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Effects of immunocastration and amino acid supplementation on fallow deer testicle development

MASTER'S THESIS

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Declaration

I hereby declare that I have completed this thesis entitled **Effects of immunocastration and amino acid supplementation on fallow deer testicle development** independently; all texts in this thesis are original, and all the sources have been quoted and acknowledged by means of complete references and according to Citation rules of the FTA.

In Prague 21st April 2023

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Thoniso Chitambala

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I would like to dedicate this work to the memory of **Flemming Jensen**, who believed in and encouraged me to follow my dreams.

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Abstract

Forty-four fallow deer (*Dama dama*) bucks (10 months of age; 22.9 ± 2.4 kg) were allocated to one of four treatment combinations (n = 11 per treatment) to investigate the combined effects of immunocastration vs intact male production under different nutrition, namely supplemented with an oat-wheat based diet either with or without rumen-protected amino acids (Lysine and Methionine at a ratio of 3:1). Improvac® (2 mL per dose) was administered to the immunocastrated bucks at weeks 1, 8 and 20 of the study, and the study concluded at week 39. At slaughter, testicles were harvested immediately, weighed, and sperm were recovered from the cauda epididymis. Smears were prepared to determine viability, testicle surface CIELab colour was measured, and testicle tissue samples were taken for histological assessment of seminiferous tubule development. To assess the relationship between testicle development, secondary sexual traits, and dimorphic body development, linear body measurements were taken, internal fat deposits were assessed, and antler size parameters were determined. The animals with a greater degree of testicle development had larger body sizes, greater forequarter development, and antler development. Immunocastration influenced testicle development, as evident in testicle surface colour (increased L* values, i.e., lighter testicles), decreased the sperm viability, and seminiferous tubule size. On the contrary, amino acid supplementation increased a* colour values (i.e., redder testicles) and increased sperm viability. Intact males fed amino acids showed the greatest seminiferous tubule development. Thus, immunocastration may be a viable welfare-friendly alternative to physical castration in deer culled from the herd for venison production to suppress testicle development and functioning, while amino acid supplementation shows potential for improving the development of yearling fallow deer bucks selected for breeding.

Key words: *castration, Dama dama. nutrition, sperm, venison, welfare*

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List of the abbreviations used in the thesis

AA: Amino Acids

AIC: Akaike Information Criterion

BCS: Body Scoring Condition

E: Intact male buck

EU: European Union

FSH: Follicle Stimulating Hormone

GnRH: Gonadotropin Releasing Hormone

GLM: Generalized Linear Model

IC: Immunocastrated male buck

LH: Luteinizing Hormone

Lys: Lysine

Met: Methionine

MGLM: Multilinear Generalised Model

1. Introduction and Literature Review

1.1. Introduction

Venison has been marketed as a high-quality meat product that is low in cholesterol and high in polyunsaturated fatty acids, proteins, and minerals (Mattiello 2009; Daszkiewicz et al. 2015; Kudrnáčová et al. 2018; Ny et al. 2022). This makes it appealing to consumers who demand a healthier alternative to commercial meat products and who are now more conscious of what they eat from a perceived health and environmental impact perspective. According to English (2008), while farmed deer is the primary source of venison and is generally obtained ethically, there are still management issues, particularly with male fallow deer.

Overall, fallow deer are seen as easily manageable animals, adapting to different environments with low feeding and handling requirements (Volpelli et al. 2003; Bykowska 2018). The biggest challenge during their production is documented as they approach rut, which is marked by a drastic increase in testosterone production to support mating activities and fighting to establish hierarchies, thus endangering each other as well as handlers (English 2008). Additionally, their antlers (or spikes during early developmental stages) pose an additional challenge (Tuckwell 1998). One method of managing this behaviour that has been practiced since the inception of commercial fallow deer farming in New Zealand is physical castration (Pollard & Wilson 2002; Tuckwell 2003), which is usually carried out at six months of age as soon as the testicles descend into the scrotum (English 2008). This practise in the livestock industries, however, has drawn outcries from welfare enthusiasts who believe that the discomfort and side effects (Melches et al. 2007; Needham et al. 2017a; Curtis et al. 2022;) that the animals face to meet human needs are unwarranted.

Immunocastration has been suggested as an alternative to physical and surgical castration in many production systems (Curtis et al. 2022). It is the administration of a vaccine (at least twice, depending on the production lifespan of the species) to an animal, which prevents the action of Gonadotropin Releasing Hormone (GnRH), usually by blocking it from binding to the pituitary gland (in the case of the Improvac® vaccine).

GnRH plays a role in the endocrine cascade which stimulates testicle development and supports functioning, by initiating the release of Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) (Gosch & Fischer 1989). FSH promotes the production of sperm by aiding in the development of the seminiferous tubules, while LH aids in the production of testosterone (Sathe & Shipley 2014; Ahmed et al. 2022); however, secretion is influenced by day length and seasonality (Tuckwell 1998; Asher et al. 2000). This anti-GnRH vaccine leads to a decrease in androgen hormone levels, atrophying the testicles and affecting development of the seminiferous tubules, thus interrupting the development of viable spermatozoa (Miller et al. 2000; Needham et al. 2017a; Ahmed et al. 2022). Immunocastration has helped ease management of male livestock by the reduction of aggressive behaviour (Simons 2008; Needham et al. 2017a). However, as there is no commercial vaccine with commercial vaccination schedule guidelines for small ruminants (only for large stock, namely cattle – Bopriva®), there is the need to further understand the efficacy of such vaccines in other species of ruminants according to their production goals.

In relation to castration and nutrition, immunocastration has shown to affect nutritional requirements and body development in different animal species (due to its suppressive effects on androgens) (Needham et al. 2017a). As immunocastration influences protein and fat metabolism, there is a high requirement of dietary protein for castrated animals. Studies have investigated the adjustment of amino acids according to the immunocastration vaccination schedules in swine, to aid in supporting feed efficiency and carcass leanness (Dunshea et al. 2013). However, the utilisation of rumen-protected amino acid nutrition in deer farming is not well-studied but may have potential for improving productivity under certain situations, especially the first limiting amino acid lysine (Lys), and methionine (Met), as described in the comprehensive review of Ny et al. (2022). Benefits of supplementation of rumen-protected Lys and Met proven in other ruminant species include improved nutrient utilization, reproduction, hair growth, milk and meat yield and quality, growth performance, growth, and survival of the young, endocrine and hormone production (Ceacero et al. 2020; Ny et al. 2022).

Thus, the purpose of this research was to investigate the effects of immunocastration as well rumen-protected lysine and methionine on testicle development in yearling fallow deer, to determine their possible benefits in deer farming.

1.2. Literature Review

The farming of deer was primarily initiated in New Zealand in the 1970s and is now practiced in other parts of the world like Europe and the Americas, approximating twelve million animals (Serrano et al. 2019). The deer are farmed both in intensive and extensive systems for their meat, known as venison (and referred to as such from here on), velvet as oriental Chinese traditional medicine, antlers and trophy hunting, and by-products, on a commercial basis (Ny et al. 2022), and for ecotourism. The deer species usually used in farming systems are white-tailed deer (*Odocoileus virginianus*), elk, sika deer (*Cervus nippon*), axis deer (*Axis axis*), red deer (*Cervus elaphus*), roe deer, wapiti (*Cervus canadensis*), fallow deer (*Dama dama*), and reindeer (*Rangifer tarandus*) amongst many others (Vos 1982). Deer farming, in comparison to other farmed animals, is preferred in landscapes that can support these animals, as deer can tolerate low quality pastures (Bykowska 2018), have more than one market for all products (Vos 1982), have a lower incidence of disease (Putman 1988), and are able to tolerate and adapt to different temperatures (Putman 1988). Thus, these advantages can make deer farming more economically profitable because monetary input is usually required (Gaines 2022).

Fallow deer are farmed for venison and antlers in countries like New Zealand (Daszkiewicz et al. 2015) and has shown great success in Europe (Ward et al. 2014) and has also been introduced in African countries such as South Africa (Hoffman & Cawthorn 2013). They are advantageous for farmers due to their medium-sized bodies, which allow for more animals per unit area compared to larger deer, like red deer (Tuckwell 2003, DIAA 2022). Originating from the Mediterranean region of Europe (Vos 1982; Putman 1988), fallow deer can be found in the wild and captivity throughout most European countries, as well as feral populations in the USA, Australia, and New Zealand (Cadman 1971; Vos 1982; English 2008). Their habitat is typically open woodlands, shrub areas, and grassy glades. They have flattened and palmate antlers with numerous points (Vos 1982) and their coats are a rich fawn with white spots in the summer, and a uniform greyish brown with little to no spotting. Fallow deer are relatively easy to manage, making them low maintenance animals to farm and are intermediate feeders, but tend to browse on twigs, leaves, and woody plants (Putman 1988). Supplemental feeding is necessary during periods of poor pasture quality, particularly during autumn or winter, with oats, hay or silage being optimal, following the quality over quantity rule. Fallow deer are

sensitive to selenium, copper, and winter death syndromes (PAHB 2015) which can be addressed with hay, ground grains, copper supplement, and good winter feed management respectively.

