e8.

Tsiplakou E, Mavrommatis A, Skliros D, Sotirakoglou K, Flemetakis E, Zervas G. The effects of dietary supplementation with rumen-protected amino acids on the expression of several genes involved in the immune system of dairy sheep. J Anim Physiol Anim Nutr 2018;102:1437e49.

Tsiplakou E, Mavrommatis A, Skliros D, Righi F, Flemetakis E. The impact of rumen-protected amino acids on the expression of key-genes involved in the innate immunity of dairy sheep. PLoS One 2020;15:e0233192.

Urbaniak M, Gut A, Krol H, Zawadzka B, Kulik M, Frankiewicz A, et al. Supplementation of mixed roughage-based diet with rumen protected methionine for lactating goats. J Anim Feed Sci 2001;10:19e26.

Volpelli LA, Valusso R, Piasentier E. Carcass quality in male fallow deer (*Dama dama*): effects of age and supplementary feeding. Meat Sci 2002;60:427e32.

Vos AD. Deer farming: guidelines on practical aspects. In: Food and agriculture organization of the united nations. FAO); 1982.

Wang Z, Wang R, Meng C, Ji Y, Sun L, Nie H, et al. Effects of dietary supplementation of N-Carbamylglutamate on lactation performance of lactating goats and growth performance of their suckling kidlets. Small Rumin Res 2019;175: 142e8.

Watanabe K, Fredeen AH, Robinson PH, Chalupa W, Julien WE, Sato H, et al. Effects of fat coated rumen bypass lysine and methionine on performance of dairy cows fed a diet deficient in lysine and methionine. Anim Sci J 2006;77: 495e502.

Wiklund E, Farouk M, Finstad G. Venison: meat from red deer (*Cervus elaphus*) and reindeer (*Rangifer tarandus tarandus*). Anim Front 2014;4:55e61.

Wu F, Li H, Jin L, Li X, Ma Y, You J, et al. Deer antler base as a traditional Chinese medicine: a review of its traditional uses, chemistry and pharmacology. J Ethnopharmacol 2013;145:403e15.

Yu ZP, Xu M, Wang F, Liu K, Yao JH, Wu Z, et al. Effect of duodenal infusion of leucine and phenylalanine on intestinal enzyme activities and starch digestibility in goats. Livest Sci 2014;162:134e40.

Zhang H, Sun LW, Wang ZY, Deng MT, Zhang GM, Guo RH, et al. Dietary N-carbamylglutamate and rumen-protected L-arginine supplementation ameliorate fetal growth restriction in undernourished ewes. J Anim Sci 2016;94: 2072e85.

Zhao K, Liu W, Lin X, Hu Z, Yan Z, Wang Y, et al. Effects of rumen-protected methionine and other essential amino acid supplementation on milk and milk component yields in lactating Holstein cows. J Dairy Sci 2019;102: 7936e47.

2.2 Immunocastration and consequences on AA requirements: a novel management tool for male cervids

2.2.1 Castration and welfare management

Castration is used for fertility and behavioural control in farm livestock, companion animals, and wildlife management (Palmer et al. 2018). In livestock production, castration is used to control indiscriminate breeding and unwanted pregnancy, reduce aggressive behaviour, improve product quality such as carcass and meat quality (i.e., reduce male taints, improve fatness in some species, and tenderness) (Needham & Hoffman 2015a,b; Campal-Espinosa et al. 2020). Castration can be done by physical (surgical, closed-crushing, elastrator bands etc) or pharmacological techniques (Stafford & Miller 2005). Physical castration has posed many ethical issues and challenges. For instance, removing the reproductive tract is a painful procedure both during and post-procedure, with complications like infection; they require costly pain mitigation and skilled labour, and reduce growth performance of the animal, etc. (Stafford & Miller 2005; Palmer et al. 2018). On the other hand, the new technique of using vaccines to block sex hormones production, i.e., IC, is more ethical and beneficial in this regard (Needham et al. 2017a; Škrlep et al. 2020).

2.2.2 Immunocastration and welfare management

The principle of IC is to manipulate androgen hormone production without invasive procedures (Palmer et al. 2018). The main concept of IC is influencing the activity of gonadotropin-releasing hormone (GnRH) which is a key hormone for the release of sexual functional hormones, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary. Once blocking FSH and LH, the production of sexual hormones such as the androgen hormone testosterone, are also supressed (Palmer et al. 2018; Campal-Espinosa et al. 2020). The two known commercial IC vaccines are Improvac® for swine and Bopriva® for cattle (Needham et al. 2017a). Improvac is the only available vaccine authorized to use in European Union (EU/2/09/095, EU 2009).

IC helps improving welfare management and livestock production in swine and ruminant species. The primarily welfare advantage of IC is the absence of pain and from further post-procedure complications (Needham et al. 2017a; Needham et al. 2019a). The reduction of androgen production from IC treatment has shown consistent results in reducing aggressive behaviour and sexual excitement in boars, bulls, ram lambs, and goat bucks (Needham et al. 2017a; Zeng et al. 2022).

2.2.3 Immunocastration and livestock production

Aside from behaviour and welfare improvement, IC also improves product quality, especially carcass and meat quality in swine and cattle. IC reduces sex odour (androstenone) in pork (boar taint) and goat meat (buck odour) (Claus et al. 2007; Zeng et al. 2022). In pig production, IC reduces aggressiveness and boar taint, and carcass and meat quality performance stands between (or better than) the physical castrates and entire males (i.e., less fat deposited than surgically castrated pigs but more than entire males) (Škrlep et al. 2020). Immunocastrated bulls increase ADG, carcass weight, and improve beef colour, compared to physical castrates or intact bulls in pasture fattening or feedlot finishing units (Amatayakul-Chantler et al. 2012,2013; Miguel et al. 2014). However, as shown in a review by Zeng et al. (2022), IC has little to no effects on weight gain, carcass traits in sheep and goats. Effects of IC on androgen hormones affects protein and fat metabolism; as a result, it has associated responses to dietary requirements, especially to the dietary protein requirements. Ultimately, this effect on metabolism and nutrient responses influences the carcass and meat quality, such as leanness and tenderness (Boler et al. 2011; Needham & Hoffman 2015a,b). Moreover, the decrease of aggressiveness, sexual behaviour, and removal of the appetite suppression effect of androgens due to IC can also encourage more feed intake (dietary protein) (Needham et al. 2017b).

The effects of IC depend on factors such as breeds, vaccination schedules, slaughtering timing after booster dose, etc. The primary dose alone does not supress the androgen production, thus booster dose is necessary (Claus et al. 2007). In most ruminant livestock, IC has shown effects on androgen suppression at least two weeks after the booster vaccination (Needham et al. 2017a). The first vaccination primes the animal's immune response while the second dose usually increases the antibody titre (Ahmed et al. 2022). However, the deviation and changes occur when the slaughter is prolonged after the second vaccination (Škrlep et al. 2020). In pigs, the second vaccination is recommended 4-6 weeks before slaughter and at least four weeks interval between the two doses (Kress et al. 2019). Similarly, Needham et al. (2016) has shown no effects of the interval between primary and secondary dose up to four weeks on growth performance and carcass traits in Dohne Merino ram, but successfully supressed the testis growth and reduced testicular steroids from one week after the second vaccination without reducing growth performance and meat production (Needham et al. 2019b, c).

IC has shown improvement in welfare management and production in ruminant livestock (cattle, sheep, goat) with consistent proof on reducing testicular steroids production, but still vary in product quality in some species and need to be more explored.

2.2.4 Application of immunocastration in cervids

The main products of deer farming include venison, trophies, and velvet antlers (Ny et al. 2022), many of which are generated from males. However, keeping only male deer in an intensive production can be challenging, especially in association with androgen hormones and aggressive behaviour which can result in fights and injuries, and further complicates practices such as pre-slaughter handling (Mulley & English 1992). This problem can also lead to loss of productivity and quality. As previously mentioned, IC has a comparable effectiveness in terms of controlling problems in male livestock compared to physical castration.

Deer castration emerged to control populations while hunting and selected culling were both not practical and or ethical (unsafe in high population area, prohibition of guns) (Palmer et al. 2018). Then, further exploration, especially regarding the effect of physical castration on antler cycle, growth performance, carcass and meat quality has been carried out. Physical castration of young prepuberal male deer (5–9-month-old) decreased growth performance (daily gain, BW), carcass weight in fallow deer bucks (Mulley & English 1985; Hogg et al. 1990; Mulley et al. 1996) and rusa deer stags (*Rusa timorensis*) (Sookhareea et al. 2001a,b). However, the castration eased handling and management, thus also reduced carcass bruising. Kim et al. (2016) also found some positive effects of castration on physicochemical compositions in *longissimus* muscle such as increased fatness, tenderness, and colour in older castrated elk (*Cervus elaphus canadensis*) stags.

In male deer, testosterone plays important roles, especially when starting the first antler growth, during rutting and fattening periods, and for the whole antler cycle (Li & Suttie 2001; Bartos et al. 2012). Therefore, this is a key topic to evaluate should IC be considered as a management tool in male deer. Lincoln et al. (1982) showed interrupting effects of GnRH antibody IC on the antler cycle (premature antler casting/permanent velvet remaining) of yearling red deer, and reduced testes size, plasma testosterone, and absence of rutting behaviour. A similar effect was observed after surgical castration of fallow deer bucks which, after casting and starting a new cycle, remained permanently in velvet (Kierdorf et al. 2004). Curtis et al. (2008) also found the suppression of anti-GnRH vaccine on testes growth, lowering sperm maturation in free ranging white-tailed deer bucks. However, due to future complications on antler growth (atrophy and risk of frost bite and antler breaking during winter), this application is not recommended in free ranging male deer. On the other hand, if the production purpose is mainly on meat production and welfare management is the priority in farm raising

conditions, this might be worth considering. Moreover, anti-GnRH vaccine effect is reversible after two years in both male and female white-tailed deer (Miller et al. 2000a,b).

With positive effects of IC on reducing sex steroids in cervids (Curtis et al. 2008), their potential effects on protein anabolism and fat catabolism should be considered (Claus et al. 2007), especially associated with metabolism, dietary protein requirements, and thus AA nutrition (Needham et al. 2017b). Therefore, AA requirements can be potentially influenced under IC, affecting protein and fat metabolism, growth performance, antler growth, carcass, and meat quality. Moreover, IC influences testosterone which can affect deer physiology, antler and/or bone growth. This can be an interesting combined welfare-friendly tool (AA & IC) to improve carcass fatness and meat quality of male deer utilised for venison production, and thus the investigation of its implementation under commercial farming practices and its effects on performance parameters should be considered.

CHAPTER 3

Effects of Lysine and Methionine supplementation on first antler growth in fallow deer (*Dama dama*)

Adapted from: Ny V, Kotrba R, Cappelli J, Bureš D, Clar MA, García AJ, Landete-Castillejos T, Bartoň L, Ceacero F. 2020. Effects of Lysine and Methionine supplementation on first antler growth in fallow deer (*Dama dama*). Small Ruminant Research **187**: 106119. https://doi.org/10.1016/j.smallrumres.2020.106119.

Authors' contribution: the first author was working on conceptualization, resources, writing - original draft, writing - review & editing. The paper was finally reviewed, commented, and edited for finally published by all authors.

Short communication

Effects of Lysine and Methionine supplementation on first antler growth in fallow deer (*Dama dama*)

Veit Ny, Radim Kotrba, Jamil Cappelli, Daniel Bureš, Mechie A. Clar, Andrés J. García, Tomás Landete-Castillejos, Luděk Bartoň, Francisco Ceacero

Department of Animal Science and Food Processing, Faculty of Tropical AgriSciences, Czech University of Life Sciences Prague, 165 00 Prague, Czech Republic

A B S T R A C T

Amino acid supplementation in cervids is an almost unexplored research area. Lysine (Lys) and Methionine (Met) are the two amino acids considered as limiting for ruminants and Lys supplementation shows good potential for increased antler growth as it is the main component of collagen and a bone tissue precursor. However, several studies in other ruminants have shown greater effects when simultaneously supplementing Lys +Met. Two experiments based on Lys and Met supplementation during first antler growth were conducted during two consecutive years. Each experiment involved 45 yearling fallow bucks distributed in three groups, balanced by weight: Exp.1: *Pasture*, *Pasture* + *Barley* (0.2 kg/animal per day), and *Pasture* + *Barley* + *Lys* (5 g/animal per day); Exp.2: *Pasture* + *Barley* (0.5 kg/animal per day), *Pasture* + *Barley* + *Lys* (9 g/animal per day), and *Pasture* + *Barley* + *Lys* + *Met* (9 and 3 g/animal per day, respectively). Lys supplementation during the first experiment did not improve any antler characteristics ($P > 0.05$). However, in the second experiment, both Lys (9 g) and Lys+ Met treatments had positive effects on the external antler characteristics ($P = 0.013$), antler weight ($P = 0.061$) and particularly on the burr perimeter ($P = 0.008$), which is crucial for antler size in following years. The results also suggest a stronger positive effect of amino acid supplementation on antler growth when the animals have a low performance in the presence of poorer pasturing situation.

Keywords: Amino acids; Copper; Antlers; Deer farming; Supplements

3.1 Introduction

For cervids, antlers are not just dead bone tissue that can be regenerated annually, but the subject of racing for primacy (Goss, 1983; Brown, 1992). Antler size is not only important for achieving a high rank, and increasing the chances of reproduction, but also for the production of velvet and as trophies (Wu et al., 2013). Nutrition affects antler growth (Baxter et al., 1999) as well as antler bone properties (Gómez et al., 2012) and diet quality has been shown to affect bone porosity, biochemical and mechanical properties of red deer antlers (Landete-Castillejos et al., 2007; 2012). Dryden (2016) reviewed protein, minerals and vitamins as the most important nutrients affecting antler growth and many studies have focused on the influence of certain key macro- and micro-mineral supplementation (Cu, Mn, Zn) and their effects on antlers (Bartoskewitz et al., 2007; Cappelli et al., 2015; Gambín et al., 2017). Clearly, this is a reasonable approach since antlers are mainly comprised of minerals. However, antlers are also composed up to 35%-45% of collagen and protein (Dobrowolska, 2002).

During antler growth, deer require high amounts of dietary protein of up to 15% (Dryden, 2016); however, microbial protein alone is not enough for supporting growth and production, especially in lactation or pregnant cattle or rapidly growing young ruminants (Kung and Rode, 1996). Specific amino acid (AA) requirements are necessary for protein synthesis, and thus the loss of essential AAs through ruminal digestion may affect the protein synthesis needed for antler growth (Dryden, 2016). The two well-known limiting AAs for ruminants are Lysine (Lys) and Methionine (Met) (Rosenberg, 1957; McDonald et al., 2011). For antler growth, Lys supplementation seems particularly promising since it is the main component of collagen and thus a precursor of bone tissue (McDonald et al., 2011). However, only one study has focused on the potential effect of AAs on antlers (Mendoza-Nazar et al., 2012), and only on their external characteristics.

Very few studies on AA supplementation have been carried out on cervids. Huang et al. (2015a) found a decrease in feed conversion ratio, but no increase in final bodyweight or average daily gains (ADG) when supplementing Met to sika deer calves. The same authors obtained very similar results when supplementing Lys + Met to calves with a poor protein diet, although supplementation did not improve the performance observed in animals with an adequate protein diet (Huang et al., 2015b). Mendoza-Nazar et al. (2012) studied Met supplementation in sub-adult red deer at different concentrations, but found no clear effects on antler beam length, brow tine length or number of tines. Unfortunately, these three studies used very small groups (always n = 4), and thus, these results are inconclusive and must be considered

with caution. Although fallow deer is the most abundant farmed deer species in Europe, no study focusing on AA supplementation has been conducted on this species. Moreover, there are no studies about the potential positive effects of AA supplementation on the mechanical properties or chemical composition of the antlers.

It was hypothesized that limiting AAs Lys and Met will improve antler characteristics, mechanical properties and chemical composition. Therefore, the objective was to study the effects of the supplementation of Lys (in two concentrations) and Lys + Met on antler growth and characteristics in a large herd of fallow deer yearlings.

3.2 Materials and methods

3.2.1 Experimental design

Two experiments were conducted during two years, 2015 and 2016, at a private deer farm in Mnich, South Bohemian region, Czech Republic (49.17 N, 14.90E; 485 masl). The experiments were carried out on yearling fallow bucks (*Dama dama*) through the addition of encapsulated Ruminally Protected Amino Acids (RPAAs) additive to their daily feed ration. Each year, 45 animals born in the previous spring were used and the fawns were kept together since weaning (around January). At the start of the experiments, the fallow bucks were separated into 3 different paddocks and balanced by weight [$P = 0.922$ for weight differences among groups in 2015, and $P = 0.427$ in 2016]. Supplementation with RPAAs started in June, slightly before antler growth starts, and continued until culling which occurred at different dates between November and February due to farming practices. Deer was kept in 2 ha paddocks and raised on good quality extensive grazing pasture during the antler growth period. The pasture is Phyto-sociologically classified as *Molinio-Arrhenatheretea* class, *Lolieto Cynosuretum* association, diagnosed by the presence of *Lolium perenne* and *Cynosurus cristatus* and dominated by the grass species of *Agrostis* sp., *Festuca* sp. and *Poa* sp (Weber et al. 2000). The paddocks were under grazing only with mowing of ungrazed plants during August and no seeding has been performed for the last 25 years. During winter, the animals received silage to compensate for the lack of pasture, but this occurred only after antler growth had finished and thus it is assumed that it had no effect on antler characteristics, as explained below. All the experimental groups also received a mineral mixture (Premin Slanisko, VVS Vermerovice s.r.o., Czech Republic; Table 3.1) *ad libitum*, and free access to drinking water throughout the experiments.

The ruminally-protected amino acids were from Kemin company (Kemin Industry, Inc., U.S.A.). In the experiment 2015, RP Lysine was LysiPEARL™, which was encapsulated by a spray-freezing encapsulation method and contains minimum 50% of L-lysine monohydrochloride. In the experiment 2016, RP Lysine was LysiGem™, contains min. 68% L-lysine monohydrochloride which was coated with hydrogenated vegetable oil (palm). The rumen bypass is 85% and 95% availability for intestine in cow. The ruminally-protected Met used was Smartamine® M, which was coated with a pH-sensitive polymer and contains min. 74% of DL-methionine with 90% protection and dissolution in vitro. The supplementation of AAs was performed on daily basis by mixing the RPAAs into the daily barley ration and

spreading it into a long feeding platform to allow access to all animals simultaneously and thereby avoiding competition (Ceacero et al., 2012). Since the animals did not compete for the supplementary feed, it is expected that each animal received a similar amount of barley and RPAAs every day. Moreover, supplemented feed was all consumed by the end of the day. Three experimental groups were used per year. During the first experiment (2015), a control group was fed during the antler growth period exclusively on pasture; a second group received 0.2 kg of barley per animal/day; the third group received the same amount of barley and 5 g of ruminally-protected Lys additive per animal/day. During the second experiment (2016), a group fed on pasture and barley (0.5 kg/animal/ day) (10.75% CP, Ceacero et al. 2020) was used as control; the second group also received 9 g of Lys additive per animal/day; the third group received 9 g of Lys and 3 g of Met additive per animal/day. However, the nutrient contents of pasture in 2016 was not analysed. The increase of barley supplementation in the second experiment to 0.5 kg was intended to compensate for the lower early body growth of animals used in 2016, which was probably due to the severe drought during their early growth in 2015 (see Discussion). The nutritional composition of this barley is shown in Table 3.1. The inclusion rates for Lys and Met were calculated based on the body weight of the animals at the beginning of the experiment, following a 3:1 ratio of Lys and Met (Schwab et al., 2004).

Due to the excitable nature of the species, no handling during the experiment was possible. Thus, the only data available was that collected at the start of the experiment (while sorting the groups) or at culling. As mentioned, the body mass of the deer was higher at the beginning of the experiment in the first year, probably due to the worse weather conditions in 2015 that affected plant growth in South Bohemian region of Czech Republic (CHMI, 2015) leading to lower pasture availability during the early growth of the fawns used in the second experiment.

Table 3.1 Nutritional composition of the silage, barley and free choice mineral supplement used during the study.

Items	Pasture[1]	Silage	Barley[2]	Premin Slanisko[3]
Crude protein (%)	12.75	5.75	11.27	-
Crude fat (%)	1.91	1.06	2.44	-
Ash (%)	8.49	4.73	2.51	-
Nitrogen-free compounds (%)	45.25	22.39	77.10	-
Lignin (%)	5.00	6.55	0.83	-
Acid detergent fibre (ADF) (%)	35.23	21.91	7.26	-
Neutral detergent fibre (NDF) (%)	65.42	30.32	30.40	-
Calcium (%)		-	-	14
Phosphorus (%)		-	-	7
Sodium (%)		-	-	21
Magnesium (%)		-	-	2
Copper (mg)		-	-	200
Manganese (mg)		-	-	1,000
Inorganic zinc (mg)		-	-	800
Organic zinc (mg)		-	-	-
Iodine (mg)		-	-	50
Cobalt (mg)		-	-	20
Selenium (mg)		-	-	10
Vitamin A (IU)		-	-	250,000
Vitamin D3 (IU)		-	-	100,000
Vitamin E (IU)		-	-	450

[1]Pasture was analysed during the first experiment (Kudrnáčová et al., 2019).

[2]*0.2 kg per animal and day in 2015; 0.5 kg in 2016*.

[3]*Source: VVS Vermerovice Ltd., Czech* Republic.

Body weight at culling was also unsuitable for the analyses because of the different timing of slaughtering between and within each year due to management purposes. For these reasons, it was decided not to include body mass at culling as control variable in the statistical analyses described below. Nevertheless, since the antler growth was already complete during the winter prior to all culling, the different culling times could not have affected the various antler characteristics. Broken antlers were excluded from the study, since mechanical tests could be performed only on antlers long enough to allow testing. Thus, 44 antlers were fully studied in 2015 (15, 14 and 15 for each group previously described) but only in 27 in 2016 (10, 9 and 8). The higher antler breakage rate in 2016 was probably due to later culling (November for the

2015 experiment; December to January for the 2016 experiment), which increased the chances of antler breakage due to multiple reasons.

3.2.2 Samples and data collection

During culling (slaughtering), the animals were rendered unconscious by mechanical stunning using a captive bolt gun according to Czech laws, and exsanguinated by severing the neck arteries. Live body mass was measured during group-sorting immediately before culling using a TruTest EziWeight scale with an accuracy of 0.1 kg. Antlers were removed after culling by cutting just below the burr using a manual saw. The length of both antlers was measured, as well as the burr perimeter. After cutting, the antlers were washed, labelled and dried at room temperature until the mass was constant (approximately four days). Antler weight was then recorded in a precision scale (± 0.01 g) and the mean value for both antlers was calculated and used within the statistical analyses.

3.2.3 Antler analyses

One antler per animal was selected for the remainder of the analyses, preferentially the left antler. The right antler was used only when the left was not suitable for mechanical analyses because of size or shape. A 5-6 cm piece was cut just above the initial pearled part of the antler. Both upper and lower complete transverse cross-sections of the piece were scanned (ScanJet 4370 Photo Scanner, HP Inc., Palo Alto, CA, USA) at 2400 × 2400 dpi and analysed using image analysis software (ImageJ). The cortical bone thickness was measured at six equally spaced points around the shaft. The area of the cortical section was measured, as well as the areas occupied by the trabecular and cortical bone. Mean values were calculated for cortical bone diameter (Ct.Wi) in cm and in percentage, and cortical bone area (Ct.Ar) in percentage.

Two bars of cortical bone were prepared for each antler to test the mechanical properties. The final size of the bars was 4.5 mm wide, 2.5 mm deep, with a variable length sufficient for a gauge length of 40 mm. A low-speed circular saw was used for initial cutting of the antler piece along the longitudinal axis. Surfaces were then abraded using a semiautomatic polishing machine (LaboPol-21, Struers Inc., Ballerup, Denmark) to create bars of the afore described size. Thereafter, samples were placed in Hank's balanced salt buffer solution (BioWhittaker, Belgium) for 48 h, and subsequently dried at 20 °C and 40% relative humidity for 72 h. This procedure aimed to standardize the humidity content. One bar was tested by three-point bending with the periosteal side in tension, in a Zwick/Roell 500 N (Germany) with head speed 32 mm/min and analysed with the software testXpert II (Zwick GmbH & Co, Ulm, Germany). The mechanical properties measured were: Young's modulus of elasticity (E; an estimate of

stiffness), bending strength (BS; calculated from the maximum load borne), and the work to peak force (W; determined by the total work done on the specimen up to the greatest load borne and divided by the central crosssectional area). Further details about these properties can be found in Currey et al. (2009). The second bar was tested for impact work (U), which measures the energy used to break an un-notched specimen by a falling pendulum. This value of U was normalized by dividing by the cross-sectional area of the specimen. Tests were carried out in a CEAST-IMPACTOR II (CEAST S.p.A., Pianezza, Italy) with a hammer with potential energy of 1 J. To calculate the density of the cortical bone, samples remaining after the mechanical test were dried out for 72 h at 60 °C, weighed with a precision scale (± 0.01 g) and measured with a precision vernier calliper (± 0.01 mm). Density was calculated by dividing the dry weight by the volume. Finally, the sample was burnt in a muffle furnace (HTC 1400, Carbolite, UK) for 6 h at 480 °C, and the ash content (%) was calculated as ash weight divided by dry weight. Another fragment after the mechanical tests was used to determine the mineral content of the antlers. Contents of Ca, P, Mg, Na, K, S, B, Cu, Fe, Mn, Sr and Zn were analysed in a specialized laboratory (CEBASCSIC; Murcia, Spain) by plasma-optical emission spectrometry, using a ICAP 6500 DUO Spectrometer/IRIS INTR.EPID II XDL (Thermo Fisher Scientific, Waltham, MA, USA). The ratio of Ca to P was also calculated.

3.2.4 Statistical analysis

Due to the differences existing in the environmental and management conditions between the two years, and the differences in the treatments used, all the statistical analyses were done separately for each year.MANOVA was used to analyse differences among treatments on the previously described variables, which were grouped as external antler characteristics (antler length, weight and burr perimeter), internal antler characteristics (Ct.Wi, Ct.Wi%, Ct.Ar, ash and density), antler mechanical properties (E, BS, W and U), and chemical composition (Ca, P, Ca/P, Mg, Na, K, S, B, Cu, Fe, Mn, Sr and Zn). One-way ANOVAs were used to detect the effects of the RPAAs supplementation treatments, within each year, for the variables described. Levene test checked for homogeneity of variances, and Tukey test was used to determine significant differences among treatments. Finally, multivariate General Linear Models studied the effects of body mass at the beginning of the experiment and treatment on the groups of variables previously described for the MANOVA analysis. The models were subsequently solved through a traditional stepwise backward selection procedure. All analyses were performed in SPSS version 20 (IBM, SPSS, USA).

3.3 Results

In the first experiment, RPAA Lys did not improve any of the antler characteristics studied (Table 3.2) and no significant differences were found amongst the three groups. The multivariate GLM revealed an effect of initial body mass on the internal antler characteristics, mechanical properties and chemical composition, which means that larger animals produced better antlers, but not bigger. However, after controlling for the body mass of the animals at the beginning of the experiment, the supplementation treatment had a marginally significant effect on chemical composition (Table 3.3).

In the second experiment, RPAAs supplementation affected more characteristics (Table 3.4), including general external antler characteristics ($P = 0.013$), antler burr perimeter ($P = 0.008$), antler weight ($P = 0.061$), and chemical composition, Cu ($P = 0.029$). The highest values were obtained in the Lys + Met group for antler burr perimeter, while the highest Cu content was found in the control group's antlers. No significant effects were found for internal and mechanical antler characteristics. However, the treatment Lys + Met tended to improve these characteristics compared to the two other groups. The multivariate GLM revealed an effect of body mass on the external antler characteristics, and mechanical properties. Moreover, the supplementation treatment had a greater effect than in the first experiment after controlling for the body mass of the animals, significantly affecting the chemical composition and marginally affecting the external antler characteristics (Table 3.5).

3.4 Discussion

Supplementation of RPAAs showed certain effects on antler characteristics, particularly within the second experiment (2016). At first glance, it may be argued that this is because in the second experiment the supplementation with RPAA was more adequate (including Met additive) and with a higher inclusion level (9 *vs* 5 g/day of Lys supplement). However, as indicated previously, the overall performance was greater during the first year probably due to better conditions during early growth. The only explanation for the differences found between the two years is thus that severe drought occurred in 2015 (CHMI, 2015), causing lower availability of pastures and leading to poor fawn growth at the start of the experiments. The lower growth in the second year may have restrained antler growth, which on the other hand could have allowed for compensatory antler growth. On the contrary, significant effects on antler characteristics were not found in animals with greater performance (2015 experiment). This is similar to the results observed by Huang et al. (2015b), who reported that supplementation of Lys + Met increased weight gains in sika deer calves only when they were

fed a low-quality diet; however, when deer were fed a high crude protein diet there was no effect on body weight or average daily gains.

