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Faculty of Tropical AgriSciences

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ASSESSING THE IMPACT OF LUPIN MEALS ON THE DEVELOPMENT OF FLECKVIEH BULLS

MASTER'S THESIS

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Declaration

I hereby declare that I have done this thesis entitled “**Assessing the Impact of Lupin Meals on the Development of Fleckvieh Bulls”** independently, all texts in this thesis are original, and all the sources have been quoted and acknowledged using complete references and according to Citation rules of the FTA.

In Prague, April 2024

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Omitoogun Bolatito Azeez

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Abstract

In response to the growing demand for sustainable protein sources in livestock farming due to increasing global population and environmental concerns, alternative feed options like Lupinus species, including white lupin and yellow lupin, have gained attention. This research investigates the impact of *Lupinus albus* (white lupin) and *Lupinus luteus* (yellow lupin) meals on the growth and development of Fleckvieh bulls, a dual-purpose cattle breed. The study aimed to assess the potential of lupin-based diets as alternative protein sources in cattle nutrition, and their effects on growth efficiency and reproductive tract development.

Thirty Fleckvieh bulls were divided into three treatment groups (one treatment per pen, with 10 animals per treatment): a control group fed a conventional diet, a group fed a diet containing *Lupinus albus* (white lupin) meal, and a group fed a diet containing *Lupinus luteus* (yellow lupin) meal as the primary protein source. The bulls' feeding behaviour, growth performance, and post-mortem reproductive tract morphology, sperm quality, and biochemical parameters of the seminal vesicle fluid were evaluated. The research revealed that bulls fed a control diet exhibited significantly higher body weights, greater average daily gains (ADG), and improved feed conversion ratios (FCR) compared to those fed lupin-based diets. However, certain parameters related to sperm quality, particularly the percentage of live sperm, showed potential positive effects with lupin diets, especially *Lupinus albus*. Although some effects of the lupin diets were observed in reproductive tract morphology, namely prostate weights and live sperm percentages, and some biochemical parameters among the diet groups suggested potential effects of lupin-based diets on reproductive gland fluid composition and sperm quality.

Given these findings, it is imperative to exercise caution in considering lupin-based diets for cattle, particularly young bulls, due to the observed effects on growth and potential impacts on reproductive parameters. Further research is needed to investigate the long-term effects of lupin species on bull development. Additionally, future studies should explore non-nutritive components, processing techniques, and optimal inclusion levels of these lupin meals that would not compromise growth performance while assessing their suitability as alternative protein sources in cattle diets.

Keywords: alternative protein; feeding behaviour; fleckvieh bulls; growth performance; *Lupinus albus*; *Lupinus luteus*; reproductive development; sperm quality

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List of the abbreviations used in the thesis

ADG: Average daily gain

AL: Ampulla length

ALP: Alkaline Phosphatase

ALT: Alanine Aminotransferase

AMP: Adenosine monophosphate

ANFs: Antinutrient factors

AST: Aspartate Aminotransferase

ATP: Adenosine triphosphate

AW: Ampulla weight

Ca: Calcium

Chol: Cholesterol

CK: Creatine Kinase

Cl: Chloride

Cu: Copper

CW: Cowper's weight

DM: Dry matter

EL: Epididymis length

EPW: Epididymis weight

FCR: Feed conversion ratio

FTW: Fresh testes weight

GI: Gonadosomatic index

GMT: Glutamate Methyltransferase

IAS: Institute of Animal Science in Prague

ICM: Intracavernosal pressure measurement

K: Potassium

LD: Lactate Dehydrogenase

LSMean: Least Square Mean

LW: Live weight

Mg: Magnesium

Na: Sodium

NEFA: Nonesterified fatty acids

NSP: Nonstarch polysaccharides

P: Phosphorus

PrL: Prostate length

PrW: Prostate weight

PUFA: Polyunsaturated fatty acid

RS: Rapeseed

Se: SAbraham um

SEM: Standard error of the mean

SFL: Sigmoid flexure length

SFW: Sigmoid flexure weight

SP: Serum Protein

STC: Seminiferous tubule circumference

STT: Seminiferous tubule thickness

SVL: Seminal vesicle length

SVW: Seminal vesicle weight

TAG: Triacylglycerol

TDN: Total digestible nutrient

TL: Testes length

TTW: Trimmed testes weight

TWID: Testes width

UFA: Unsaturated fatty acids

WL: White lupin

YL: Yellow lupin

Zn: Zinc

1. Introduction and Literature Review

1.1. Introduction

In modern livestock farming, the pursuit of sustainable and efficient protein sources has become increasingly imperative. With the growing global human population and environmental concerns associated with traditional livestock farming practices, there is a pressing need to explore alternative protein sources for animal feeds that can meet nutritional demands while mitigating ecological impacts (Berger et al. 2013). Recent data from the Food and Agriculture Organization of the United Nations (FAO) underscores the magnitude of the livestock industry's contribution to global protein production. In 2021, world meat production reached a staggering 357 million tonnes, marking a significant 53% increase, or 124 million tonnes, compared with 2000 (FAO 2023). Notably, the production growth rate between 2020 and 2021 surged to 4%, the highest observed over the 2000–2021 period. It is however not surprising that the chicken, pig, and cattle dominated nearly 90% of global production between 2000 and 2021 despite the diversity of livestock species raised for meat production.

Amidst this backdrop, alternative protein sources have garnered attention as viable solutions to address the challenges confronting modern livestock farming. Among these alternatives, *Lupinus albus* (white lupin), *Lupinus angustifolius* (narrow-leafed lupin), and *Lupinus luteus* (yellow lupin) have emerged as promising contenders (Gresta et al. 2003). The chemical composition of lupins varies by species, with white lupin (*Lupinus albus*), boasting a nutrient-rich profile (Sofia, 2008). These lupin species offer a myriad of advantages across various facets of agricultural production. Notably, lupins are not merely utilised as animal feed additives but also hold significance as rotation crops due to their adaptability to diverse environments and minimal cultivation requirements (Bolland & Brennan 2008; Fumagalli et al. 2014). Lupins have also garnered attention for their nutritional value, grain productivity, and suitability for cultivation in marginal lands (Berger et al. 2013). Their nitrogen-fixing capabilities and adaptive features make them well-suited for cultivation in infertile soils, thereby contributing to sustainable agricultural practices (Dijkstra et al. 2003).