The average birth weight for fallow deer fawns is 3.8 - 4.2 kg for males and 3.6 kg - 4 kg for females (Putman 1988). As males are dimorphic, they grow faster than females. Putman (1988) records that adult fallow deer can grow up to 70-80 cm in height and a weight of 35 -65 kg for females and 90 cm in height, and a weight of 70 -100 kg for males which resonates with the information provided by Vos (1982). Male fallow deer reach puberty between 14 -16 months (McElligott et al. 2001; Ros-Santaella et al. 2019) and have motile, fertile sperm (Chapman & Chapman 1970; Mulley 2007), however, they do not normally mate until they are about four years old, at appoint where they are sexual mature enough to dominate in stand offs with other males (McElligott et al. 2001). They go through rut between September and November in the Northern Hemisphere and in April and May in the Southern Hemisphere (Sathe & Shipley 2014; Mulley 2007).

During rut, males mark their territories and have rutting stands (Vos 1982), making them difficult to handle (Tuckwell 2003). Bucks rapidly gain weight (marked by increased fat deposition as well as increased neck muscle mass) during the spring and summer months and reach their peak in autumn before the rut. However, during the rut, they tend to focus less on feeding and can lose up to 30% of their total body weight (PAHB 2015). They then must regain condition once the rut is over and usually overwinter in poor body condition (Asher 1993). As a photoperiodic species (Gosch & Fisher 1989), fallow deer undergo annual cyclic changes in testicle development. According to Asher (1993), LH release from the pituitary gland is regulated by changes in day length, with lower LH concentrations occurring during the non-breeding season, increasing in concentration as the breeding season approaches in the autumn. These LH secretions promote testicle growth, activity, and testosterone secretion, which in turn increase testicle size, spermatogenic activity and the number of viable spermatozoa (Asher 1993). As the rutting season ends and LH levels decrease, testosterone levels drop, and the opposite happens: testicles regress and produce low levels of spermatozoa (Gosch & Fisher 1989).

Antler growth is also linked to the testicle-testosterone cycle as noted by Bartos et al. (2012) and Fletcher et al. (2016). The casting of antlers occurs when testosterone levels

drop (Gosch & Fisher 1989), and new antlers begin to grow as testosterone levels increase towards the rutting season (Chapman & Chapman 1970). The mineralization of the velvet occurs during this period (Bartos et al. 2012), and hard antler are retained through the rutting period (Tajchman 2022). Testosterone also affects other dimorphic features of bucks such as the size of their neck muscle mass, which increases and decreases following testosterone levels (Sathe & Shipley 2014). Tuckwell (2003) records that once male fallow deer reach puberty at about 16 months, they become difficult to handle (if not castrated), and farmers typically choose to slaughter them at this point. Dressing percentages from 52 – 58% are recorded in bucks of 12 -24 months of age, with an average carcass weight of 24 kg and with an average fat content of 7%; older bucks can produce a carcass weight of up to 42 kg (Tuckwell 2003).

1.2.1. Venison, antlers, and by-products

The growing demand for venison is due to increasing consumer concerns about "healthier, better" diets, leading to an estimated market value of over 1.5 billion US dollars (Allan et al. 2015) with New Zealand as the largest producer and exporter worldwide (USDA 2023). Venison farming practices typically have a low utilisation of antibiotics, growth promotors, and intensive farming practices (Hoffman & Wiklund 2006) making it a preferred choice. The advantage that venison has over beef or lamb is that it has low cholesterol, high protein, minerals, and polyunsaturated fatty acids (Hoffman & Wiklund 2006; Kudrnáčová et al. 2018). Red deer and fallow deer are the most common deer species for venison farming with good quality carcasses of low-fat content obtained at 15 to 16 months in bucks and 18 months in does (Tuckwell 2003). Dressing percentages of 58 – 60% were indicated in bucks slaughtered at 12, 18 and 27 months old (Vos 1982).

Antlers are the most distinguishing feature of deer, growing from the pedicle located on the skull (Tajchman et al. 2022; Ceacero et al. 2023); solely growing in the male of these species, except for reindeer where both male and females have antlers (Vos 1982; Fletcher et al. 2016; Tajchman et al. 2022). Antlers differ from horns of the Bovidae, as they are a 'living' entity that is covered by a vesiculated skin known as velvet, and deer with antlers who have not yet shed their velvet are called "in velvet" (Fletcher et al. 2016). The velvet sheds gradually once the antler calcifies, and the deer aid the

shedding process by rubbing their antlers on trees (Vos 1982). Once all the velvet is shed, the antler is classified as hard antler, and these antlers are retained during the breeding season until they are cast (fall off) usually at the end of the breeding season (Chapman & Chapman 1970; Asher 1993). Antlers in general are considered trophies depending on size and development, according to species. Hunting of deer for their trophies is an age-old practice that was mostly done by the wealthy but is now accessible to many, especially in game estate enclosures. Many do it for sport/competition and are awarded accordingly, although there are mixed reactions about hunting deer in general, especially because of the ability to find different meats opposed to through hunting.

According to Vos (1982), antlers can be utilized in various ways, including the production of buttons, pipes, knife handles, letter openers and walking sticks. The velvet from the antlers is used for medicinal purposes (Tuckwell 1998), in the form of powder or thin slices (DINZ 2009). Vos (1982) notes that typically, the antlers are removed from deer when they are in the velvet stage, approximately 1 cm above the pedicle, under either local or general anaesthetic. Medicinal products derived from the hard antler and velvet are graded according to quality, freshness, and degree of damage (Vos 1982). These include: Pantocrin, derived from axis deer antlers and used for preventive health care and Rantarin obtained from reindeer velvet antlers which is believed to possess anti-inflammatory and anti-stress properties and hypotensive properties (Vos 1982; Kawtikwar et al. 2010).

Other by-products of deer farming include the penis (pizzle) testicles, sinews, tails, hearts, livers, tongues, and teeth which are used for soups, gourmet dishes as well as jewellery respectively (Vos 1982). Deer skins are commonly usually used to produce suede leather for clothing, gloves, handbags, and moccasins, while reindeer hides make excellent parkas, trousers, shoes (Gore et al. 2002).

1.2.2. Deer management, handling, and welfare

The management of deer is relatively easy, but handling can be a challenge due to their timid and nervous nature, especially fallow deer (Vos 1982; Tuckwell 1998). Therefore, it is essential to minimize stress levels by incorporating all handling activities as closely as possible. For example, the weighing the animals can be incorporated into the restraint systems so that the animals are weighed every time they enter the system.

Other activities that might induce stress in deer through handling include ear tagging, vaccination, drenching, weighing and removal of velvet or spikes amongst many others (PAHB 2015).

To ensure the best welfare for the deer, it is ideal to have paddocks which will be utilised for activities such as mating, fawning, weaning, and growing; and that each is equipped with fresh water and shelter (Mattiello 2009); and the animals should have access to yards, trees for shade, shelter, and a source of food. Trees also prevents erosion and should be included as they contribute to a tranquil environment (Asher 1993; PAHB 2015). Nutritional requirements must be carefully considered for farmed deer in both extensive and intensive systems (Pollard & Wilson 2002). When grazing in pastures, it is important to consider the types of grasses and toxic plants present (Pollard & Wilson 2002). In regions with mild winter or tropical climates, supplemental feeding is not typically necessary (Vos 1982). Conversely, in areas with harsh winter, indoor-housed deer require roughage sources. For intensively farmed deer, supplementary feeding (Volpelli et al. 2003; Dryden 2011, 2016;) should be provided throughout the year to meet the protein and energy requirements of pregnant and lactating females and the mineral requirements of males during antler growth. A body scoring system is also important to ensure proper growth (Audigé et al. 2003; Bykowska 2018;). Additionally, equal access to food should be guaranteed for all animals, regardless of sex. Therefore, some feeders can have barriers to prevent antlered males from feeding there.

In farms that have a raceway that leads to pens, restraint areas and a yard, it is also important to utilise fences as deer like fallow deer can easily jump (DINZ, 2018) and to also ensure that the part that leads into the year is boarded up to prevent injury. As the animals move through the system and even into the paddocks, there might a tendency to be aggressive and fight as different hierarchies are mixed. The Public Animal Health Branch (2015) recommends that as the animals enter the light-controlled pens the lights are turned off, especially when penning the older sires. There should also be avoidance of mixing males and females, different age groups as well as placement of bucks in hard antler in paddocks with other males for the same reason (DINZ 2018).