Table 3.2 MANOVAs showing lack of significant differences of diet supplementation with ruminally protected Lys (mean value), on antler the characteristics of fallow deer.

	Experimental groups (2015)				
Parameters	Pasture	Pasture + Barley	Pasture + Barley + Lys	SEM	*P*-value
External antler characteristics (Wilks' $\lambda = 0.943$, $P = 0.886$)					
Antler length (cm)	28.88	26.29	27.03	0.86	0.461
Antler weight (g)	83.6	80.2	81.4	3.0	0.895
Antler burr (cm)	24.02	23.91	23.63	0.36	0.897
Internal antler characteristics (Wilks' $\lambda = 0.817$, $P = 0.643$)					
Ct.Wi (cm)	0.372	0.340	0.328	0.018	0.304
Ct.Wi (%)	0.420	0.461	0.395	0.017	0.300
Ct.Ar (%)	0.666	0.683	0.617	0.017	0.283
Ash (%)	56.79	56.55	55.61	0.37	0.400
Density (kg/dm^3)	1.530	1.600	1.540	0.026	0.531
Mechanical properties (Wilks' $\lambda = 0.873$, $P = 0.716$)					
Young´s Modulus (GPa)	10.92	12.16	11.70	0.36	0.370
Bending Strength (MPa)	224.2	250.1	245.7	7.4	0.327
Work (KJ/m^2)	27.36	29.02	29.12	0.93	0.689
Impact (KJ/ m^2)	16.10	15.28	15.49	0.58	0.842
Chemical composition (Wilks' $\lambda = 0.219$, $P = 0.149$)					
Ca (g/100g)	16.66	16.95	16.95	0.17	0.726
P (g/100g)	11.20	11.43	11.59	0.18	0.668
Ca/P	1.490	1.490	1.471	0.012	0.754
Mg (g/100g)	0.3770	0.3891	0.3720	0.0037	0.158
Na (g/100g)	0.5403	0.554	0.548	0.0050	0.407
K (g/100g)	0.0344	0.0340	0.0344	0.0010	0.991
S (g/100g)	0.2330	0.2365	0.2367	0.0024	0.771
B (mg/Kg)	0.629	0.664	0.624	0.035	0.885
Cu (mg/Kg)	0.378	0.357	0.453	0.024	0.239
Fe (mg/Kg)	13.9	18.5	15.5	1.5	0.477
Mn (mg/Kg)	16.64	17.05	16.56	0.16	0.439
Sr (mg/Kg)	153.6	148.2	147.4	2.4	0.533
Zn (mg/Kg)	47.25	46.28	46.39	0.73	0.843

Ct.Wi: Cortical bone diameter; Ct.Ar: Cortical bone area; Ca/P: Ca to P ratio. Lys: Lysine supplement (5 g/day).

Zinc is involved in bone mineralization through the enzyme alkaline phosphatase (Hove et al., 1940) and its presence in antlers in high levels is an indicator of inadequate mineralization (Yamaguchi, 1998; Landete-Castillejos et al., 2012). In both experiments Zinc content was low, and very low variability was observed for both years, amongst and within treatments, indicating

completion of the mineralization process. This means that all the animals involved in the experiment could "build" their antlers under no physiological constraints, which is important to adequately understand our results: if the antlers were grown under no constraint, the differences observed may be indeed caused by the treatments, and not because of the different performance observed between years.

The supplementation with RPAAs also produced differences in the mineral content of the antlers, some of which play a role in the cartilage formation and further mineralization, like Mn. Rats with low Mn content in their diet had bones with low Ca content (Strause et al., 1986) and similarly, supplementation of Mn produced antlers with greater Ca content in red deer (Cappelli et al., 2015).

In our study, Mn and Ca contents were also correlated, both across years and within experiments, but contrary to Landete-Castillejos et al. (2010) or Cappelli et al. (2015) the variability in these minerals did not produce differences in the mechanical properties.

Table 3.3 Multivariate General Linear Models showing the effect of body mass at the beginning of the experiment and treatment (different diet supplementation regimes; see text) on the antler characteristics of fallow deer yearlings in the first experiment described (2015).

	Multivariate General Linear Models (First experiment)	
Parameters	Wilk´s λ (*P*-value)	Pillai´s Trace (*P*-value)
External Antler Characteristics – Not significant		
Internal Antler Characteristics		
Intercept	0.356 (<0.001***)	0.644 (<0.001***)
Body mass (kg)	0.727 (0.028*)	0.273 (0.028*)
Treatment	(^{ns})	(^{ns})
Mechanical properties		
Intercept	0.785 (0.046*)	0.215 (0.046*)
Body mass (kg)	0.643 (0.001**)	0.357 (0.001**)
Treatment	(^{ns})	(^{ns})
Chemical composition		
Intercept	0.002 (<0.001***)	0.998 (<0.001***)
Body mass (kg)	0.412 (0.006**)	0.588 (0.006**)
Treatment	0.335 (0.080†)	0.811 (0.093†)

***, **, * and † indicates significance at $P<0.001$, 0.01, 0.05 and 0.1, respectively.

Cu also plays an essential role in the maturation of collagen, specifically, the synthesis of lysine-derived crosslinks, decreasing bone fragility (Opsahl et al., 1982). It has been recently proposed that there is a potential positive effect of Cu supplementation on antlers (cortical thickness;

Gambín et al., 2017). However, both treatments in the current study within the second experiment produced a significant decrease in Cu content, but this had no consequences for antler structure or mechanical properties. Thus, an indirect effect of RPAAs supplementation on antler growth mediated by interaction with key minerals is not supported.

Table 3.4 MANOVAs showing significant differences of diet supplementation (mean value), on the antler characteristics of fallow deer yearlings.

Experimental groups (2016)					
Parameters	Pasture + Barley	Pasture + Barley +Lys	Pasture + Barley + Lys + Met	SEM	*P*-value
External antler characteristics (Wilks' λ = 0.495, *P* = 0.013)					
Antler length (cm)	22.3	27.2	27.7	1.3	0.158
Antler weight (g)	55.0	58.6	74.5	3.7	0.061
Antler burr (cm)	19.63[b]	21.88[ab]	23.85[a]	0.58	**0.008**
Internal antler characteristics (Wilks' λ = 0.594, *P* = 0.326)					
Ct.Wi (cm)	0.442	0.441	0.424	0.019	0.924
Ct.Wi (%)	0.496	0.525	0.488	0.021	0.754
Ct.Ar (%)	0.726	0.751	0.726	0.021	0.867
Ash (%)	58.1	53.7	56.3	1.1	0.250
Density (kg/dm^3)	1.410	1.508	1.627	0.041	0.093
Mechanical properties (Wilks' λ = 0.724, *P* = 0.510)					
Young´s Modulus (GPa)	18.92	19.25	22.26	0.76	0.162
Bending Strength (MPa)	223.3	236.7	263.1	8.9	0.190
Work (KJ/m^2)	29.2	31.3	34.1	1.4	0.359
Impact (KJ/ m^2)	14.43	13.42	15.31	0.63	0.504
Chemical composition (Wilks' λ = 0.022, *P* = 0.131)					
Ca (g/100g)	25.24	25.51	26.06	0.34	0.666
P (g/100g)	11.01	10.95	11.28	0.18	0.764
Ca/P	2.230	2.335	2.311	0.012	0.420
Mg (g/100g)	0.569	0.581	0.608	0.010	0.268
Na (g/100g)	0.683	0.721	0.733	0.011	0.137
K (g/100g)	0.0829	0.0814	0.0819	0.0011	0.849
S (g/100g)	0.1985	0.1986	0.1908	0.0035	0.614
B (mg/Kg)	0.683	0.989	0.707	0.151	0.670
Cu (mg/Kg)	0.480[a]	0.384[b]	0.318[c]	0.026	**0.029**
Fe (mg/Kg)	25.4	19.9	18.8	3.5	0.716
Mn (mg/Kg)	26.30	26.30	27.96	0.25	0.510
Sr (mg/Kg)	202.8	217.9	225.8	5.1	0.174
Zn (mg/Kg)	45.78	46.19	47.75	0.75	0.566

[a,b] Means statistical differences between groups ($P < 0.05$), highlighted in bold.

Ct.Wi: Cortical bone diameter; Ct.Ar: Cortical bone area; Ca/P: Ca to P ratio.

Lys: Lysine supplement 9 g/day; Met: Methionine supplement 3 g/day.

The analyses also show that Lys and Lys + Met supplementation positively affected antler size after controlling for body characteristics (initial body mass): marginal effect on chemical composition was observed during the first experiment, whilst significant effect on chemical composition and marginally significant effect on external antler characteristics during the second were found. This confirms that supplementation of AAs has a direct effect on antlers. Thus, the only two available results on this matter (Mendoza-Nazar et al., 2012 and this study), both suggest that the effects of RPAAs on antler is direct, and not mediated by body mass.

Table 3.5 Multivariate General Linear Models showing the effect of body mass at the beginning of the experiment and treatment (different diet supplementation regimes; see text) on the antler characteristics of fallow deer yearlings in the second experiment described (2016).

	Multivariate General Linear Models (Second Experiment)	
Parameters	Wilk´s λ (*P*-value)	Pillai´s Trace (*P*-value)
External Antler Characteristics		
Intercept	0.565 (0.024*)	0.435 (0.024*)
Body mass (kg)	0.551 (0.020*)	0.449 (0.020*)
Treatment	0.518 (0.084†)	0.556 (0.069†)
Internal Antler Characteristics – Not significant		
Mechanical properties		
Intercept	0.829 (0.497^{ns})	0.171 (0.497^{ns})
Body mass (kg)	0.590 (0.050*)	0.410 (0.050*)
Treatment	(^{ns})	(^{ns})
Chemical composition		
Intercept	<0.001 (<0.001***)	1.000 (<0.001***)
Body mass (kg)	(^{ns})	(^{ns})
Treatment	0.030 (0.033*)	1.566 (0.050*)

***, **, * and † indicates significance at $P<0.001$, 0.01, 0.05 and 0.1, respectively.

3.5 Conclusions

In summary, our results indicate that RPAAs have limited effects on first antler growth in the present pasturing conditions. However, these effects are more intense when Lys + Met is supplemented, producing antlers with greater burr, as well as longer and heavier. Future studies need to test the effect of RPAAs with measurements of feed intake and analyse similar effects in adult animals, which can become an interesting feeding solution for the production of velvet and trophies.

Declarations of interest: none

Acknowledgements

This study was supported by the Faculty of Tropical AgriSciences – CZU Prague (IGA-IGA20195011, CIGA 20185006) and by the Ministry of Agriculture of the Czech Republic (MZE-RO0718). The authors wish to thank Pavel Friedberger, his family and farm staff for the assistance and access to the animals, Eva Kudrnáčová for field assistance, Pablo Gambín for lab assistance, and Tersia Needham for language edition. The authors state that there are no existing financial and personal conflicts of interest.

3.6 References

Bartoskewitz, M.L., Hewitt, D.G., Laurenz, J.C., Pitts, J.S., Bryant, F.C., 2007. Effect of dietary copper and zinc concentrations on white-tailed deer antler growth, body size, and immune system function. Small Ruminant Res. 73, 87–94. https://doi.org/10. 1016/j.smallrumres.2006.11.005.

Baxter, B.J., Andrews, R.N., Barrell, G.K., 1999. Bone Turnover Associated with Antler Growth in Red Deer (*Cervus elaphus*). Anat. Rec. 256, 14–19. https://doi.org/10.1002/(SICI)10970185(19990901)256:13.0.CO.

Brown, R.B., 1992. The Biology of Deer. Springer-Verlag, New York.

Cappelli, J., Garcia, A., Ceacero, F., Gomez, S., Luna, S., Gallego, L., Gambin, P., LandeteCastillejos, T., 2015. Manganese Supplementation in Deer under Balanced Diet Increases Impact Energy and Contents in Minerals of Antler Bone Tissue. PLOS ONE. 10, 1–15. https://doi.org/10.1371/journal.pone.0132738.

Ceacero, F., García, A.J., Landete-Castillejos, T., Bartošová, J., Bartoš, L., Gallego, L., 2012. Benefits for dominant red deer hinds under a competitive feeding system: food access behavior, diet and nutrient selection. PLOS ONE 1–7. https://doi.org/10.1371/journal.pone.0032780.

Ceacero, F., Clar, MA., Ny, V., Kotrba, R., 2020. Differential effects of ruminally protected amino acids on fattening of fallow deer in two culling periods. Animal 14, 648-655. https://doi.org/10.1017/S1751731119002325.

Currey, J.D., Landete-Castillejos, T., Estevez, J.A., Ceacero, F., Olguin, A., Garcia, A., Gallego, L., 2009. The mechanical properties of red deer antler bone when used in fighting. J. Exp. Biol. 212, 3985–3993. https://doi.org/10.1242/jeb.032292.

Czech Hydrometerological Institute CHMI, 2015. Drought in Czech Republic in 2015. (accessed 3 July, 2018). http://portal.chmi.cz/files/portal/docs/meteo/ok/SUCHO/ zpravy/en_drought2015.pdf.

Dobrowolska, S.A., 2002. Chemical composition of the red deer (*Cervus elaphus*) antlers, with a particular reference to the toxic metal contents. Z. Jagdwiss. 48, 148–155. https://doi.org/10.1007/BF02192402.

Dryden, G.Mc.L., 2016. Nutrition of antler growth in deer. Anim. Prod. Sci. 56, 962–970. https://doi.org/10.1071/AN15051.

Gambín, P., Serrano, M.P., Gallego, L., García, A., Cappelli, J., Ceacero, F., LandeteCastillejos, T., 2017. Does Cu supplementation affect the mechanical and structural properties and mineral content of red deer antler bone tissue? Animal 11, 1312–1320. https://doi.org/10.1017/S1751731116002767.

Gómez, J.A., Landete-Castillejos, T., García, A.J., Gallego, L., 2006. Importance of growth during lactation on body size and antler development in the Iberian red deer (*Cervus elaphus hispanicus*). Livest. Sci. 105, 27–34. https://doi.org/10.1016/j.livsci.2006.04. 028.

Gómez, J.A., Ceacero, F., Landete-Castillejos, T., Gaspar-López, E., García, A.J., Gallego, L., 2012. Factors affecting antler investment in Iberian red deer. Anim. Prod. Sci. 52, 867–873. https://doi.org/10.1071/AN11316.

Goss, J.R., 1983. Deer Antlers: Regeneration, Function, and Evolution, first ed. Academic Press, New York.

Hove, E., Elvehjem, C.A., Hart, E.B., 1940. The effect of Zinc on alkaline phosphatases. J. Biol. Chem. 134, 425–442.

Huang, J., Sun, W.L., Li, C.Y., Liu, H.L., Zhang, T.T., Bao, K., Fan, Y.Y., Li, G.Y., Wang, K.Y., 2015a. Effects of *DL*-methionine supplement on growth performance and amino acid digestion and plasma concentrations in sika deer calves (*Cervus nippon*). Anim. Prod. Sci. 56, 1002–1007. https://doi.org/10.1071/AN15042.

Huang, J., Zhang, T.T., Kun, B., Li, G.Y., Wang, K.Y., 2015b. Effect of supplementation of lysine and methionine on growth performance, nutrients digestibility and serum biochemical indices for growing sika deer (*Cervus nippon*) fed protein deficient diet. Ital. J. Anim. Sci. 14, 61–65.

Kierdorf, U., Stoffels, E., Stoffels, D., Kierdorf, H., Szuwart, T., Clemen, G., 2003. Histological studies of bone formation during pedicle restoration and early antler regeneration in Roe Deer and Fallow deer. Anat. Rec. A. 273, 741–751. https://doi. org/10.1002/ar.a.10082.

Kierdorf, U., Kierdorf, H., Szuwart, T., 2007. Deer antler regeneration: Cells, concepts, and controversies. J. Morphol. 268, 726–738. https://doi.org/10.1002/jmor.10546.

Kudrnáčová, E., Bureš, D., Bartoň, L., Kotrba, R., Ceacero, F., Hoffman, L.C., Kouřimská, L., 2019. The effect of barley and Lysine supplementation of pasture-based diet on growth, carcass composition and physical quality attributes of meat from farmed fallow deer (*Dama dama*). Animals. 9, 1–13. https://doi.org/10.3390/ani9020033.

Kung, L., Rode, L.M., 1996. Amino acid metabolism in ruminants. Anim. Feed Sci. Technol. 59, 167–172. https://doi.org/10.1016/0377-8401(95)00897-7.

Landete-Castillejos, T., Estevez, J.A., Martínez, A., Ceacero, F., Garcia, A., Gallego, L., 2007. Does chemical composition of antler bone reflect the physiological effort made to grow it? Bone. 40, 1095–1102. https://doi.org/10.1016/j.bone.2006.11.022.

Landete-Castillejos, T., Currey, J.D., Estévez, J.A., Fierro, Y., Calatayud, A., Ceacero, F., García, A.J., Gallego, L., 2010. Do drastic weather effects on diet influence changes in chemical composition, mechanical properties and structure in deer antlers? Bone. 47, 815–825. https://doi.org/10.1016/j.bone.2010.07.021.

Landete-Castillejos, T., Currey, J.D., Ceacero, F., García, A.J., Gallego, L., 2012. Does nutrition affect bone porosity and mineral tissue distribution in deer antlers? The relationship between histology, mechanical properties and mineral composition.
Bone. 50, 245–254. https://doi.org/10.1016/j.bone.2011.10.026.

McDonald, P., Edwards, R.A., Greenhalgh, J.F.D., Morgan, C.A., Sinclair, L.A., Wilkinson, R.G., 2011. Animal nutrition, seventh ed. Pearson Education Limited, London.

Mendoza-Nazar, P., Mendoza-Martínez, G.D., Herrera-Haro, J., Ruiz-Sesma, B., BárcenaGama, R., Tarango-Arámbula, L., 2012. Effect of ruminally protected methionine on body weight gain and growth of antlers in red

deer (*Cervus elaphus*) in the humid tropics. Trop. Anim. Health. Prod. 44, 681–684. https://doi.org/10.1007/s11250011-9956-4.

Opsahl, W., Zeronian, H., Ellison, M., Lewis, D., Rucker, R.B., Riggins, R.S., 1982. Role of copper in collagen cross-linking and its influence on selected mechanical properties of chick bone and tendon. J. Nutr. 112, 708–716. https://doi.org/10.1093/jn/112.4. 708.

Rosenberg, H.R., 1957. Amino Acids in Feeds, Methionine and Lysine Supplementation of Animal Feeds. J. Agric. Food Chem. 5, 694–700. https://doi.org/10.1021/ jf60079a008.

Schwab, C.G., Ordway, R.S., Whitehouse, L., 2004. Amino Acid Balancing in the Context of MP and RUP Requirement. Proc. 15th Annual Florida Ruminant Nutrition Symposium. Gainesville, Florida 10–25.

Strause, L.G., Hegenauer, J., Saltman, P., Cone, R., Resnick, D., 1986. Effects of long-term dietary manganese and copper deficiency on rat skeleton. J. Nutr. 116, 135–141. https://doi.org/10.1093/jn/116.1.135.

Weber, H.E., Moravec, J., Theurillat, J.P., 2000. International code of phytosociological nomenclature. J Veg Sci 11, 739–768. https://doi.org/10.2307/3236580.

Wu, F., Li, H., Jin, L., Li, X., Ma, Y., You, J., Li, S., Xu, Y., 2013. Deer antler base as a traditional Chinese medicine: A review of its traditional uses, chemistry and pharmacology. J. Ethnopharmacol 145, 403–415. https://doi.org/10.1016/j.jep.2012.12. 008.

Yamaguchi, M., 1998. Role of Zinc in bone formation and bone resorption. J. Trace. Elem. Exp. Med. 11, 119–135. https://doi.org/10.1002/(SICI)1520-670X.

CHAPTER 4

Differential effects of ruminally protected amino acids on fattening of fallow deer in two culling periods

Adapted from: Ceacero F, Clar MA, **Ny V,** Kotrba R. 2020. Differential effects of ruminally protected amino acids on fattening of fallow deer in two culling periods. Animal **14**:648-655. https://doi.org/10.1017/S1751731119002325.

Authors' contribution: Veit Ny participated in conceptualization, resources, writing - original draft, writing - review & editing. The paper was finally reviewed, commented, and edited for finally published by all authors.

Original Article

Differential effects of ruminally protected amino acids on fattening of fallow deer in two culling periods

F. Ceacero , M. A. Clar, **V. Ny**, R. Kotrba

Department of Animal Science and Food Processing, Faculty of Tropical AgriSciences, Czech University of Life Sciences Prague, 165 00 Prague, Czech Republic

A B S T R A C T

A well-balanced amino acid profile in the feedstuffs for livestock is essential to support adequate growth. This is well studied for monogastric species but still not well understood for ruminants and especially for the most unconventional species, like cervids. This study investigated the influence of ruminally protected lysine (**Lys**) and methionine (**Met**) supplementation during the fattening period, as well as two slaughter seasons (late autumn (**LA**) v. late winter (**LW**), on the growth, carcass traits, body condition and blood plasma metabolites of fallow deer (*Dama dama*). Forty-five yearlings of fallow deer bucks were allocated into three groups, balanced by weight (40.2 ± 2.7 kg). The deer were pasture-fed and supplemented with barley, free-choice mineral premix, silage during the winter period, and varying levels of ruminally protected Lys and Met supplement: no amino acids (Control), 9 g/day of Lys, and 9 g/day of Lys plus 3 g/day of Met (*Lys+Met*). Animals were slaughtered in two separate seasons, LA (six animals per group), and LW (nine animals per group). Animals culled in LA had higher average daily weight gain than LW (P = 0.002), due to the reduced growth during winter typical for seasonal cervids in temperate zone, mediated by the photoperiod. Dressing percentage was significantly higher in LW and in the *Lys+Met* group (*P* = 0.002). Body condition score (*P* = 0.024), kidney fat index (**KFI**) (*P* = 0.005), and internal fat (*P* < 0.001) increased significantly with *Lys+Met* supplementation. During LW, KFI (*P* = 0.004) and kidney fat (*P* = 0.001) were also significantly higher than in LA. Blood creatinine concentration increased significantly for deer receiving Lys (*P* = 0.002) and *Lys+Met* (*P* <

0.001). Also, triglycerides level increased in Lys group ($P < 0.001$). These findings highlight the effects of Lys and Met supplementation on the growth and internal fat storage for winter survival, suggesting a different use of the supplemented resource according to the season. Also, the observed effects on protein and fat metabolism of fallow deer may influence the production of farmed animals, and offer interesting insights about the physiology of the species.

Keywords: body condition, creatinine, fat storage, urea, metabolism

Implications

Deer farming for meat production is becoming economically important as an alternative red meat source. Feeding deer with high-quality protein and balanced amino acid content is important for adequate muscle growth and production, particularly for winter survival when food is scarce and cervids show reduced appetite. We found out that supplementation of ruminally protected lysine and methionine improved growth and production of deer during fattening period. This supplementation is important for compensating the limitation in amino acids and adverse effects of seasonal changes. The results also help to understand seasonal physiological changes in the species.

4.1 Introduction

Fallow deer is one of the most common deer species farmed in Europe for meat production (Kotrba, 2016) and significantly contributes to the meat industry in many regions (Kudrnáčová et al., 2019). Nowadays, noticeable increased demands for venison are reported in many parts of the world (Hoffman and Cawthorn, 2013). Nutrition is crucial to support these increasing demands, since it affects productivity, reproductive performance and health of farmed animals. As expected in a capital breeder (Jönsson, 1997), fallow deer accumulate substantial amounts of subcutaneous fat during summer and autumn until required for reproduction, and thus, body reserves (Ninov, 2003) and body condition (Tollefson et al., 2010) show strong seasonal variations. However, food consumption is lower during winter mediated by the reduced photoperiod (Scott et al., 2013), and the animals may need to mobilize body reserves.

Lysine (**Lys**) and methionine (**Met**) are recognized as the limiting amino acids for ruminants (Merchen and Titgemeyer, 1992; Kung and Rode, 1996; Schwab, 1996). Lysine is important in synthesis of proteins, such as milk, growth, pregnancy and maintenance (Ordway and Aines, 2010), and Met is especially required for growth and development in young ruminants (National Research Council, 2007).

Protein and amino acids from diet are mainly crucial for muscle growth, but can also be used as an energy source if they are supplied in excess. Many studies have shown the improvement of body condition and carcass yield due to the elevation of dietary protein or amino acids. Volpelli et al. (2002) reported that measurements such as carcass weight and dressing percentage of male fallow deer were higher when supplemented with concentrate (16.1% CP) compared to pasture-fed animals. Similar performance was observed on reindeer (*Rangifer tarandus*; Wiklund et al., 2003a) and red deer (*Cervus elaphus*; Wiklund et al., 2003b). Recently, Kudrnáčová et al. (2019) found better growth performance and carcass dressing in animals fed on barley. In this experiment, Lys supplementation also showed higher effects on these parameters than animals grazing only pasture. Lysine supplementation resulted in a higher proportion of separable fat, higher proportion of shoulder muscles, but a lower proportion of rump muscles measured as a proportion from right carcass side and compared to animals grazing only pasture with no supplementation.

High-protein diet also has effects on blood biochemistry metabolites. Tomkins and McMeniman (2006) reported higher plasma urea nitrogen concentrations in Timor deer (*Rusa timorensis*) fed by higher CP diet, while animals fed low-protein diets were found to have higher

permanent loss rate of urea nitrogen. Methionine and Lys plasma concentrations increased in Targhee lambs (Oke et al.,1986) and Holstein cows (Polan et al., 1991) after providing ruminally protected Met and ruminally protected Lys in the diet. In sika deer (*Cervus nippon*), increased total protein (**TP**) in serum concentration was observed when amino acids were added in the diet, and decreased when CP level was reduced (Huang et al., 2015b).

Few studies about supplementation of amino acids in cervids have been conducted. Mendoza-Nazar et al. (2012) supplemented ruminally protected Met up to 4.5 g/day to red deer, and did not detect any effects on blood metabolites despite small effects on weight gain and antler beam length were found. Another study on Lys and Met supplementation of sika deer was based on different protein levels (Huang et al., 2015b) and showed improvement of average daily weight gain (**ADG**) and digestibility only in animals with an adequate protein diet. Methionine supplementation increased the TP and albumin (**ALB**) metabolites while Lys increased globulin (**GLB**). The same authors studied Met supplementation on sika deer calves and found increase of digestibility of amino acids and its plasma metabolites. However, no improvement on feed conversion ratio was found, although ADG increased during early days of supplementation (Huang et al., 2015a). Finally, Kudrnácová et al. (2019) showed the effect of ruminally protected Lys on growth performance, dressing percentage and carcass traits of farmed fallow deer.

This study aimed to investigate the effects of ruminally protected Lys and Met supplementation in a herd of farmed fallow deer yearlings during the fattening period. We hypothesized that ruminally protected Lys and Met supplementation will improve growth, body condition score (**BCS**), dressing percentage and fat deposits, and will modify the metabolic profile for certain blood plasma metabolites related to protein and fat metabolism. The supplementation of ruminally protected amino acids (**RPAAs**) means an improvement in protein quality, and thus, it is expected to affect the use of protein: greater use for body growth and less for fat stores. Moreover, due to the previously explained lower feed intake by cervids during winter, and its consequences on the mobilization of fat stores, we also aimed to determine differential patterns in the metabolic use of the supplemented amino acids along two periods: autumn (growing period) and winter (low consumption period).

4.2 Materials and methods

4.2.1 Study area and experimental animals

The study was conducted in a private deer farm in Mnich, South Bohemia Region, Czech Republic, GPS coordinates 49.2N, 14.9E, with an altitude of 485 m above sea level. Forty-five farmed yearlings' of fallow deer at age of 12 months were separated into three groups with balanced weight (average 40.2 ± 2.7 kg). Each group consisted of 15 spikers kept in two hectares paddocks for the whole period of the study. Animals were identified using ear tags in both ears. The experiment started on 30 June 2016 and terminated in two separate culling periods: first on 8 December 2016 (late autumn (**LA**); six animals per group) and second on 16 February 2017 (late winter (**LW**); nine animals per group).