Historically, lupins have been incorporated into the diets of poultry, swine, and sheep, showcasing their versatility and suitability across different livestock systems (Nalle et al. 2011; Berger et al. 2013). They present a cost-effective and sustainable option for livestock farmers, particularly in supplementing dairy diets, as evidenced by the preference shown by Australian dairy farmers due to factors such as cost-effectiveness and ease of storage (White & Staines 2007). In addition, supplementation of ruminant diets with lupins has demonstrated positive effects on growth and reproductive efficiency, comparable to conventional cereal grain supplements (Volek & Marounek 2008). Lupins offer significant promise as a sustainable protein source for livestock nutrition, characterized by high protein levels and nutritional benefits. However, feeding lupins to ruminants poses challenges, particularly concerning their alkaloid content. Certain lupin varieties, such as *Lupinus luteus* cv. Cardiga, contain alkaloids like lupanine and sparteine, which can reach levels potentially harmful to animal health if not carefully managed (Cabrita et al. 2024). However, their utilization requires consideration of anti-nutritional factors, such as the specific carbohydrate composition of lupin grains, characterized by low starch levels and high non-starch polysaccharides (NSP) and raffinose oligosaccharides, which can impede energy utilization and decrease feed intake and digestibility in animals (White & Staines 2007; Erbas et al. 2005). Additionally, the presence of quinolizidine alkaloids in lupin grain contributes to a bitter taste, although current cultivars generally exhibit low levels of alkaloids that do not significantly affect feed intake (Pieper et al. 2016; Nalle et al. 2012). Strict adherence to maximum tolerable levels of alkaloids in lupins is necessary to prevent toxicity in animals, necessitating careful selection of cultivars and agricultural practices (Cabrita et al. 2024).

The utilisation of dual-purpose livestock breeds also offers opportunities to improve the sustainability and diversification of production systems. One such example is the Fleckvieh cattle, originating from Germany and Austria, and used nowadays for milk and meat production. Generally, meat quality from dairy breeds of cattle has been reported to be very poor (Varnam & Sutherland 1995). However, the Fleckvieh cattle exhibit superior growth rates and dairy production, efficient feed conversion, and desirable carcass characteristics compared to traditional beef breeds such as Hereford and Angus cattle. (Sölkner et al. 2012). Therefore, feeding practices play a pivotal role in maximising the breed's potential, especially for the finishing of bulls culled as they are generally in excess within production systems (especially dairy systems). Understanding the nutritional requirements and formulating well-balanced diets are crucial for optimising meat production in dual-purpose cattle, like Fleckvieh (Sölkner et al. 2012).

The use of lupins as alternative protein sources for livestock presents a compelling avenue to address the dependence on imported sourecs, like soybeans, a prevalent example of protein sources commonly used in animal feed formulations (Abraham et al. 2019). This thesis aims to investigate how incorporating *Lupinus albus* (white lupin) and *Lupinus luteus* (yellow lupin) into the diets of Fleckvieh bulls, a dual-purpose cattle breed, compared to traditional rapeseed diets regarding the influence of these alternative protein sources on the growth performance and reproductive development of Fleckvieh cattle.

1.2. Literature Review

1.2.1. Introduction to lupins as an animal feed ingredient

The Lupinus genus encompasses a wide diversity of species, ranging between 200 and 600, with primary centres of diversity in South America, western North America, the Mediterranean region, and Africa (RIRDC 2011). The cultivation of Lupinus species holds a deep historical significance, with their domestication and utilization documented across diverse regions globally (Carvajal-larenas et al. 2016). *Lupinus albus* (white lupin) stands out as one of the earliest cultivated species, particularly esteemed in the Mediterranean for its seeds, which were processed for consumption and valued for their soil-enhancing properties (Udall et al. 2005). Similarly, *Lupinus mutabilis* (andean lupin) played a pivotal role in pre-Columbian South American agriculture, serving as a dietary staple (Carvajal-larenas et al. 2016). Notably, *Lupinus angustifolius* (narrow-leafed lupin) gained traction in Europe and Australia as a grain crop, appreciated for its adaptability to cooler climates and nutritional value (Udall et al. 2005). Likewise, *Lupinus luteus* (yellow lupin) emerged as a valuable livestock feed due to its protein-rich seeds and adaptability to diverse environments (Carvajal-larenas et al. 2016). Early cultivars of lupins, such as *Lupinus angustifolius* 'Unicrop', *Lupinus albus* 'Ultra', and *Lupinus luteus* 'Sonet', were characterized by relatively high levels of toxic and bitter alkaloids, posing challenges such as depressed growth, reduced feed utilization, and potential toxic effects (Olkowski et al. 2001). The new varieties of *Lupinus albus*, *Lupinus angustifolius*, and *Lupinus luteus*, particularly 'Alyi parus', 'Mitchurinskiy', and 'Pilgrim', show promising traits such as high grain productivity and quality comparable to soybeans, while also showing reduced toxicity and alkaloid content, offering potential as sustainable protein sources for livestock (Yagovenko et al. 2022).

Lupins offer a promising solution to address the escalating demand for sustainable protein sources in animal feed systems (Bolland & Brennan 2008). These legumes present advantages across various facets of livestock farming, as evidenced by recent literature. In terms of profitability, lupins boast high grain productivity and require minimal input, which can potentially enhance economic returns for farmers (Bolland & Brennan 2008). Furthermore, their dual functionality as both a concentrate and forage feed in ruminant diets offers flexibility in feeding strategies, contributing to balanced nutrition for livestock (Borreani et al. 2009). Many lupin species also exhibit comparable nutritional quality to that of soybeans, making them a viable alternative protein source in animal feed formulations (Sedláková et al. 2016). Beyond their utility in animal nutrition, lupins demonstrate significant benefits for soil health and agricultural sustainability. They have the capacity for nitrogen fixation, enriching soil fertility, and reducing reliance on synthetic fertilisers (Sedláková et al. 2016). Additionally, lupins exhibit adaptability to poor soils and serve as excellent rotation crops, promoting crop diversity, reducing disease incidence, and contributing to overall agricultural sustainability (Fumagalli et al. 2014). In the realm of dairy farming, lupins have garnered favour as supplementary feed due to their cost-effectiveness and ease of storage and handling (White & Staines 2007). Research has underscored the suitability of *Lupinus albus* for the Mediterranean crop-livestock food chain, while *Lupinus luteus* shows promise for similar applications (Chiofalo et al. 2012).

Suchý et al. (2006) and Stanek et al. (2006) conducted analyses comparing yellow lupin (*Lupinus luteus*) and narrow-leaved lupin (*Lupinus angustifolius*) protein to soy protein, uncovering distinctive amino acid compositions. Lupin protein displayed higher arginine content but lower levels of methionine, cysteine, lysine, threonine, and tryptophane compared to soy protein (Suchý et al. 2006; Stanek et al. 2006). This amino acid profile, characterised by a notable abundance of arginine, sets lupin protein apart.

In addition to minerals, *Lupinus albus* boast an average content of carotenoids, including β-carotene, lutein, zeaxanthin, tocopherols, and other bioactive components with stimulating potential (Msika et al. 2006). Within the lipidic fraction of *Lupinus albus*, lupeol, a triterpene alcohol, has been identified as a component with the potential to improve the renewal of epidermal tissue (Msika et al. 2006).Yanez et al. (1983) highlight *Lupinus albus*as a rich source of unsaturated fatty acids, particularly oleic and linoleic acids, constituting up to 80%. The nutritional profile of lupin oil reveals a favourable ω-3 to ω-6 fatty acids ratio, ranging from 1:1.7 to 10.8 across varieties registered in the Czech Republic based on independent analyses. Zralý et al. (2008) and Písaříková and Zralý (2009) narrow their focus to specific lupin varieties, the *Lupinus polyphyllus* and *Lupinus albus* varieties, revealing nuanced fat content variations. The *Lupinus polyphyllus* variety displays a fat content of 107.7 g/kg, while the *Lupinus albus* variety showcases 79.1 and 102.3 g/kg in whole and dehulled seeds, respectively. Straková et al. (2006) conducted a comprehensive analysis of lupin seeds, revealing substantial variability in nutritional constituents among approved varieties in the Czech Republic. Lupin seeds from *Lupinus albus*, *L. angustifolius*, and *L. luteus* exhibited diverse compositions of proteins, fats, and fibre, influenced by factors such as variety and climatic conditions (Straková et al. 2006).