1.2.3. Male deer farming challenges

Male deer are usually farmed together for meat production and for breeding stock. However, their production presents challenges when the testosterone levels increase during puberty and breeding season. The breeding season varies depending on the hemisphere and species, with each species exhibiting unique behaviours during this period. Aggressive behaviour in males during breeding season includes grinding their teeth, vocalisation, lowering head and stomping feet amongst many others (WSNZ 2015) states that whereas female deer, on the other hand, exhibit aggressive behaviour mostly during calving and to unfamiliar handlers (Cadman 1971).

In the wild, males will have to fight for territories where sometimes an incumbent male is usurped by a newcomer, taking over the territory and the females within that territory. To mark their territories and attract mates, some species like the Iberian Red Deer spray urine and microbial secretions on their bellies, which gives them a dark belly (De la Peña et al. 2019). The belly mark can get as large as 70 cm and the stronger it smells, the more the male is trying to represent its dominance, age, range, and physical condition (De la Peña et al. 2019). Additionally, this chemical signalling is also important in conditions of low light or for females in faraway distances. Fallow deer tend to reduce their intake during the 3 – 4-week breeding period (Asher 1993), which leads to loss in weight and thus affecting the farmers input initially. Additionally, they also release a scent from their eye gland to mark territory and tend to vocalise through groans/growls/snorts (Cadman 1971) to attract their female counterparts.

As the behaviour is not lost even in farming systems, once the breeding season approaches, the male fallow deer bucks fight, lock antlers and vocalisation which takes up a lot of their energy, hence the weight loss (Cadman 1971). They are at risk of sustaining injuries such as loss of eyes or bodily damage that can result in lameness or damage to their skin and this can lead to economic losses for farmers as the damaged skin or antler cannot be sold and bruising must be cut from the carcass and disposed of, or even worse, loss of the deer. Antlers getting interlocked is an unfortunate circumstance that can lead to slow death as the deer becomes unable to defend itself.

1.2.4. Castration: challenges and opportunities for deer farming

Castration is an age-old practice (Ahmed et al. 2022) that has been carried out in almost all types of livestock (AMVA 2014) and by simple definition, is the alteration of a male animal's testicle (AMVA 2014; Ahmed et al. 2022). It can be done physically (non-surgically or surgically), chemically and/or through hormonal suppressive methods like immunocastration (Curtis et al. 2022).

This procedure is done for different reasons such as increasing wool production, curbing aggressive behaviour (Ahmed et al. 2022), making handling easier, increasing fat deposition of the carcass often desired in some cultures and controlling of unnecessary breeding or spreading of undesired genetics, especially in herds where male and female animals are combined – as reviewed by Needham et al. (2017a). If any legislation does exist in a specific country, as many are lacking such legislation, it is generally recommended to be performed at a young age, with the perception that this helps to ease pain, stress levels, and loss of blood in animals.

Non-surgical methods include the use of an elastrator which is a small but strong rubber ring (elastic band) at the base of the scrotum, cutting off blood supply to the testicle and causing them to fall off in a few weeks (AMVA 2014; Needham et al. 2017a;). Another non-surgical physical practice is use of a clamping tool, such as a burdizzo, which crushes the sperm ducts and blood vessels of the animal. These methods are commonly used in cattle, sheep, and goats (Melches et al 2007; AMVA 2014). Surgical methods involve making an opening in the scrotum with a scalpel or a sharp knife. It is typically done in pigs since their testicles are closely held to the body but is also be performed in cattle, sheep, and goats (AMVA 2014). A combination of the three methods can be utilised such as applying a burdizzo clamp, followed by rubber ring placement and then surgically removing scrotal tissue nine days after the application of a rubber ring (AMVA 2014).

Surgical castration has the advantage of being a more reliable method, as the removal of testicle is assured and when done correctly, and it is less painful and stressful to the animal (Huston 2015). The wounds heal faster than non-surgical methods. However, it requires a skilled handler and carries the risks of bleeding, swelling and infection. Instruments and the area must be properly cleaned and disinfected to reduce the risk of infection and fly strike (Huston 2015). Non-surgical castration has advantages

such as less blood loss, less risk of infection and relative ease of performance (Huston 2015). However, the method used may result in complications such as incomplete clamping of spermatic cord or the possibility of missing a testicle (English 2008). There is also a risk of tetanus in cattle and in deer, and tetanus vaccination is recommended 6 - 8 weeks prior to castration and proper placement of the rubber band is essential to prevent breakage (English 2008). Chemical methods, such as injecting toxic agents like formaldehyde and lactic acid (Ahmed et al. 2022) into the testicle parenchyma, can cause irreversible damage and loss of functioning. However, this method requires additional time, skilled personnel, and requires twice the healing time compared to surgical methods (AVMA 2014). Immunocastration, which is considered a welfare-friendly alternative, and which works through the immune system of the animal, induces antibody production against GnRH and has shown success in increasing live weight gain, dressing percentage, hot carcass weight, and average daily gain in certain livestock in comparison to surgical methods (Needham et al. 2017a,b). It has shown great success in swine and ovine production; however, it is a developing science when it comes to larger ruminants (Curtis et al. 2022).

In Australia, fallow deer are the only farmed deer commonly castrated because they are considered dangerous unless castrated (English 2008). Castration should take place before antler pedicle development (Chapman & Chapman 1970) and the descent of the testicles at about six months (English 2008). The most common method is the application of elastrator rings, along with two tetanus vaccinations four to six weeks apart, with the second dose given on the day of castration (English 2008). Castration can cause loss of appetite and signs of discomfort for a few days, but it is helpful in preventing antler growth (for deer intended for venison production) if carried out before pedicle development (Fletcher et al. 2016). In older deer, castration might not be as effective as it not guaranteed to reduce aggression (Miller et al. 2000; Simons 2008).

Castration has positive impacts, as mentioned, but it also has negative impacts on animals, as reported by Melches et al. (2007) and AVMA (2014). Physical castration, the most common method, has several negative effects, on including acute, and chronic pain as well as physiologic stress. Acute pain resulting from physical methods can last up to four hours without pain mitigation (Needham et al. 2017a). Chronic pain, which lasts for several weeks after castration, typically results in reduced weight gain and growth

(AMVA 2014; Needham et al. 2017a). Surgical and burdizzo clamp methods cause immediate pain, while elastrator bands delay immediate pain due to blood supply interruption (Melches et al. 2007; Zeng et al. 2022). Castrated animals exhibit responses such as struggling, kicking, tail swishing and reduced activity, among others (AMVA 2014). Burdizzo castration causes more severe inflammation than band castration (AVMA 2014). Although, elastrator rings have shown greater success in young cattle calves of 3 -4 months, they still produce acute and chronic pain (as shown by behaviour and cortisol levels) due to inflammation (and in extreme cases, sepsis) at the ring placement site. This could be the main reason why the practice is more utilised in calves (AMVA 2014) and lambs (Melches et al. 2007) than surgical methods. Surgical castration can result in complications such as haemorrhages, excessive swelling, infection, poor wound healing, and failure (Melches et al. 2007). In addition, physical castration is often performed in combination with other managerial practices, such as weaning, vaccinations, deworming etc, which only adds to the stress of the animal (Etim et al 2013).

Castration is a stressful experience for livestock, with all methods resulting in varying levels of blood cortisol concentrations. Stress levels are lower when local anaesthesia is applied together with castration procedures (Melches et al. 2007). However, without anaesthesia, surgical castration produces the highest rise in cortisol levels, followed by burdizzo clamp, elastrator band and immunocastration presenting the lowest levels (AMVA 2014). Cortisol levels are generally lower in immunocastrated animals, with increases immediately after handling and injection, but no evidence of acute of chronic pain, has been demonstrated in serum cortisol levels and behaviour in sheep (Needham et al. 2017b).

In deer, castration has been successful (English 2008), but Fletcher et al. (2016) has sighted that for deer that are not castrated as soon as the testicle descend into the scrotum and are castrated much later in life tend to grow antlers that will remain in velvet throughout the life of the deer. In unlikely cases, with late castration, deer may develop perruques (an exaggerated antler growth) which can either be treated by a veterinarian or lead to the death of the deer (English 2008). As elastrator bands are the preferred method of castrating deer (English 2008), the abovementioned issues are applicable, including chronic pain, increased risk of infection of bacteria and/or parasites, etc. This is further exacerbated by the fact that deer are typically not handled as frequently as livestock and

are not as easy to monitor due to their flighty nature. Thus, monitoring of recovery after the procedure, wound healing and castration success is more challenging, together with the possible application of anti-inflammatories and pain mitigation drugs (which pose their own challenges regarding food safety), which may be required for physical castration procedures in the future. Thus, alternatives like immunocastration may be a more viable and welfare-friendly option for deer farming.