4.2.2 Diet and treatments

The dietary sources were characterized according to seasons. During summer and autumn, the primary source of diet was grazing on a good-quality pasture (12.70% CP, referenced from the first experiment because we did not analyse the pasture of the second this experiment). During winter, in order to replace pasture once this resource was scarce, grass silage (5.75% CP) was provided *ad libitum*. During both seasons, the animals received a supplementary feeding composed of 0.5 kg of barley (10.75% CP), free choice mineral supplement (Premin Slanisko; VVS Vermerovice Ltd, Verměřovice Czech Republic), and RPAA supplementation. The chemical composition of the diets is shown in Table 4.1. Supplementation of RPAAs was performed on a daily basis by mixing the RPAA additive into the daily barley ration and spreading it into a long feeding trough to allow access to all animals simultaneously and thereby avoiding competition (Ceacero et al., 2012). Moreover, supplemented feed was all consumed by the end of the day.

Since the animals did not compete for the supplementary feed, it is expected that each animal received a similar amount of barley and RPAAs every day. Similarly, it can be expected that all animals consumed a similar amount of pasture (in autumn) and silage (in winter).

The amount of ruminally protected Lys (LysiGem™; Kemin Industries, Inc., Des Moines, IA, USA) and ruminally protected Met (Smartamine® M; Kemin Industries, Inc., Des Moines, IA, USA) supplement was calculated according to different dietary treatments for each group: no amino acid supplementation (Control group), 9 g/deer per day of Lys (Lys group), and 9 g/deer per day of Lys plus 3 g/deer per day of Met (*Lys+Met* group), respectively.

The inclusion rates for Lys and Met were calculated based on the metabolic BW (following Savage et al., 2004) of the animals at the beginning of the experiment, the inclusion

rate suggested for red deer by Mendoza-Nazar et al. (2012) for BW effects after Met supplementation and a 3 : 1 ratio of Lys and Met as proposed by Schwab (1996).

4.2.3 Data collection

The animals were weighed at the beginning and the end of the experiment in a Tru-Test EziWeigh scale (Tru-Test Group, Auckland, New Zealand) situated under a handling box along the handling premises, with an accuracy of 0.1 kg. ADG was calculated thereafter. Animals were slaughtered in a restraint box by stunning with captive bolt, weighed right after (slaughter weight – used for the calculation of slaughter characteristics) and then bled out by cutting both neck arteries. Carcasses were then transported and processed at the abattoir located 20 km from the farm. After evisceration, carcasses were chilled for 12 h to 4°C. Chilled carcasses were weighed (carcass weight), and dressing percentage was calculated relative to the weight after culling.

Body condition was determined through BCS (Audigé et al., 1998), kidney fat index (**KFI**; Riney, 1955) and internal fat depots. Body condition score was determined through hands-on palpation while the animal was on relaxed position, performed by the same person and following the BCS chart for deer by Audigé et al. (1998) with an interval of 0.5 and the range of score from 1 (lean) to 5 (fat). The left and right kidneys and the fat attached from the rear of the stomach cavity were removed. Both kidneys were weighed to the nearest 0.01 g with attached fat and separately, to determine the KFI following Riney (1955). Internal fat resulted from combining heart fat, scrotum fat and kidney fat, which were collected and weighed separately.

Table 4.1 Nutrient composition of feedstuffs used during the experiment.

Composition	Pasture [a]	Barley	Silage	Mineral supplement[b]
Crude protein (%)	12.75	10.75	5.75	–
Crude fat (%)	1.91	3.34	1.06	–
Crude fibre (%)	31.61	8.84	17.62	–
Ash (%)	8.49	2.88	4.73	–
Nitrogen-free compounds (%)	45.25	61.25	22.39	–
Lignin (%)	5.00	4.51	6.55	–
Acid detergent fibre (%)	35.23	10.91	21.91	–
Neutral detergent fibre (%)	65.42	23.60	30.32	–
Calcium (%)		–	–	14
Phosphorus (%)		–	–	7
Sodium (%)		–	–	21
Magnesium (%)		–	–	2
Copper (mg)		–	–	200
Manganese (mg)		–	–	1000
Zinc (mg)		–	–	800
Iodine (mg)		–	–	50
Cobalt (mg)		–	–	20
Selenium (mg)		–	–	10
Vitamin A (mJ)		–	–	250 000
Vitamin D_3 (mJ)		–	–	100 000
Vitamin E (mg)		–	–	450

[a] Pasture was analysed during a preliminary experiment all along the previous summer (Kudrnáčová et al., 2019).

[b] Premin Slanisko; VVS Vermerovice Ltd.

Blood samples were collected twice. At the beginning of the experiment, blood was drawn from vena jugularis by 20-gauge vacuette needles. The second blood sampling was performed during slaughter directly when animals were bled out by cutting neck arteries. Blood samples were collected in heparinized tubes (VetTest® Tube Sarstedt Li-Heparin 1.3 ml). After collection, heparinized tubes were inverted to ensure that blood was mixed thoroughly and did not coagulate. Blood samples were stored in a cooler box with frozen packs during

transportation to the laboratory. On the same day, plasma was extracted by allowing the blood samples to cool down at room temperature. Subsequently, blood samples were centrifuged at 12 000 × g RCF for 2 min, and plasma samples were extracted and transferred to untreated microtubes for storage at −18°C. Frozen blood plasma samples were thawed at room temperature, mixed by inversion and centrifuged at 12 000 × g RCF for 2 min to ensure homogeneity and remove fibrin particles that may have formed during the storage. Plasma samples were then analysed for the concentration of blood plasma metabolites related to protein and fat metabolism (creatinine (**CREA**), urea/blood urea nitrogen (**BUN**), TP, ALB, GLB, and triglycerides (**TRIG**)) using VetTest® Chemistry Analyzer (IDEXX Laboratories, Westbrook, ME, USA) with a standard commercial kit. VetTest® Chemistry Analyzer has no reference ranges for cervids. Thus, we used the physiological reference intervals for *Dama dama* from Teare (2013).

4.2.4 Statistical analyses

All data were initially tested for normality (Shapiro–Wilks test). Non-parametric tests were applied to non-normally distributed data. One-way ANOVA was used to compare the differences between treatments on initial, final weight and ADG. Levene's test for homogeneity of variances was considered for these analyses. For comparing differences between culling periods, independent samples Student's t tests were applied to BCS, KFI, and Mann–Whitney U test was used for internal fat percentage. Box-plot was used for a graphical representation of these analyses. Most of the variables used in the statistical tests showed adequate CV ($CV_{Initial\ Body\ Mass}$ = 6.63%; $CV_{Final\ Body\ Mass}$ = 8.05%; CV_{BCS} = 12.11%; CV_{CREA} = 13.87%; CV_{ALB} = 5.40%; CV_{BUN} = 16.16%; CV_{TP} = 4.92%; CV_{GLB} = 7.35%), except KFI CV_{KFI} = 49.31% and TRIG CV_{TRIG} = 185.25%.

Generalized linear mixed models were used to determine the effects of treatment and culling season on growth (slaughter weight, carcass weight and dressing percentage), body condition (BCS, KFI and internal fat) and blood plasma metabolites (CREA, BUN, TP, ALB, GLB and TRIG). The fixed effects used in the models were dietary treatments, culling seasons, initial weight, BCS and the interaction between treatment and culling season. Collinearity between the variables entering the models was tested by calculation of the variance inflation factor (VIF), and all the results obtained were satisfactory (maximum VIF obtained: $VIF_{Initial\ Body\ Mass}$ = 1.206, VIF_{BCS} = 1.206; VIF_{KFI} = 1.103). All analyses were performed in SPSS version 25 (IBM, SPSS, Armonk, NY, USA), and results are expressed as (mean ± SD).

4.3 Results

Mean growth among treatments in both culling seasons is shown in Table 4.2. Initial weight of fallow deer showed no significant differences among groups, but also no significant differences were observed among treatments in the final weight, weight gain and ADG in the LA and LW. However, significant differences were found in the ADG between seasons, being higher in animals culled in LA (33.4 ± 13.2 v. 20.3 ± 12.8; t = 3.329; df = 43; P = 0.002). No significant differences were found in weight gain between culling seasons ($P > 0.05$). The supplementation of RPAAs affected neither slaughter nor carcass weight, although carcass weight was higher in animals culled in LW. However, dressing percentage was found significantly different among treatments and between culling seasons, being higher in LW and in the *Lys+Met* supplementation treatment (Table 4.3).

Table 4.2 Mean (SD) values and significant differences (one-way ANOVA) in growth performance of fallow deer (*Dama dama*) supplemented with Lys and Met during the fattening period, and culled on different seasons: LA (n = 18) and LW (n = 27).

		Treatments				
Parameters	Season	Control	Lys	*Lys+Met*	SEM	*P*-Value
Initial weight (kg)	Summer	39.6 (2.6)	40.2 (2.6)	41.0 (2.9)	0.400	0.331
Final weight (kg)	LA	45.4 (3.8)	45.9 (1.8)	47.4 (3.2)	0.759	0.530
	LW	43.6 (3.3)	44.9 (4.6)	44.8 (4.1)	0.700	0.762
Weight gain (kg)	LA	5.50 (3.1)	5.82 (1.2)	4.84 (2.0)	0.502	0.745
	LW	4.31 (3.7)	4.66 (2.8)	4.81 (2.4)	0.559	0.937
ADG (g)	LA	34.1 (19.1)	36.1 (7.6)	30.1 (12.2)	2.789	0.745
	LW	19.0 (16.2)	20.5 (12.3)	21.3 (10.9)	3.226	0.932

ADG = average daily weight gains; LA = late autumn; LW = late winter; Lys = lysine; Met = methionine. Treatments: Control = pasture with barley and mineral supplementation; Lys = 9 g/day per deer Lys supplement; *Lys+Met* = 9 g/day per deer Lys supplement and 3 g/day per deer Met supplement. Culling periods: LA and LW.

On the contrary, supplementation of RPAAs significantly affected body condition parameters such as BCS, KFI and percentage of internal fat (see Table 4.4 for this and following results in the paragraph). All of them increased significantly in the *Lys+Met* group compared to the Control one. However, the Lys group only showed significant differences with the Control group in internal fat. Regarding the culling date, deer slaughtered during LW had significantly higher KFI but lower BCS. No differences for the percentage of internal fat were found between both culling periods (Figure 4.1).

Blood plasma concentration of BUN decreased significantly only in the Lys group and was higher in LA than in LW. Blood plasma concentration of TP was lower in both Lys and *Lys+Met* groups. Blood plasma concentration of ALB significantly decreased in the *Lys+Met* group, was higher in LW and was positively related to body condition. Blood plasma concentration of GLB was not affected by the treatments, culling period or body condition. Finally, the fat-related metabolite TRIG increased in the Lys group.

Table 4.3 Generalized linear mixed models explaining growth parameters of fallow deer (*Dama dama*) supplemented with Lys and Met during the fattening period, and culled on different seasons.

Parameter	Model term	*β*	*t*	*P*-value
Slaughter weight (kg)	Intercept	9.104 ± 5.936	1.534	0.133
	Initial weight	0.868 ± 0.145	6.006	<0.001***
Carcass weight (kg)	Intercept	−3.474 ± 4.977	−0.698	0.489
	Initial weight	0.668 ± 0.121	5.510	<0.001***
	LW	2.073 ± 0.657	3.156	0.003**
Dressing %	Intercept	42.317 ± 4.456	9.497	<0.001***
	Initial weight	0.191 ± 0.110	1.738	0.090
	Treatment			
	Lys+Met	3.581 ± 1.096	3.269	0.002**
	LW	5.449 ± 0.968	5.631	<0.001***

LA = late autumn; LW = late winter; Lys = lysine; Met = methionine.

For different treatments, the Control group was used as a category of reference. For culling season, LA was used as a reference. Culling periods: LA and LW. Treatments: Control = pasture with barley and mineral supplementation; Lys = 9 g/day per deer Lys supplement; Lys+Met = 9 g/day per deer Lys supplement and 3 g/day per deer Met supplement. *P*-value is significantly different indicated by $^{**}P < 0.01$ and $^{***}P \leq 0.001$.

Table 4.4 Generalized linear mixed models explaining body condition fallow deer (*Dama dama*) supplemented with Lys and Met during the fattening period, and culled on different seasons.

Measurement	Model term	*β*	*t*	*P*-value
BCS	Intercept	1.88 ± 0.917	2.045	0.047*
	Initial weight	0.041 ± 0.023	1.801	0.079
	Treatment- *Lys+Met*	0.340 ± 0.145	2.354	0.024*
	Culling-LW	−0.589 ± 0.119	−4.931	<0.001***
KFI (%)	Intercept	80.5 ± 22.0	3.659	0.001***
	Treatment- *Lys+Met*	75.3 ± 25.4	2.966	0.005**
	Culling-LW	65.4 ± 21.2	3.091	0.004**
Internal fat (%)	Intercept	0.536 ± 0.057	9.408	<0.001***
	Treatment- Lys	0.191 ± 0.081	2.370	0.022*
	Treatment- *Lys+Met*	0.320 ± 0.081	3.981	<0.001***

BCS = body condition score; KFI = kidney fat index; Internal fat= kidney fat + scrotum fat + heart fat; LA = late autumn; LW= late winter; Lys= Lysine; Met = methionine.

For different treatments, the Control group was used as a category reference. For culling season, LA was used as a reference. Culling periods: LA and LW. Treatments: Control = pasture with barley and mineral supplementation; Lys = 9 g/day per deer Lys; *Lys+Met* = 9 g/day per deer Lys and 3 g/day per deer Met.

P-value is significantly or marginally different indicated by *P < 0.05, **P < 0.01 and ***P < 0.001.

All the analysed blood metabolites related to protein and fat metabolism were within the reference intervals for the species (Teare, 2013). Generalized linear mixed models showed significant effects of the treatments on blood metabolites related to protein and fat metabolism. Blood plasma concentration of CREA increased both in Lys and in *Lys+Met* groups compared to Control (see Table 4.5 for this and following results) and was negatively related to BCS.

Table 4.5 Generalized linear mixed models explaining blood plasma metabolites concentration in fallow deer (*Dama dama*) supplemented with Lys and Met during the fattening period, and culled on different seasons.

Metabolites	Model term	β	t	P-value
	Intercept	121 ± 10	12.026	<0.001***
CREA (μmol/l)	Treatment-Lys	11.5 ± 3.6	3.190	0.002**
	Treatment-*Lys+Met*	25.2 ± 3.7	6.890	<0.001***
	BCS	−7.39 ± 3.14	−2.353	0.021*
BUN (mmol/l)	Intercept	7.74 ± 0.16	48.377	<0.001***
	Treatment-Lys	−1.39 ± 0.34	−4.074	<0.001***
	Culling-LW	−0.373 ± 0.20	−1.884	0.063
TP (g/l)	Intercept	66.1 ± 0.36	183.782	<0.001
	Treatment-Lys	−1.72 ± 0.97	−1.760	0.082
	Treatment-*Lys+Met*	−2.58 ± 0.9	−2.650	0.010**
ALB (g/l)	Intercept	22.1 ± 1.4	15.993	<0.001
	Treatment-*Lys+Met*	−1.10 ± 0.40	−2.768	0.007**
	Culling-LW	1.23 ± 0.31	3.940	<0.001***
	BCS	1.57 ± 0.40	3.883	<0.001***
GLB (g/l)	Ns			
TRIG (mmol/l)	Intercept	0.018 ± 0.006	2.732	0.008**
	Treatment-Lys	0.101 ± 0.028	3.664	<0.001***

CREA = creatinine concentration; BUN = blood urea nitrogen concentration; TP = total protein concentration; ALB = albumin concentration; GLB = globulin concentration; TRIG = triglycerides concentration; BCS = body condition score; LA = late autumn; LW = late winter; Lys = lysine; Met = methionine. For different treatments, the Control group was used as a category reference. For culling season, LA was used as a reference. Culling periods: LA and LW. Treatments: Control = pasture with barley and mineral supplementation; Lys = 9 g/day per deer Lys; *Lys+Met* = 9 g/day per deer Lys and 3 g/day per deer Met.

P-value is significantly or marginally different indicated by *$P < 0.05$,**$P < 0.01$ and ***$P < 0.001$.

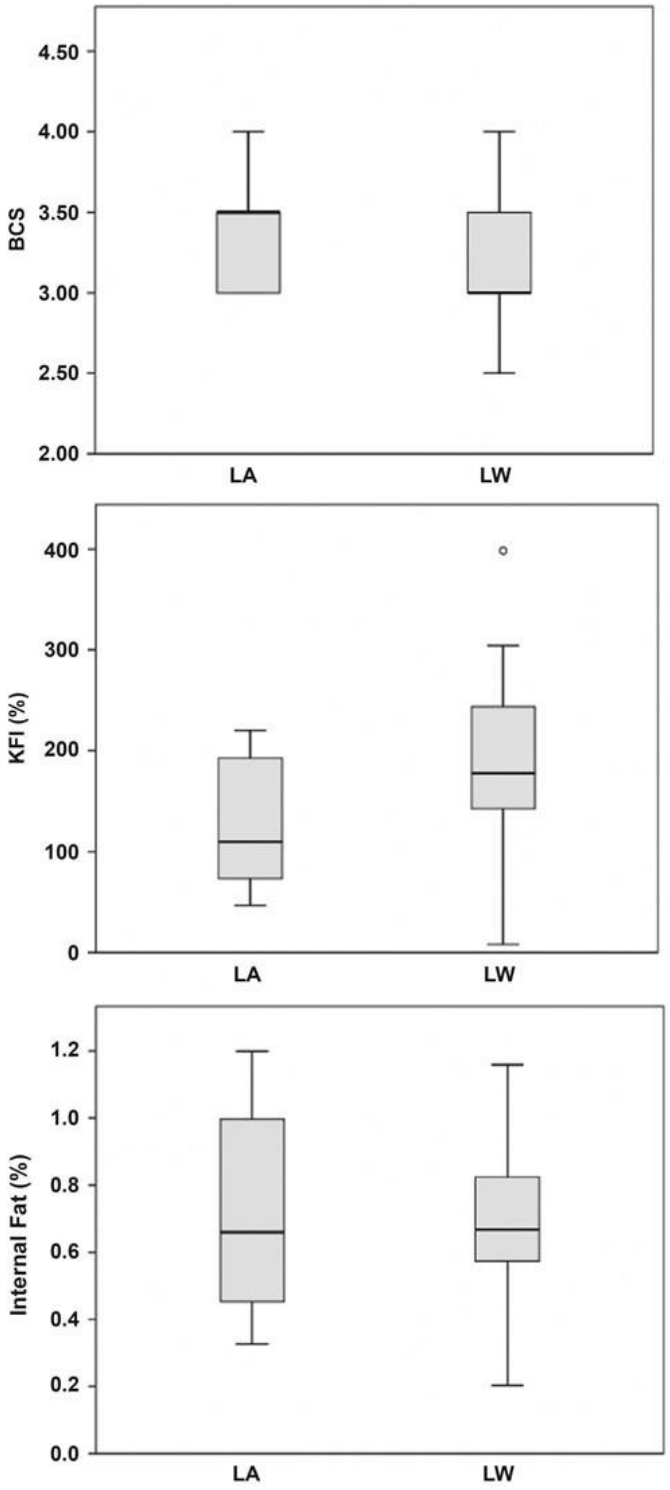

Figure 4.1 Box-plot of the studied body condition parameters (BCS, KFI and internal fat) after the fattening period of fallow deer (*Dama dama*) culled on different season: LA (*n= 18*) and LW (*n= 27*). Independent samples Student's t tests showed differences for BCS (*P*= 0.006) and KFI (*P*= 0.012). Mann–Whitney *U* test found no differences in internal fat depots (*P*= 0.729). BCS = body condition score; KFI = kidney fat index; LA = late autumn; LW = late winter.

4.4 Discussion

Amino acid supplementation did not have any effects on growth (final weight, carcass weight and ADG), although it affected dressing percentage, body condition and blood biochemistry (CREA, BUN, TP, ALB and TRIG). For cervids, weight gain is seasonal-dependent due to changes of photoperiod, with lower voluntary feed intake and weight gain during winter connected to the shorter photoperiod (Shin et al., 2000). As expected, culling season (LA *v.* LW) significantly affected ADG, carcass weight, dressing percentage, BCS, KFI and some blood metabolites like BUN and ALB.

From a commercial point of view, the most interesting result is that dressing percentage was positively influenced by dietary Lys and Met in both culling seasons. Dressing percentage of fallow deer (Volpelli et al., 2002), reindeer (Wiklund et al., 2003a) and red deer (Wiklund et al., 2003b) was reported to be higher when fed with commercial concentrate than with pasture. Thus, similar effects under high-quality protein supplementation were also expectable.

Moreover, this kind of effects is more likely to occur in still-growing animals, since their metabolic rate is higher (Barboza et al., 2009). Thus, animals that received better nutrition during this stage performed better. The reasons behind the increased carcass weight and dressing percentage in the LW are more unclear, and discussed next in combination with other results.

The RPAA supplementation treatments did not affect body growth (final weight, carcass weight and ADG). However, *Lys+Met* supplementation improved all the body condition indicators studied: BCS, KFI and percentage of internal fat; while Lys supplementation just had a significant but weaker effect on the percentage of internal fat. It has already been demonstrated that protein supplementation positively affects body condition in fallow deer (Hutchison et al., 2012). Here, we show an improvement of body condition through supplementation of amino acids, especially in the *Lys+Met* group. However, since no significant effect in BW was noticed, it is possible that these amino acids were mainly metabolized as a source of energy (fat deposition; Barboza et al., 2009). Nevertheless, the deposition of fat varied in time: the percentage of internal fat remained stable between LA and LW, KFI increased in LW, even if CP content of the main feedstuff was much lower (good-quality pasture with 12.70% CP in autumn v. grass silage with 5.75% CP in winter). Similar results have been already reported: Kuroiwa et al. (2017) observed slightly higher KFI in winter than in summer in sika deer even if food availability and intake were lower, and Tollefson et al. (2010) showed that body fat percentage started to increase from early autumn through winter

in mule deer (*Odocoileus hemionus*). On the contrary, BCS, which is based on palpation of both muscles and external fat deposits, decreased along winter. Since carcass weight and dressing percentage also increased in LW (which means no depletion of muscles), it suggests that while external fat stores were being used during the winter, fat was simultaneously being deposited around the kidneys for future utilization as an energy source (Stevens et al.,2002). Considering these results and the seasonal adaption of cervids to low feed intake during winter (Scott et al., 2013), it may be hypothesized a physiological strategy focused on storing internal fat during the winter 'no-growth' period in order to subsequently support enhanced growth (for growing animals), late gestation (for females) and antler growth (for males) during early spring, when protein supply in the wild is maximum (White, 2012). This winter 'no-growth' period is also clearly supported in our experiment by the lack of differences in weight between LA and LW, as so as by the sharp decrease in ADG.

Supplementation of RPAAs increased the blood plasma CREA and TRIG concentrations, and decreased blood plasma BUN, TP and ALB concentrations. An increase of CREA may reflect both increased muscle mass and increased rate of proteolysis (Russell and Roussel, 2007; Caldeira et al., 2007a). However, if the first were true a positive correlation with BCS should be expected (Caldeira et al., 2007a), but on the contrary BCS negatively explained blood CREA levels, supporting the increased proteolysis. Blood TP concentration measures all the proteins found in the blood. ALB is the largest fraction of serum TP, positively related to BCS (Caldeira et al., 2007b), and is influenced by nutrition and the metabolic status of the animals (Adachi et al., 1997; Caldeira et al., 2007b; Huang et al., 2015b). Blood urea nitrogen is a by-product of protein catabolism, and thus greater levels are observed when dietary protein is increased (Caldeira et al., 2007a; Tomkins and McMeniman, 2006). Thus, increased BUN, TP and ALB concentration reflects the amount of protein available. In sika deer, increased BUN, TP and ALB concentration was also observed when Met was supplemented (Huang et al., 2015b). However, our results showed the opposite pattern: the *Lys+Met* supplementation treatments negatively affected BUN, TP and ALB. Finally, the increase in plasma TRIG is associated with lipid metabolism and can be used to monitor body condition (Serrano et al., 2008), since it is associated with the amount of stored fat in body tissues (Caldeira et al., 2007a and 2007b). Kudrnáčová et al. (2019) recently found a decrease in intramuscular fat in fallow deer supplemented with Lys during the fattening period during summer. In our experiment, Lys supplementation positively affected TRIG concentration, supporting increased metabolism of lipids. Moreover, certain seasonal effect was observed: in LW, BUN was lower and ALB was higher than in LA.

Altogether, and contrary to the expected, the results suggest that amino acid supplementation decreased the metabolism of proteins but boosted that of lipids. One explanation may be related to the physiological peculiarities already described for cervids, which may prioritize storing fat reserves to body growth during the winter period. On the other hand, concentrations of TP and ALB, at least, have been found to be affected by the methods of capture (Marco and Lavin, 1999), which could have affected our results.

As previously suggested, our results also support that supplementation of Lys and Met positively influences the metabolism of cervids, improving the body condition particularly during winter. The season of harvesting of farmed deer also influences the productivity and physiology of the animals, and how they use the available protein resources. Further research on amino acid supplementation should focus on early growth, physiological monitoring, antler growth and reproduction, seasonal effects and ecological and economic importance of supplementation. Moreover, inclusion rates for cervids should be determined since all the studies focusing on RPAA supplementation in cervids (Mendoza-Nazar et al., 2012; Huang et al., 2015a and 2015b; and this study) used higher amounts than those recommended for dairy cows according to the metabolic BW of the animals used, while the results obtained for deer are clear.

4.5 References

Adachi K, Kawano H, Tsuno K, Nomura Y, Katsura N, Arikawa A, Tsuji A and Onimaru T 1997. Values of the serum components in Japanese Black beef steers at farms with high productivity and low frequencies of disease and death in Miyazaki prefecture. Journal of Veterinary Medical Science 59, 873–877.

Audigé L, Wilson PR and Morris RS 1998. A body condition score system and its use for farmed red deer hinds. New Zealand Journal of Agricultural Research 41, 545–553.

Barboza PS, Parker KL and Hume ID 2009. Integrative wildlife nutrition. SpringerVerlag, Berlin, Heidelberg, Germany.

Caldeira RM, Belo AT, Santos CC, Vazques MI and Portugal AV 2007a. The effect of body condition score on blood metabolites and hormonal profiles in ewes. Small Ruminant Research 68, 233–241.

Caldeira RM, Belo AT, Santos CC, Vazques MI and Portugal AV 2007b. The effect of long-term feed restriction and over-nutrition on body condition score, blood metabolites and hormonal profiles in ewes. Small Ruminant Research 68, 242–255.

Ceacero F, Clar-Serrano MA, Kotrba R, Ny V, and Faltus O 2018. Effects of lysine and methionine supplementation on fattening and blood protein metabolites in Fallow Deer (*Dama dama*). In Presented at the 9th International Deer Biology Conference, 5–10 August 2018, Estes Park, USA.

Ceacero F, García AJ, Landete-Castillejos T, Bartošová J, Bartoš L and Gallego L 2012. Benefits for dominant red deer hinds under a competitive feeding system: food access behavior, diet and nutrient selection. PLoS ONE 7, e32780.

Hoffman LC and Cawthorn D 2013. Exotic protein sources to meet all needs. Meat Science 95, 764–771.

Huang J, Sun WL, Li CY, Liu HL, Zhang TT, Bao K, Fan YY, Li GY and Wang KY 2015a. Effects of DL-methionine supplement on growth performance and amino acid digestion and plasma concentrations in sika deer calves (*Cervus nippon*). Animal Production Science 56, 1002–1007.

Huang J, Zhang TT, Kun B, Li GY and Wang KY 2015b. Effect of supplementation of lysine and methionine on growth performance, nutrients digestibility and serum biochemical indices for growing sika deer (*Cervus nippon*) fed protein deficient diet. Italian Journal of Animal Science 14, 3640.

Hutchison CL, Mulley RC, Wiklund E and Flesch JS 2012. Effect of concentration feeding on instrumental meat quality and sensory characteristics of fallow deer venison. Meat Science 90, 801–806.

Jönsson KI 1997. Capital and income breeding as alternative tactics of resource use in reproduction. Oikos 78, 57–66.

Kotrba R 2016. Game management under ranching and farming condition in the Czech Republic and Europe. In Proceedings of the 9th International Wildlife Ranching Symposium, Windhoek, Namibia, p. 15.

Kudrnáčová E, Bureš D, Bartoň L, Kotrba R, Ceacero F, Hoffman L and Kouřimská L 2019. The effect of barley and Lysine supplementation of pasture-based diet on growth, carcass composition and physical quality attributes of meat from farmed fallow deer (*Dama dama*). Animals 9, 1–13.

Kung L and Rode LM 1996. Amino acid metabolism in ruminants. Animal Feed Science and Technology 59, 167–172.