Musco et al. (2017) compared three lupin species (*Lupinus albus*, *Lupinus luteus*, and *Lupinus angustifolius*) and their respective varieties, and intriguing findings emerged regarding their nutritional and dietetic characteristics. Among the six varieties of *Lupinus albus*, Multitalia and Lublanc exhibited particularly favourable nutritional profiles despite having the highest alkaloid content (Musco et al. 2017). Their high protein levels and low structural carbohydrate content, along with favourable fatty acid compositions, suggest promising dietary alternatives in livestock feeding. Notably, alkaloid content did not significantly affect the *in vitro* degradability or fermentation process of *Lupinus albus* varieties, indicating potential suitability for animal diets. Conversely, *Lupinus luteus* varieties demonstrated intermediate chemical compositions but boasted the most favourable dietetic aspects, such as high polyunsaturated fatty acid (PUFA) content and low alkaloid levels (Musco et al. 2017). These characteristics, coupled with moderate fermentation patterns, position *Lupinus luteus* as a promising dietary option with potential health benefits for livestock. However, varieties of *Lupinus angustifolius* appeared less appealing nutritionally, with lower protein and fat content and higher levels of saturated fatty acids. Despite being considered 'erucic acid-free oil', *Lupinus angustifolius* varieties exhibited less favourable nutritional indices compared to other lupin species (Musco et al. 2017).

1.2.2. Lupins as a feed source for ruminants: effects on growth and performance

Investigations by Abraham et al. (2019) delved into the potential of Lupinus species, notably *Lupinus albus* (white lupin) and *Lupinus angustifolius* (narrow-leafed lupin), as alternative protein sources for livestock, with a specific focus on ruminants and cattle. This research addresses the European Union's heavy reliance on imported soybeans for livestock feed, presenting lupins as promising solutions that offer both economic and environmental advantages (Abraham et al. 2019). Modern cultivars of lupin grains, particularly those of *Lupinus albus* and *Lupinus angustifolius*, have been developed with reduced levels of anti-nutritional factors, enhancing their suitability as protein sources in ruminant and cattle diets (Abraham et al. 2019). Breeding efforts have concentrated on stabilising yields, increasing stress resistance, and improving seed quality to enhance lupin protein quality while mitigating anti-nutritional factors. These efforts highlight the potential of Lupinus species, especially *Lupinus albus* and *Lupinus angustifolius*, as valuable protein sources in livestock feed formulations, warranting further research and breeding endeavours to maximise their utilisation in the livestock farming sector and reduce reliance on imported soybean feed (Abraham et al. 2019).

In studies focusing on ruminants and cattle, various investigations have been conducted to evaluate the nutritional composition of lupin meals as a potential feed source. Among commercially and agriculturally significant lupins, white lupin (*Lupinus albus*) stands out as a large-seeded annual legume crop, valued for both human consumption and animal feed due to its high protein content (Arfaoui et al. 2021). Arfaoui et al. (2021) specifically examined the potential of white lupine seeds (*Lupinus albus*) as a substitute for soybean meal in the diets of lambs, with a specific focus on the nutritional composition of lupin meals. Their research revealed that the introduction of *Lupinus albus* seeds as an additional protein source did not significantly impact intake, indicating that lupin meals may offer comparable palatability and nutritional value to soybean meal in lamb diets. Additionally, the lupin incorporation showed no significant effect on ADG, suggesting that *Lupinus albus* seed meals can sustain adequate growth performance in lambs and could safely replace soybean meal in Barbarine lamb diets. Additionally, Arfaoui et al. (2021) found that *Lupinus albus* seed incorporation improved diet digestibility, a crucial factor in optimising nutrient utilisation and animal health. The research findings underscore the potential of lupin meals as beneficial additions to livestock feed formulations, offering favourable nutritional characteristics compared to conventional protein sources (Arfaoui et al. 2021).

El Otman et al. (2013) evaluated the effects of incorporating *Lupinus angustifolius* grain into goat kids' diets on growth performance, carcass characteristics, and meat quality. Four concentrate rations with varying levels of lupin inclusion were fed to different groups of kids. Results indicated that lupin inclusion of up to 35% dry matter (DM) of the concentrate did not negatively affect final weight, ADG, carcass yield, gastric pouch weight, adipose tissue, bone tissue importance, carcass length or thigh length, However, it significantly improved Semimembranosus muscle and also impacted carcass colour (El Otman et al. 2013). In terms of meat quality, lupin incorporation led to a significant reduction in moisture content but did not affect minerals, protein, or fat content. Overall, the study concluded that lupin can be included in up to 35% DM of the concentrate source in goat kids' diets without adverse effects on growth performance, carcass characteristics, or meat quality (El Otman et al. 2013).

A recent study by Um et al. (2024) examined the effects of lupin meals, including *Lupinus albus*, *Lupinus luteus*, and *Lupinus angustifolius*, on various parameters of Hanwoo steers, cattle breed primarily raised for beef production in Korea. They found that supplementation with lupin flakes did not significantly impact meat colour, myoglobin content, or the meat moisture, crude protein, ether extract, and crude ash contents of Hanwoo steers (Um et al. 2024). Additionally, increasing dietary lupin flake supplementation did not alter ADG, formula feed and rice straw intake, crude protein intake, or FCR. However, it did increase TDN intake (Um et al. 2024). Furthermore, the study revealed a linear increase in carnosine levels in the strip loin of late-fattening Hanwoo steers with escalating lupin flake supplementation. Although there was a slight, non-significant elevation in muscle creatine content, trends suggested an increase in anserine and creatinine levels in muscle tissue with higher lupin flake supplementation levels (Um et al. 2024). These findings suggest a potential influence of lupin flake supplementation on dipeptide composition in the strip loin of late-fattening Hanwoo steers (Um et al. 2024). Moreover, the research indicated that lupin meal supplementation led to a significant reduction in octanoic and decanoic acid contents while increasing palmitoleic acid, unsaturated fatty acids (UFA), and the n-6/n-3 ratio in the strip loin. This suggests potential dietary influences on the fatty acid composition and nutritional profiles of beef derived from Hanwoo steers supplemented with lupin meals (Um et al. 2024). The chemical composition of beef, a pivotal determinant of quality, appears to remain largely unaffected by lupin supplementation. Compounds such as carnosine, anserine, creatine, creatinine, and nucleic acids reveal potential impacts on taste improvement, antioxidant effects, and energy metabolism (Peiretti et al. 2012). Fatty acids, particularly oleic acid, emerge as critical factors influencing beef characteristics, with lupin flake's richness in unsaturated fatty acids offering implications for flavour. Thus, while lupin flake supplementation did not significantly affect growth performance, carcass characteristics, or meat composition, it positively influenced carnosine, anserine, creatinine, adenosine triphosphate (ATP), and adenosine monophosphate (AMP) contents, enhancing the taste and flavour of beef (Um et al. 2024).