1.2.5. Immunocastration

Immunocastration is an alternative to traditional castration that induces an immune response against (mainly) hypothalamic or pituitary hormones. It is the administration of a vaccine that works to produce antibodies against GnRH which suppresses the release of FSH as well as LH, thus reducing testosterone production, such a vaccine is thus known as an anti-GnRH vaccine (Needham et al. 2017a; Ahmed et al. 2022). There are other vaccines that are not anti-GnRH but against specific pituitary hormones, however, anti-GnRH immunization is considered more effective, as it suppresses the hypothalamic release of the forementioned hormones affecting spermatogenesis (Simons 2008). Animals that are immunocastrated are considered intact until the second vaccination and subsequent vaccinations thereafter (Dunshea et al. 2013). This is because the initial priming dose is preparatory for the immune system whereas the subsequent vaccines produce a large and rapid increase in anti-GnRH antibodies (Zeng et al. 2022). The scheduling/timing of the vaccinations also plays a big role in the effect of the vaccine, of which commercial manufacturer guidelines are only available for swine and cattle (Needham et al. 2017b).

There are various vaccines that are commercially available but only Improvac® is permitted for use in the European Union since 2009 (ECU 2009), Bopriva® and GonaCon™ are other vaccines that are also registered and utilised in some countries (Gupta & Minhas 2017). Immunocastration aligns with animal welfare clauses, causing slight discomfort during vaccination (Needham et al. 2017b; Curtis et al. 2022), but it has the same benefits as traditional castration methods and has shown great success both in production and wildlife animals in terms of welfare management, suppressing testicle functioning and thus decreasing agonistic behaviours and indiscriminate breeding, as well as improved growth rate and feed efficiency (Gupta & Minhas 2017). In swine,

immunocastration has reduced sexual and aggressive behaviour (Karaconji et al. 2015) due to its suppressive effects on testosterone concentration levels (Theubet et al. 2010; Wicks 2013), as well as androsterone and skatole in swine (Dunshea et al. 2001; Needham et al. 2017a; Škrlep et al. 2020), reducing boar taint and improving meat quality (Zamaratskaia et al. 2008; Lugar et al. 2016). In cattle, it decreased testicle development (Janet et al. 2012), and testosterone serum levels (Theubet et al. 2010) without significantly affecting body weight. It has also been used in wildlife population management in elephants and was shown to reduced testicle function as well as serum testosterone levels (Leuders et al. 2014).

Studies related to testicle development indicated disruption of testicle tissue development and functioning, indicated by smaller seminiferous tubule circumference and epithelium thicknesses, as shown in lambs immunocastrated with Improvac®, regardless of the vaccination schedule used (Needham et al. 2019). The surface CIELab colour of the testicles may also indicate changes in testicle activity, with differences found in testicle colour for both lamb (Needham et al. 2016) and swine (Needham et al. 2015b) compared to non-castrated males. However, this method is still not well-studied regarding its relationship with testicular activity and therefore it is necessary to further investigate these parameters.

In deer, physical castration is already discouraged for bucks intended for venison as they recorded a lower body weight of 9 to 16% in castrated bucks (Vos 1982) and a reduced carcass weight and dressing proportion in comparison to intact males (Mulley & English 1985). However, immunocastration on the other hand has shown potential in deer management through research conducted by Miller et al. (2000) where white tailed deer showed regressed testicles as well as reduced interest in females in oestrus a similar finding for Curtis et al. (2008) and can be applied at a later age than physical castration, thus potentially not decreasing growth rates as significantly as physical castration.

As the hormonal effects on general body development are diminished after immunocastration (Dunshea et al. 2013), this can lead to a reduction in potential lean tissue growth, weight gain efficiency if an adjusted diet is not implemented, especially in swine where an increased feed intake and subsequently fat deposition is seen after the second vaccination (Brunius 2011). Thus, adjustment of nutrition after the second vaccination could be beneficial to prevent excessive fat deposition in some species, like

swine. Needham et al. (2015) showed that balanced protein requirements of swine differ after immunocastration, however, in ruminants, protein nutrition is primarily affected by rumen microbes. Ruminal microbes have an impact on amino acids, thus there is utilisation of rumen-protected amino acids to ensure maximum absorption of proteins is recommended (Ny et al. 2022). In ruminant species, rumen-protected Lys supplementation increases carcass and muscle yield, fat storage for the winter period, and improves the nutritional and marbling quality of venison in fallow deer bucks (Ny et al. 2022). Rumen-protected Met has been found to improve milk yields, and protein digestion and amino acid absorption in young ruminant animals (Ny et al. 2022;). However, it has only demonstrated a limited impact on weight gain, especially beyond the first months of life. Nonetheless, it has in shown a positive impact on antler beam length in deer (Ny et al. 2022). When used in combination, both rumen-protected amino acids have demonstrated improvement in antler growth, plasma fat and protein metabolites, and fat storage during winter in deer (Ceacero et al. 2020; Ny et al. 2023). Thus, adjustment of supplementation may be necessary for immunocastrated ruminants to avoid excessive fat deposition, but no information is available regarding this in deer.

On the other hand, in terms of testicular development, cattle bulls on high protein diets had higher average daily gains, larger scrotal circumferences, greater body weight and high body score condition. These findings also positively impacted sperm volume, motility, concentration, and total count than animals on a low protein diet (Rekwot et al. 1988). Similar findings were observed in fallow deer by Ros-Santaella et al. (2019). Thus, improving the amino acid nutrition of fallow deer bucks through supplementing rumen-protected amino acids may be beneficial for non-castrated bucks selected for breeding purposes, and not for venison production.

Regardless of the increasing demand for venison products, a thorough review in literature shows gaps of research related to welfare-friendly alternatives for castration in male deer to aid in their management for venison production, and its consequent effects on other management parameters, like nutrition. As reviewed, physical castration if preferred as a routine management method for agonistic behaviours in deer (not only livestock species like swine, cattle, and sheep), however, immunocastration presents a better option regarding animal welfare and ethics. This is because it aligns with the EU regulations when it comes to welfare management of animals in general, does not have a

withdrawal period, and there is no need to oral administration, suiting their timid nature and reducing the need of handling. Immunocastration also aligns in a way with organic farming (FVE 2020), highlighting its potential use in organic deer farming, should it be such as case. Regarding protein supplementation, Dryden (2011, 2016) highlights the importance of protein supplementation in deer production as most research focuses on increasing the crude protein in ruminants on a high-low basis but not much research has been done when it comes to limiting amino acids in fallow deer. This shows that there could a potential that is not being explored that could be beneficial to venison-antler producers that can add onto the pastured and/or supplemented diets that the deer are currently exposed to. Considering all factors discussed related to immunocastration and rumen-protected amino acid supplementation, it is of interest to observe their effects and potential application in fallow deer production, to provide possible further recommendations to producers for deer management tools that align with current animal welfare principles and consumer expectations regarding ethical venison production.

2. Aims

This research aimed to investigate the combined effects of immunocastration and rumen-protected amino acid supplementation (namely lysine and methionine) on the testicle development and functioning in yearling fallow deer bucks, as well as differential body development and secondary sexual traits like antler development, to determine if it may be a viable management tool for venison producing farmers.

2.1. Research questions

1. To what extent does immunocastration disrupt testicle tissue development and sperm quality in yearling fallow deer?
2. What is the influence of supplementing rumen-protected Lys and Met to yearling fallow deer on testicle development and functioning?
3. What is the relationship between development and secondary sexual traits and dimorphic body development in fallow deer?

2.2. Hypotheses

Firstly, H_0: Immunocastration will not affect testicle surface colour, morphometry, and sperm quality of yearling fallow deer.

H_1: Immunocastration will affect testicle surface colour, morphometry, and sperm quality of yearling fallow deer.

Secondly, H_0: The supplementation of rumen-protected Lys and Met to yearling fallow deer will not influence testicle development and sperm quality.

H_1: The supplementation of rumen-protected Lys and Met to yearling fallow deer will improve testicle development and sperm quality.

Thirdly, H_0: The degree of testicle development will not influence the development of secondary sexual traits and dimorphic body development of yearling fallow deer.

H_1: The degree of testicle development will influence secondary sexual traits and dimorphic body development of yearling fallow deer.

3. Materials and Methods

All experimental procedures conducted during this study were approved by the Institutional Animal Care and Use Committee at the Czech University of Life Sciences, Prague (Permit: 63479_2016-MZE-17214). The experiment was carried out at a private deer farm in South Bohemia, Czech Republic (49.17 N, 14.90E; 485 m.a.s.l.), and formed part of a larger project investigating the effects of rumen-protected amino acid supplementation, with and without immunocastration, on the productivity of fallow deer, regarding their antler development and meat production performance.

3.1. Animals, feeding and experimental design

At the beginning of the experiment, forty-four male fallow deer (~ ten months old) were divided into two 2-hectar paddocks (balanced for body weight, at an average live weight of 22.9 ± 2.4 kg). Within each paddock, the animals were then further allocated into two castration treatment groups: half were immunocastrated animals (IC; n = 11 per paddock) and half remained non-castrated intact males (E; n = 11 per paddock). In the second month of the study, a supplementation of rumen-protected amino acids was included into the diet of the animals within one paddock. Thus, four treatment combinations were utilised in the study, under a factorial design, as follows: non-castrated intact males without amino acid supplementation (E-Control), non-castrated intact males with amino acid supplementation (E-AA), immunocastrated males without amino acid supplementation (IC-Control) and immunocastrated males with amino acid supplementation (IC-AA).