Kuroiwa A, Kuroe M and Yahara T 2017. Effects of density, season, and food intake on sika deer nutrition on Yakushima Island, Japan. Ecological Research 32, 369–378.

Marco I and Lavin S 1999. Effect of the method of capture on the haematology and blood chemistry of red deer (*Cervus elaphus*). Research in Veterinary Science 66, 8184.

Mendoza-Nazar P, Mendoza-Martínez GD, Herrera-Haro J, Ruiz-Sesma B, Bárcena-Gama R and Tarango-Arámbula L 2012. Effect of ruminally protected methionine on body weight gain and growth of antlers in red deer (*Cervus elaphus*) in the humid tropics. Tropical Animal Health and Production 44, 681–684.

Merchen NR and Titgemeyer EC 1992. Manipulation of amino acid supply to the growing ruminant. Journal of Animal Science 70, 3238–3247.

National Research Council (NRC) 2007. Nutrient requirements of small ruminants: sheep, goats, cervids, and new world camelids. National Academy Press, Washington, DC, USA.

Ninov NP 2003. Somatometric characteristics of the fallow deer (*Dama dama* L., 1758) in Bulgaria. Acta Zoologica Bulgarica 55, 43–52.

Oke BO, Loerch SC and Deetz LE 1986. Effects of rumen-protected methionine and lysine on ruminant performance and nutrient metabolism. Journal of Animal Science 62, 1101–1112.

Ordway R and Aines G 2010. Feeding Lysine: a nutritionist and dairy producer's perspective. In Proceedings of the High Plains Dairy Conference, 2010, Texas, US, pp. 109–116.

Polan CE, Cummins KA, Sniffen CJ, Muscato TV, Vicini JL, Crooker BA, Clark JH, Johnson DG, Otterby DE, Guillaume B, Muller LD, Varga GA, Murray RAand Peirce-Sandner SB and 1991. Responses of dairy cows to supplemental rumen protected forms of methionine and lysine. Journal of Dairy Science 74, 2997–3013.

Riney T 1955. Evaluating condition of free-ranging red deer (Cervus elaphus), with special reference to New Zealand. New Zealand Journal of Science and Technology 36, 430–463.

Russell KE and Roussel AJ 2007. Evaluation of the ruminant serum chemistry profile. Veterinary Clinics of North America: Food Animal Practice 23, 403–426.

Savage VM, Gillooly JF, Woodruff WH, West GB, Allen AP, Enquist BJ and Brown JH 2004. The predominance of quarter-power scaling in biology. Functional Ecology 18, 257–282.

Schwab CG 1996. Rumen-protected amino acids for dairy cattle: progress towards determining lysine and methionine requirements. Animal Feed Science and Technology 59, 87–101.

Scott IC, Asher GW, Barrell GK and Juan JV 2013. Voluntary food intake of pregnant and non-pregnant red deer hinds. Livestock Science 158, 230–239.

Serrano E, Gonzalez FJ, Granados JE, Moco G, Fandos P, Soriguer RC and Perez JM 2008. The use of total serum proteins and triglycerides for monitoring body condition in the Iberian wild goat (*Capra pyrenaica*). Journal of Zoo and Wildlife Medicine 39, 646–649.

Shin HT, Hudson RJ, Gao XH and Suttie JM 2000. Nutritional requirements and management strategies for farmed deer – review. Asian-Australasian Journal of Animal Sciences 13, 561–573.

Stevens DR, Webster JR, and Corson ID 2002. Effects of seasonality and feed quality on the feed requirements and live weight gain of young deer-a review. New Zealand Grassland Association (NZGA): Research and Practice Series 9, 17-23.

Teare JA 2013. *Dama dama*: ISIS physiological reference intervals for captive wildlife. Retrieved on 15 March 2017 from https://zims.species360.org/Main.aspx.

Tollefson TN, Shipley LA, Myers WL, Keisler DH and Dasgupta N 2010. Influence of summer and autumn nutrition on body condition and reproduction in lactating mule deer. Journal of Wildlife Management 74, 974–986.

Tomkins NW and McMeniman NP 2006. The effect of different levels of dietary crude protein on urea metabolism of rusa deer (*Cervus timorensis*). Small Ruminant Research 66, 187–196.

Volpelli LA, Valusso R and Piasentier E 2002. Carcass quality in male fallow deer (*Dama dama*): effects of age and supplementary feeding. Meat Science 60, 427–432.

White TC 2012. The inadequate environment: nitrogen and the abundance of animals. Springer-Verlag, Berlin & New York.

Wiklund E, Johansson L and Malmfors G 2003a. Sensory meat quality, ultimate pH values, blood parameters and carcass characteristics in reindeer (*Rangifer tarandus tarandus* L.) grazed in natural pastures or fed a commercial feed mixture. Food Quality and Preference 14, 573–581.

Wiklund E, Manley TR, Littlejohn RP and Stevenson-Barry JM 2003b. Fatty acid composition and sensory quality of Musculus longissimus and carcass parameters in red deer (*Cervus elaphus*) grazed on natural pasture or fed a commercial feed mixture. Journal of the Science of Food and Agriculture 83, 419–424.

CHAPTER 5

Effects of immunocastration and supplementary feeding level on the performance and blood biochemical markers of farmed yearling fallow deer (*Dama dama*)

Adapted from: Ny V, Needham T, Bartoň L, Bureš D, Kotrba R, Musa AS, Ceacero F. 2023. Effects of immunocastration and supplementary feeding level on the performance and blood biochemical markers of farmed yearling fallow deer (*Dama dama*). Journal of Animal Physiology and Animal Nutrition. https://doi.org/10.1111/jpn.13807.

Authors' contribution: the first author, **Veit Ny** participated in data collection, laboratory analyses, data curation, writing-original draft, review and editing. The paper was finally reviewed, commented, and edited for finally published by all authors.

Immunocastration and supplementary feeding level on the performance and blood biochemical markers of farmed yearling fallow deer (*Dama dama*)

Veit Ny[1,2], Tersia Needham[1], Luděk Bartoň[2], Daniel Bureš[2], Radim Kotrba[1,3], Abubakar S. Musa[1], Francisco Ceacero[1]

[1] Department of Animal Science and Food Processing, Faculty of Tropical AgriSciences, Czech University of Life Sciences Prague, 165 00 Prague, Czech Republic

[2] Department of Cattle Breeding, Institute of Animal Science, 104 00 Prague, Czech Republic

[3] Department of Ethology, Institute of Animal Science, 104 00 Prague, Czech Republic

A B S T R A C T

In cervids, blood biochemical markers may reflect changes in various physiological and environmental factors, especially in response to changes in metabolism following nutrient supplementation or the manipulation of hormone production. Decreasing androgen production through immunocastration (IC) to ease the husbandry of male animals is currently a more ethically acceptable method than physical castration, but it is unexplored in fallow deer. Forty yearling male fallow deer were grouped into four treatment combinations: IC on high (200g commercial pellets + 600g concentrate mixture of 90% oats and 10% wheat grains) or low (100g commercial pellets + 300g concentrate mixture of 90% oats and 10% wheat grains) level of feed supplementation, or non-castrated bucks on a high or low level of feed supplementation. Immunocastrated animals were vaccinated at the start of the study (week 1) and again during week 3 of the study. Diet affected all body growth parameters (slaughter weight, daily gain, carcass weight, dressing percentage, and body condition score). Fallow deer from all treatments showed increasing concentrations of fat and energy blood biochemical markers over the study period, including plasma glucose (GLU) and triglyceride (TRIG), and decreased cholesterol (CHOL) and lipase (LIPA) concentrations. The higher level of supplementary feeding decreased plasma albumin (ALB) and creatinine (CREA), and increased globulin (GLOB) concentrations. On the other hand, IC and lower-level supplementation reduced growth performance. Overall, IC can be an interesting tool for welfare management of yearling stags for slaughter; however, the advantage appears to only be in well-fed animals, as low-level of feeding can further reduce growth performance in immunocastrated animals. Further studies should evaluate the carcass performance of animals under similar treatment conditions to ascertain the effects on muscle and fat yields.

KEYWORDS: Castration; Cervid; Supplementary feeding; Venison; Welfare

5.1 Introduction

Deer farming produces animals for trophy hunting, venison, velvet, and other by-products (Hoffman & Wiklund, 2006; Serrano et al., 2019). New Zealand is the leading country for deer farming, especially for venison, with their venison market value estimated at ~ $1.5B USD and which generates about 75% of the annual revenue from all deer products in New Zealand (Allan et al., 2015). Of all deer products produced internationally, venison produced in New Zealand contributes 55% of the generated revenue world-wide (Serrano et al., 2019). Moreover, deer farming in Europe has increased over the last decade, with the main venison producer in Europe being Spain (Serrano et al., 2019). According to the latest survey conducted in 2010 by the European Federation of Deer Farmers Associations, the estimated numbers of farms in the Europe Union was 10, 000 with a total number of 280,000 deer (Kotrba & Bartoš, 2010). Deer utilization is also increasing in other parts of the word across the USA, England, Australia, and Asia (Serrano et al., 2019; Spencer, 2020).

Improving nutrition and feeding, especially protein (Dryden, 2011), to maximise production yield and quality is as important in deer as in other livestock species (Volpelli, Valusso, & Piasentier, 2002; Ny, Needham, & Ceacero, 2022). Blood biochemical markers may be used as indicators of metabolic changes in response to different nutritional conditions, especially those related to protein and fat metabolites. For instance, low nutritional levels can affect nutrient digestion and metabolism, depressing protein and/or fat metabolism, thereby decreasing markers such as blood urea nitrogen (BUN), creatinine (CREA), and total protein (TP) concentrations (Poljičak-Milas et al., 2004; McCusker, Shipley, Tollefson, Griffin, & Koutsos, 2011). On the other hand, feeding high protein diets has shown to increase blood albumin (ALB) and TP concentrations in reindeer (Rangifer tarandus tarandus; Säkkinen et al., 1999). Fallow deer (Dama dama) raised on adequate nutrition or feed supplementation also have higher concentrations of blood ALB, CREA, cholesterol (CHOL), TP, and triglycerides (TRIG) concentrations compared to non-supplemented pasture-fed deer (Slavica et al., 2000; Poljičak-Milas et al., 2004; Poljičak-Milas, Slavica, Janicki, Marenjak, & Kolić, 2006).

Changes in androgenic hormones can also affect protein metabolism (Bruss, 2008). In male deer, testosterone concentrations rise at the start of the first antler growth when reaching puberty and during the fattening period (Bartos, Bubenik, & Kuzmova, 2012). Therefore, both direct and indirect effects of nutrition can affect testosterone production and protein anabolism under adequate feeding levels, and thus ultimately the blood biochemical profile of the animal (Fennessy & Suttie, 1985Bruss, 2008). While elevated testosterone production can increase

blood TP metabolites and TRIG concentrations due to increased protein anabolism (Eckersall, 2008) and decreased fat deposition (Bruss, 2008; Kun et al., 2021), it can also affect the excitability of the animal, leading to an increase in blood GLU (Säkkinen et al., 1999; Poljičak-Milas et al., 2004; Das, Choubey, Gupta, Saini, & Swarup, 2010). In deer farming, large groups are maintained under mostly extensive conditions, with male animals forming a large proportion of the animals slaughtered for meat production, simply due to the need for fewer male breeding replacements in the remaining herd. However, this creates numerous management issues related to the androgenic effects of testosterone, particularly during rutting, which can result in fighting and injury, and further complicates handling, especially the pre-slaughter handling of male spikers (Mulley & English, 1992).

While castration is utilised to control many behavioural issues associated with male livestock production, this is not necessarily common practice within the deer industry, despite the need for decreasing aggressive behaviour in male fallow deer (Mulley & English, 1992). Immunocastration is ethically more acceptable than physical castration due to numerous welfare concerns linked to physical castration methods. It has improved management and production in many livestock species (Needham, Lambrechts, & Hoffman, 2017a), making it an attractive solution for managing male spiker herds used for meat production. As immunocastration suppresses testosterone, oestradiol, and IGF-1 levels (Claus, Lacorn, Danowski, Pearce, & Bauer, 2007), the animal's potential for protein anabolism and fat catabolism are likely to decrease, affecting their nutrient responses, particularly dietary protein (Needham, Hoffman, & Gous, 2017b). Typically, this effect is seen after the second vaccination is given (from up to two weeks after), when GnRH-antibody titres rise substantially, and testosterone concentrations subsequently decrease. These metabolic changes are reflected in the increased fat deposition of immunocastrated livestock species, especially in fast-growing monogastric animals, like pigs (Needham & Hoffman, 2015). However, the effects of immunocastration on the nutrient responses and metabolic status of ruminants remains poorly characterised, and currently non-existent in fallow deer, despite its potential benefits for animal husbandry and meat quality.

Therefore, it could be expected that providing a high dietary feeding level should improve the growth performance of fallow deer, and should be reflected by changes in blood biochemical markers primarily linked with protein and fat metabolism. Furthermore, immunocastration might alter protein and biochemical markers due to the decreased anabolic potential of these animals. Thus, the aim of this study was to determine the combined effects of varying dietary feeding levels and immunocastration on the growth performance and nutrient

utilisation (blood protein and fat biochemistry markers) of yearling male fallow deer during finishing.

5.2 Material and methods

All experimental procedures and use of animals were approved by the Institutional Animal Care and Use Committee at the Czech University of Life Sciences, Prague within its competence (Permit: 63479_2016-MZE-17214).

5.2.1 Animals, diets and experimental design

The study was carried out from June to October 2019, at a private deer farm at Mnich, in the South Bohemian region of the Czech Republic (49.17N, 14.90E; 485 masl). The timeline of immunocastration vaccinations, the start of feed supplementation, and sampling is illustrated in Figure 5.1. In week 1, forty yearling male fallow deer (13 months old) were individually ear-tagged and grouped into four treatment combinations (balanced for body weight, average live weight 39.9 ± 2.45 kg). The treatments combinations (10 animals per treatment) included immunocastrated animals fed on either a high (High x IC) or low (Low x IC) feed supplementation level, or control non-castrated bucks fed on either a high (High x Co) or low (Low x Co) feed supplementation level. The animals were extensively grazing on pasture with young (5-15 cm long) green grass in two separate paddocks until the supplementation started; the first paddock was utilized for the animals (10 immunocastrates and 10 controls) fed a high level of feed supplementation and the other for the animals (10 immunocastrates and 10 controls) fed a low level of supplementation.

The feed supplementation started from week 4 (14 months old) and continued to week 17 and 18, for approximately 100 days. The low-level supplemented group received 100g commercial pellets + 300g concentrate mixture (90% oats + 10% wheat) and the high-level group received 200g commercial pellets + 600g concentrate mixture (90% oats + 10% wheat) per deer per day. The concentrate supplementation was given on daily basis, mimicking typical commercial farming practice in Europe, and it was observed to be completely consumed before the end of each day. The concentrate was spread in wooden troughs (4.5 m length, 0.22 m width) placed in paddocks, allowing access to all animals simultaneously to avoid competition and monopolisation of the resource by the dominant individuals. Moreover, supplemented feed was all consumed by the end of the day.

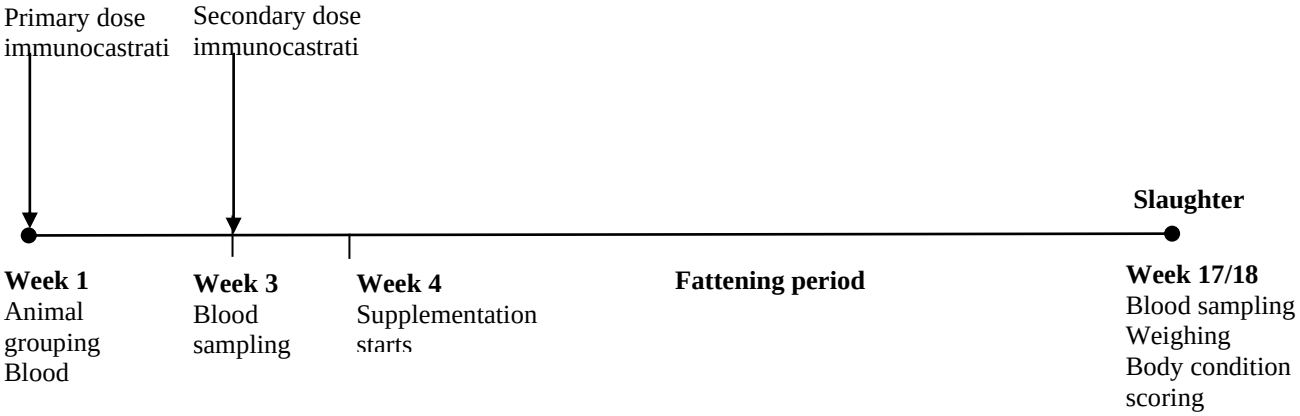

Figure 5.1 The immunocastration vaccination schedule, blood sampling, feed supplementation period, and data collection points from the study onset (week 1) until the slaughter (week 17/18).

The pellets were obtained from a commercial deer feed manufacturer, Mikrop Čebín a.s. The ingredients of the commercial pelleted feed were soybean meal, rapeseed meal, alfalfa meal, and a premixture of minerals. The nutrient composition of the concentrate mixture and pasture were analysed, following the methodology of Kudrnáčová et al. (2019), and is shown in Table 5.1. The immunocastration procedure was performed twice; the first dose was given in week 1 and the second dose was given two weeks after (week 3). The immunocastrated group were subcutaneously injected with 2mL Improvac per dose (Zoetis Animal Health, New Jersey, USA), using a Sterimatic safety vaccination gun (Sterimatic Worldwide Ltd, UK).

Table 5.1 Mean chemical compositions of the pasture and concentrate supplemented to fattening fallow deer bucks.

Composition (g/kg Dry Matter)	Concentrate mixture	Pasture*
Crude protein	18.56	12.28
Crude fat	3.58	1.04
Crude fibre	14.19	33.14
Ash	7.37	4.81
Nitrogen-free compounds	56.31	48.73
Acid detergent fibre	13.24	35.61
Neutral detergent fibre	35.55	69.72

*Pasture was collected at the beginning, in the middle and at the end of the experiment, and from three different locations in each paddock; the analyses were done for each collection and the mean values are shown above.

5.2.3 Sample collection and analysis

Body weight was measured at the beginning (week 1) and at the end of the experiment (week 17/18) to determine the average daily gain (ADG) over the entire experimental period. Blood was collected from all animals (n = 40) at three time points (week 1, week 3, week 17), using an 18G needle, from the jugular vein and into vacutainer tubes for separation of plasma. Subsequently, blood samples were centrifuged at 24 000×g RCF for 15 min, and plasma samples were transferred to microtubes for storage at −18°C until analyses. At the time of analysis, five animals per treatment were randomly selected and their frozen blood plasma samples for the study period were thawed at room temperature, mixed by inversion, and centrifuged at 12 000×g RCF for 2 minutes to ensure homogeneity and remove fibrin particles that may have formed during storage. A sub-sample of seven animals per treatment group were randomly selected for blood biochemical profile analysis. Plasma samples were then analysed for the concentrations of biochemical markers related to protein and fat metabolism, including ALB, BUN, CHOL, CREA, GLU, lipase (LIPA), TP, and TRIG, using a VetTest Chemistry Analyzer (IDEXX Laboratories, Westbrook, ME, USA) and standard commercial kits. The subtraction of ALB from TP determined the concentration of globulin (GLOB). As the VetTest Chemistry Analyzer has no reference ranges for cervids, the physiological reference ranges for *Dama dama* from Teare (2013) and Vengušt and Bidovec (2002) were utilised.

The animals were slaughtered over two days (during weeks 17/18) according to the capacity of the abattoir facility, where half of the animals from each treatment group were slaughtered per day. Animals were slaughtered in a restraint box by stunning with a captive bolt

gun and exsanguinated by severing both neck arteries. Live weight was recorded at the point of slaughter (i.e., slaughter weight). The body condition score (BCS) was also measured at slaughter, through palpation and following the BCS chart for deer detailed by Audigé, Wilson and Morris (1998), with an interval of 0.5 and a scoring range from 1 (lean) to 5 (fat). Then, carcasses were transported for processing at the abattoir, located 20 km from the farm, where carcasses were eviscerated and weighed (hot carcass), and chilled for 12 h at 4°C. The dressing percentage was calculated using the hot carcass weight, dressing % = (Hot carcass weight/Live weight)*100.

5.2.4 Statistical analysis

Performance data collected at slaughter (slaughter weight, carcass weight, ADG, dressing percentage, and BCS) were analysed through General Linear Models (GLM), with castration status, nutrition level, and their interaction included in the model as fixed effects (Table 5.2). General Linear Mixed Models (GLMM) were used for testing the influence of diets, IC treatments and the diet*IC interaction on the blood biochemical markers studied (Table 5.3). Period was also included in the model and entered the models as a repeated measure for each individual. For both GLM and GLMM models, pairwise comparisons through least significant differences were used to detect differences due to the nutrition level within each IC-treatment in the variables analysed, independently of their potential interaction already considered in the main models. The full models are shown in Tables 5.2 and 5.3. A significance level of 5 % was used throughout. All the analyses were performed in SPSS version 28.0 (IBM, SPSS, USA).

5.3 Results

For the body growth parameters, there were no significant interactions between castration status and nutritional level (Table 5.2). As expected, nutritional level affected all studied body growth parameters (slaughter weight, carcass weight, ADG, dressing percentage, and BCS); however, castration status had no effect (Table 5.2). Those animals which received a high level of supplementary feeding had higher slaughter weights ($P = 0.005$), carcass weights ($P = 0.002$), ADG ($P < 0.001$), dressing percentages ($P = 0.039$) and BCS ($P < 0.001$).

A significant interaction was found between castration status and nutritional level for the ALB ($P = 0.026$; Figure 5.2) and GLOB ($P = 0.041$; Figure 5.3) concentrations. Regarding the main effects, some of the selected blood biochemistry parameters linked to the metabolism of fat and protein varied over the study period (Table 5.3). CHOL, and LIPA concentrations decreased, GLU and TRIG increased, while BUN showed an irregular pattern. Animals under a high level of supplementary feeding showed decreased ALB, CREA and GLU concentrations, while LIPA and GLOB increased. Immunocastration had a lesser effect, decreasing CREA but increasing GLOB.

Pairwise comparisons were extracted from the models to determine the effects of supplementary feeding separately for the IC and control groups. Supplementation increased carcass weight ($P = 0.018$ for control; $P = 0.033$ for IC), ADG ($P = 0.001$ vs $P < 0.001$), and GLOB ($P < 0.001$ vs $P = 0.004$) and decreased ALB ($P < 0.001$ vs $P = 0.007$) and CREA ($P < 0.001$ vs $P = 0.007$) concentrations.

The effect was generally greater for the control group than the IC group, except for ADG, which is slightly greater for the IC group. Moreover, supplementary feeding resulted in significantly increased slaughter weight ($P = 0.029$) and decreased BUN ($P = 0.039$), GLU ($P = 0.011$) and TRIG ($P = 0.013$) concentrations just for the control group. The contrary (significant effect of supplementary feeding just for the IC group) was observed for LIPA ($P = 0.010$) concentrations and BCS ($P < 0.001$).

Table 5.2 General Linear Model showing the combined effects of immunocastration, supplementary feeding and their interaction on growth patterns of fattening fallow deer bucks (*n=40*) measured at slaughter. Statistical differences of the pairwise comparison for the effect of diet within the control (Co) and immunocastrated (IC) groups are also shown.

	R^2	Castration		Diet		SE	P-values		
		Co	IC	Low	High		Castration	Diet	IC x Diet
Slaughter weight (kg)	0.208	47.45	46.93	45.98	48.40	0.44	0.521	0.005	0.830
Carcass weight (kg)	0.245	26.52	26.09	25.43	27.18	0.29	0.415	0.002	0.857
ADG (g)	0.528	67.13	60.93	51.48	76.58	2.90	0.142	<0.001	0.295
Dressing (%)	0.127	55.87	55.56	55.28	56.14	0.20	0.433	0.039	0.928
BCS	0.343	2.75	2.68	2.55	2.88	0.05	0.367	<0.001	0.136

Note: significant *p-Values* are indicated by $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$.

Abbreviations: IC- Immunocastrated; Co- Control; High- High level of supplementary feeding; Low- Low level of supplementary feeding; SE – pooled standard error of the means.

Figure 5.2 Interaction of castration and diet on blood ALB concentrations of immunocastrated and control (non-castrated) fattening fallow deer bucks (n=28). High- High level of supplementary feeding; Low- Low level of supplementary feeding. Error bars indicate the standard deviation of the mean values.

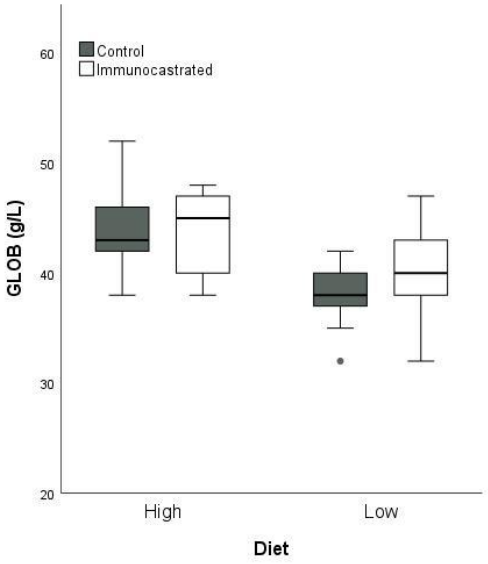

Figure 5.3 Interaction of castration and diet on blood GLOB concentrations of immunocastrated and control (non-castrated) fattening fallow deer bucks (n=28). High- High level of supplementary feeding; Low- Low level of supplementary feeding. Error bars indicate the standard deviation of the mean values.

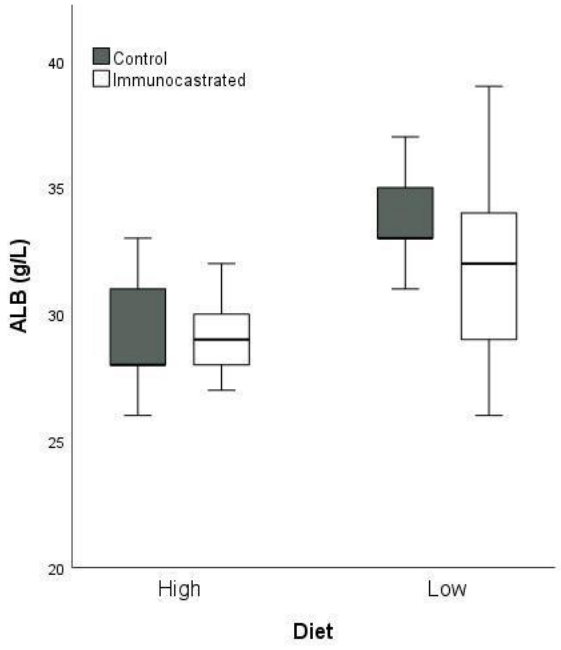

Table 5.3 Generalized Linear Mixed Models showing the effects of immunocastration, supplementary feeding and their interaction on blood biochemistry markers of fattening fallow deer bucks (*n=28*) measured along the experiment (1- start, 2- week 3 and at 3- culling). Statistical differences of the pairwise comparison for the effect of diet within the control (Co) and immunocastrated (IC) groups are also shown.

	Castration		Diet		Period [1]			SE	P-values			
	Co	IC	Low	High	1	2	3		Castration	Diet	IC x Diet	Period
ALB (g/L)	29.06	29.63	33.82	31.82	30.92	30.70	31.63	0.35	0.210	<0.001	0.026	0.382
BUN (mmol/L)	7.43	7.67	7.95	7.63	7.91^{a}	6.91^{b}	8.19^{a}	0.11	0.830	0.164	0.107	<0.001
CHOL (mmol/L)	1.51	1.48	1.51	1.49	1.62^{a}	1.48^{ab}	1.40^{b}	0.03	0.594	0.933	0.871	0.002
CREA (µmol/L)	101.33	91.96	148.65	114.80	115.15	104.88	117.26	3.87	0.003	<0.001	0.167	0.230
GLU (mmol/L)	6.19	6.33	6.90	6.57	5.15^{b}	6.19^{ab}	8.15^{a}	0.17	0.613	0.014	0.218	<0.001
LIPA (U/L)	170.31	181.18	147.21	129.80	179.56^{a}	182.08^{a}	109.75^{b}	8.61	0.816	0.010	0.316	<0.001
TP (g/L)	72.85	73.22	72.30	72.09	72.29	71.91	73.65	0.33	0.902	0.198	0.651	0.083
TRIG (mmol/L)	0.07	0.12	0.14	0.09	0.04^{c}	0.06^{b}	0.11^{a}	0.01	0.679	0.467	0.132	<0.001
GLOB (g/L)	43.67	43.86	37.73	40.87	41.19	41.30	42.09	0.45	0.021	<0.001	0.041	0.501

Note: significant *P-values* are indicated by $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$.