However, several studies, including those by Vicenti et al. (2009), Sami et al. (2010) and Um et al. (2024), have reported a decrease in the ADG of beef cattle with lupin meal supplementation. In contrast, Kwak and Kim (2001) investigated the effects of flaked *Lupinus angustifolius* seed inclusion levels on the growth of Korean native bulls; their results indicated no significant alterations in ADG, concentrate intake, rice straw intake, total feed intake, or feed efficiency compared to the control group. However, at higher inclusion levels, , there was an increase in concentrate and total feed intake, alongside a decrease in rice straw intake, suggesting potential dietary adjustments (Kwak & Kim 2001). Additionally, their research noted tendencies for reduced average daily total feed cost per kilogram, attributed partially to the substitution of *Lupinus angustifolius* for soybean meal (Kwak&Kim 2001). These findings contribute to understanding lupin supplementation effects on growth parameters and feed cost, offering insights for optimising feed formulations in beef production systems.

Despite positive results of feeding some lupin species to ruminants, the inclusion of lupin seeds in ruminant diets requires cautious management due to the presence of anti-nutritional factors like alkaloids and lectins, which could compromise animal health if not properly addressed through processing techniques (Siger et al. 2012). While studies have demonstrated promising outcomes regarding lupin meals' suitability for ruminant and cattle diets, variability in results necessitates further investigation into factors such as lupin variety and supplementation levels. Future research efforts should aim to better understand lupin's mechanisms of action on ruminant nutrition, ultimately optimising its integration into feed formulations to enhance animal performance and health (Siger et al. 2012).

1.3. Nutritional and anti-nutritional considerations: potential health impacts of lupin meals in animals

A study by Arnoldi et al. (2015) on the health benefits of lupin meals indicates that lupin may provide some useful health benefits in the areas of hypercholesterolemia, diabetes, and hypertension prevention in Hanwoo cattle. The literature on the potential hypotensive effects of lupin protein is notably limited, with only one study conducted by Pilvi et al. (2006) investigating its impact. This study utilised the Goto-Kakizaki rat model, known to develop hypertension when subjected to a salt-rich diet containing 6% NaCl. The protein sources in the diet comprised either white lupin (*Lupinus albus*) or a soy protein isolate, both at 20% weight/weight. Following a two-week treatment period, the results showed a significant reduction in systolic blood pressure to the control group (casein). Specifically, the lupin group exhibited a systolic blood pressure reduction of 18.6 mmHg, while the soy group showed a reduction of 12.0 mmHg (Pilvi et al. 2006). The authors attributed the attenuation of hypertension to the improved vascular function observed in both the lupin and soy groups compared to the control (Soy) group. Notably, the study highlighted a distinctive aspect, with lupin protein demonstrating enhanced endothelium-dependent vasodilatation, a phenomenon not observed with soy protein. This limited but significant study underscores the potential of lupin protein, particularly white lupin (*Lupinus albus*), which may mitigate hypertension and offer unique vascular benefits compared to soy protein, enhancing overall cardiovascular health and productivity while preventing metabolic disorders (Pilvi et al. 2006). The hypocholesterolemic property of the heat-treated *Lupinus albus* has been investigated using the hyperlipidaemic rat model (Chango et al. 1998). Small but significant decreases in total serum cholesterol and triacylglycerol (TAG) levels were observed in animals treated with *Lupinus albus* (total cholesterol change −17.8%) versus control animals (Chango et al. 1998).

Alkaloids are one of the main antinutrients factors (ANFs) present in lupin seeds, along with saponins, tannins, and alpha-galactosides. The presence of these ANFs may interact with each other, potentially exacerbating their negative effects on animal health and nutrient utilization. For example, interactions between alkaloids and oligosaccharides such as stachyose may lead to metabolic interference and metabolic troubles in livestock (Reinhard et al. 2006). The alkaloid content in lupin seeds varies among different species and cultivars. Reinhard et al. (2006) observed significant variability in alkaloid content across lupin varieties, with levels ranging from negligible amounts to potentially toxic concentrations. Notably, lupanine is the most common alkaloid found in *Lupinus albus* varieties, while *Lupinus luteus* and *Lupinus angustifolius* generally exhibit lower alkaloid content (Reinhard et al. 2006). High levels of alkaloids, particularly in *Lupinus albus* varieties, may pose toxicity risks to animals. Concerns about alkaloid toxicity have led to recommendations to limit dietary alkaloid content to ensure animal health and performance.

However, recent sweet lupin varieties have lower alkaloid content, reducing the risk of toxicity in livestock diets (Reinhard et al. 2006). In a comprehensive literature review, Aniszewski et al. (2001) found that newly developed sweet varieties of lupin, in contrast to bitter varieties, exhibit significantly reduced proportions of anti-nutritional quinolizidine alkaloids, present only in trace amounts, thereby rendering the seeds and derived products suitable for human consumption and feeding ruminants and mono-gastric animals. Additionally, Gefrom et al. (2013) investigated the impact of lactic acid fermentation during ensiling on alkaloids, oligosaccharides, and tannins in field bean, pea, and lupin grains, observing an overall reduction in tannins and oligosaccharides. However, Green et al. (2012) cautioned against the teratogenic piperidine alkaloids found in plants like hemlock, lupin, and tobacco, emphasizing their acute toxicity to adult livestock and potential to induce musculoskeletal deformities in neonatal animals. Furthermore, Stanek et al. (2015) demonstrated in rats that concentrations of alkaloids in blue lupin (*Lupinus angustifolius*), administered over 28 days, led to reduced feed intake and significantly impaired growth, highlighting the potential negative impact of alkaloids on animal health. In the present study, our objective is to address these gaps in knowledge by examining the influence of different inclusion levels of *Lupinus albus* and *Lupinus luteus* on the growth performance and reproductive parameters of Fleckvieh bulls. This research endeavour will thereby enhance our comprehension of the impact of lupin supplementation on the health and productivity of Fleckvieh bulls.

2. Aims of the Thesis

This study aims to investigate the effects of incorporating *Lupinus albus* (white lupin) and *Lupinus luteus* (yellow lupin) into the diets of Fleckvieh bulls, a dual-purpose cattle breed, in comparison to traditional rapeseed diets. The specific objectives were:

- to evaluate the impact of incorporating *Lupinus albus* (white lupin) and *Lupinus luteus* (yellow lupin) as alternative protein sources on the growth performance (ADG, FCR) and feeding behaviour of Fleckvieh bulls;
- to evaluate the impact of incorporating *Lupinus albus* (white lupin) and *Lupinus luteus* (yellow lupin) as alternative protein sources on the reproductive tract development of Fleckvieh bulls.