All animals were fed a mixed grain diet of oats and wheat (90 oats:10 wheat) at 250 g per animal per day throughout the experiment. The AA supplementation was rumen-protected lysine (Lys) at 6.3 g of the supplement per animal per day which is equivalent to 4.3 g L-Lysine HCL or 4.1 g rumen by pass (95% rumen by pass) (LysiGem, Kemin Industry, Inc., USA) and methionine (Met) at 2.1 g per animal per day, equal to a 1.6 g DL-Methionine or 1.4 g ruminal bypass (90% rumen by pass) (Kessent, Kemin Industry, Inc., USA), following a 3:1 ratio of Lys to Met respectively (Schwab et al. 2004). The mixture of grains and AA was fed in long feeders, to best minimize competition and monopolization of the resource by dominant individuals (Ceacero et al. 2020) and feeding

was observed to support this. All feed was observed to be eaten by the end of each day. To avoid a pasture effect, the groups were rotated twice from one paddock to another during the experimental period. The most abundant species of plants in the paddocks were *Lolium perenne* and *Cynosurus cristatus*, as well as common grass species including *Agrostis* sp., *Festuca* sp. and *Poa* sp. *Trifolium repens*, and weed species *Urtica dioica* and *Cirsium arvense* (Bureš et al. 2020). Pasture samples from both paddocks were collected three times during the study period to analyse the nutrient composition. The nutrient composition of the grain mixture and pasture were analysed following the methodology of Kudrnáčová et al. (2019) and the results are shown in Table 1.

Table 1. Chemical composition of the pasture and concentrate supplemented to yearling 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

The deer allocated to the immunocastrated treatment were vaccinated using three 2mL doses of Improvac® (Zoetis Animal Health, New Jersey, USA) injected subcutaneously into the area above the shoulders with a Sterimatic safety needle guard system (Sterimatic Worldwide Ltd, UK). The priming dose was injected in the beginning of the experiment in March (week 1). The latter two doses acted as boosters, with the first booster applied seven weeks after the priming dose in April (week 8) and the second booster; three months after the first booster dose in July (week 20) (Figure 1). A second booster was utilised to ensure the duration of the immunocastration effect until slaughter, based on commercial vaccination recommendations for cattle, as no recommendations are available for deer species.

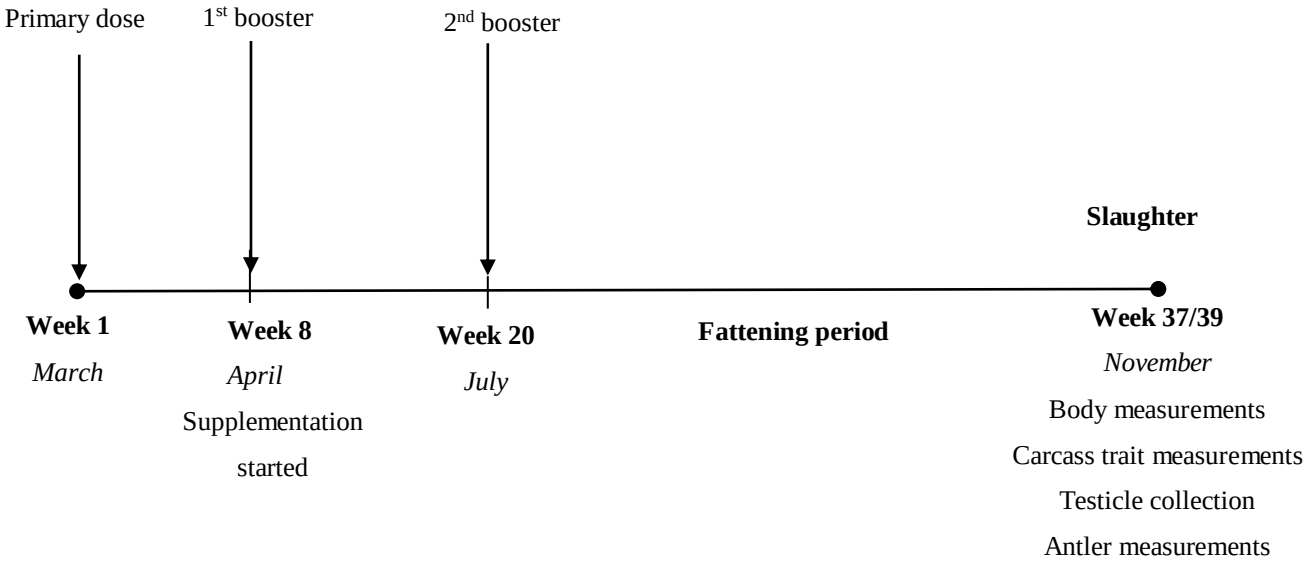

Figure 1. The trial timeline showing the immunocastration vaccination schedule, and the feed supplementation period, for the respective treatments and data collection at slaughter.

3.2. Slaughtering and carcass measurements

After seven months of AA supplementation (mid-November; Figure 1), all deer were slaughtered using captive bolt stunning in a restraint system, followed by exsanguination by severing the jugular veins and carotid arteries. Before slaughter, the body condition of each buck was scored from a range of 1 to 5 using the methodology of Audigé et al. (1998) and live weight was also recorded (average slaughter weight: 39.55 ± 0.89 kg). The animals were slaughtered over two days, where half of the animals from each treatment group were randomly selected and slaughtered per day, according to the capacity of the abattoir facility and management schedule of the commercial deer farm. This approach also allows for the finishing of slaughter animals before winter, when the animals would start using excess nutrition to increase the fat stores and subsequently prepare for their first rut in the season thereafter (Ceacero et al. 2020). Linear measurements were taken on the carcasses, including height at the withers, body length, heel length, neck circumference, chest circumference, and leg circumference were measured using a flexible tape measure.

After the measurements, carcasses were transported for processing at the abattoir, located 20 km from the farm, where carcasses were eviscerated and weighed (hot carcass

weight) before chilling at 4°C. The dressing percentage was calculated as: (hot carcass weight/slaughter weight) × 100. The internal fat deposits such as kidney fat, heart fat, and stomach fat were removed and weighed (Serrano et al. 2008). Antlers were cut from the skulls using an electric saw and the length of each antler was measured, and then weighed (Ceacero et al. 2023).

Immediately after slaughter, both testicles were removed from scrotum, and each was trimmed of excess tissues and epididymis, and weighed together (trimmed testicle weight) using a precision scale with accuracy ± 0.01 g (Kern, Verkon, Germany). Thereafter, one gram of cauda epididymis was removed, placed in a microtube with 3 mL of physiological saline and macerated (Needham et al. 2017b). Then, 10 µl was pipetted onto a clean labelled microscopic slide followed by 20 µl of nigrosine-eosin staining media, to produce a smear slide. After drying, the percentage of dead versus alive sperm were determined by evaluating 150 sperm per sample (at 100x magnification) (Nikon Eclipse E200, Nikon, Japan). Then, another 10 µl of the extracted fluid was further diluted with 300 µl of physiological saline. Finally, 10 µl of the diluted sample was transferred to each side of a Neubauer Chamber (0.100 mm depth, 0.0025 mm^2, Assistent, Germany) and the number of sperm cells on each of the four grids on both sides of the chambers were counted, using an optical microscope (at 40x magnification) (Nikon Eclipse E200, Nikon, Japan). The final sperm concentration per gram of cauda epididymal tissue was then calculated following the protocol of WHO (2010) and EMS (2020).

The testicles were then cut in half, perpendicular to the longitudinal axis of the testicle and the colour of the cut surface was measured (6 measurements/sample) using a calibrated portable spectrophotometer (CM-2500d, Minolta, Osaka, Japan; aperture size of 8 mm including the specular component and 0% UV; illuminant/observer of D65/10◦; zero and white calibration) (Needham et al. 2019). The results were expressed by the *L* (lightness)*, *a* (redness)* and *b* (yellowness)* co-ordinates of the CIELab colorimetric space. Following the protocol of Needham et al. (2019), tissue samples were cut from each testicle and preserved in 10% buffered formalin for further processing for histological evaluation.

At the State Veterinary Institute Prague, the preserved testicle tissue samples were washed with water and dehydrated in a graded alcohol series before being paraffin embedded. Tissue samples were sliced into 5 µm thick sections, placed onto microscope

slides, and stained with haematoxylin and eosin (Needham et al. 2017b, 2019). The seminiferous tubule circumference and epithelium thickness of 120 seminiferous tubules per deer were measured using an optical microscope (Nikon Eclipse E200, Nikon, Japan) connected to digital ds-f1 (at 4x magnification), and the image analysis software NIS-Elements AR 3.2. (Nikon Instruments Europe B.V., Amsterdam, Netherlands).