[1] Differences after pairwise contract between the three periods studied are indicated by superscripts. Abbreviations: IC- Immunocastrated; Co- Control; High- High level of supplementary feeding; Low- Low level of supplementary feeding; SE- pooled standard error of the means.

5.4 Discussion

The higher supplementary feeding level used in the present study improved growth parameters related to body mass gain, body condition, and carcass weight. Similar studies on commercial supplementary feeding have shown increased body growth and carcass in fallow deer (Volpelli et al., 2002), reindeer (Wiklund, Johansson, & Malmfors, 2003) and red deer (Wiklund, Manley, Littlejohn, & Stevenson-Barry, 2003). These effects were reflected within the blood biochemical markers, which were further influenced by seasonal variations in metabolism. This effect of season was particularly evident for biochemical markers involved in energy and fat metabolism, including TRIG, LIPA, CHOL, and GLU. TRIG is a long-chain fatty acid that indicates adipose tissue development and fat deposition in the animal body as a reserve energy source (Bruss, 2008; Kun et al., 2021), and is associated with fat storage in animal tissues (Caldeira, Belo, Santos, Vazques, & Portugal, 2007a; Caldeira et al., 2007b; Serrano et al., 2008). GLU is stored as glycogen in the liver and muscles or converted to fatty acids and triglycerides in the liver, adipocytes, and muscles (Kaneko, 2008; Rui, 2014). Thus, plasma GLU concentrations typically reflect the physiological state of the animal body, as increased plasma GLU may indicate an increased tissue metabolic rate (Kaneko, 2008), and is generally positively related to growth rate (Kaneko, 2008; Kun et al., 2021). Both TRIG and GLU increased during the experimental period, likely in preparation for the upcoming rutting and winter periods by increasing fat storage (i.e., fattening period; Stanisz et al., 2019; Ceacero et al., 2020; Kun et al., 2021).

However, plasma GLU concentrations are also impacted by physiological stress, as under stressful conditions, glycogenolysis (breaking down glycogen from liver or muscle sources) is increased (Sapolsky, Romero, & Munck, 2000). Under the lower nutritional conditions in the present study, GLU concentrations were elevated, perhaps as a result of potential nutritional stress that is exacerbated by the seasonal effect on increased fat storage necessary for winter (Kannan, Terril, Kouokou, Gelaye, & Amoah, 2002). Lipase is an enzyme which is secreted from the liver, adipocytes, or vascular endothelial cells (Bruss, 2008). It is involved in TRIG breakdown to lipoprotein, glycerol, and free fatty acids for lipid transportation in the circulatory system (Bruss, 2008; Pirahanchi & Sharma, 2019). Its secretion can be influenced by the availability of plasma GLU concentration, which increases when there is lower concentration of plasma GLU. This agrees with the present results which shows increased plasma GLU together with decreasing plasma LIPA concentration (Kaneko, 2008). Moreover, CHOL is also mobilized by LIPA and resides primarily as lipoprotein in blood plasma, which increases when

the animal starts to utilize body fat stores (Säkkinen et al., 1999; Espenshade, 2013). Therefore, reduced plasma LIPA and CHOL concentrations might be because the animal is in the phase where deer do not utilize fat from their tissues yet, but are rather increasing lipogenesis (increased TRIG) for fat deposition for later utilization in winter.

When the supplementation rate was increased, LIPA and GLOB concentrations increased, while ALB and CREA concentrations decreased, and animals improved their ADG and BCS (closer to 3). Increased plasma LIPA under a high supplementation rate might be related to increasing hormone-sensitive LIPA which changes depending on plasma GLU concentration (Kaneko, 2008). It is known that when there is less availability of plasma GLU, LIPA is produced for lipolysis of fat to fatty acids (Brockman, 2013). Lower plasma GLU concentrations in high-rate supplementary fallow deer group might trigger higher hormone-sensitive LIPA levels in this group compared to low level supplementation (Bruss, 2008).

Good BCSs in deer is determined by more prominent muscle and fat development on the area of wings pine bone, sacral spinous process, and rump area (Audigé et al., 1998), and thus BCS can be an essential indicator of fat deposition and muscle gain in response to dietary changes (Caldeira et al., 2007a, 2007b). Differences in plasma CREA may reflect differing rates of proteolysis for muscle growth and/or kidney functioning (Russell & Roussel, 2007). Previous studies have shown a correlation between BCS and plasma CREA, where animals with BCS on the extreme ends of the evaluation scale (low and high) had elevated CREA concentrations likely due to increased proteolysis, while those animals with average (and thus more optimal) BCSs had lower CREA concentrations and likely a more balanced metabolic state due to no dramatic decrease or increase in proteolysis (Caldeira et al., 2007a, 2007b).

Both ALB and GLOB contribute towards plasma TP (Russell & Roussel, 2007), and may be utilized as indicators of changes in protein metabolism and liver functioning (ALB). Decreased ALB in plasma may reflect hypoproteinaemia in animals fed a protein-deficient diet (Tennant & Center, 2008), but it also reflects liver functioning, as the liver is a major site of ALB synthesis (Bruss, 2008) and any burden on the liver (such as oversupplying dietary protein) can also decrease ALB synthesis. Thus, decreased ALB and increased GLOB concentrations can indicate an imbalance in hepatic synthesis and degradation of ALB (Tennant & Center, 2008). However, while McCusker et al. (2011) found that feeding a high protein supplement diet cause hepatic burden in young mule deer, no negative effects on body growth were reported. In the case of the present study, growth rates were improved under higher supplementation levels, and thus these minor changes in ALB and GLOB had no significant effect on the performance of the animals. Furthermore, these changes in ALB and GLOB were

not reflected in the TP fraction, which was comparable under both supplementation levels, as similarly found in other deer species fed supplementary concentrates (Säkkinen et al., 1999; Das et al., 2010).

Immunocastration also had minor effects on GLOB, but more pronounced effects on CREA, which decreased in comparison to control males, which may reflect decreased protein anabolism as a result of androgen suppression (Needham & Hoffman, 2015; Huenchullan, Vidal, Larraín , & Saénz, 2021). However, this was not reflected in the growth and slaughter performance parameters, where the immunocastrates had the equivalent performance as that of the control males. In other species, particularly pigs, immunocastration decreases lean growth rate, resulting in lighter carcasses compared to non-castrated males, and increases fat deposition. However, no negative effects on the slaughter performance of other immunocastrated game species (Needham, Kotrba, Hoffman, & Bureš, 2020) have been reported. This result is not unexpected, as fallow deer have a slower rate of growth than commercial livestock species, and thus changes in androgens are likely to take longer to affect these parameters than the study period used. Furthermore, the extent of immunocastration on these parameters is influenced by the vaccination schedule, with vaccination early in the life of the animal having a more pronounced effect (Amatayakul-Chantler et al., 2013).

When the data was separated by sex and analysed individually for the effect of nutrition, immunocastrates tended to show less variation in their blood biochemical markers in response to different levels of supplementary feeding. This could reflect differences in the nutrient requirements of IC animals, due to decreased androgen production and alterations in metabolism (Needham et al., 2017a). However, when the effects of nutrition on the growth parameters is considered for each sex independently, immunocastrates showed poorer ADGs, BCSs, and carcass weights under low nutritional conditions. This is likely the combined effect of lower nutrition with decreased anabolic growth potential from immunocastration (Mulley, Asher, Flesh, O'Neill, & Ferguson, 2000; Needham et al., 2017b). Studies on the physical castration of deer have also found a decrease in body condition, and depressed growth rate, in response to interrupted testosterone production (Mulley & English, 1985; Hogg, Catcheside, & Mercer, 1990; Sookhareea, Woodford, & Dryden, 2001; Sookhareea, Taylor, Dryden, & Woodford, 2001).

5.5 Conclusions

Overall, blood biochemical markers related to protein and fat metabolism reflect changes in nutritional supplementation level and physiological growth changes in yearling fallow deer. Moreover, immunocastration also affects blood biochemical markers, likely reflecting changes in protein metabolism (decreased anabolism) as a consequence of androgen suppression. Therefore, immunocastration can be an interesting tool for welfare management in yearling fallow deer; however, the advantage is only realized in animals fed a higher level of supplementation feeding, as low-level feeding can reduce growth performance in immunocastrated animals. Further studies should evaluate the carcass performance of animals under similar treatment conditions to ascertain the effects on muscle and fat yields.

Animal welfare statement

The authors confirm that the ethical policies of the journal have been adhered to, and the appropriate ethical review committee approval has been received. The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes. All experimental procedures and use of animals were approved by the Institutional Animal Care and Use Committee at the Czech University of Life Sciences, Prague within its competence (Permit: 63479_2016-MZE-17214).

Declaration of competing interest

The authors declare no conflict of interest.

Author contributions

Veit Ny: Data collection, Laboratory analyses, Data curation, Writing-original draft; **Tersia Needham**: Conceptualization, Funding acquisition, Methodology, Data collection, Writing-review and editing, Language editing; **Luděk Bartoň**: Conceptualization, Funding acquisition, Data collection, Writing-review and editing; **Daniel Bureš**: Conceptualization, Funding acquisition, Methodology, Funding acquisition, Data collection, Writing-review and editing; **Radim Kotrba**: Methodology, Funding acquisition, Data collection, Writing-review and editing; **Abubakar S. Musa**: Data collection, Laboratory analyses, Writing-review and editing; **Francisco Ceacero:** Conceptualization, Funding acquisition, Methodology, Data collection, Data analyses, Writing-review and editing.

5.6 References

Allan, P., Cook, R., Lawrence, D., Pearse, T., Stevens. D., & Somerville, J. (2015). Deer Farming in Southland – Information on Environmental Management Approaches and Comments on the Draft Water and Land Plan. Prepared for Environment Southland by the New Zealand Deer Farmers Association, Southland Branch. Retrieved from https://www.deernz.org/sites/dinz/files/Comments%20on%20Draft%20Water%20and%20Land%20Plan%20-%20NZDFA%20Southland%20Branch%202015-10-30.pdf.

Amatayakul-Chantler, S., Hoe, F., Jackson, J.A., Roca, R.O., Stegner, J.E., King, V., & Howard, R. (2013). Effects on performance and carcass and meat quality attributes following immunocastration with the gonadotropin releasing factor vaccine Bopriva or surgical castration of *Bos indicus* bulls raised on pasture in Brazil. *Meat Science*, *95*(1), 78-84. doi:10.1016/j.meatsci.2013.04.008.

Audigé, L., Wilson, P.R., & Morris, R.S. (1998). A body condition score system and its use for farmed red deer hinds. *New Zealand Journal of Agricultural Research*, *41*(4), 545–553. doi:10.1080/00288233.1998.9513337.

Bartos, L., Bubenik, G.A., & Kuzmova, E. (2012). Endocrine relationships between rank-related behaviour and antler growth in deer. *Frontiers in Bioscience*, *4*, 1111-1126. doi:10.2741/445.

Brockman, H.L. (2013). Lipases. In William, J., Lennarz, M., & Daniel, L. (Ed.), *Encyclopaedia of Biological Chemistry* (pp. 729-732). Academic Press.

Bruss, M.L. (2008). Chapter 4: Lipids and Ketones. In Kaneko, J.J., Harvey, J.W., & Bruss, M.L. (Ed.), *Clinical biochemistry of domestic animals* (pp. 81-115). Academic press.

Caldeira, R.M., Belo, A.T., Santos, C.C., Vazques, M.I., & Portugal, A.V. (2007a). The effect of body condition score on blood metabolites and hormonal profiles in ewes. *Small Ruminant Research*, *68*, 233-241. doi:10.1016/j.smallrumres.2005.08.027.

Caldeira, R.M., Belo, A.T., Santos, C.C., Vazques, M.I., & Portugal, A.V. (2007b). The effect of long-term feed restriction and over-nutrition on body condition score, blood metabolites and hormonal profiles in ewes. *Small Ruminant Research*, *68*(3), 242-255. doi:10.1016/j.smallrumres.2005.08.026.

Ceacero, F., Clar, M.A., Ny, V., & Kotrba, R. (2020). Differential effects of ruminally protected amino acids on fattening of fallow deer in two culling periods. *Animal*, *14*(3), 648-655. doi:10.1017/S1751731119002325.

Claus, R., Lacorn, M., Danowski, K., Pearce, M.C., & Bauer, A. (2007). Short-term endocrine and metabolic reactions before and after second immunization against GnRH in boars. *Vaccine*, *25*(24), 4689–4696. doi:10.1016/j.vaccine.2007.04.009.

Das, A., Choubey, M., Gupta, S.P., Saini, M., & Swarup, D. (2010). Feed consumption, nutrient utilization, faecal pellet characteristics and serum metabolite profile of captive spotted deer (*Axis axis*) fed diets containing different roughages. *Small Ruminant Research*, *94*, 185-189. doi:10.1016/j.smallrumres.2010.07.015.

Dryden, G.M. (2011). Quantitative nutrition of deer: energy, protein, and water. *Animal Production Science*, *51*(4), 292-302. doi:10.1071/AN10176.

Eckersall, P.D. (2008). Chapter 5: Proteins, Proteomics, and the Dysproteinemias. In Kaneko, J.J., Harvey, J.W., & Bruss, M.L. (Ed.), *Clinical biochemistry of domestic animals* (pp. 117-55). Academic press.

Espenshade, P.J. (2013). Cholesterol Synthesis and Regulation. In William J., Lennarz, M., & Daniel, L. (Ed.), *Encyclopaedia of Biological Chemistry* (pp. 516-520). Academic Press.

Fennessy, P.F., & Suttie, J.M. (1985). Antler growth: nutritional and endocrine factors. In Fenessy, P.F., & Drew, K.R. (Ed.), *Biology of Deer Production* (pp. 242-250). The Royal Society of New Zealand, Wellington.

Hoffman, L.C., & Wiklund, E. (2006). Game and venison – meat for the modern consumer. *Meat Science*, *74*(1), 197-208. doi:10.1016/j.meatsci.2006.04.005.

Kaneko, J.J. (2008). Chapter 3: Carbohydrate Metabolism and Its Diseases. In Kaneko, J.J., Harvey, J.W., & Bruss, M.L. (Ed.), *Clinical biochemistry of domestic animals* (pp. 45-80). Academic press.

Kannan, G., Terrill, T. H., Kouakou, B., Gelaye, S., & Amoah, E. A. (2002). Simulated preslaughter holding and isolation effects on stress responses and live weight shrinkage in meat goats. *Journal of Animal Science*, *80*(7), 1771-1780. doi:10.2527/2002.8071771x.

Kotrba, R., & Bartoš, L. (2010). Survey on deer farming in Europe. *Federation of European Deer Farmers Association (FEDFA)*. Retrieved from http://www.fedfa.org/en/fedfa-org.-data/documents-2767790.

Kudrnáčová, E., Bureš, D., Bartoň, L., Kotrba, R., Ceacero, F., Hoffman, L. C., & Kouřimská, L. (2019). The effect of barley and Lysine supplementation of pasture-based diet on growth, carcass composition and physical quality attributes of meat from farmed fallow deer (*Dama dama*). *Animals*, *9*(2), 33. doi:10.3390/ani9020033.

McCusker, S., Shipley, L. A., Tollefson, T. N., Griffin, M., & Koutsos, E. A. (2011). Effects of starch and fibre in pelleted diets on nutritional status of mule deer (*Odocoileus hemionus*) fawns. *Journal of Animal Physiology and Animal Nutrition*, *95*(4), 489-498. doi:10.1111/j.1439-0396.2010.01076.x.

Mulley, R. C., & English, A. W. (1985). The effects of castration of fallow deer (*Dama dama*) on body growth and venison production. *Animal Science*, *41*(3), 359-361. doi:10.1017/S0003356100036412.

Mulley, R.C., & English, A.W. (1992). Evaluation of carcass composition changes to fallow bucks castrated prepuberally. In Brown, R.D. (Ed), *The Biology of Deer* (pp. 238-243). Springer., New York.

Mulley, R. C., Asher, G. W., Flesh, J. S., O'Neill, K. T., & Ferguson, J. (2000). Energy intake and patterns of growth for male and female fallow deer of two genotypes, between 10 and 21 months of age. *Animal Science*, *70*(2), 335-342. doi:10.1017/S1357729800054795.

Needham, T., & Hoffman, L. C. (2015). Physical meat quality and chemical composition of the *Longissimus thoracis* of entire and immunocastrated pigs fed varying dietary protein levels with and without ractopamine hydrochloride. *Meat Science*, *110*, 101-108. doi:10.1016/j.meatsci.2015.06.017.

Needham, T., Lambrechts, H., & Hoffman, L. (2017a). Castration of male livestock and the potential of immunocastration to improve animal welfare and production traits: Invited Review. *South African Journal of Animal Science*, *47*(6), 731-742. doi:10.1016/j.meatsci.2015.06.017.

Needham, T., Hoffman, L. C., & Gous, R. M. (2017b). Growth responses of entire and immunocastrated male pigs to dietary protein with and without ractopamine hydrochloride. *Animal*, *11*(9), 1482-1487. doi:10.1017/S1751731117000271.

Needham, T., Kotrba, R., Hoffman, L.C., & Bureš, D. (2020). Ante-and post-mortem strategies to improve the meat quality of high-value muscles harvested from farmed male common eland (*Taurotragus oryx*). *Meat Science*, *168*, 108183. doi:10.1016/j.meatsci.2020.108183.

Ny, V., Needham, T., & Ceacero, F. (2022). Potential benefits of amino acid supplementation for cervid performance and nutritional ecology, with special focus on lysine and methionine: A review. Animal Nutrition, 11, 391-401. doi:10.1016/j.aninu.2022.09.001.

Pirahanchi, Y., & Sharma, S. (2019). *Biochemistry, Lipase.* StatPearls Publishing, Treasure Island.

Poljičak-Milas, N., Slavica, A., Janicki, Z., Robić, M., Belić, M., & Milinković-Tur, S. (2004). Serum biochemical values in fallow deer (*Dama dama* L.) from different habitats in Croatia. *European Journal of Wildlife Research*, *50*(1), 7-12. doi:10.1007/s10344-003-0003-2.

Poljičak-Milas, N., Slavica, A., Janicki, Z., Marenjak, T.S., & Kolić, E. (2006). Comparison of serum biochemical parameters between red (*Cervus elaphus*) and fallow deer (*Dama dama* L.) in Moslavina Region of Croatia. *Veterinarski Arhive*, *76*(Supplement), S229-238.

Russell, K.E., & Roussel, A.J. (2007). Evaluation of the ruminant serum chemistry profile. *Veterinary Clinics of North America: Food Animal Practice*, *23*(3), 403-426. doi:10.1016/j.cvfa.2007.07.003.

Sapolsky, R.M., Romero, L.M., & Munck, A.U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr Rev*, *21*, 55–89. doi:10.1210/edrv.21.1.0389.

Säkkinen., H., Timisjärvi. J., Eloranta, E., Heiskari, U., Nieminen, M., & Puukka, M. (1999). Nutrition-induced changes in blood chemical parameters of pregnant reindeer hinds (*Rangifer tarandus tarandus*). *Small Ruminant Research*, *32*(3), 211-221. doi:10.1016/S0921-4488(98)00184-9.

Serrano, M.P., Maggiolino, A., Pateiro, M., Landete-Castillejos, T., Domínguez, R., García, A., Franco, D., Gallego, L., Palo, P.D., & Lorenzo, J.M. (2019). Carcass characteristics and meat quality of deer. In Lorenzo, J.M., Munekata, P.E., Barba, F.J., & Toldrá, F. (Ed.), *More than Beef, Pork and Chicken–The production, processing, and quality traits of other sources of meat for human diet* (pp. 227-268). Springer.

Slavica, A., Janicki, Z., Rafaj, R.B., Kolić, E., Manojlović, L., & Dežđek, D. (2000). Biochemical blood analysis of the fallow deer (*Dama dama* L.) from the Brijuni islands, Croatia. *Veterinarski Arhive*, *70*(Supplement), S193-199.

Sookhareea, R., Woodford, K.B., & Dryden, G.M. (2001). The effect of castration on growth and body composition of Javan rusa stags. *Asian-Australasian Journal of Animal Sciences*, *14*(5), 608-614. https://doi.org/10.5713/ajas.2001.608.

Sookhareea, R., Taylor, D.G., Dryden, G.M., & Woodford, K.B. (2001). Primal joints and hind-leg cuts of entire and castrated Javan rusa (*Cervus timorensis russa*) stags. *Meat Science*, *58*(1), 9-15. https://doi.org/10.1016/S0309-1740(00)00124-8

Spencer, A. (2020, January). *Cervidor to China. In: Deer Industry News Southern Safari for Hawke's Bay AP*. Retrieved from https://www.deernz.org/assets/News-and-publications/Deer-Industry-News-Magazine/DIN-2018/Deer-Industry-News-Issue-99-Dec-Jan-2019.pdf.

Stanisz, M., Skorupski, M., Ślósarz, P., Bykowska-Maciejewska, M., Składanowska-Baryza, J., Stańczak, Ł., Krokowska-Paluszak, M., & Ludwiczak, A. (2019). The seasonal variation in the quality of venison from wild fallow deer (*Dama dama*)–a pilot study. *Meat Science*, *150*, 56-64. https://doi.org/10.1016/j.meatsci.2018.12.003.

Teare, J. A. (2013). *Dama dama*: ISIS Physiological Reference Intervals for Captive Wildlife. Retrieved from https://zims.species360.org/Main.aspx.

Tennant, B.C., & Center, S.A. (2008). Chapter 13: Hepatic functions. In Kaneko, J.J., Harvey, J.W., & Bruss, M.L. (Ed.), *Clinical biochemistry of domestic animals* (pp. 379-412). Academic press.

Vengušt, G., & Bidovec, A. (2002). Some serum chemistry values of fallow deer (*Dama dama* L.) in Slovenian hunting enclosures. *Veterinarski Arhive*, *72*, 205-212.

Volpelli, L.A., Valusso, R., & Piasentier, E. (2002). Carcass quality in male fallow deer (*Dama dama*): effects of age and supplementary feeding. *Meat Science*, *60*(4), 427-432. https://doi.org/10.1016/S0309-1740(01)00156-5.

Wiklund, E., Johansson, L., & Malmfors, G. (2003). Sensory meat quality, ultimate pH values, blood parameters and carcass characteristics in reindeer (*Rangifer tarandus tarandus* L.) grazed in natural pastures or fed a commercial feed mixture. *Food Quality and Preference*, *14*, 573–581. doi:10.1016/S0950-3293(02)00151-9.

Wiklund, E., Manley, T.R., Littlejohn, R.P., & Stevenson-Barry, J.M. (2003). Fatty acid composition and sensory quality of Musculus longissimus and carcass parameters in red deer (*Cervus elaphus*) grazed on natural pasture or fed a commercial feed mixture. *Journal of the Food Science and Agriculture*, *83*(5), 419–424. https://doi.org/10.1002/jsfa.1384.

CHAPTER 6

Combined effects of supplementation of amino acids and immunocastration in first antler growth of farmed fallow deer (*Dama dama*)

Adapted from: Ceacero F, **Ny V,** Kotrba R, Bartoň L, Čupić S, Bureš D, Turek J, Komárková M, Needham T. 2023. Combined effects of supplementation of amino acids and immunocastration in first antler growth of farmed fallow deer (*Dama dama*). Animal Production Science. https://doi.org/10.1071/AN22258.

Authors' contribution: Veit Ny participated in data collection, laboratory analyses, data curation, writing-original draft, review and editing. The paper was finally reviewed, commented, and edited for finally published by all authors.

Combined effects of supplementation of amino acids and immunocastration in first antler growth of farmed fallow deer (*Dama dama*)

Francisco Ceacero[1,*], **Veit Ny**[1,2], Radim Kotrba[1,3], Luděk Bartoň[2], Stipan Čupić[4], Daniel Bureš[2], Jiři Turek[4], Martina Komárková[1], Tersia Needham[1].

[1]Department of Animal Science and Food Processing, Faculty of Tropical AgriSciences, Czech University of Life Sciences Prague, 165 00 Prague, Czech Republic.

[2]Department of Cattle Breeding, Institute of Animal Science, 104 00 Prague, Czech Republic.

[3]Department of Ethology, Institute of Animal Science, 104 00 Prague, Czech Republic.

[4]Department of Game Management and Wildlife Biology, Faculty of Forestry and Wood Sciences, Czech University of Life Sciences Prague, 165 00 Prague, Czech Republic.

A B S T R A C T

Context: Amino acid supplementation and immunocastration are two husbandry practices with an increasing interest in the worldwide growing industry of deer farming. Amino acids (AAs) optimise nutrition and feed costs and improve the quality of products such as venison, velvet or antlers. Immunocastration (IC) reduces agonistic behaviours, which produce carcass damage and reduced growth. Thus, both treatments have positive effects on body growth, but may interfere with hormonal regulation, subsequently affecting antler growth.

Aims: This study aimed to evaluate the combined use of both practices and their impact on body and antler growth.

Methods: Forty-four yearling male fallow deer, approximate 10 months old, were subjected to the following four combinations based on both treatments: control–control, AA–control, IC–control, and AA–IC. Immunocastration treatment started in March 2020 and was repeated after 50 and 130 days. All groups received 250 g per animal and day of grains, and those under AA supplementation received ruminally protected lysine 6.3 g and ruminally protected methionine 2.1 g per animal and day. Biometric data, antlers and metatarsus were collected at slaughter in November. Antlers and metatarsus were analysed through computer-tomography scanning and mineral spectrometry.

Key results: Supplementation of AAs scarcely affected antler growth, although an indirect effect through improved body condition cannot be rejected. Immunocastration showed significant but not dramatic effects: IC animals had lighter antlers, with a lower amount of cortical bone and less mineralisation (density and calcium and phosphorus concentrations) in the base of the antler than did control animals. In contrast, the effects were scarce at the top of the antler and the metatarsus.

Conclusions: The results suggest a reduction but not total suppression of testosterone, with almost negligible effects on body growth and internal bones, thus not raising animal welfare issues.

Implications: Both techniques can be used simultaneously in deer farming, especially in farms with venison-production goals. Future research should focus on optimising the vaccination schedule for the main farmed deer species to ensure that the welfare benefits are well-balanced with productivity.

Keywords: calcium, cortical bone, metatarsus, phosphorus, RP-Lysine, RP-Methionine, testosterone, zinc.

6.1 Introduction

Optimising nutrition and feeding, especially protein (Dryden 2011), is essential to maximise the production of farmed cervids (Volpelli et al. 2002; Hutchison et al. 2012). For that reason, amino acid (AA) supplementation has become a growing topic for improving the nutrition of ruminants, including cervids (Ny et al. 2022), in connection with the rapid increase in deer farming during the past decades (Fletcher 2014).

Antlers are one of the most valuable deer products, as either a trophy or for velvet production (Fletcher 2014). AAs play an important role in their growth, given that antlers comprise up to 35–45% collagen (Dobrowolska 2002). Thus, during antler growth, deer require high amounts of dietary protein of up to 15% (Dryden 2016); however, diet quality not only affects antler growth (Baxter et al. 1999; Shin et al. 2000), it also has direct effects on body growth and puberty onset (Gomez et al. 2006) and, subsequently, on antler bone properties (Landete-Castillejos et al. 2007, 2012; Gomez et al. 2012). Few studies have focused on the potential impact of AAs on antler development and mineral density. Mendoza-Nazar et al. (2012) studied the effects of methionine supplementation on subadult red deer at different concentrations, finding specific effects on antler beam length but no apparent effects on the brow tine length or the number of tines. In contrast, Ny et al. (2020) used a larger sample size (over 10 animals per group) and concluded that supplementing ruminally protected AAs (RP-AAs) to fallow deer produced longer and heavier antlers with a greater burr diameter, with this effect being stronger in the experimental groups raised in low-quality pastures.

Large only-male groups are common in modern deer farming enterprises, creating numerous management issues connected to increased androgen levels (Mulley and English 1992), such as fighting and injuries, which may lead to deaths, carcass losses, or meat-quality issues. Castration has been used in many common livestock species to control the behavioural problems associated with increased testosterone concentrations and decreases the aggressive behaviour of farmed male fallow deer (Mulley and English 1992). Immunocastration (IC) is a more ethically acceptable tool for castration (Needham et al. 2017). However, its effects have been little studied in cervids, although it may significantly improve the welfare of farmed cervids (Mattiello 2009). Testosterone concentrations increase at the onset of puberty, leading to the start of pedicle development and first antler growth (Suttie et al. 1995; Li and Suttie 2001; Bartoš et al. 2012). Immunocastration suppresses testosterone and insulin-like growth factor 1 (IGF-1; Claus et al. 2007), the two hormones with the most significant role in antler growth (Bartoš et al. 2012). Thus, IC may have significant direct adverse effects on antler growth.