2.1. Research Question

Will the use of *Lupinus albus* (white lupin) and *Lupinus luteus* (yellow lupin) be a suitable alternative protein source for the diet of young bull cattle without an impact on their growth performance and reproductive development compared to conventional diets?

2.2. Hypotheses

H0: the use of *Lupinus albus* (white lupin) or *Lupinus luteus* (yellow lupin) as alternative protein sources for the diet of Fleckvieh bulls will not influence the growth performance and reproductive development compared to a conventional diet.

H1: the use of *Lupinus albus* (white lupin) or *Lupinus luteus* (yellow lupin) as alternative protein sources for the diet of Fleckvieh bull will influence the growth performance and reproductive development compared to a conventional diet.

3. Materials and Methods

3.1. Experimental design, animals and slaughter

The experimental procedure was approved by the Ministry of Agriculture of the Czech Republic (No. MZE-58151/2022-13143). Thirty (30) Fleckvieh bulls, all offspring of twenty different sires, procured from a commercial herd at 232 ± 8 days were transported to the experimental stable of the Institute of Animal Science in Prague (IAS) located at GPS (50°1´54″N, 14°36´15″E). Upon reaching an average age of 358 ± 8 days and a live weight (LW) of 413 kg ± 31.8 kg, the bulls were divided into three treatment groups, each comprising ten animals, distributed to balance age and LW per treatment. They had unlimited access to food via the Vytelle GrowSafe Combine Automatic Feeder system which tracked their feed consumption and visit frequency each day. The animals underwent a 63-day feeding trial of *ad libitum* access to diets with identical protein and calorie content but varied protein sources (Table 1): yellow lupine (YL; *Lupinus luteus* cv Salut), white lupine (WL; *Lupinus albus* cv Amiga), and rapeseed (RS). Following a 22-day adaptation phase, the formal test period commenced (9.2.2023). Regular weighing sessions were conducted throughout the study period on the following dates: 9.2.2023, 23.2.2023, 9.3.2023, 23.3.2023, 6.4.2023, 13.4.2023, and at slaughter (starting on 17.4.2023). The ADG and feed intake was calculated during the period that all animals were present in the facilities, before the serial slaughter started, i.e., from 9.2.2023 to 13.4.2023. The FCR was calculated from the weight gain and feed intake over the feeding period.

For analysis of the dietary chemical composition performed the IAS, feed samples were subjected to drying at 55 °C for 48 hours. Residual moisture content was assessed via oven drying for 6 hours at 105 °C. Ash content was determined following a 6-hour exposure to 550 °C, while ether extract was obtained through extraction with petroleum ether using a Soxtec 1043 (FOSS Tecator AB, Höganäs, Sweden). Nitrogen was measured using the Kjeldahl method (Kjeltec AUTO 1030 Analyzer, Höganäs, Sweden) following the AOAC Official Method (2005), with crude protein (CP) calculated as N x 6.25. Acid detergent fibre content was assessed using AOAC Official Method (2005), while neutral detergent fibre content was analysed using sodium sulphite and α-amylase treatment according to Van Soest et al. (1991), resulting in an ash-free presentation. Fibre fractions were determined using Fibertec 2010 (FOSS Tecator AB, Höganäs, Sweden).

Table 1. Ingredient and nutrient composition of diets fed to Fleckvieh bulls (N = 30, 10 animals per treatment) for 60 days before slaughter.

Item	**Treatment group**		
	YL[a]	**WL[b]**	**RS[c]**
Ingredient (g/kg DM)			
Maize silage	504.9	501.3	502.2
Alfalfa silage	87.8	87.2	87.3
Wheat straw	34.3	34.0	34.1
Wheat grain meal	263.0	261.1	261.6
Oat grain meal	26.1	25.9	26.0
Yellow lupine grain meal	70.1		
White lupine grain meal		76.8	
Rapeseed meal			75.1
Vitamin-mineral supplement with urea[d]	13.8	13.7	13.7
Nutrient			
Dry matter (g/kg fresh weight)	523.4	524.7	522.9
CP (g/kg DM)	132.6	131.7	131.7
OM (g/kg DM)	946.9	948.0	942.5
Ether extract (g/kg DM)	34.6	39.6	29.7
NDF (g/kg DM)	327.2	316.3	318.8
ADF (g/kg DM)	192.7	190.7	195.3
PDI (g/kg DM)[e]	86.4	86.0	86.4
NEF (MJ/kg DM)[f]	6.64	6.63	6.52

[a] YL, yellow lupine meal; [b] WL, white lupine meal; [c] RS, rapeseed meal ; [d] Contained per 1 kg: CP-867 g, Ca—145 g, P—10 g, Na—60 g, Mg—30 g, S-6 g, Cu— 600 mg, Mn—2400 mg, Zn—4000 mg, Se—18 mg, I—60 mg, Co—12 mg, Vitamin A—300 000 IU, Vitamin D3—60,000 IU, Vitamin B1—180 mg, Vitamin E —720 mg; [e] Protein digested in the small intestine (Vérité and Peyraud, 1989; Vermorel, 1989); [f] Net energy of fattening (Vermorel, 1989).

3.2. Slaughter, reproductive tract assessment, and sample analysis

The bulls were slaughtered at the IAS experimental slaughterhouse located 2 km from the stable. The two heaviest animals from each group were selected for slaughter on each of five slaughter days (one slaughter day per week, starting on 17.4.2023), due to the capacity of the abattoir and to enable the data collection and processing. The animals were stunned with a captive bolt pistol and killed by exsanguination. Within an hour after slaughter, the entire reproductive tract, inclusive of the accessory glands, was harvested at the slaughterhouse for subsequent morphometric assessments, encompassing measurements of the width, length, circumference, and weight of the different components. These assessments were conducted employing a digital calliper (Z22855, Milomex Ltd, United Kingdom) and a weighing scale (Kern and Sohn GmbH kb 2000–2 N; D 72336, Balingen, Germany).

After the bull had undergone complete exsanguination and thorough bleeding, the scrotal sac with testes were excised form the animal by gripping the inguinal area and incisions were made using a sterile scalpel. After this, the scrotal sacs were removed, followed by the dissection out of both the testes with their epididymis. Once the tissues were separated, the testes with epididymis were weighed (as a pair; fresh testes weight), and their respective length, weight, and width (on both right and left sides) were documented, following the methodology described by Abu et al. (2016). After trimming of the epididymis, the testes were weighed again as a pair (trimmed testes weight), and each epididymis was weighed separately (and averaged; epididymis weight).