3.3. Statistical Analyses

IBM SPSS Statistics 28 software was used to generate several Multivariate General Linear Models (MGLM) to assess effects of supplementation and immunocastration treatments on the testicles´ colour, morphometry, and sperm quality variables. Normality of the dependent variables was tested using Wilk's Lambda. Sperm concentration and viability data were not normally distributed and were thus transformed using the log transformation and square root functions, respectively. Weight and body condition were also included in the model since both variables are well-known to affect testicle development, especially around puberty (Putman 1988; McElligott et al. 2001; Sathe & Shipley 2014). Variance Inflation Factor showed no multicollinearity between body weight and two poxy variables for body condition: body condition score (BCS) (Audigé et al. 1998) and percentage of internal fat (Serrano et al. 2008), even if the three variables were significantly correlated. Preliminary models were tested using weight and BCS or weight and internal fat; the second option consistently showed lower AIC values, and thus internal fat was selected as an indicator of body condition for building the final models. Descriptive statistics were also used to calculate estimated mean values and standard error for the variables grouped by nutrition, castration status and the interaction between nutrition and castration status.

Relationships among the studied testicle variables, and between them and selected animal characteristics (withers height, body length, heel length, neck circumference, chest circumference, leg circumference, carcass weight, dressing percentage, kidney fat, heart fat, stomach fat, antler length, and antler weight), were analysed using partial correlations. Statistical tests used the following significance levels: $P < 0.05$, $P < 0.01$ and $P < 0.001$.

4. Results

Whole body weight at slaughter and internal fat percentage affected the GLM in terms of the testicle' morphometry (trimmed testicle weight, seminiferous tubule circumference, seminiferous tubule epithelium thickness). There was a significant interaction between castration status and nutrition for seminiferous tubule circumference ($P < 0.05$; Table 2) as well as for the percentage of live sperm ($P < 0.05$; Table 2).

Table 2. Multivariate GLMs showing the effects of the supplementation of amino acids, immunocastration and their interaction on testicle colour, morphometry, and sperm quality in fallow deer bucks.

	n	R^2	Nutrition	Castration Status	Nutrition x Castration Status	Body weight	Internal fat (%)
COLOUR							
Wilk´s λ			***0.869***	***0.669****	***0.779****	***0.911***	***0.983***
L*	40	0.377	ns	**	ns	ns	ns
a*	40	0.182	*	ns	ns	ns	ns
b*	40	0.040	ns	ns	ns	ns	ns
MORPHOMETRY							
Wilk´s λ			***0.548****	***0.360****	***0.845***	***0.446****	***0.713****
Testicle weight (g)	39	0.601	*	*	ns	***	ns
ST circumference (µm)	39	0.507	**	**	*	**	*
ST epithelium thickness (µm)	39	0.597	**	***	ns	**	***
SPERM QUALITY							
Wilk´s λ			***0.803****	***0.754****	***0.889***	***0.484****	***0.847***
Sperm concentration (cells/g)	37	0.561	ns	ns	ns	*******	ns
Live sperm (%)	37	0.453	*	**	ns	ns	*

Significance at $P < 0.05$, $P < 0.01$, and $P < 0.001$ levels are indicated by *, **, and ***, respectively.
ns: non-significant; ST: seminiferous tubule.

Immunocastration influenced the testicle cut surface colour, having higher ($P < 0.01$; Table 2) *L** values than non-castrated males (i.e., having lighter testicle colour) (Table 3). Immunocastrated deer also had a decrease in seminiferous tubule epithelium thickness ($P < 0.001$; Table 2) compared to non-castrated males (Table 3). Despite this, immunocastrated deer had slightly higher trimmed testicle weights ($P < 0.05$), and whilst being statistically significant, this difference is rather biologically negligible.

Nutrition affected the *a** values of the cut surface testicle colour ($P < 0.05$; Table 2), with animals fed supplementary amino acids having higher *a** values (i.e., the testicles were redder) (Table 3). Additionally, amino acid supplementation increased seminiferous tubule epithelium thickness ($P < 0.01$; Table 2 and 3). On the contrary, control animals (i.e., not fed supplementary amino acids) had higher trimmed testicle weights ($P < 0.05$; Table 2 and 3).

Table 3. The estimated mean values and standard error (SE) of the testicle colour, morphometry, and sperm quality parameters from immunocastrated and non-castrated fallow deer bucks (n = 44), with and without (control) amino acid supplementation.

	Nutrition		**Castration Status**		**Nutrition x Castration Status**			
	AA	**Control**	**E**	**IC**	**E-AA**	**E-Control**	**IC-AA**	**IC-Control**
L*	62.63 ± 0.34	63.53 ± 0.34	62.37 ± 0.34	63.79 ± 0.34	62.28 ± 0.48	62.47 ±0.49	62.98 ± 0.48	64.60 ± 0.48
a*	3.96 ± 0.14	3.52 ± 0.14	3.79 ± 0.14	3.68 ± 0.14	4.00 ± 0.20	3.59 ± 0.20	3.93 ± 0.20	3.45 ± 0.20
b*	12.68 ± 0.16	12.70 ± 0.16	12.64 ± 0.16	12.74 ± 0.16	12.55 ± 0.22	12.72 ± 0.22	12.81 ± 0.22	12.67 ± 0.22
ST circumference (µm)	490.68 ± 5.01	468.07 ± 5.14	489.73 ± 4.93	469.03 ± 5.07	493.79^{a} ± 7.04	485.67^{a}± 7.14	487.58^{a} ± 7.00	450.48^{b} ± 7.38
ST epithelium thickness (µm)	39.43 ± 0.67	36.33 ± 0.69	40.28 ± 0.66	35.48 ± 0.68	41.49 ± 0.94	39.07 ± 0.96	37.37± 0.94	33.60 ± 0.99
Testicle weight (g)	31.89 ± 1.07	35.05 ± 1.10	31.64 ± 1.05	35.29 ± 1.08	30.35 ± 1.50	32.93 ± 1.52	33.43± 1.49	37.16 ± 1.57
Sperm concentration (cells/g) #	3.93±0.44 x10^{8}	5.74 ± 7.19 x10^{8}	4.90± 6.56 x10^{8}	4.68x± 5.80 x10^{8}	4.16 ± 6.96 x10^{8}	5.73± 1.13 x10^{8}	3.70 ± 5.65 x10^{8}	5.76x ± 9.59 x10^{8}
Live sperm (%) #	21.72 ± 2.80	9.50 ± 1.12	22.85 ± 2.82	9.00 ± 0.84	32.93 ± 2.03	11.66 ± 1.68	10.51 ± 1.07	7.34 ± 1.14

abc: indicates significance differences for the interaction terms between castration and nnutrition and castration status.
# indicates that variables were back transformed.
AA: supplemented with amino acids, Control: not supplemented with amino acids; E: intact; IC: immunocastrated; E-AA: intact and supplemented with amino acids; E-Control: intact and not supplemented with amino acids; IC-AA: immunocastrated and supplemented with amino acids; IC-Control: immunocastrated and not supplemented with amino acids; ST: seminiferous tubule.

Table 4. Pearson's correlation coefficients (r) between the characteristics of testicle and sperm quality parameters indicating reproductive functioning, in fallow deer bucks (n = 44).

	L*	***a****	***b****	**ST circumference (µm)**	**ST epithelium thickness (µm)**	**Testicle weight (g)**	**Sperm concentration (sperm/g)**
b*		ns					
L*		-0.598***	0.374*				
ST circumference (µm)	-0.424**	0.312*	ns				
ST epithelium thickness (µm)	-0.398**	0.337*	ns	0.814***			
Testicle weight (g)	ns	ns	ns	0.331*	ns		
Sperm concentration (cells/g)	ns	ns	ns	0.322*	ns	0.637***	
Live sperm (%)	ns	ns	ns	ns	ns	ns	ns

Significance at $P < 0.05$, $P < 0.01$, and $P < 0.001$ levels are indicated by *, **, and *** respectively.
ns: non-significant; ST: seminiferous tubule.

Seminiferous tubule circumference and thickness showed weak positive correlations with *a** values ($P < 0.05$; Table 4) and weak to moderate correlations with *L** values ($P < 0.01$; Table 4); i.e., testicle with larger seminiferous tubule circumferences and thicker epithelium had cut surface colours that were less bright (darker) and more red. Seminiferous tubule parameters showed a strong positive correlation with each other ($P < 0.001$; Table 4) indicating that the larger the tubule circumference, the thicker the tubule epithelium. Trimmed testicle weight showed a strong positive correlation with sperm concentration ($P < 0.001$; Table 4); weak positive correlation with seminiferous tubule circumference whereas sperm concentration showed a positive weak correlation with seminiferous tubule circumference i.e., larger, well-developed testicle had larger seminiferous tubule circumferences and a higher sperm concentration.