However, the magnitude of this effect would be difficult to predict because different castration methods (physical and chemical) have previously shown conflicting results on testosterone concentrations and antler development (or not) in different cervid species (Lincoln et al. 1982; Lincoln 1987; Bartoš et al. 2001; Barrell et al. 2009). Moreover, reduced testosterone production may also affect protein metabolism because testosterone increases total plasma protein (Eckersall 2008). Physical castration of deer has been shown to decrease body condition and growth rate due to interrupted testosterone production (Mulley and English 1985; Hogg et al. 1990; Sookhareea et al. 2001). Similarly, IC of yearling fallow deer appears to affect their responses to diet quality, especially under low nutritional conditions (the situation where the supplementation of AAs has demonstrated more substantial positive effects both on growth and antlers). This study aimed to measure the combined effects of AA supplementation and IC in yearling fallow deer to confirm whether both techniques can be used simultaneously without dramatically affecting antler growth. Effects on internal bones (metatarsus) were also included in the study.

6.2 Material and Methods

The study was conducted at a private fallow deer farm in Mnich, South Bohemian Region, Czech Republic (49.17 N, 14.90E; 485 m.a.s.l.). Experimental procedures and use of animals were approved by the Institutional Animal Care and Use Committee at the Czech University of Life Sciences, Prague, within its competence (Permit: 63479_2016-MZE-17214). Fallow deer are currently the most abundant farmed deer species in Europe (Hoffman and Cawthorn 2014) in extensive, pasture-based systems. Forty-four yearling male fallow deer, around ten months old at the start of the experiment (average live weight 22.9 ± 2.4 kg), were grouped into four combinations based on two treatments, namely, supplementation of AAs and IC.

The groups were balanced for body weight at the beginning of the experiment and were kept in two 20 000-m^2 paddocks, each with one of the nutritional treatments. To avoid a pasture effect, the groups switched the paddock in a monthly basis along the experiment. Half of the animals in each group were subjected to the IC treatment. The four groups will be hereafter referred to as control–control, AA–control, IC–control, and AA–IC. Thus, 11 animals were allocated to each treatment.

The study started in early March 2020, when the groups were created, and the first IC treatment was performed. The animals were subcutaneously injected with 2 mL of Improvac per dose (Zoetis Animal Health, New Jersey, USA) by using a Sterimatic safety vaccination gun (Sterimatic Worldwide Ltd, UK). Improvac is a vaccine containing a synthetic peptide analogue of gonadotropin-releasing hormone (GnRH), in response to which the animal's immune system creates antibodies against GnRH, thus preventing it from attaching to the GnRH-specific receptors on the anterior pituitary gland. This way, the hypothalamic–pituitary–gonadal axis is interrupted. The vaccine does not pose any health or safety issues for animal production and is authorised for use in the EU and many other countries. The treatment was repeated after 50 (ending April) and 130 (middle July) days. Both groups received daily supplementation of a mixture of oats and wheat (90:10) at 250 g per animal per day (Table 6.1). From the second IC treatment date onward, one of the groups started receiving a mixture of AAs daily, using the whole grains as a carrier with the AA spreading on the top. Lysine (Lys) and methionine (Met) have been identified as the limiting AAs for ruminants (Merchen and Titgemeyer 1992; Kung and Rode 1996). Lysine is essential for the protein synthesis process, and it is very important for the production of collagen (Ny et al. 2020), whereas Met is especially required for growth and development in young ruminants (National Research Council 2007). The mixture of grains and RP-AAs was offered in long feeders so as to avoid

competition and monopolisation of the resource by the dominant individuals (Ceacero et al. 2012). Moreover, supplemented feed was all consumed by the end of the day. Feeding unprotected AAs to ruminants is not recommended (Kung and Rode 1996). Thus, RP-AA supplementation remains a better solution for improving the protein nutrition of ruminants. Ruminally protected lysine (RP-Lys; LysiGem, Kemin Industry, Inc., USA) was added at a rate of 0.21 g per kg of metabolic bodyweight (MBW; Savage et al. 2004; Tedeschi et al. 2015) and day (i.e. 6.3 g of Lys supplement per animal and day). Ruminally protected methionine (RP-Met; Kessent, Kemin Industry, Inc., USA) was added at a rate of 0.07 g per MBW kg and day (i.e. 2.1 g of Met supplement per animal and day). The doses and inclusion rates for RP-Lys and RP-Met were calculated following a 3:1 ratio suggested in previous studies (Schwab et al. 2004; Ceacero et al. 2020; Ny et al. 2020). Animals were slaughtered in mid-November 2020 by stunning with a captive bolt gun in a restraint box, followed by exsanguination by severing the jugular veins and carotid arteries. That way, the supplementation finished before winter, when the animals would start using the protein to increase the fat stores (Ceacero et al. 2020). Liveweight and body condition score (BCS, measured on a 1–5 scale adjusted to 0.25 scores; Audigé et al. 1998) were recorded before slaughter. Antlers were cut after culling, measured for size (length and burr circumference), and weighed. The right-leg metatarsus bone was also collected, cleaned of tendons and meat, measured for size (length and circumference at the centre), and weighed. Both samples (antler and metatarsus) were then kept in the laboratory at room temperature for drying before further analyses.

Table 6.1 Chemical compositions of the pasture and concentrate supplemented to fattening fallow deer bucks.

Composition (g/kg dry matter)	**Concentrate mixture**	**Pasture**
Crude protein	18.56	12.28
Crude fat	3.58	1.04
Crude fibre	14.19	33.14
Ash	7.37	4.81
Nitrogen-free compounds	56.31	48.73
Acid detergent fibre (ADF)	13.24	35.61
Neutral detergent fibre (NDF)	35.55	69.72

Antler and bones were subjected to computer tomography (CT) scanning on a Siemens Somatom Scope Power multidetector, using the spiral scanning technique with 5 mm wide source sections, 16 mm × 0.6 mm collimation detectors and a pitch factor of 1.0. Automatic exposure and the 4D care function were used to optimise dose distribution. Subsequently, 2 mm sections were subjected to a reconstruction algorithm, highlighting the density interface for high resolution and contrast of small structures. The resulting images are reconstructed into a 3D MPR-multiplanar image, i.e. in all planes perpendicular to each other (axial, sagittal, coronary). The scans were further processed in ImageJ software (see https://imagej.nih.gov/ij/index.html). Cortical bone thickness, areas and bone densities were measured at two levels in the antler, namely, 0.6 cm above the burr (antler base) and 1 cm below the tip (antler top). Each metatarsus was measured just at its central point. Four axis lines (45°, all meeting in the centre of the sample) were drawn, and the plot (Figure 6.1) and raw data showing the mineral density along each line were extracted using the plot profile function.

The outer side of the sample was defined as the point where the bone density drops by more than 50% from one measure to another. The same criterion was adopted for defining the inner side of the metatarsus samples due to the sharp decrease in density between bone and marrow. However, this cannot be applied to determining the internal side of the antler cortical bone beacause there is a smooth transition from cortical to trabecular bone. Through visual inspection of the samples, we selected 300 g/mm^2 as the threshold for defining the end of the cortical area. Through this methodology, we measured four diameter and eight cortical thickness values per sample. The means were calculated and further used to determine the cortical-bone area percentage.

Average and maximum bone density were calculated using the histogram function after applying the 300 mg/cm^3 threshold to each picture.

Concentrations of the three most important minerals for bone biology (calcium (Ca), phosphorus (P), and zinc (Zn) ; Landete-Castillejos et al. 2012) were measured at each studied sample point (i.e. the base of the antlers, the top of the antlers, and metatarsus) using a Vanta ED-FXR spectrometer (Olympus, Waltham, MA, USA). Each sample was polished slightly on its outer side to reach the pure bone using a LaboPol-35 polishing machine (Struers, Ballerup, Denmark) with 80 and 180 grit sandpaper. The samples were then cleaned in an Elmasonic S30H ultrabath (Elma, Singen, Germany; 5 min at 50°) to remove any stains, dried for 10 min at room temperature, and measured in the spectrometer (5 min; two scans per sample, and the average used for further analyses).

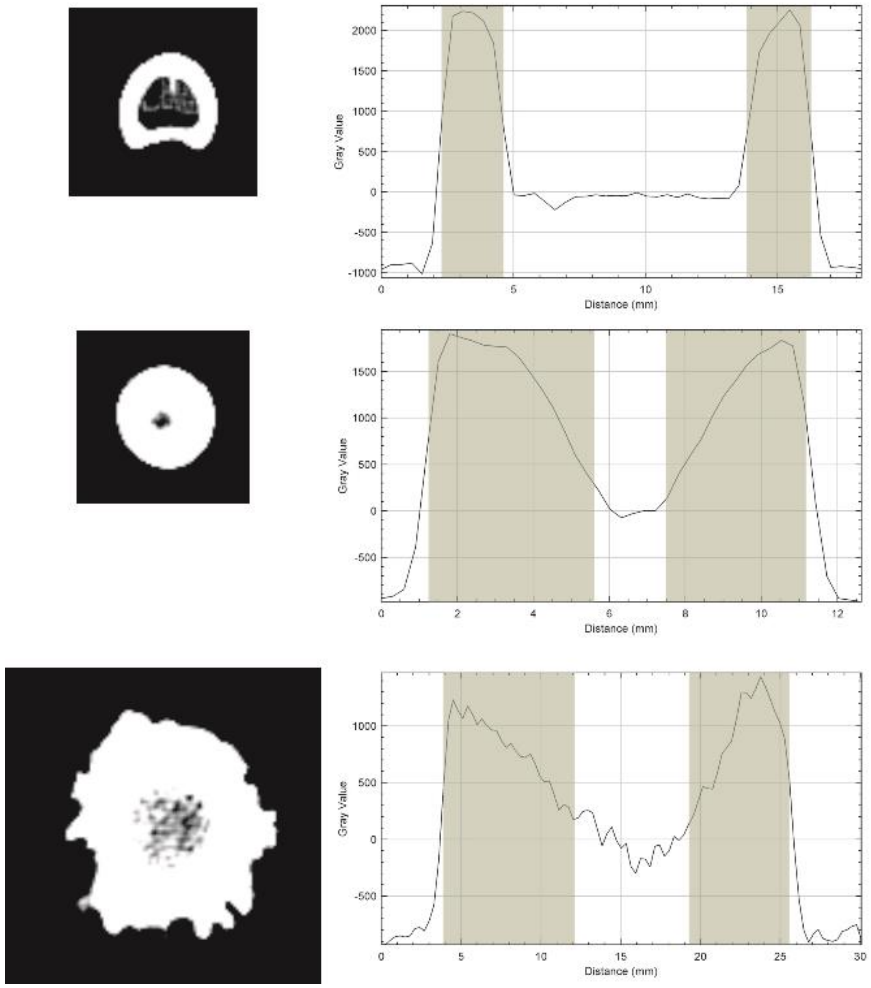

Figure 6.1 Plots showing the variations in bone density across the transversal axis. Note the sharp transition between bone and marrow in the metatarsus sample (top graph) but the smooth transition between the cortical and trabecular bone in the antler top (middle graph) and base (bottom graph).

6.3 Statistical analyses

Shapiro-Wilks tests confirmed normality for most of the variables used in the analyses, except for cortical area percentage at the base of the antler, and area, cortical area percentage, and Ca and Zn contents of the top of the antlers. Normality for these variables was confirmed after visual inspection of the histograms and Q-Q plots, and no further transformation of variables was undertaken.

Pearson correlation coefficients were calculated among all the studied variables. BCS and bodyweight were correlated (n = 44, r = 0.338, P = 0.025). Since BCS showed low variability (ranging 2.25-3.50, with 45% of the animals scoring 3.00) and low correlation with the antler variables (see Results), it was decided to use only bodyweight in the subsequent analyses.

Multivariate general linear models (GLMs) were used for testing the influence of AA supplementation, IC treatment, and their interaction into the following five groups of the studied response variables: general body growth (bodyweight and BCS), external characteristics of the antler (length, weight and burr circumference), internal characteristics of the antler (area, cortical thickness, percentage of cortical area, average and maximum bone density, Ca, P and Zn concentrations) at the base and top levels, and metatarsus (length, area, cortical thickness, percentage of cortical area, average and maximum bone density, and Ca, P and Zn concentrations). Bodyweight was also included in the last five models (antler characteristics and metatarsus) to control this important parameter. All analyses were performed in SPSS ver. 28.0 (IBM, SPSS, USA). A significance level of 5 % and a marginal significance level of 10 % were used throughout.

6.4 Results

The multivariate GLMs (Table 6.2) showed no general influence of AA supplementation on antler and metatarsus characteristics. It showed a marginally significant effect on BCS, being higher in the supplemented group (2.977) than in the control group (2.807). However, no effect was detected on bodyweight, the variable used as a covariate in the following analyses. Surprisingly, AA supplementation negatively affected the area at the top of the antler (β = -3.489), being significantly lower in the supplemented group.

Table 6.3 Pearson's correlation coefficients (*r*) among the characteristics of the top of the antler.

	Cortical thickness	Cortical area	Average bone density	Maximum bone density	Ca	P	Zn
Area	n.s.	-0.472**	n.s.	n.s.	n.s.	n.s.	n.s.
Cortical thickness		0.920***	0.661***	0.737***	n.s.	n.s.	n.s.
Cortical area			0.700***	0.757***	n.s.	n.s.	n.s.
Average bone density				0.803***	n.s.	n.s.	n.s.
Maximum bone density					n.s.	n.s.	n.s.
Ca						0.881***	n.s.
P							n.s.

Significance at $P < 0.01$, and $P < 0.001$ levels are indicated by **, and *** respectively. n.s., not significant.

Table 6.4 Pearson's correlation coefficients (*r*) between the characteristics of the metatarsus.

	Area	Cortical thickness	Cortical area	Average bone density	Maximum bone density	Ca	P	Zn
Length	0.743***	0.566***	0.337*	n.s.	n.s.	n.s.	-0.409*	n.s.
Area		0.665***	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Cortical thickness			0.992***	n.s.	n.s.	n.s.	n.s.	Ns.
Cortical area				n.s.	n.s.	n.s.	n.s.	n.s.
Average bone density					0.693***	n.s.	n.s.	n.s.
Maximum bone density						n.s.	n.s.	0.474**
Ca							0.867***	n.s.
P								n.s.

Significance at $P < 0.05$, $P < 0.01$, and $P < 0.001$ levels are indicated by *, **, and *** respectively. n.s., not significant.

The external characteristics oof the antler (weight, length, and burr circumference) all showed strong positive correlations with one another (all $P < 0.001$). Numerous correlations were also found among the studied variables at each level, namely, the top (Table 6.4) and base (Table 6.5) of antler, and metatarsus (Table 6.6), which further justifies the use of multivariate GLM in the following analyses. All these correlations were positive, except that between antler

area and percentage of cortical area at the top of the antler, which was negative. Certain correlations also existed among variables at different levels, the most interesting being the negative correlation between Zn concentration at the top of the antler and the cortical thickness (r = -0.400, P = 0.016), cortical area (r = -0.405, P = 0.014), average (r = -0.445, P = 0.007) and maximum bone density (r = -0.689, P < 0.001), and P (r = -0.370, P = 0.029) and Ca (r = -0.447, P = 0.007) concentrations in the base of the antler (i.e. Zn concentration decreased in the top when the antler had good quality in the base). Moreover, Zn concentration in the top of the antler did not correlate with any other studied variable, including the others at the top of antler.

Bodyweight strongly affected the external antler characteristics, including having positive effects on each of the following individual variables: antler length (β = 0.616), weight (β = 1.461) and burr circumference (β = 0.200). It also had a general effect on internal antler characteristics at the top, but not individually for any variable nor for the variables measured at the base. For the metatarsus, it had a marginally significant general effect. Bodyweight positively affected its area (β = 0.214), cortical thickness (β = 0.035), percentage of cortical area (β = 0.002), and marginally the Zn concentration (β = 0.820).

Table 6.5 Average values and standard error (s.e.) recorded for each group for the studied variables from the antler base (AB), antler top (AT) and metatarsus (Met).

	Control-control	**AA-control**	**IC-control**	**AA-IC**	**s.e.**
Bodyweight (kg)	41.39	39.02	37.66	39.36	0.65
Body condition score	2.84	3.02	2.77	2.93	0.04
Antler length (cm)	11.94	10.73	8.84	10.17	0.68
Antler weight (g)	26.00	19.66	14.90	20.43	1.55
Burr circumference (cm)	8.33	8.14	7.93	8.46	0.23
AB – area (mm^2)	71.38	68.70	64.22	69.46	1.85
AB – cortical thickness (mm)	9.70	8.58	4.42	6.11	0.52
AB –cortical area (%)	86.3	79.3	44.9	56.4	5.1
AB – average bone density (mg/cm^3)	1108.6	1049.6	865.6	914.8	25.9
AB – maximum bone density (mg/cm^3)	1644.4	1642.2	1496.7	1522.5	17.7
AB – Ca (%)	17.93	17.36	15.27	15.35	0.32
AB – P (%)	7.64	7.68	6.93	6.85	0.11
AB – Zn (ppm)	54.33	53.00	58.56	53.89	1.19
AT – area (mm^2)	24.39	18.05	21.39	17.64	0.84
AT – cortical thickness (mm)	2.07	1.97	2.46	2.05	0.16
AT – cortical area (%)	60.1	71.6	76.2	74.9	4.8
AT – average bone density (mg/cm^3)	1021.4	1019.2	1208.3	1163.3	48.9
AT –maximum bone density (mg/cm^3)	1621.3	1646.2	1658.9	1702.7	38.5
AT – Ca (%)	19.29	18.52	19.33	18.72	0.34
AT – P (%)	8.02	8.05	8.22	7.67	0.17
AT – Zn (ppm)	105.4	79.4	163.0	126.3	10.5
Met – length (cm)	19.21	18.79	19.05	19.45	0.19
Met – area (mm^2)	46.60	44.82	44.70	45.66	0.37
Met – cortical thickness (mm)	2.60	2.38	2.48	2.47	0.05
Met – cortical area (%)	17.5	16.6	17.4	17.0	0.5
Met – average bone density (mg/cm^3)	2035.8	2081.6	2031.8	2021.1	11.3
Met – maximum bone density (mg/cm^3)	2514.9	2530.7	2478.5	2451.5	14.5
Met – Ca (%)	21.97	21.47	21.94	21.64	0.12
Met – P (%)	8.69	8.71	8.88	8.67	0.07
Met – Zn (ppm)	72.89	71.88	77.60	73.22	1.77

Table 6.6 Multivariate GLMs showing the effects of the supplementation of amino acids, immunocastration and their interaction on antler and metatarsus characteristics in fallow deer bucks.

	n	R^2	Amino acids	Castration	Interaction	Body weight
Growth and condition						
Wilk´s λ			0.892	0.953	0.928	-
Bodyweight (kg)	44	0.098	n.s.	n.s.	n.s.	-
Body condition score	44	0.103	†	n.s.	n.s.	-
Antler – external characteristics						
Wilk´s λ			0.965	0.754*	0.727*	0.555***
Antler length (cm)	36	0.410	n.s.	n.s.	n.s.	***
Antler weight (g)	36	0.540	n.s.	*	†	***
Burr circumference (cm)	36	0.336	n.s.	n.s.	n.s.	***
Antler base – internal characteristics						
Wilk´s λ			0.817	0.407**	0.790	0.637
Area (mm^2)	35	0.317	n.s.	n.s.	**	n.s.
Cortical thickness (mm)	35	0.487	n.s.	***	n.s.	n.s.
Cortical area (%)	35	0.379	n.s.	***	n.s.	n.s.
Average bone density (mg/cm^3)	35	0.421	n.s.	***	n.s.	n.s.
Maximum bone density (mg/cm^3)	35	0.407	n.s.	***	n.s.	n.s.
Ca (%)	35	0.421	n.s.	***	n.s.	n.s.
P (%)	35	0.354	n.s.	***	n.s.	n.s.
Zn (ppm)	35	0.096	n.s.	n.s.	n.s.	n.s.
Antler top – internal characteristics						
Wilk´s λ			0.384***	0.637	0.816	0.537*
Area (mm^2)	36	0.353	***	n.s.	n.s.	n.s.
Cortical thickness (mm)	36	0.111	n.s.	n.s.	n.s.	n.s.
Cortical area (%)	36	0.044	n.s.	n.s.	n.s.	n.s.
Average bone density (mg/cm^3)	36	0.087	n.s.	n.s.	n.s.	n.s.
Maximum bone density (mg/cm^3)	36	0.024	n.s.	n.s.	n.s.	n.s.
Ca (%)	36	0.421	n.s.	n.s.	n.s.	n.s.
P (%)	36	0.354	n.s.	n.s.	n.s.	n.s.
Zn (ppm)	36	0.096	n.s.	*	n.s.	n.s.
Metatarsus						
Wilk´s λ			0.655	0.568	0.630	0.550†
Length (cm)	35	0.068	n.s.	n.s.	n.s.	n.s.
Area (mm^2)	35	0.263	n.s.	n.s.	n.s.	*
Cortical thickness (mm)	35	0.273	n.s.	n.s.	n.s.	**
Cortical area (%)	35	0.185	n.s.	n.s.	n.s.	*
Average bone density (mg/cm^3)	35	0.158	n.s.	n.s.	n.s.	n.s.
Maximum bone density (mg/cm^3)	35	0.170	n.s.	†	n.s.	n.s.
Ca (%)	35	0.085	n.s.	n.s.	n.s.	n.s.
P (%)	35	0.063	n.s.	n.s.	n.s.	n.s.
Zn (ppm)	35	0.020	n.s.	n.s.	n.s.	†

Significance at $P < 0.1$, $P < 0.05$, $P < 0.01$, and $P < 0.001$ levels are indicated by †, *, **, and ***, respectively. n.s., not significant.

6.5 Discussion

This work studied the combined effects of the supplementation of AAs and IC as a management tool on first antler growth in fallow deer. Immunocastration showed mild negative effects, while AA supplementation showed weak positive effects. Few studies have focused on AA supplementation and antler growth. Mendoza-Nazar et al. (2012) found a weak quadratic effect of RP-Met on the antler beam length of adult red deer. Ny et al. (2020) found that RP-Lys alone did not improve any antler characteristics and had only a marginal positive effect on its chemical composition; however, a combined treatment with RP-Lys plus RP-Met improved the burr perimeter and antler weight. These studies also suggested a stronger effect of AA supplementation on antlers in settings with a low nutritional quality, which was not the case in the present study (Table 6.2). Considering that the supplementation level used in this study has already previously shown positive results, the pasture quality in the study year may explain the weak effect found. However, it cannot be discarded a certain effect on body growth caused by the apparent reduction in testosterone concentrations. In contrast, the supplementation of AAs had a marginal effect on BCS. Thus, certain indirect effect of better antlers in animals with better condition cannot be rejected. The role of AAs on deer performance is still understudied, and the few studies available show contradictory effects. Huang et al. (2015a, 2015b) found very weak but positive effects on average daily gains (ADG) in sika deer, and Mendoza-Nazar et al. (2012) reported positive effects on total gain and ADG in red deer only for certain supplementation levels; however, it must be indicated that both studies used low numbers of animals per treatment (four per group). In contrast, recent studies in fallow deer using a larger sample size (over 10 per group) found no effects on weight gain and carcass weight (RP-Lys; Kudrnáčová et al. 2019), nor ADG, bodyweight or carcass weight (RP-Lys and RP-Met; Ceacero et al. 2020), but this last study also reported an increase in BCS. It is well known that deer need to reach a minimum threshold bodyweight and condition to start pedicle growth (Fennessy and Suttie 1985; Puttoo et al. 1998; Baxter et al. 1999; Dryden 2011), due to the high nutrient demand during velvet growth (Goss 1983; Price et al. 2005; Jeon et al. 2011). Thus, the effect of AA supplementation on BCS may still indirectly affect antler growth.

Immunocastration was the treatment that showed the strongest effects in the current experimental setting. Immunocastration interrupts the hormonal cascade of the hypothalamic–pituitary–gonadal (HPG) axis through gonadotropin-releasing hormone (GnRH) agonists, reducing the concentrations of androgens, including testosterone. But it also indirectly affects other hormones involved in antler growth, such as IGF-1 concentrations (Padula 2005; Claus

et al. 2007; Bartoš et al. 2009; Gaspar-López et al. 2010). However, there are conflicting results about how strongly it affects testosterone concentrations in cervids (Lincoln 1987; Bartoš et al. 2001; Barrell et al. 2009). In this study, no key processes of antler development were affected, including pedicle development, antler growth and velvet shedding. Just the mineralisation process was apparently negatively affected, with this process being mediated by testosterone. The main effect was observed on antler weight. The effect was strongest at the level of antler base, where the cortical bone was reduced and less mineralised (lower Ca and P concentrations; lower bone density). Surprisingly, these effects did not occur at the level of antler top, which may be due to the lower requirements to build a functional bone at this level, combined with the extra resources available after the low investment at the base. Moreover, the effects on the internal bone were also very limited, with only a marginally significant effect on maximum bone density. Testosterone, but also growth hormone, affects the density of internal bones (see Prakasam et al. 1999, using rats as model species), being a habitual treatment in humans for reducing bone density problems (Snyder et al. 1999). Even if the reduced testosterone production may also affect protein metabolism (high testosterone concentrations have an anabolic effect, increasing the total plasma protein; Eckersall 2008), no effect on body growth was observed in the present study. These results suggest a reduction but not complete suppression of testosterone (unclear for IGH-1 since antler length was not affected), with almost negligible effects on body growth and internal bones, thus not raising animal welfare issues. It is also important to highlight that IC is especially interesting for reducing intra-specific aggressivity (Asa and Porton 2005), especially in farmed species, thus improving welfare.

However, a stronger decrease in testosterone production and subsequent effects on antler growth were expected. This highlights the need for further research about its use in cervids in terms of doses and schedule. Depending on the vaccination schedule, the timing of testosterone suppression and its subsequent effects may be manipulated; however, a recent research in dwarf bucks has shown that different vaccination schedule, including a long-term one with six doses every 6 months, failed to fully suppress the testosterone concentrations (Giriboni et al. 2022). Interestingly, two reindeers physically castrated close after birth developed full antlers and normal antler cycle later on (Lincoln et al. 1982). Thus, future research should focus on optimising the vaccination schedule for fallow deer to ensure that the welfare benefits are balanced with its effects on productivity, related to the individual production goals of the farming enterprise (i.e. antler, venison, or both). It is also important to ascertain the effects of IC on the second and following antlers (Kierdorf et al. 2004).

Other results that deserve certain attention include the negative correlation between total and cortical area at the top of the antler (i.e. the percentage of cortical bone gets reduced as the cross-cut area of the antler increases), which has biomechanical implications, suggesting that a higher investment in the resources-demanding cortical bone is not necessary in wide antlers for keeping adequate mechanical performance.

Changes in the Zn concentration were also quite informative in this study; while the concentration was very constant at base of the antler and the metatarsus bone, it increased at the top of the antler in IC animals, suggesting unfinished mineralisation process (Landete-Castillejos et al. 2012), probably due to the lower testosterone signal. This can be confirmed by the negative correlations between Zn concentration at the top of the antler and cortical thickness and area, average and maximum bone density, and P and Ca concentrations at the base of the antler, while it did not correlate with any other measurement recorded at the top of the antler. This means that Zn concentration just decreased at the top of very well-formed antlers at their base. Moreover, the correlation between Zn concentration and maximum density in the metatarsus indicates stronger bone remodelling activity in animals with higher levels of bone calcification.

From the results, it can be concluded that further research is necessary to determine the nutritional conditions, combinations, and levels of AA supplementation that may have positive benefits on the body and antler growth, since the scarce published results are still not conclusive. AA supplementation rates under different pasture-quality scenarios seem essential to finally determine when and under which dosage the AA supplementation may be economically worthy for production purposes. In contrast, and considering that the antler cycle was not dramatically altered, IC under the schedule and doses used in this study seems a promising practice for deer farming if a reduction in agonistic interactions and effects on future antler cycles is confirmed. Future research should focus on finding adequate doses and vaccination schedules, which may be different for different deer species, and exploring the long-term impact. Indeed, the just reduced mineralisation may be even interesting for velvet production.

Acknowledgements

The authors wish to thank **Pavel Friedberger´s** family and farm staff for the assistance during the study and for providing access to the animals, and **Jericó C. Mituda** and **Hajra Munir** for assistance with samples´ processing in the lab. The authors state that no existing financial and personal conflicts of interest exist.