Further dissection was conducted on the intact testes, cutting each testis in half, a crossectionl slice was cut, and then two triangular tissue sections were taken for preservation for histological evaluation. Tissue samples from the mid-section of the right testes were taken and preserved in 10% buffered formaldehyde (25mL) in preparation for histological assessment (Bai et al. 2017) at the Veterinary University of Brno, Czech Republic. Subsequently, fixed tissue samples were processed, embedded in paraffin wax, sectioned at a thickness of 4μm, and stained with hematoxylin and eosin. Images of the stained tissue samples were captured using 1.72μm/px (1280 x 960 pixels; NIS Element Software and 10x magnification, NIKON Eclipse E600, Japan). Then, 100 seminiferous tubules per testis were measured for tubule circumference and epithelium thickness using

NIS-Element AR3.2 (NIKON Instruments Europe BV, Amsterdam, Netherlands) and averaged for the left and right testes per animal.

The remaining reproductive tract was also extracted from the pelvic area during processing at the abattoir according to the methodology of Consolacion et al. (2024). Subsequently, the accessory glands were separated from the penis, and dissection extended from the prepuce to the base of the penis's sigmoid flexure. Measurements of the prepuce, penis sheath, sigmoid flexure length, and Intracavernosal pressure measurement were taken (prepuce + penis + sigmoid flexure length+ICM), followed by weighing using a Kern and Sohn GmbH kb 2000–2 N scale (D-72336, Balingen, Germany). The lengths of the accessory glands were determined using a calliper, after which they were weighed as well (Consolacion et al. 2024). The averages of all bilateral glands were calculated for each measurement. The seminal vesicle glands were collected to determine the biochemical composition of their fluid (described further below).

3.3. Spermatozoa harvesting and quality assessments

The harvesting of spermatozoa was conducted by making an incision around the caudal epididymis, with precautions taken to prevent blood from penetrating around the epididymis. Approximately 1g of one cauda epididymis was removed and placed into a labelled microtube. Subsequently, 1mL of sperm media 37°C (Tyrode's albumin lactate pyruvate) was added to the 1g tissue, and maceration was performed using sterilized scissors. The microtube was then gently shaken and placed in a water bath maintained at a temperature between 35-37 °C for various assessments of sperm quality. These assessments included live sperm percentage and sperm morphometric assessment based on the methodology described by Garcia-Herreros et al. (2007), acrosome integrity evaluated according to Watson (1975), membrane functionality assessed using the hypo-osmotic swelling test as outlined by Soler et al. (2022), and sperm concentration determined following the methods detailed by Ny et al. (2020).

3.4. Biochemical sample collection and analyses

In this study, biochemical parameters were assessed from the seminal vesicle glands at the Veterinary University of Brno, Czech Republic. Following dissection, phosphate buffer solution was added to the dissected parts to collect the analyte after centrifugation, and samples were stored at approximately -18 °C for preservation. Total Protein, Creatinine, Urea, Cholesterol, Alkaline Phosphatase (ALP), Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Creatine Kinase (CK), Glutamate Methyltransferase (GMT), Lactate Dehydrogenase (LD), Calcium (Ca), Magnesium (Mg), Phosphorus (P), and Chloride (Cl) were analysed using diagnostic kits from DiaSys kits (Diagnostic Systems GmbH, Holzheim, Germany) and the photometric method via an automatic biochemical analyzer (Konelab 20XT, ThermoFisher Scientific, Waltham, MA, USA), utilizing commercial BioVendor kits (BioVendor – Laboratorní medicína a.s., Brno, Česká Republika).

Sodium (Na), Potassium (K), Copper (Cu), and Zinc (Zn) were measured using a Solaar AASeries Spectrometer (Thermo Scientific). SAbraham um (Se) levels were determined using the hydride technique (VP 100 Vapour System). Vitamin E levels were assessed using High-Performance Liquid Chromatography (HPLC) after ethanol deproteination and extraction into hexane. This method ensures the extraction of Vitamin E from the samples while removing proteins. A HPLC equipped with a UV-Visible detector separates and quantifies the Vitamin E compounds based on their absorption properties (Boxhammer et al. 2016).

3.5. Statistical Analysis

Data was analysed in Statistica 13 (StatSoft Inc.). The normality of the residuals was confirmed and the restricted maximum likelihood method was utilised to evaluate the treatment effects. In the case of the live data, fixed effects included the effect of diet and time whilst random effects included bull nested in diet. Initial body weight was used as a covariate. Data collected at slaughter included the fixed effect of diet and the random effect of bull nested in the diet. The body weight at slaughter and age at slaughter were used as covariates. In the case of significant interaction effects (diet x time) or main effect (diet), the means were compared using Fisher's Least Significant Difference post-hoc tests. All results are reported as the Least Square Mean (LSMean) ± standard error of the mean (SEM). A significance level of $P \leq 0.05$ was used throughout.

4. Results

The body weights of the cattle were not significantly different at the first body weight collection period (9.2.2023); however, at each subsequent data point they were significantly different (Figure 1). Since the second body weight collection period (23.2.2023), the cattle fed the control diet (rapeseed) had greater body weights than those fed the lupin diets (23.2.2023, P = 0.042; 9.3.2023, P = 0.004; 23.3.2023, P = 0.004; 6.4.2023, P = 0.006; 13.4.2023, P = 0.002). Furthermore, at the last body weight collection point before the slaughtering started (13.4.2023), the cattle fed white lupins had greater body weights than those fed yellow lupins. In all cases, the body weight increased over the study period for the treatments, except for between the last two data collection periods (i.e., from 6.4 to 13.4.2023), where the cattle fed yellow lupin meal maintained their average body weight.