Table 5. Pearson's correlation coefficients (r) between the characteristics of reproduction parameters and body linear measurement, carcass traits, and antlers in fallow deer bucks (n = 44).

	Testicle weight (g)	Sperm concentration (sperm/g)	ST circumference (µm)	ST epithelium thickness (µm)	*L**	*a**
Withers height (cm)	0.358*	0.400**	ns	ns	-0.338*	ns
Body length (cm)	0.494***	0.455**	ns	ns	ns	ns
Heel length (cm)	0.523***	0.405**	ns	ns	ns	ns
Neck circumference (cm)	0.304*	ns	0.308*	ns	ns	ns
Chest circumference (cm)	0.656***	0.423**	ns	ns	-0.461**	ns
Leg circumference (cm)	ns	ns	ns	ns	ns	ns
Carcass weight (kg)	0.663***	0.698***	ns	ns	ns	ns
Dressing percentage (%)	0.357*	ns	ns	ns	ns	ns
Kidney fat (%)	ns	ns	ns	ns	ns	ns
Heart fat (%)	ns	ns	-0.313*	-0.511***	ns	ns
Stomach fat (%)	ns	ns	ns	ns	ns	ns
Antler length (cm)	ns	0.409*	0.503**	ns	-0.429**	0.400*
Antler weight (g)	0.390*	0.390*	0.577***	0.403*	-0.571***	0.402*

Significance at $P < 0.05$, $P < 0.01$, and $P < 0.001$ levels are indicated by *, **, and ***, respectively.
ns: non-significant; ST: seminiferous tubule.

Trimmed testicle weight showed a moderate positive correlation with carcass weight, chest circumference, heel length ($P < 0.001$; Table 5), and body length ($P < 0.01$; Table 5). It also showed a weak positive correlation ($P < 0.05$; Table 5) with withers height, neck circumference, dressing percentage, and antler weight.

Sperm concentration showed a strong positive correlation with carcass weight ($P < 0.001$; Table 5), moderate positive correlation with withers height, body length, heel length, chest circumference ($P < 0.01$; Table 5), as well as antler length ($P < 0.05$; Table 5) and weak positive correlation with antler weight ($P < 0.05$; Table 5).

Seminiferous tubule circumference showed a moderate positive correlation with antler weight ($P < 0.001$; Table 5), and antler length ($P < 0.01$; Table 5). It also showed a weak positive correlation with neck circumference ($P < 0.05$; Table 5), and a weak negative correlation with heart fat ($P < 0.05$; Table 5). Seminiferous tubule thickness showed a weak negative correlation with heart fat ($P < 0.01$; Table 5) and a positive moderate correlation with antler length ($P < 0.05$; Table 5).

For the testicle colours, *L** values showed weak to moderate negative correlations with antler weight ($P < 0.001$; Table 5), antler length, chest circumference ($P < 0.01$; Table 5), and withers height ($P < 0.05$; Table 5); *a** values showed moderate positive correlation to antler length and antler weight ($P < 0.05$; Table 5).

5. Discussion

This study assessed the effects of immunocastration and amino acid supplementation on the testicle development of fallow deer bucks. The fallow deer's reproductive cycle is based on seasonal changes which also influence the release of androgen hormones. Puberty in bucks is reached between 14 and 16 months, a stage at which motile, fertile spermatozoa have been observed by Mulley (2007), although the bucks are not used for breeding until they reach about four years of age. It is important to note that the bucks used in this study were yearlings, and many of the findings can be attributed to their age and stage of the reproductive cycle.

In pigs and sheep, immunocastration reduced testicle size (Needham et al. 2015, 2016; Škrlep et al. 2020; Ahmed et al. 2022). In the present study, immunocastration affected seminiferous tubule development of the immunocastrated bucks, indicating tissue atrophy and thus reduced testicle activity. These effects were seen in the size reduction of the circumference and epithelium of the seminiferous tubules (Appendix 1), where spermatogenesis occurs in mammals. These findings were anticipated and are comparable to many other species, including deer, sheep, and swine (Needham et al. 2015, 2016, 2017b; Giriboni 2022). Testicle size of the castrated deer, however, was not affected by immunocastration in this present study. Considering that the deer were about 16 months when slaughtered, i.e., around the period of puberty and before their first rut, this could have impacted the lack of effects of immunocastration on testicle size. Unlike some livestock breeds, testicle size in fallow deer is highly related to season – testicle size increases to support spermatogenesis as they approach the breeding season (or rut) and decrease when out of season (Chaplin & White 1972). The testicle size might also not be an ideal measurement for the effect of immunocastration as observed in swine by Zamaratskaia et al. (2008), however, in deer, it could be because of the rut effect.

Colour parameters of the deer testicle surface in the present study indicated that testicles with larger seminiferous tubule circumferences and thicker epithelium also had redder and darker a* values. This was the opposite for the immunocastrated deer which had higher *L** values of their testicle surface colour, likely indicating lower testicle activity. It is possible that the link between colour and reduced testicle activity may be due to reduced blood supply to the testicle. Similar results were illustrated by Needham

et al. (2015) in pigs, where testicle tissue atrophy was also associated with lighter, less red testicle colour. However, whilst this trend is observed in other studies, the association needs further investigation as information on testicle surface colour in most livestock species is currently limited, and it is thus difficult to draw clear conclusions in this regard.

Immunocastration also decreased the percentage of live sperm. In animal production, ideal levels of viable sperm for production should be at about 70% (Sathe & Shipley 2014), proving that even though the differences in the percentage of live sperm between the treatments were significant in the present study, the percentage was still quite low even in the Control group – possibly because of levels of testosterone (Chapman & Chapman 1970) linked to age and seasonality (Gosch & Fisher 1989) as they would enter their first rut. Additionally, it was observed (no data shown) that the morphology of the sperm showed immature spermatozoa in all samples, as expected in bucks at this age (Sathe & Shipley 2014). As an observational note, the immunocastrated deer appeared to have a higher prevalence of immature spermatozoa, which also showed abnormalities such as coiled tails (Appendix 2). When considering the correlation analyses, fallow deer with heavier testicles had more developed seminiferous tubules (i.e., larger circumferences), increased sperm concentrations, and higher sperm concentrations, as expected. This is also observed when weight and body condition are under control in the model, so these findings are independent of these variables in this study.

As fallow deer are sexually dimorphic animals (Serrano et al. 2008), it is easy to see the impact that a higher rate of testosterone can have on the secondary male characteristics. The animals with a greater degree of testicle development were positively associated with larger body sizes, greater forequarter development, and antler development. The specific hormone that oversees antler growth in deer is believed to be IGF-1 (Bartos 2012), whereas testosterone's main influence is on pedicle development and mineralisation of the antler. Similar results were reported by Malo et al. (2008) in Iberian red deer, Simons (2008) and Bartos (2012) in fallow deer, and Miller et al. (2000) in white-tailed deer. As observed by Ceacero et al. (2023), it is possible to conclude that there was no total cessation in the production of testosterone and IGF-1. Immunocastration vaccination schedules for other species focuses on suppressing testosterone to a level low enough to prevent agonistic behaviours, and in the case of swine, to suppress androgen production enough to allow for boar taint clearance, and thus

does not rely on the complete elimination of testosterone production. The antler growth was not interrupted in the present study however, immunocastration affected mineralisation and thus antler weight which is linked to testosterone production as evidenced by Ceacero et al. (2023). However, it is impossible to deduce by how much the testosterone levels had dropped, as serum testosterone was not analysed. Serum androgen levels are challenging to study due to their frequent fluctuations, thus requiring frequent and standardised blood sampling, which is not possible in species that are difficult to handle frequently, such as fallow deer. Whilst the effects of immunocastration on serum testosterone levels have been established over a range of species, in-depth studies should be considered utilising samples that better represent longer-term hormone levels, such as faecal hormone metabolites.

Amino acid supplementation is a novel topic in deer nutrition, particularly for reproduction. Lys and Met are the most studied AA, and limiting AAs, for high-producing ruminants and necessary for the rapid growth of young ruminants (Kung & Rode 1996; Ny et al. 2022). Improving dietary protein and AA profiles in deer nutrition is important for growth performance and production, especially during antler growth (Dryden 2011, 2016). A positive effect of AA supplementation was seen for seminiferous tubule circumference and thickness, and live sperm (%) in the present study. Lys has an essential role in Lys acetylation in spermatogenesis (Pang & Rennert 2013), and Met is also important for methylation function in early spermatogenesis (Menezo et al. 2020). Alkhashab et al. (2021) focused on reproductive parameters in rams fed rumen-protected Met supplementation and found a significant increase in the percentage of live sperm, testicle size, and serum testosterone levels. Additionally, in the present study, the AA supplementation influenced the redness of the testicle surface colour, which could further indicate that AA supplementation might improve spermatogenesis activity. The indirect effect of AAs can also be evidenced throughout the improvement of body growth and antler growth, as deer need to reach a threshold body weight to start their antler growth (secondary sexual traits) and the onset of puberty (Fennessy & Suttie 1985; Baxter et al. 1999; Bartos 2012). As indicated in the correlations, the reproductive parameters, including seminiferous tubule circumference, thickness, and percentage of live sperm were positively related to most linear body measurements (indicating dimorphic growth) and antler growth. Thus, in contrast to immunocastration, supplementation of rumen-protected Lys and Met could prove beneficial for yearling fallow deer who are selected

for breeding purposes and not for venison production, to support development and reproductive functioning later in their productive life.