Declaration of funding

This work was supported by the internal grants of the Faculty of Tropical AgriSciences (IGA-20223107) and the Ministry of Agriculture of the Czech Republic (MZE-RO0718).

Data Availability Statement

The data is available through the corresponding author upon reasonable request.

Conflict of Interest Statement

The authors declare no conflicts of interest.

6.6 References

Asa CS, Porton IJ. (2005) *Wildlife Contraception: Issues, Methods, and Applications*. JHU Press. p.300.

Audigé L, Wilson PR, Morris RS (1998) A body condition score system and its use for farmed red deer hinds. *New Zealand Journal of Agricultural Research* **41**, 545–553.

Barrell GK, Schaafsma SCT, Ridgway MJ, Wellby M, Miller LA (2009) Effects of a synthetic gonadotrophin-releasing hormone agonist, leuprolide, on rut-associated events in male red deer. *Animal Production Science* **49**, 1120.

Bartoš L, Kierdorf U, Siler J, Bubenik GA (2001) Spontaneous mineralisation, partial velvet shedding and antler sequestration in castrated fallow deer bucks (*Dama dama*). Antler science and product technology. Edmonton (Canada): ASPTRC, 87-95.

Bartoš L, Schams D, Bubenik GA (2009) Testosterone, but not IGF-1, LH, prolactin or cortisol, may serve as antler-stimulating hormone in red deer stags (*Cervus elaphus*). *Bone* **44**, 691-698.

Bartoš L, Bubenik GA, Kuzmova E (2012) Endocrine relationships between rank-related behaviour and antler growth in deer. *Frontiers in Bioscience* **4**, 1111-1126.

Baxter BJ, Andrews RN, Barrell GK (1999). Bone turnover associated with antler growth in red deer (*Cervus elaphus*). *Anatomical Records* **256**, 14-19.

Ceacero F, García AJ, Landete-Castillejos T, Bartošová J, Bartoš L, Gallego L (2012). Benefits for dominant red deer hinds under a competitive feeding system: food access behavior, diet and nutrient selection. *PLoS One* **7**, e32780.

Ceacero F, Clar MA, Ny V, Kotrba R (2020). Differential effects of ruminally protected amino acids on fattening of fallow deer in two culling periods. *Animal*, **14**, 648-655.

Claus R, Lacorn M, Danowski K, Pearce MC, Bauer A (2007) Short-term endocrine and metabolic reactions before and after second immunisation against GnRH in boars. *Vaccine* **25**, 4689–4696.

Dobrowolska SA (2002) Chemical composition of the red deer (*Cervus elaphus*) antlers, with a particular reference to the toxic metal contents. *Zeitschrift für Jagdwissenschaft* **48**, 148–155.

Dryden GM (2011) Quantitative nutrition of deer: energy, protein, and water. *Animal Production Science* **51**, 292-302.

Dryden GM (2016) Nutrition of antler growth in deer. *Animal Production Science* **56**, 962-70.

Eckersall PD (2008) Proteins, proteomics, and the dysproteinemias, in: Kaneko JJ, Harvey JW, Bruss ML (Eds.), *Clinical biochemistry of domestic animals*. Academic press, pp. 81-115.

Fennessy PF, Suttie JM (1985) Antler growth: nutritional and endocrine factors. In: Fenessy PF, Drew KR, editors. *Biology of Deer Production*. Wellington: The Royal Society of New Zealand, p. 242-50.

Fletcher J (2014) *Deer*. Reaktion Books, London, UK.

Gaspar-López E, Landete-Castillejos T, Estévez JA, Ceacero F, Gallego L, García AJ (2010) Biometrics, testosterone, cortisol and antler growth cycle in Iberian red deer stags (*Cervus elaphus hispanicus*). *Reproduction in Domestic Animals* **45**, 243-249.Gómez JA, Landete-Castillejos T, García AJ, Sánchez PJ, Estévez JA, Gallego L (2006) Importance of growth during lactation on body size and antler development in the Iberian red deer (*Cervus elaphus hispanicus*). *Livestock Science* **105**, 27-34.

Giriboni J, Martínez-Nevado E, García J, Velázquez R, Toledano-Díaz A, Ungerfeld R, Santiago-Moreno J (in press) Single or repeated immunization against GnRH fails to completely abolish spermatogenesis in dwarf bucks (*Capra hircus*). Zoo Biology.

Gómez JA, Ceacero F, Landete-Castillejos T, Gaspar-López E, García AJ, Gallego L (2012) Factors affecting antler investment in Iberian red deer. Animal Production Science **52**, 867–873.

Goss RJ (1983) *Deer antlers: regeneration, function, and evolution*. New York: Academic Press, INC.

Huang J, Sun WL, Li CY, Liu HL, Zhang TT, Bao K, Fan YY, Li GY, Wang KY (2015a) Effects of DL-methionine supplement on growth performance and amino acid digestion and plasma concentrations in sika deer calves (*Cervus nippon*). *Animal Production Science* **56**, 1002-7.

Huang J, Zhang TT, Kun B, Li GY, Wang KY (2015b) Effect of supplementation of lysine and methionine on growth performance, nutrients digestibility and serum biochemical indices for growing sika deer (*Cervus nippon*) fed protein deficient diet. *Italian Journal of Animal Science* **14**, 61-5.

Hutchison CL, Mulley RC, Wiklund E, Flesch JS (2012) Effect of concentrate feeding on instrumental meat quality and sensory characteristics of fallow deer venison. *Meat Science* **90**, 801-806.

Hoffman LC, Cawthorn D (2014) Species of Meat Animals: Game and Exotic Animals. In: Carrick D, Michael D, editors. *Encyclopaedia of Meat Sciences* Vol. 3, Oxford: Elsevier, p. 345-56.

Kierdorf U, Kierdorf H, Schultz M, Rolf HJ (2004) Histological structure of antlers in castrated male fallow deer (*Dama dama*). *The Anatomical Record Part A: Discoveries in Molecular, Cellular, and Evolutionary Biology* **281**, 1352-1362.

Kudrnáčová E, Bureš D, Bartoň L, Kotrba R, Ceacero F, Hoffman LC, Kouřimská L (2019) The effect of barley and Lysine supplementation of pasture-based diet on growth, carcass composition and physical quality attributes of meat from farmed fallow deer (*Dama dama*). Animals 9, 33.

Jeon BT, Cheong SH, Kim DH, Park JH, Park PJ, Sung SH, Thomas DG, Kim KH, Moon SH (2011) Effect of antler development stage on the chemical composition of velvet antler in elk (*Cervus elaphus canadensis*). *Asian-Australasian Journal of Animal Science* **24**, 1303-13.

Kung L, Rode LM (1996) Amino acid metabolism in ruminants. *Animal Feed Science and Technology* **59**, 167-72.

Landete-Castillejos T, Estevez JA, Martínez A, Ceacero F, Garcia A, Gallego L (2007) Does chemical composition of antler bone reflect the physiological effort made to grow it? *Bone* **40**, 1095–1102.

Landete-Castillejos T, Currey JD, Ceacero F, García AJ, Gallego L (2012) Does nutrition affect bone porosity and mineral tissue distribution in deer antlers? The relationship between histology, mechanical properties and mineral composition. *Bone* **50**, 245–254.

Li C, Suttie JM (2001) Deer antlerogenic periosteum: a piece of postnatally retained embryonic tissue? *Anatomy and Embryology* **204**, 375-388.

Lincoln GA (1987) Long-term stimulatory effects of a continuous infusion of LHRH agonist on testicular function in male red deer (*Cervus elaphus*). *Reproduction* **80**, 257-261.

Lincoln GA, Tyler NJ (1992) Antler growth in male and female reindeer calves occurs in the absence of the gonads. In: The Biology of Deer (pp. 493-498). Springer, New York, NY.

Mattiello S (2009) Welfare issues of modern deer farming. *Italian Journal of Animal Science* **8**, 205-17.

Mendoza-Nazar P, Mendoza-Martínez GD, Herrera-Haro J, Ruiz-Sesma B, Bárcena-Gama R, Tarango-Arámbula L (2012) Effect of ruminally protected methionine on body weight gain and growth of antlers in red deer (*Cervus elaphus*) in the humid tropics. *Tropical Animals Health and Production* **44**, 681-4.

Mulley RC, English AW (1985) The effects of castration of fallow deer (*Dama dama*) on body growth and venison production. *Animal Science* **41**, 359-361.

Mulley RC, English AW (1992) Evaluation of Carcass Composition Changes to Fallow Bucks Castrated Prepuberally, in: Brown RD (Eds), *The Biology of Deer*. Springer., New York, pp. 238-243.

Needham T, Lambrechts H, Hoffman L (2107) Castration of male livestock and the potential of immunocastration to improve animal welfare and production traits: Invited Review. *South African Journal of Animal Science* **47**, 731-742.

Ny V, Kotrba R, Cappelli J, Bureš D, Clar MA, García AJ, Landete-Castillejos T, Bartoň L, Ceacero F (2020). Effects of Lysine and Methionine supplementation on first antler growth in fallow deer (*Dama dama*). *Small Ruminant Research* **187**, 106119.

Ny V, Needham T, Ceacero F (2022). Potential benefits of amino acid supplementation for cervid performance and nutritional ecology, with special focus on lysine and methionine: A review. *Animal Nutrition* **11**, 391-401.

Ny V, Needham T, Bartoň L, Bureš D, Kotrba R, Musa AS, Ceacero F (in press). Effects of immunocastration and supplementary feeding level on the performance and blood biochemical markers of farmed yearling fallow deer (*Dama dama*). *Journal of Animal Physiology and Animal Nutrition*.

Padula AM (2005) GnRH analogues—agonists and antagonists. *Animal Reproduction Science* **88**, 115-126.

Prakasam G, Yeh JK, Chen MM, Castro-Magana M, Liang CT, Aloia JF (1999) Effects of growth hormone and testosterone on cortical bone formation and bone density in aged orchiectomized rats. *Bone* **24**, 491-497.

Price JS, Allen S, Faucheux C, Althnaian T, Mount JG (2005) Deer antlers: a zoological curiosity or the key to understanding organ regeneration in mammals? *Journal of Anatomy* **207**, 603-18.

Puttoo M, Dryden GMcL, McCosker JE (1998) Performance of weaned rusa (Cervus timorensis) deer given concentrates of varying protein content with sorghum hay. *Australian Journal of Experimental Agriculture* **38**, 33–9.

Savage VM, Gillooly JF, Woodruff WH, West GB, Allen AP, Enquist BJ, Brown, JH (2004) The predominance of quarter-power scaling in biology. *Functional Ecology* **18**, 257-82.

Schwab CG, Ordway RS, Whitehouse L (2004) Amino Acid Balancing in the Context of MP and RUP Requirement. Proc. 15th Annual Florida Ruminant Nutrition Symposium. Gainesville, Florida 10–25.

Shin HT, Hudson RJ, Gai XH, Suttie JM (2000) Nutritional requirements and management strategies for farmed deer-review. *Asian-Australasian Journal of Animal Science* **13**, 561-73.

Snyder PJ, Peachey H, Hannoush P, Berlin JA, Loh L, Holmes JH, Dlewati A, Staley J, Santanna J, Kapoor SC, Attie MF, Haddad Jr. JH, Strom, BL (1999) Effect of testosterone treatment on bone mineral density in men over 65 years of age. *The Journal of Clinical Endocrinology and Metabolism* **84**, 1966-1972.

Sookhareea R, Woodford KB, Dryden GM (2001) The effect of castration on growth and body composition of Javan rusa stags. *Asian-Australasian Journal of Animal Science* **14**, 608-614.

Suttie JM, Gluckman PD, Butler JH, Fennessy PF, Corson ID, Laa FJ (1985) Insulin-like growth factor 1 (IGF-1) antler-stimulating hormone? *Endocrinology* **116**, 846-8.

Tedeschi LO, Fox DG, Fonseca MA, Cavalcanti LF. 2015. Models of protein and amino acid requirements for cattle. *Revista Brasileira de Zootecnia* **44**, 109-32.

Volpelli LA, Valusso R, Piasentier E (2002) Carcass quality in male fallow deer (*Dama dama*): effects of age and supplementary feeding. *Meat Science* **60**, 427-432.

CHAPTER 7

General discussion

This thesis combines important findings on the effects of AA nutrition, especially RP-Lys and RP-Met, on the nutritional ecology of yearling male fallow deer. Different experiments were conducted under real commercial farming conditions, thus producing practical information for deer farmers. This study covers all the aspects stated in the aims. Primarily, the thesis is focused on the effects of RP-Lys and RP-Met on growth performance, such as body weight, body condition, ADG, antler and bone growth, and carcass performance. The supplementation ratio used was 3:1 of Lys and Met (3 Lys, 1 Met), following Schwab et al. (2004), and based on metabolic body weight. The RPAA additives supplemented (addressed as RP-Lys and RP-Met) were in the form of small granules, mixed with grains (whole barley grains or concentrates), and have different percentages of AA available (L-Lys HCl or DL-Met) and rumen bypass or absorbable (according to cow rumen bypass). The L-Lys available in RP-Lys was 50% - 68% with 85% - 95% rumen bypass while RP-Met contains 75% of DL-Met with 90% rumen bypass. Therefore, the levels of RP-Lys as additives supplemented were 5 g, 9 g, and 6.3 g/deer/day, equivalent to 2.5 g, 6.1 g, 4.3 g L-Lys HCl present in the additives, and 2.1 g, 5.8 g, 4.1 g rumen bypass, respectively. The level of RP-Met as additives were 3 g and 2.1 g/deer/day, equivalent to 2.2 g, 1.6 g DL-Met, and equal to 2 g, 1.4 g rumen bypass. The goal of the experimental settings and supplementation was to mimic the real deer farming practices. Therefore, the diet was mainly based on pasture and grain supplements to support basal metabolism for maintenance and rumen microbes, and RPAAs as additive supplements.

Only a few studies have examined the effects of AA supplementation on growth performance and antler growth in deer. However, the results are inconsistent, and the research carried out was based on a small number of animals (four per group) and none were on fallow deer (Mendoza-Nazar et al. 2012; Huang et al. 2015a,b). In our studies, larger sample sizes were used (≈ 10 - 15 deer/group) in the same fallow deer farm. However, this kind of experimental setting is challenging to closely control the data collection, thus limitations like controlling for the feed or pasture intake per individual are inevitable. Despite this difficulty, the AA supplementation generally showed interesting effects on deer production.

The RP-Lys and RP-Met supplementation improved first antler growth, carcass dressing percentage, and maintaining good body condition in yearling fallow deer bucks. Moreover, the positive effects of AA supplementation appeared only in animals under the poor nutritional

condition of low pasture quality (affecting early growth of animal) and during growing periods. Therefore, the RP-Lys and RP-Met supplementation can potentially improve the productivity of deer farms for velvet, trophy hunting, and to some extent, venison production. AA supplementation did not have a large effect on growth performance, such as weight gain or body condition. Moreover, supplementation during the winter did not increase carcass yield for venison production but promoted internal fat storage instead.

The nutritional reflection/requirement of protein or AA supplementation is influenced by androgen suppression after immunocastration. Immunocastration decreased protein anabolism and appeared to reduced nutritional stress, as reflected in blood biochemical markers' variation to dietary protein supplementation. IC also reflected physiological changes, and slightly reduced antler growth and mineralisation compared to control bucks, especially at the antler base. These two methods could be simultaneously used for deer farming to support improved welfare and production, especially in deer farms focused on venison production.

The effects of AA supplementation on antler growth and carcass traits can be direct or indirect. During antler growth, deer require high dietary protein, but the available AAs (undegradable AAs) are even more important for supporting this stage (Dryden 2016). In farm conditions, providing a protein-rich diet is not very cost-effective if it will be degraded by rumen microbes or excreted. Therefore, AA supplementation improves production, especially in the rumen-protected form (Kung & Rode 1996). RP-Lys and/or RP-Met can directly build up the collagenous matrix for velvet and antler growth, and body protein for carcass performance (Shin et al. 2000; McDonald et al. 2011). The influence can also be mediated through body condition and nutrient-hormone interactions, especially androgen hormones such as testosterone to reach maturity sooner, reaching minimum threshold body weight, and initiate antler growth (Li & Suttie 2001; Gaspar-Lopez et al. 2010; Ceacero et al. 2019). Our studies found that AA supplementation increased antler weight and burr perimeter, probably also affected by improving body condition. It has potential for velvet production because the velvet antler is mainly composed of a cartilage matrix (Price et al. 2005); thus, AA supplementation, especially RP-Lys, is crucial for velvet growth. Moreover, the increase in burr perimeter is important for future antler growth, with the potential for increasing size and number of tines and producing good trophy hunting stags (Chapman 1975; Schmidt et al. 2001; Dryden 2016).

The effects of AA supplementation are limited in body growth (body weight, ADG, body condition) and no effect on carcass weight was found. Some studies on other ruminants, such as cattle, sheep, and goats, also found inconsistent results and mainly no effects of AA supplementation on growth performance (Watanabe et al. 2006; Lee et al. 2015). Mendoza-

Nazar et al. (2012) found limited effect of RP-Met on body gain in adult red deer stags. Antler growth is an essential feature in male deer, and it is the fastest growing mammal tissue (2-4 cm/day) making up 1 to 5% of body weight (Goss 1983; Gómez et al. 2012). Thus, nutrient intake is prioritised for this production over increasing muscles for body gain and carcass trait, especially protein and AA nutrition (Dryden 2016). Our experiments were carried out during the antler growth period, so the AAs might have been used to invest more in the first antler growth. From a practical standpoint, AA supplementation seems to provide more benefits to velvet antler production than venison production due to physiology's drain of nutrition for the first antler growth (Brown 1999). Therefore, a well-balanced diet of protein, energy, and minerals must be formulated with AA supplementation to improve venison production.

In our study, the nutritional effects at early growth seemed to influence the AA supplementation at a later stage. Hence, supplementation during an early stage of deer or at the perinatal level might improve gestation, lactation and thus improve growth and production of calves, yearlings, and the future antler cycles (Dryden 2016). Huang et al. (2015a) found that RP-Met increased ADG only in early growth stages in sika deer calves. Moreover, studies in dairy cattle showed that AA supplementation (RP-Lys, RP-Met and/or RP-His) increased milk yield and composition (Socha et al. 2005; Giallong et al. 2016; Zhao et al. 2019), which can contribute to supporting early growth and development of calves, as well as future body size. Red deer calves feeding on milk rich in protein, or when minerals were provided to the mother, increased their spike-antler weight (Gómez et al. 2006; Ceacero et al. 2010). Supplementation of RP-Met and/or RP-Lys to young sika deer calves also improved nutrient digestibility, weight gain, and general growth (Huang et al. 2015a,b). These findings can be beneficial for application in velvet production and trophy hunting. Some other aspects of AA supplementation that should be further explored include the supplementation of essential AAs that involve reproduction to improve the breeding success to start early antler growth and improve velvet antler and trophy hunting stags. For instance, Lys is involved in the lysine acetylation during spermatogenesis (Pang & Rennert 2013), and Arg is a building block of the nucleoprotein in sperm during spermatogenesis and supporting reproductive health in both male and female animals (De Chávez et al. 2015; Kaya et al. 2019; Hegazy et al. 2021). Moreover, supplementation of RP-Lys and RP-Met during velvet antler growth can also be interesting as Si et al. (2021a,b) found that Arg supplementation increased serum IGF-1, an important hormone involved in promoting antler growth and increased velvet antler weight in sika deer bucks. Thus, it has potential for application in velvet farming.

Deer farms generally rely on pasture for raising and fattening (Volpelli et al. 2003). Therefore, pasture quality and resource availability are important for growth and production (Mattiello 2009). Pasture is only sometimes a reliable nutritional source and is insufficient, especially in intensive farming conditions; thus, supplementary feeding is required according to physiological changes and nutrient requirements (Nicol & Barry 2003). Supplementary feeds such as concentrates have improved deer growth and carcass dressing percentage (Volpelli et al. 2003; Wiklund et al. 2003a,b). When pasture resource is scarce due to seasonal or environmental changes, direct effects on nutrient intake or competitive pressure in deer stock can suppress growth and production (Mattiello 2009; Dryden 2011). In our study, AA supplementation during the fattening period improved antler growth only in fawns raised on low pasture quality, indicating that the effects appeared only in growing animals. Some studies also found similar effects of AA supplementation only in animals fed low-quality diets (Huang et al. 2015b). As the antler is one of the deers' features that more clearly reflects their nutritional ecology (Picavet & Balligand 2016), our findings suggest the benefits of AA supplementation in deer farming, especially for velvet production with low protein dietary regimes, compared to the requirements during antler growth.

We need to also consider that the response to the RPAA supplements depends on AA or protein requirements and dietary protein provided. That includes the dietary protein from grains and concentrates on top of that from the pasture (CP ~12%), to maintain the basal metabolism, energy needs and support gut microbes (Dryden 2011; Dryden 2021). Regarding pasture and feed intake, there are some limitations due to the challenges in controlling pasture and feed intake in this farm-setting research. Controlling diet selection of grazing animals and the nutrient composition of consumed forage is often unknown in a farm (Kunkle et al. 2000). It was barely possible to control forage quality and selection preferences (only nutrient analysis of pasture). Fallow deer have an excitable nature (Brown 1992), and it is difficult to observe their behaviour or feeding patterns without interrupting their voluntary feeding activity. However, the supplemented feed plus RPAA was all consumed by the end of the day.

Forage availability, quality, intake, and digestibility can be affected by many parameters such as season, climate, forage maturity, forage selection, etc. (Dryden 2021), which was out of the scope of the management. However, a balanced diet of grain supplementation with pasture during spring and summer (experimental period) can cover the first limiting nutrient requirements of energy and ruminally available nitrogen. For instance, one of our experiments was during the drought in 2015 (CHMI 2015), which could affect the availability of pasture and maybe also the grazing frequency of animals (due to hot temperatures), thus affecting the

overall intake and performance (Mulley 2003). This might cause a deficiency of N and AA availability for animals. Therefore, the response of RPAA was evidenced in improved antler growth. Moreover, fallow is an intermediate feeder (Hofmann 1989), opportunistic, and selects high nutrient quality when available. Studies in white-tailed deer also found that they were more selective plant species to meet nutritional requirements when resources are limited (i.e., drought, Lashley & Harper 2012; poor soil quality forage, Lashley et al. 2015).

Our experiments were carried out during the growing period of fallow deer spikers throughout summer, when a higher nutrient intake is required, especially dietary protein. However, some studies found that feeding various protein levels (7% vs 20%) did not affect antler growth, but recommended undegradable protein or AA supplementation as a more promising approach (Dryden 2011). Therefore, regardless of completely controlling pasture quality and dietary intake in farm conditions, RPAA additive can provide informative results and be easily used by farmers (farmers cannot analyse pasture yearly or watch over how much pasture the animals consume). For some farmers, forage quality is meaningless unless they have a different contribution to animal performance (Moore et al. 1991). There are limitations due to challenges in the experimental setting in this research; however, in the overall picture and from a practical standpoint, the AA supplementation in pasture farms with a low protein regime combined with grain supplement can benefit deer production during the first antler growth period.

Timing of the supplementation concerning the physiological changes at different life stages and environmental changes are important for product optimisation (Dryden 2011) and the importance for economic. Cervids in temperate regions show a seasonal-dependent growth pattern in which deer increase fat stores during the winter (Shin et al. 2000; Scott et al. 2013); therefore, supplementation during this time only adds to fat depositions and not for building body mass. Regardless of the seasonal effect, AA supplementation increased body condition and dressing percentage (Ceacero et al. 2020). However, the AA supplementation until winter was not used to build muscle but to increase fat reserves, as shown in internal fat storage and fat plasma biochemical markers (Caldeira et al. 2007a,b ; Serrano et al. 2008). Even though the AAs helped maintain good body condition, when farming deer for venison production it is more important to improve body mass for increasing meat yield. Therefore, from a practical point of view, AA supplementation during winter is not recommended, especially for venison production.

The combination of AA supplementation or protein nutrition with immunocastration showed a potential application for venison and velvet farming, especially under intensive

systems. Immunocastration reduces agonistic behavioural interactions, affects protein and fat metabolism, and thus influences AA requirements (Needham et al. 2017a,b). With concentrate supplementation, the immunocastration showed less variation in blood biochemical markers, and likely reflected the reduced nutritional stress in this group compared to the control group. Immunocastration suppresses testosterone and IGF-1, which are important for bone growth (Claus et al. 2007; Bartoš et al. 2009; Gaspar-Lopez et al. 2010). In our study, immunocastration slightly reduced antler growth, cortical bone, and showed less mineralisation (at antler base), but did not completely interrupt the antler growth, as pedicle development already occurred before vaccination. AA supplementation was carried out under normal pasture conditions (not poor pasture) and had limited effects on antlers and bone growth, but indirect effects through body condition cannot be disregarded, which would be important for velvet antler production (Dryden 2011; Price et al. 2005; Jeon et al. 2011). Therefore, the combined effect of immunocastration with AA supplementation can also be applied to optimising product performance without completely interrupting the antler growth process, which is a welfare-friendly tool for deer farms for venison or velvet, especially in intensive deer farming systems. Future studies regarding the doses of AA additives and immunocastration vaccination schedules, and the observation up to the second antler cycle should be carried out to see the potential timeline for applying both AA supplementation and immunocastration. Moreover, the aggressivity and sexual behaviour during these combined treatments should also be monitored to describe possible welfare effects linked to animal behaviour.

CHAPTER 8

Overall conclusions

Keeping in mind the challenges and limitations in the common Central Europe deer farming practices for controlling feeding behaviour, feed intake, pasture availability and quality to interpret the results more conclusively, from the series of experiments performed and the five studies presented in this thesis, the following conclusions can be extracted:

- AA supplementation can improve deer farming for velvet, trophy hunting, and venison.
- AA supplementation during antler growth in yearling fallow deer bucks contributes mostly to the antler growth rather than improving body growth and carcass traits.
- RP-Lys and RP-Met should be supplemented to growing animals.
- RP-Lys and RP-Met should not be provided during the winter period.
- RP-Lys and RP-Met supplementation can compensate in extensive farming with poor pasture or under intensive deer farming.
- Immunocastration can be used to improve deer farming for velvet production and venison, whilst improving welfare at the same time.
- Future studies should focus on the effects of the supplementation of RP-Lys and RP-Met (or with addition Arg, His) on hinds during the gestation and lactation periods, and the subsequent effects on early growth of the fawns until the first and second antler cycles (i.e., expand to other production phases of the overall cycle).
- The effects of AA supplementation on males´ reproductive parameters should also be explored.
- Future studies on schedules of immunocastration during AA supplementation settings should be extended up to the second antler cycle, and must monitor agonistic and sexual behaviours.
- Future studies on the economical values of RPAA supplementation and income, especially for high quality products and intensive deer farming should be evaluated.

CHAPTER 9

General references

Ahmed S, Jiang X, Liu G, Sadiq A, Farooq U, Wassie T, Saleem AH, Zubair M. 2022. New trends in immunocastration and its potential to improve animal welfare: a mini review. Tropical Animal Health and Production **54**(369) DOI: 10.1007/s11250-022-03348-8.

Alharthi AS, Batistel F, Abdelmegeid MK, Lascano G, Parys C, Helmbrecht A, Trevisi E, Loor JJ. 2018. Maternal supply of methionine during late-pregnancy enhances rate of Holstein calf development in utero and postnatal growth to a greater extent than colostrum source. Journal of animal science and biotechnology **9**(83) DOI: 10.1186/s40104-018-0298-1.

Amatayakul-Chantler S, Hoe F, Jackson JA, Roça RD, Stegner JE, King V, Howard R, Lopez E, Walker J. 2013. Effects on performance and carcass and meat quality attributes following immunocastration with the gonadotropin releasing factor vaccine Bopriva or surgical castration of *Bos indicus* bulls raised on pasture in Brazil. Meat science **95**:78-84.

Amatayakul-Chantler S, Jackson JA, Stegner J, King V, Rubio LM, Howard R, Lopez E, Walker J. 2012. Immunocastration of *Bos indicus*× *Brown Swiss* bulls in feedlot with gonadotropin-releasing hormone vaccine Bopriva provides improved performance and meat quality. Journal of animal science **90**:3718-3728.

Bartoš L, Schams D, Bubenik GA. 2009. Testosterone, but not IGF-1, LH, prolactin or cortisol, may serve as antler-stimulating hormone in red deer stags (*Cervus elaphus*). Bone **44**:691-698.

Bartoš L, Bubenik GA, Kuzmova E. 2012. Endocrine relationships between rank-related behaviour and antler growth in deer. Frontiers in Bioscience **4**:111-1126.