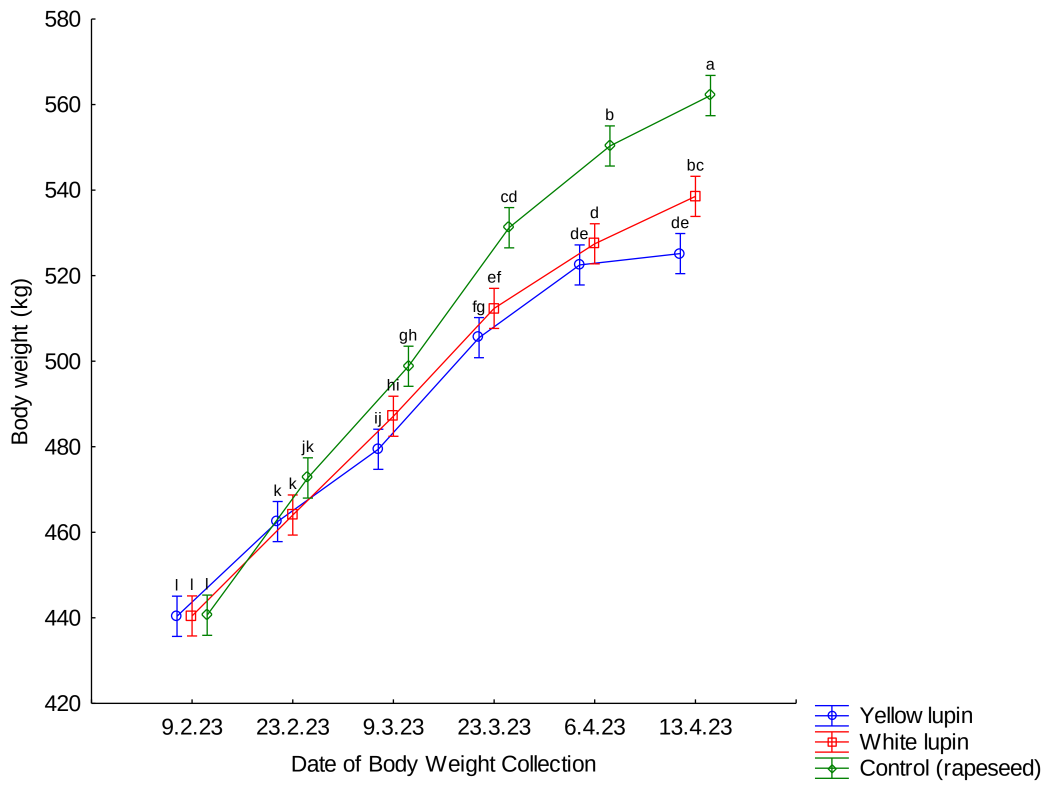

Figure 1. The effect of diet on the body weight of Fleckvieh bulls over the study period prior to the commencement of serial slaughtering. Vertical bars denote ± SEM. Time points are indicated by calendar dates in the year 2023 on which the bodyweight data was recorded.

In Table 2, the body weight at slaughter, average daily gain, average daily feed intake, feed conversion ratio, and feeding behaviour data from the start of the trial until the serial slaughtering took place (i.e., from 9.2.2023 to 13.4.2023) are presented. Significant differences were observed across the dietary groups. Specifically, cattle fed the control diet (rapeseed meal) had significantly higher body weights at slaughter compared to those on lupin diets (P = 0.0005). Additionally, the control group exhibited superior ADG and FCR compared to both lupin diet groups (P = 0.004 and P = 0.002, respectively). However, there were no significant differences in ADFI, visits to the feeder per day, or duration of time spent per visit among the three diet groups (P> 0.05). These results indicate that the type of protein source in the lupin diet significantly influenced the growth performance and feed efficiency of the Fleckvieh cattle, with the control diet yielding the best outcomes in terms of body weight gain and feed conversion efficiency (Table 2).

Table 2. The body weight at slaughter, average daily gain, average daily feed intake, feed conversion ratio and feeding behaviour data from the start of the trial until the serial slaughtering took place (i.e., from 9.2.2023 to 13.4.2023) for Fleckvieh cattle fed one of three diets with either yellow lupin meal, white lupin meal, or rapeseed meal (control) as the primary protein source.

Parameter	Yellow lupin	White lupin	Control	P-value
Body weight at slaughter	$558.3^{b} \pm 6.94$	$571.1^{b} \pm 11.17$	$604.8^{a} \pm 9.03$	**0.0005**
ADG (kg/d)	$1.3^{b} \pm 0.12$	$1.6^{b} \pm 0.11$	$1.9^{a} \pm 0.09$	**0.004**
ADFI (kg/d)	23.6 ± 0.61	21.9 ± 0.91	24.8 ± 0.68	0.058
FCR	$14.4^{a} \pm 1.57$	$12.0^{b} \pm 0.43$	$10.8^{c} \pm 0.22$	**0.002**
Visits to feeder/day	62.8 ± 7.91	59.4 ± 4.51	55.1 ± 5.90	0.727
Duration in sec/visit at feeder	97.8 ± 17.82	97.0 ± 11.17	109.27 ± 10.80	0.933

ADG: average daily gain; ADFI: average daily feed intake; FCR: feed conversion ratio

There were no significant differences observed in many reproductive parameters across the dietary groups (Table 3), except for minor differences in the weight of the prostate gland (P = 0.05) and live sperm percentage (P = 0.05). Catlle fed the lupin diets had lower prostate weights than those fed the control diet (Table 3). Cattle fed the white lupin diet also exhibited significantly higher percentages of live sperm compared to those fed the yellow lupin diet, while both did not differ significantly from the control diet (Table 3).

Table 3. The reproductive tract morphological measurements, testis histology, and sperm quality for Fleckvieh cattle fed one of three diets with either yellow lupin meal, white lupin meal, or rapeseed meal (control) as the primary protein source.

Parameter	**Yellow lupin**	**White lupin**	**Control**	**P-value**
Penis +prepuce+sigmoid flexure weight+ICM (kg)	1.21± 0.07	1.3 ± 0.04	1.3 ± 0.06	0.437
Penis +prepuce+sigmoid flexure length-ICM (cm)	90.4 ± 2.0	92.9 ± 1.47	92.0 ± 1.3	0.643
Gonadosomatic index (%)	0.13 ± 0.010	0.11 ± 0.007	0.12 ± 0.006	0.578
Fresh testes weight (g)	958.6 ± 56.67	970.8 ± 47.63	1024.2 ± 42.91	0.876
Trimmed testes weight (g)	693.5 ± 49.16	657.5 ± 49.26	713.8 ± 29.92	0.566
Testis length (mm)	122.7 ± 5.18	124.3 ± 3.46	131.7 ± 2.27	0.745
Testis width (mm)	72.2 ± 2.28	67.1 ± 2.05	70.1 ± 1.39	0.091
Epididymis weight (g)	38.7 ± 1.98	43.9 ± 7.12	42.4 ± 1.86	0.670
Epididymis length (cm)	21.1 ± 0.61	20.5 ± 0.7	21.4 ± 0.46	0.635
Seminal vesicle weight (g)	31.4 ± 2.2	32.7 ± 4.11	33.2 ± 0.96	0.892
Seminal vesicle length (mm)	95.3 ± 3.27	96.6 ± 3.08	98 ± 4.23	0.894
Cowper's weight (g)	8.3 ± 1.12	8.8 ± 0.77	9.6 ± 0.97	0.988
Prostate weight (g)	100.8^{b} ± 2.92	94.7^{b} ± 2.5	119^{a} ± 6.35	**0.050**
Prostate length (mm)	127.0 ± 3.27	121.2 ± 3.95	138 ± 2.83	0.095
Ampulla weight (g)	9.0 ± 0.50	8.9 ± 0.9	7.6 ± 0.54	0.235
Ampulla length (mm)	132.4 ± 8.12	139.1 ± 12.4	114.3 ± 10.91	0.210
Testis histology				
Seminiferous tubule circumference (um)	664.3 ± 18.39	670.6 ± 11.12	704.1 ± 22.86	0.132
Seminiferous tubule thickness(um)	50.1± 2.16	48.7 ± 2.50	54.4 ± 2.00	0.442
Sperm quality				
Sperm concentration	$2.42 \times 10^{8} \pm 3.14 \times 10^{7}$	$2.48 \times 10^{8} \pm 3.14 \times 10^{7}$	$2.51 \times 10^{8} \pm 3.14 \times 10^{7}$	0.978
Live sperm %	68.7^{b} ± 7.26	88.3^{a} ± 2.63	84.4^{ab} ± 1.54	**0.050**
Swollen membrane (%)	93 ± 1.43	87.3 ± 2.46	90.8 ± 2.49	0.233

ICM: Intracavernosal pressure measurement

Some significant differences were observed in seminal vesicle fluid ALP, Na, and Cu concentrations among the diet groups (Table 4). Cattle fed the white lupin meal had greater concentrations of ALP (P = 0.049) and Cu (P = 0.032) than the other dietary groups. Additionally, cattle from both lupin-based diets had greater Na concentrations in the seminal vesicle fluid than the control group (P = 0.008).