6. Conclusions

Both tools (immunocastration and amino acid supplementation) show potential for utilisation in commercial deer farming systems for venison production, where suboptimal breeding males are culled for meat purposes, and immunocastration can be applied to control the various welfare issues already highlighted. In the case of selective breeding male replacements, amino acid supplementation may support their further development. Immunocastration showed interruption of testicle functioning, and thus could have implications on the behaviour of yearling fallow deer, which should be further investigated. The implementation of immunocastration in replacement of physical castration may thus help alleviate the pressure that farmers of deer face in relation to animal management from a welfare perspective.

Recommendations for future studies include the optimisation of a vaccination schedule that is specific for deer producers, that considers their specific goals regarding to antler development and venison production. An optimisation of the vaccination schedule would identify the timing that would be best for vaccination administrations to take advantage of lean muscle growth driven by hormones but at the same time reduce antagonistic behaviour easing management at critical periods of increased androgen production. This would help producers fully optimise venison production and/or velvet antlers production and at the same time ensure ease of handling Such research requires the support of in-depth production and behavioural data. Regarding the utilisation of rumen-protected amino acids, it would be necessary to further deepen their study to understand their benefits in fallow deer, especially related to testicle development and reproduction success, as well as body development not only in males but also females. Studies of the effect of other limiting amino acids in fallow deer is also recommended, to further expand the understanding, role, and potential benefits of this in deer farming. Finally, should this study be replicated, a detailed look at the testosterone levels through faecal hormone metabolite monitoring throughout the study as well as vaccination at a younger age (preferably before pedicle development) could be applied.

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Appendices

List of the Appendices:

Appendix 1: Atrophy of the seminiferous tubules showing reduced epithelium thickness of the IC buck vs non- atrophied seminiferous tubule of an E buck.

Image 1: IC buck

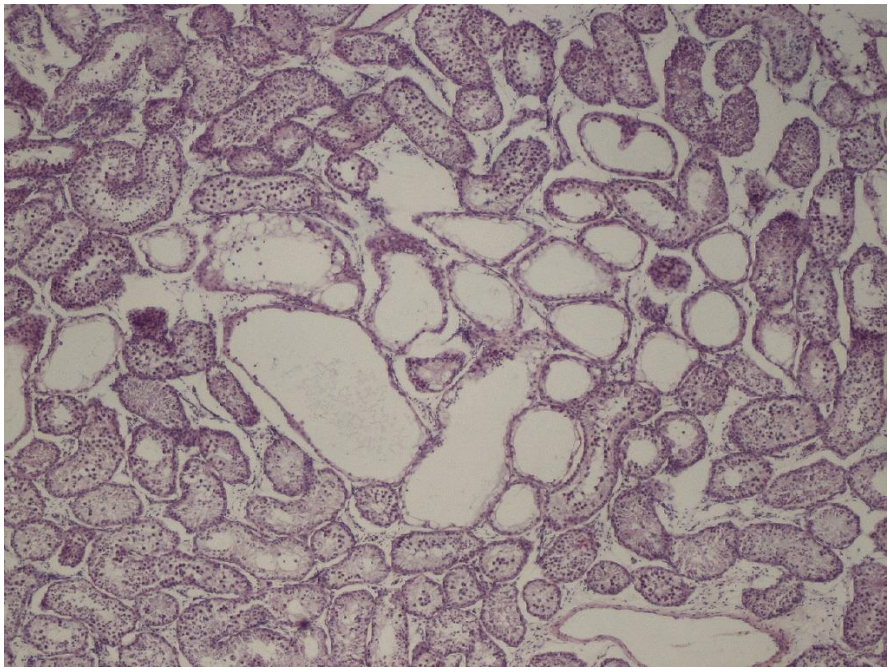
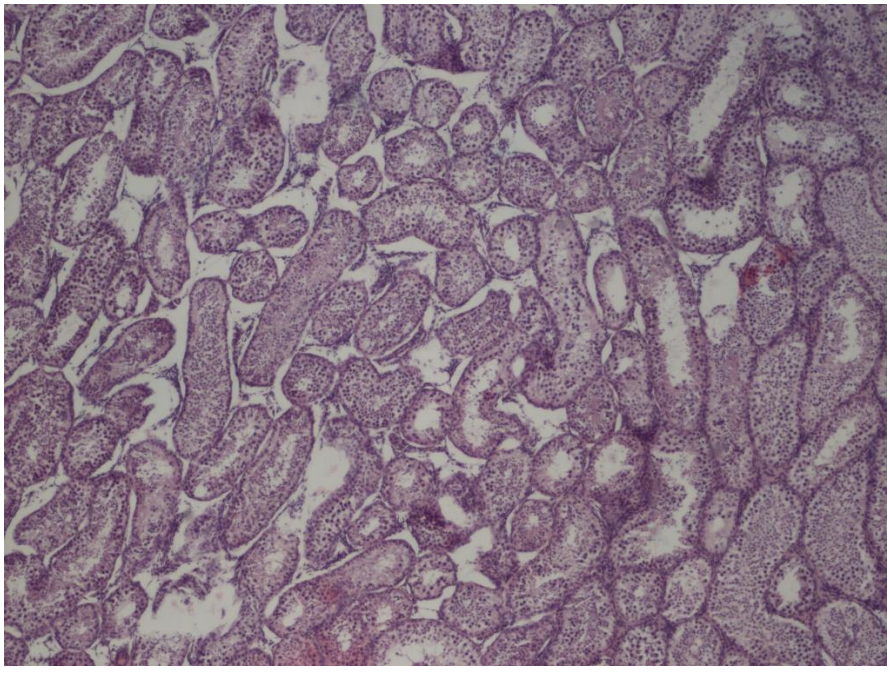


Image 2: E buck



Appendix 2: Sample of spermatozoa from (IC) buck (showing coiled tails) and spermatozoa from intact (E) buck. The darker stained sperm are dead, and the lighter (whiter are alive).

Image 3: IC Buck

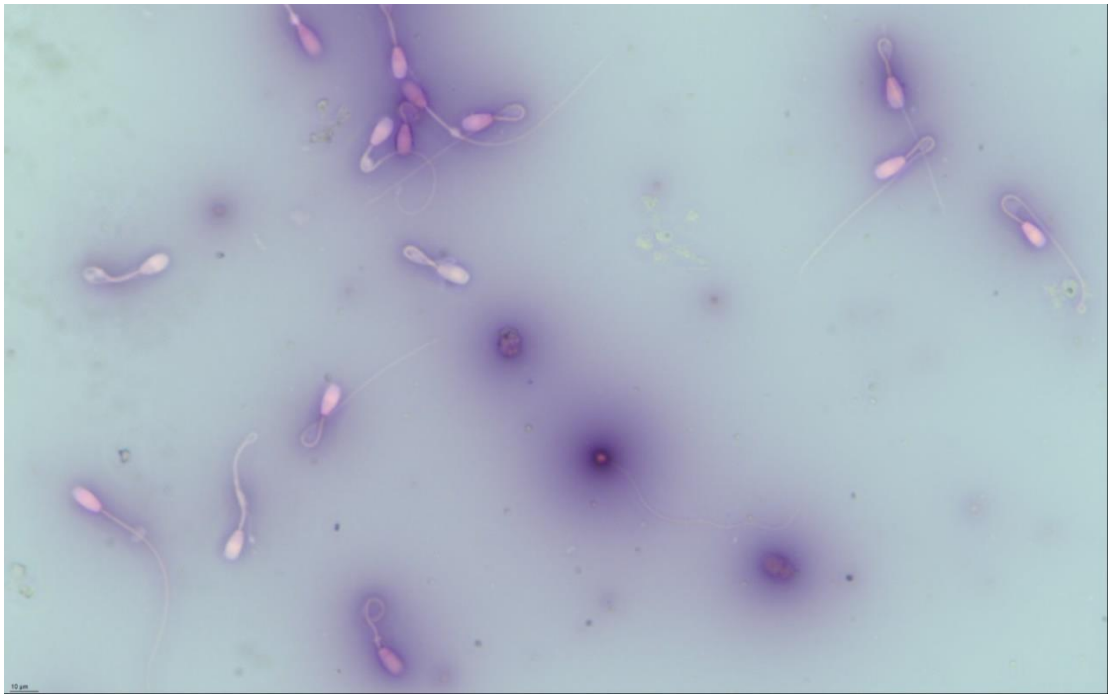


Image 4: E Buck