Bartošová J, Bartoš L, Kotrba R, Ceacero F. 2012. Pre-orbital gland opening in farmed red deer (*Cervus elaphus*) during stressful handling. Journal of Animal Science **90**:3200-3206.

Boler DD, Kutzler LW, Meeuwse DM, King VL, Campion DR, McKeith FK, Killefer J. 2011. Effects of increasing lysine on carcass composition and cutting yields of immunologically castrated male pigs. Journal of animal science **89**:2189-2199.

Brown RB. 1992. The Biology of Deer. Springer-Verlag, New York.

Caldeira RM, Belo AT, Santos CC, Vazques MI and Portugal AV. 2007a. The effect of body condition score on blood metabolites and hormonal profiles in ewes. Small Ruminant Research **68**:233–241.

Caldeira RM, Belo AT, Santos CC, Vazques MI and Portugal AV. 2007b. The effect of long-term feed restriction and over-nutrition on body condition score, blood metabolites and hormonal profiles in ewes. Small Ruminant Research **68**:242–255.

Campal-Espinosa AC, Junco BJ, Fuentes AF, Calzada AL, Bover CA. 2020. Contraception and immunocastration vaccines. Use in veterinary medicine. Revista Colombiana de Ciencia Animal-RECIA (12:e760) DOI: 10.24188/recia.v12.n2.2020.760.

Ceacero F, Landete-Castillejos T, García AJ, Estévez JA, Gaspar-López E, Gallego L. 2010. Effects of ad libitum mineral consumption in Iberian red deer hinds and calves. Animal Production Science **50**:37-44.

Ceacero F, Landete-Castillejos T, Bartošová J, García AJ, Bartoš L, Komárková M, Gallego L. 2014. Habituating to handling: Factors affecting preorbital gland opening in red deer calves. Journal of Animal Science **92**:4130-4136.

Ceacero F, Villagrán M, Gambín-Pozo P, García AJ, Cappelli J, Ungerfeld R. 2019. Better antlers when surrounded by females? The social context influence antler mineralisation in pampas deer (*Ozotozeros bezoarticus*). Ethology Ecology & Evolution **31**:358-368.

Ceacero F, Clar MA, Ny V, Kotrba R. 2020. Differential effects of ruminally protected amino acids on fattening of fallow deer in two culling periods. Animal **14**:648-655.

Ceacero F, Ny V, Kotrba R, Bartoň L, Čupić S, Bureš D, Turek J, Komárková M, Needham T (in press). Combined effects of supplementation of amino acids and immunocastration in first antler growth of farmed fallow deer (*Dama dama*). Animal Production Science.

Chapman DI. Antlers–bones of contention. 1975. Mammal review **5**:121-172.

Claus R, Lacorn M, Danowski K, Pearce MC, Bauer A. 2007. Short-term endocrine and metabolic reactions before and after second immunization against GnRH in boars. Vaccine **25**:4689-4696.

Curtis PD, Pooler RL, Richmond ME, Miller LA, Mattfeld GF, Quimby FW. 2002. Comparative effects of GnRH and porcine zona pellucida (PZP) immunocontraceptive vaccines for controlling reproduction in white-tailed deer (*Odocoileus virginianus*). Reproduction (Cambridge, England) Supplement **60**:131-41.

Curtis PD, Richmond ME, Miller LA, Quimby FW. 2008. Physiological effects of gonadotropin-releasing hormone immunocontraception on white-tailed deer. Human-Wildlife Conflicts **2**:68-79.

Czech Hydrometerological Institute CHMI, 2015. Drought in Czech Republic in 2015. (accessed 3 July, 2018). http://portal.chmi.cz/files/portal/docs/meteo/ok/SUCHO/zpravy/en_drought2015.pdf.

De Chávez JA, Guzmán A, Zamora-Gutiérrez D, Mendoza GD, Melgoza LM, Montes S, Rosales-Torres AM. 2015. Supplementation with rumen-protected L-arginine-HCl increased fertility in sheep with synchronised estrus. Tropical animal health and production **47**:1067-1073.

D'Mello JPF. Amino acids in animal nutrition. 2nd ed. London: CAB International; 2003.

Dong L, Zhong ZX, Cui HH, Wang SN, Luo Y, Yu LH, Loor JJ, Wang HR. 2020. Effects of rumen-protected betaine supplementation on meat quality and the composition of fatty and amino acids in growing lambs. Animal **14**:435-444.

Dryden GM. 2011. Quantitative nutrition of deer: energy, protein, and water. Animal Production Science **51**:292-302.

Dryden GM. 2016. Nutrition of antler growth in deer. Animal Production Science **56**:962-970.

Dryden GM. 2021. Fundamentals of Applied Animal Nutrition. CABI.

European Commission Union. 2009. Register of Veterinary Medicinal Products. Available online: https://www.ema.europa.eu/en/medicines/veterinary/EPAR/improvac. Accessed 20 December 2022.

Gaspar-Lopez E, Landete-Castillejos T, Estevez JA, Ceacero F, Gallego L, García AJ. 2010. Biometrics, testosterone, cortisol and antler growth cycle in Iberian red deer stags (*Cervus elaphus hispanicus*). Reproduction in Domestic Animals **45**: 243–249.

Giallongo F, Harper MT, Oh J, Lopes JC, Lapierre H, Patton RA, Parys C, Shinzato I, Hristov AN. 2016. Effects of rumen-protected methionine, lysine, and histidine on lactation performance of dairy cows. Journal of dairy science **99**:4437-4452.

Gómez JA, Landete-Castillejos T, García AJ, Gallego L. 2006. Importance of growth during lactation on body size and antler development in the Iberian red deer (*Cervus elaphus hispanicus*). Livestock Science **105**:27–34.

Gómez JA, Ceacero F, Landete-Castillejos T, Gaspar-López E, García AJ, Gallego L. 2012. Factors affecting antler investment in Iberian red deer. Animal Production Science **52**:867–873.

Goss RJ. 1983. Deer antlers: regeneration, function, and evolution. New York: Academic Press.

Hegazy MM, Sakr AE, El-Aziz A, Ayman H, Swelum AA. 2021. Effect of adding different concentrations of L-arginine to Tris-yolk extender on the quality of sub-fertile ejaculates in buffalo. Tropical Animal Health and Production **53**:1-7.

Hogg B., Catcheside LM, Mercer GJK. 1990. Carcass composition in male fallow deer: age and castration effects on dissected tissue distribution. Animal Science **51**:405-413.

Hofmann RR. 1989. Evolutionary steps of ecophysiological adaptation and diversification of ruminants: a comparative view of their digestive system. Oecologia **178**: 443-457.

Huang J, Sun WL, Li C Y, Liu HL, Zhang TT, Bao K, Fan YY, Li GY, Wang KY. 2015a. Effects of DL-methionine supplement on growth performance and amino acid digestion and plasma concentrations in sika deer calves (*Cervus nippon*). Animal Production Science **56**: 1002-1007.

Huang J, Zhang TT, Kun B, Li GY, Wang KY. 2015b. Effect of supplementation of lysine and methionine on growth performance, nutrients digestibility and serum biochemical indices for growing sika deer (*Cervus nippon*) fed protein deficient diet. Italian Journal of Animal Science **14**:61-65.

Hutchison CL, Mulley RC, Wiklund E, Flesch JS. 2012. Effect of concentrate feeding on instrumental meat quality and sensory characteristics of fallow deer venison. Meat Science **90**:801-806.

Jeon BT, Cheong SH, Kim DH, Park JH, Park PJ, Sung SH, Thomas DG, Kim KH, Moon SH. 2011. Effect of antler development stage on the chemical composition of velvet antler in elk (*Cervus elaphus canadensis*). Asian-Australasian Journal of Animal Science **24**:1303-1313.

Kaya SO, Gur S, Kaya E. 2019. The effect of L-arginine on erection time, sperm quality and the seminal plasma arginase activity in rams. Revue De Medecine Veterinaire **170**:73-79.

Kierdorf U, Kierdorf H, Schultz M, Rolf HJ. 2004. Histological structure of antlers in castrated male fallow deer (*Dama dama*). The Anatomical Record Part A: Discoveries in Molecular, Cellular, and Evolutionary Biology: An Official Publication of the American Association of Anatomists **281**:1352-1362.

Kim SW, Kim KW, Park SB, Kim MJ, Yim DG. 2016. Quality characteristics and composition of the longissimus muscle from entire and castrate elk in Korea. Asian-Australasian Journal of Animal Sciences **29**:709-715.Kress K, Millet S, Labussière É, Weiler U, Stefanski V. 2019. Sustainability of pork production with immunocastration in Europe. Sustainability **11**(3335) DOI:10.3390/su11123335.

Kung L, Rode LM.1996. Amino acid metabolism in ruminants. Animal Feed Science and Technology **59**:167–172.

Kunkle WE, Johns JT, Poore MH, Herd DB. 2000. Designing supplementation programs for beef cattle fed forage-based diets. Journal f Animal Science **77**:1-11.

Kress K, Millet S, Labussière É, Weiler U, Stefanski V. 2019. Sustainability of pork production with immunocastration in Europe. Sustainability **17**:3335.

Lashley MA, Harper CA. 2012. The effects of extreme drought on native forage nutritional quality and white-tailed deer diet selection. Southeastern Naturalist **11**:699-710.

Lashley MA, Chitwood MC, Harper CA, Moorman CE, DePerno CS. 2015. Poor soils and density-mediated body weight in deer: forage quality or quantity?. Wildlife Biology **21**:213-219.

Lee C, Hristov AN, Cassidy TW, Heyler KS, Lapierre H, Varga GA, De Veth MJ, Patton RA, Parys C. 2012. Rumen-protected lysine, methionine, and histidine increase milk protein yield in dairy cows fed a metabolizable protein-deficient diet. Journal of dairy science **95**:6042-6056.

Lee C, Giallongo F, Hristov AN, Lapierre H, Cassidy TW, Heyler KS, Varga GA, Parys C. 2015. Effect of dietary protein level and rumen-protected amino acid supplementation on amino acid utilization for milk protein in lactating dairy cows. Journal of Dairy Science. **98**:1885-1902.

Li C, Suttie JM. 2001. Deer antlerogenic periosteum: a piece of postnatally retained embryonic tissue? Anatomy and Embryology **204**:375-388.

Lincoln GA, Fraser HM, Fletcher TJ. 1982. Antler growth in male red deer (*Cervus elaphus*) after active immunization against LH-RH. Reproduction **66**:703-708.

Ma H, Zhang W, Zhu X, Song W, Liu J, Jia Z. 2010. Effects of rumen-protected tryptophan on growth performance, fibre characteristics, nutrient utilization and plasma essential amino acids in Cashmere goats during the cashmere slow growth period. Livestock Science **131**:227-233.

Ma H, Cheng J, Zhu X, Jia Z. 2011. Effects of rumen-protected tryptophan on performance, nutrient utilization and plasma tryptophan in cashmere goats. African Journal of Biotechnology **10**:5806-5811.

Mattiello S, Mattiangeli V, Bianchi L, Carenzi C. 1997. Feeding and social behaviour of fallow deer (*Dama dama* L.) under intensive pasture confinement. Journal of animal science **75**:339-3347.

Mattiello S. 2009. Welfare issues of modern deer farming. Italian Journal of Animal Science **8**:205-217.

McDonald P, Edwards RA, Greenhalgh JFD, Morgan CA, Sinclair LA, Wilkinson RG. 2011. Animal nutrition. 7th ed. London: PEARSON.

Mendoza-Nazar P, Mendoza-Martínez GD, Herrera-Haro J, Ruiz-Sesma B, Bárcena-Gama R, Tarango-Arámbula L. 2012. Effect of ruminally protected methionine on body weight gain and growth of antlers in red deer (*Cervus elaphus*) in the humid tropics. Tropical Animal Health and Production **44**:681-684.

Miguel GZ, Faria MH, Roca RO, Santos CT, Suman SP, Faitarone AB, Delbem NL, Girao LV, Homem JM, Barbosa EK, Su LS. 2014. Immunocastration improves carcass traits and beef color attributes in *Nellore* and *Nellore× Aberdeen Angus* crossbred animals finished in feedlot. Meat Science **96**:884-891.

Miller LA, Johns BE, Killian GJ. 2000a. Immunocontraception of white-tailed deer with GnRH vaccine. American Journal of Reproductive Immunology **44**:266-274.

Miller LA, Johns BE, Killian GJ. 2000b. Long-term effects of PZP immunization on reproduction in white-tailed deer. Vaccine **18**:568-574.

Moore JE, Kunkle WE, Brown WF. 1991. Forage quality and the need for protein and energy supplements. Florida Beef Cattle Short Course. University of Florida.

Mulley RC, English AW. 1985. The effects of castration of fallow deer (*Dama dama*) on body growth and venison production. Animal Science **41**:359-361.

Mulley RC, English AW. 1992. Evaluation of carcass composition changes to fallow bucks castrated prepuberally. The biology of deer. Springer, New York.

Mulley RC, English AW, Thompson JM, Butterfield RM, Martin P. 1996. Growth and body composition of entire and castrated fallow bucks (*Dama dama*) treated with zeranol." Animal Science **63**:159-165.

Mulley RC. 2003. The feed requirements of adult red deer. New Zealand Grassland Association (NZGA): Research and Practice Series **9**:51-55.

Needham T, Hoffman LC. 2015a. Carcass traits and cutting yields of entire and immunocastrated pigs fed increasing protein levels with and without ractopamine hydrochloride supplementation. Journal of Animal Science **93**:4545-4556.

Needham T, Hoffman LC. 2015b. Physical meat quality and chemical composition of the *Longissimus thoracis* of entire and immunocastrated pigs fed varying dietary protein levels with and without ractopamine hydrochloride. Meat science **110**:101-108.

Needham T, Lambrechts H, Hoffman LC. 2016. The influence of vaccination interval on growth, carcass traits and testicle parameters of immunocastrated ram lambs. Small Ruminant Research **145**:53-57.

Needham T, Lambrechts H, Hoffman LC. 2017a. Castration of male livestock and the potential of immunocastration to improve animal welfare and production traits: Invited Review. South African Journal of Animal Science **47**:731-742.

Needham T, Hoffman LC, Gous RM. 2017b. Growth responses of entire and immunocastrated male pigs to dietary protein with and without ractopamine hydrochloride. Animal **11**:1482-1487.

Needham T, Lambrechts H, Hoffman LC. 2019a. Extending the interval between second vaccination and slaughter: I. Effects on growth, scrotal size and stress responses of immunocastrated ram lambs. Animal **13**:1952-1961.

Needham T, Lambrechts H, Hoffman LC. 2019b. Extending the interval between second vaccination and slaughter: II. Changes in the reproductive capacity of immunocastrated ram lambs. Animal **13**:1962-1971.

Needham T, Lambrechts H, Hoffman LC. 2019c. Influence of immunocastration vaccine administration interval on serum androgen concentrations and testis activity in ram lambs. Small Ruminant Research **170**:82-90.

Nicol AM, Barry TN. 2003. Pastures and forages for deer growth." NZGA: Research and Practice Series **9**: 25-40.

Ny V, Kotrba R, Cappelli J, Bureš D, Clar MA, García AJ, Landete-Castillejos T, Bartoň L, Ceacero F. 2020. Effects of Lysine and Methionine supplementation on first antler growth in fallow deer (*Dama dama*). Small Ruminant Research **187**(106119) DOI: 10.1016/j.smallrumres.2020.106119.

Ny V, Needham T, Ceacero F. 2022. Potential benefits of amino acid supplementation for cervid performance and nutritional ecology, with special focus on lysine and methionine: A review. Animal Nutrition **11**: 391-401.

Ny V, Needham T, Bartoň L, Bureš D, Kotrba R, Musa AS, Ceacero F (in press). Effects of immunocastration and supplementary feeding level on the performance and blood biochemical markers of farmed yearling fallow deer (*Dama dama*). Journal of Animal Physiology and Animal Nutrition.

Palmer C, Pedersen HG, Sandøe P. 2018. Beyond castration and culling: Should we use non-surgical, pharmacological methods to control the sexual behaviour and reproduction of animals?. Journal of Agricultural and Environmental Ethics **31**:197-218.

Pang AL, Rennert OM. Protein acetylation and spermatogenesis. 2013.Reproductive system & sexual disorders: current research **5**, Suppl 1. doi:10.4172/2161-038x.s1-005.

Phillip LE, Oresanya TF, Jacques JS. 2007. Fatty acid profile, carcass traits and growth rate of red deer fed diets varying in the ratio of concentrate: dried and pelleted roughage, and raised for venison production. Small Ruminant Research **71**:215-221.

Picavet PP, Balligand M. 2016. Organic and mechanical properties of Cervidae antlers: a review. Veterinary research communications **40**:141-147.

Price JS, Allen S, Faucheux C, Althnaian T, Mount JG. 2005. Deer antlers: a zoological curiosity or the key to understanding organ regeneration in mammals?. Journal of anatomy **207**:603-618.

Schmidt KT, Stien A, Albon SD, Guinness FE. 2001. Antler length of yearling red deer is determined by population density, weather and early life-history. Oecologia **127**:191–197.

Schwab CG, Ordway RS, Whitehouse L. 2004. Amino Acid Balancing in the Context of MP and RUP Requirement. Page 10-25. Proc. 15th Annual Florida Ruminant Nutrition Symposium. Gainesville, Florida.

Scott IC, Asher GW, Barrell GK, Juan JV. 2013. Voluntary food intake of pregnant and non-pregnant red deer hinds. Livestock Science **158**:230–239.

Serrano E, Gonzalez FJ, Granados JE, Moco G, Fandos P, Soriguer RC, Perez JM. 2008. The use of total serum proteins and triglycerides for monitoring body condition in the Iberian wild goat (*Capra pyrenaica*). Journal of Zoo and Wildlife Medicine **39**: 646–649.

Serrano MP, Maggiolino A, Pateiro M, Landete-Castillejos T, Domínguez R, García A, Franco D, Gallego L, Palo PD, Lorenzo JM. 2019. Carcass characteristics and meat quality of deer. Page 227-268 in Lorenzo JM, Munekata PES, PD, Barba FJ, Toldrá F, editors. More than Beef, Pork and Chicken–The Production, Processing, and Quality Traits of Other Sources of Meat for Human Diet. Springer Nature Switzerland AG.

Serrano MP, Maggiolino A, Landete-Castillejos T, Pateiro M, Barbería JP, Fierro Y, Domínguez R, Gallego L, García A, De Palo P, Lorenzo JM. 2020. Quality of main

types of hunted red deer meat obtained in Spain compared to farmed venison from New Zealand. Scientific Reports **10**:1-9.

Shin HT, Hudson RJ, Gao XH, Suttie JM. 2000. Nutritional requirements and management strategies for farmed deer – review. Asian-Australasian Journal of Animal Sciences **13**:561–573.

Si H, Han Y, Liu H, Lou Y, Li Z. 2021b. Effects of rumen-protected arginine supplementation on the plasma amino acids and gut microbiota of sika deer (*Cervus nippon*). Animal Feed Science and Technology **273**:114828. https://doi.org/10.1016/j.anifeedsci.2021.114828.

Si H, Liu H, Nan W, Li G, Li Z, Lou Y. 2021a. Effects of arginine supplementation on serum metabolites and the rumen bacterial community of sika deer (*Cervus nippon*). Frontiers in Veterinary Science **5**:630686. https://doi.org/10.3389/fvets.2021.630686.

Škrlep M, Tomašević I, Mörlein D, Novaković S, Egea M, Garrido MD, Linares MB, Peñaranda I, Aluwé M, Font-i-Furnols M. 2020. The use of pork from entire male and immunocastrated pigs for meat products—An overview with recommendations. Animals **10**(1754) DOI: 10.3390/ani10101754.

Socha MT, Putnam DE, Garthwaite BD, Whitehouse NL, Kierstead NA, Schwab CG, Ducharme GA, Robert JC. 2005. Improving intestinal amino acid supply of pre-and postpartum dairy cows with rumen-protected methionine and lysine. Journal of Dairy Science **3**:1113-1126.

Sookhareea R, Woodford KB, Dryden GM. 2001a. The effect of castration on growth and body composition of *Javan rusa* stags. Asian-Australasian Journal of Animal Sciences **14**:608-614.

Sookhareea R, Taylor DG, Dryden GM, Woodford KB. 2001b. Primal joints and hind-leg cuts of entire and castrated Javan rusa (*Cervus timorensis russa*) stags. Meat science **58**:9-15.

Stafford KJ, Mellor DJ. 2005. The welfare significance of the castration of cattle: a review. New Zealand Veterinary Journal **53**:271-278.

Teixeira PD, Tekippe JA, Rodrigues LM, Ladeira MM, Pukrop JR, Kim YHB, Schoonmaker JP. 2019. Effect of ruminally protected arginine and lysine supplementation on serum amino acids, performance, and carcass traits of feedlot steers. Journal Animal Science **97**:3511-3522.

Volpelli LA, Valusso R, Piasentier E. 2002. Carcass quality in male fallow deer (*Dama dama*): effects of age and supplementary feeding. Meat Science **60**:427-432.

Volpelli LA, Valusso R, Morgante M, Pittia P, Piasentier E. 2003. Meat quality in male fallow deer (*Dama dama*): effects of age and supplementary feeding. Meat Science **65**:555–562.

Watanabe K, Fredeen AH, Robinson PH, Chalupa W, Julien WE, Sato H, et al. 2006. Effects of fat coated rumen bypass lysine and methionine on performance of dairy cows fed a diet deficient in lysine and methionine. Anim Science Journal **77**:495–502.

Wiklund E, Johansson L, Malmfors G. 2003a. Sensory meat quality, ultimate pH values, blood parameters and carcass characteristics in reindeer (*Rangifer tarandus tarandus* L.) grazed in natural pastures or fed a commercial feed mixture. Food Quality and Preference **14**:573–581.

Wiklund E. Manley TR. Littlejohn RP, Stevenson-Barry JM. 2003b. Fatty acid composition and sensory quality of Musculus longissimus and carcass parameters in red deer (*Cervus elaphus*) grazed on natural pasture or fed a commercial feed mixture. Journal of the Food Science and Agriculture **83**:419–424. doi:10.1002/jsfa.1384.

Zeng FM, Yi DI, Wassie T, Jing HJ, Ahmed S, Liu GQ, Jiang XP. 2022. Recent advances in immunocastration in sheep and goat and its animal welfare benefits: A review. Journal of Integrative Agriculture **21**:299-309.

Zhao K, Liu W, Lin X, Hu Z, Yan Z, Wang Y, Shi K, Liu GM, Wang Z. 2019. Effects of rumen-protected methionine and other essential amino acid supplementation on milk and milk component yields in lactating Holstein cows. Journal of dairy science **102**:7936-7947.

CHAPTER 10

Curriculum Vitae

Ing. Veit NY

Prague, Czech Republic

+420 776 895 861

nyv@ftz.czu.cz / ny_veit@yahoo.com

Date of birth: 15/04/1993

Nationality: Khmer

EDUCATION

2018- Present **PhD student Czech University of Life Sciences Prague (CULS)**

Focus: Feeding amino acids for deer production and ecology

Feed additive animal science, animal nutrition, biochemistry, bioinformation

2016 – 2018 **Master of Animal Science and Food Processing (CULS)**

Focus: Animal feeding and production

Food processing technology (food safety, dairy products, food preservation, meat processing)

2013 – 2016 **Bachelor of Chemical Engineering and Food Technology**

Institut de Technologie du Cambodge (ITC), Phnom Penh, Cambodia

Focus: Food microbiology, Food processing, Food preservation, Biotechnology, Analytical Chemistry, Biochemistry Chemistry

WORK EXPERIENCE

2018 - Present **Part-time Research assistant**

Institute of Animal Science Prague (IAS)

Meat quality analysis (Physical and Sensorial analysis), feeding ruminant (cervids)

2018 - Present **Part-time Research assistant**

Food Research Institute Prague (VUPP)

Research and development of medicinal herbs and plants for food

Food preservation methods (biotechnology)

Jul – Aug 2014 **Quality controller**

C.P. CAMBODIA CO., LTD

Quality control of raw materials, whole production line of chicken parts and meat ball production

SCIENTIFIC PUBLICATIONS AND CONFERENCES

Publications

Ny V, Needham T, Ceacero F. 2022. Potential benefits of amino acid supplementation for cervid performance and nutritional ecology, with special focus on lysine and methionine: A review. Animal Nutrition **11**:391-401. https://doi.org/10.1016/j.aninu.2022.09.001.

Ny V, Houška M, Pavela R, Tříska J. 2021. Potential benefits of incorporating *Astragalus membranaceus* into the diet of people undergoing disease treatment: An overview. Journal of Functional Foods **77**(104339). https://doi.org/10.1016/j.jff.2020.104339.

Ny V, Kotrba R, Cappelli J, Bureš D, Clar MA, García AJ, Landete-Castillejos T, Bartoň L, Ceacero F. 2020. Effects of Lysine and Methionine supplementation on first antler growth in fallow deer (*Dama dama*). Small Ruminant Research **187**(106119. https://doi.org/10.1016/j.smallrumres.2020.106119.

Ceacero F, Clar MA, **Ny V,** Kotrba R. 2020. Differential effects of ruminally protected amino acids on fattening of fallow deer in two culling periods Animal **14**:648-655. https://doi.org/10.1017/S1751731119002325.

Ny V, Needham T, Bartoň L, Bureš D, Kotrba R, Musa AS, Ceacero F. 2023. Effects of immunocastration and supplementary feeding level on the performance and blood biochemical markers of farmed yearling fallow deer (*Dama dama*). Journal of Animal Physiology and Animal Nutrition. https://doi.org/10.1111/jpn.13807.

Ceacero F, **Ny V,** Kotrba R, Bartoň L, Čupić S, Bureš D, Turek J, Komárková M, Needham T.2023. Combined effects of supplementation of amino acids and immunocastration in first antler growth of farmed fallow deer (*Dama dama*). Animal Production Science. https://doi.org/10.1071/AN22258.

Smekalova L, Chaloupkova P, Nemejc K, **Ny V** (in press). Satisfaction with acquired transferable competences among university students in Cambodia. Asia Pacific Education Review. https://doi.org/10.1007/s12564-023-09821-9.

Conferences

- Poster presentation: "73rd Annual Meeting of European Federation of Animal Science, EAAP". Effects of immunocastration and supplementary feeding level on the performance and blood biochemical markers of farmed yearling fallow deer (*Dama dama*). 2022 (Porto, Portugal).
- Poster presentation: "The annual interdisciplinary conference on research in tropical and subtropical agriculture, natural resource management and rural development (TROPENTAG)". 2022 (Prague, Czech Republic).
- Attendant: "24th Congress of the European Society of Veterinary and Comparative Nutrition, ESVCN". 2020 (Online).
- Poster presentation: "71st Annual Meeting of European Federation of Animal Science, EAAP". Effects of Lysine and Methionine supplementation on first antler growth in fallow deer (*Dama dama*). 2020 (Online).

- Co-author: "67th International Congress of Meat Science & Technology, ICoMST". The effect of immunoscatration and supplementary feeding level on *m. longissimus lumborum* quality of farmed fallow deer (*Dama dama*). 2021 (Krakow, Poland).
- Co-author: "Animal Science Days". Behavioural of farmed common eland after immunocastration. 2019 (Prague).
- Attendant: "SIMPLE project, Bridge the gap between academic and professional sector". 2019 (Bangkok, Thailand).
- Co-author: "Czech and Slovak Ethological Society, ČSEtS". Influence of Immunocastration on temperament and habituation of common eland to routing handling. 2019 (Bratislava, Slovakia).
- Co-author: "9th International Deer Biology Conference, Effects of Lysine and Methionine Supplementation on Fattening and Blood Protein Metabolites in Fallow Deer (*Dama dama*). 2018 (Colorado, USA).

Seminars, workshops, internship

- Amino Acid Academy Workshop, EAAP 2021. Paris (France).
- Wildlife Research and Conservation 2019. Berlin (Germany).
- Workshop: "Work with Information and Databases", "Advanced Academic Writing".
- Internship at the University of Life Sciences in Lublin: helped with the preparation a grant proposal under Weave-UNISONO Program between Poland and Czech, "Implementation of modern feeding technologies for optimizing the sustainable production of alternative farming species", 2021. Lublin (Poland).
- Pedagogy: animal nutrition and feed additives, animal feeding in tropic and sub-tropics, practical animal handling, sampling and processing, practical laboratory work, feed formulation during PhD study.

AWARDS AND SCHOLARSHIPS

- Rector Award for outstanding research and publication, 4th place, November 28th, 2022.
- Scholarship from EAAP to join the 73rd EAAP Annual Meeting in Porto, 2022.
- Scholarship from European Union to study master's degree in Prague (Erasmus Mundus 2, ALFABET project 2016-2018).