Table 4. Biochemical parameters of the fluid from the (seminal vesicle gland) for Fleckvieh cattle fed one of three diets with either yellow lupin meal, white lupin meal, or rapeseed meal (control) as the primary protein source.

Parameter	Yellow lupin	White lupin	Control	P-value
Total Protein (g/l)	68.2 ± 1.85	72.7 ± 0.45	69.6 ± 8.86	0.685
Creatine (mmol/l)	244.4 ± 62.7	372.1 ± 170.3	180.6 ± 19.65	0.489
Urea (mmol/l)	13.5 ± 3.96	14.5 ± 3.46	8.3 ± 2.14	0.422
ALP (µkat/l)	86.3^{b} ± 73.7	151.5^{a} ± 0.50	112.8^{b} ± 20.49	**0.049**
ALT (µkat/l)	8.4 ± 1.45	10.4 ± 0.25	9.0 ± 0.80	0.604
AST (µkat/l)	350.5 ± 73.50	365 ± 18.00	293.3 ± 42.84	0.187
CK (µkat/l)	641.5 ± 190.50	285.5 ± 6.50	430.3 ± 140.89	0.255
GMT(µkat/l)	156 ± 17.00	186.5 ± 10.50	177 ± 18.04	0.232
LD(µkat/l)	103.3 ± 21.03	106.4 ± 6.29	133.9 ± 13.41	0.775
Na (mmol/l)	58.1^{a} ± 3.79	52.4^{a} ± 1.25	46.6^{b} ± 1.42	**0.008**
K (mmol/l)	51.1 ± 7.63	59.6 ± 4.93	49.4 ± 8.17	0.636
Ca (mmol/l)	2.9 ± 0.15	4.1 ± 0.40	3.2 ± 0.45	0.156
P (mmol/l)	21 ± 0.05	18.6 ± 0.51	22.1 ± 2.03	0.731
Cl (mmol/l)	119.3 ± 3.85	107.3 ± 1.20	136.8 ± 12.10	0.537
Cu (µg/l)	9.7^{b} ± 3.66	15.7^{a} ± 2.41	7.3^{b} ± 0.52	**0.032**
Mg (µg/l)	5.4 ± 0.20	5.3 ± 0.27	5.1 ± 0.30	0.096
Chol (mmol/l)	5.3 ± 0.70	4.9 ± 0.05	6.2 ± 1.33	0.987
Vit E (mmol/l)	7.2 ± 2.12	6.9 ± 2.64	10.2 ± 0.30	0.760
SeSP(µg/l)	226 ± 66.82	478.5 ± 67.23	338.7 ± 55.81	0.386
ZnSP(µg/l)	113.2 ± 14.87	133.1 ± 16.91	85 ± 35.90	0.447

ALP: Alkaline Phosphatase; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; Ca: Calcium; Chol: Cholesterol; CK: Creatine Kinase; Cl: Chloride; Cu: Copper; GMT Glutamate Methyltransferase; LD: Lactate Dehydrogenase; Mg: Magnesium; Na: Sodium; K: Potassium; P: Phosphorus; Se: SAbraham um; SP: Serum Protein Vit E:Vitamin E; Zn: Zinc

5. Discussion

Lupinus species, including *Lupinus albus* (white lupin), and *Lupinus luteus* (yellow lupin), offer advantages such as high protein content, adaptability to diverse environments, and soil-enriching properties (Bolland & Brennan 2008; Sedláková et al. 2016). These legumes present a promising solution to address the increasing demand for locally available sustainable protein sources in animal feed systems, offering benefits in terms of profitability, nutritional quality, and soil health (Sedláková et al. 2016).

In terms of growth performance, the study reveals a significant interaction between diet and time, with Fleckvieh bulls on the control diet demonstrating superior growth performance compared to lupin-supplemented diets over the evaluated period (Table 2). Furthermore, there were differences in the growth performance between the cattle fed the lupin-based diets, with those fed yellow lupin meal having poorer FCRs and final bodyweights. This observation emphasises the crucial role of dietary composition in influencing the physiological responses and growth trajectories of cattle (Arfaoui et al. 2021). The disparities in growth performance between the control and lupin-supplemented diets can be attributed to various factors inherent to the nutritional profiles of the respective feed sources. The control diet, primarily composed of rapeseed meal, is well-known for its high protein content and balanced amino acid profile, which are essential for supporting optimal growth and muscle development in cattle (Arfaoui et al. 2021; Suchý et al. 2006; Stanek et al. 2006). On the other hand, while lupin varieties such as *Lupinus albus* and *Lupinus luteus* offer substantial protein content, their amino acid compositions may not perfectly align with the nutritional requirements of Fleckvieh bulls, resulting in suboptimal growth outcomes (Arfaoui et al. 2021; Suchý et al. 2006; Stanek et al. 2006).

Furthermore, the observed differences in growth performance between the control and lupin-supplemented diets may also arise from variations in feed palatability and digestibility (Arfaoui et al. 2021). The observed variations in taste between diets containing rapeseed meals and lupin may not be the only factor influencing feed consumption in Fleckvieh bulls. Despite no significant differences in feed consumption data, the lupin diets exhibited a poorer FCR, indicating potential concerns regarding digestion efficiency, nutrient balance, and the presence of ANFs. Although the diets were formulated to fulfill nutrient requirements, variations in digestibility could impact the actual nutrients provided to the animals. Carrying out a digestibility trial would yield valuable insights into the digestibility of nutrients in each diet and help clarify the underlying reasons behind the observed disparities in growth performance. Additionally, the presence of anti-nutritional factors in lupin seeds, such as alkaloids and lectins, might have impaired nutrient absorption and utilisation in the cattle's gastrointestinal tract, thereby impacting their growth performance (Arfaoui et al. 2021; Reinhard et al. 2006). These findings underscore the importance of considering not only the nutritional composition but also the digestibility of alternative protein sources like lupins when formulating diets for cattle to optimise growth and performance outcomes.

El Otman et al. (2013) focused on *Lupinus angustifolius*, Stanek et al. (2015) also used *Lupinus angustifolius*, and Um et al. (2024) utilized *Lupinus albus* and *Lupinus luteus* in their investigations. Although different lupin species were used in these trials, the findings regarding growth performance remain significant. For instance, El Otman et al. (2013) reported similar growth performance in kids fed Lupinus angustifolius, indicating its suitability as a feed ingredient. Similarly, Stanek et al. (2015) found consistent growth outcomes in lambs fed different varieties of Lupinus angustifolius. However, Um et al. (2024) observed variations in growth performance between *Lupinus albus* and *Lupinus luteus* diets, suggesting the importance of considering specific lupin species in diet formulations. In Um et al.'s study, Fleckvieh bulls fed *Lupinus albus* exhibited superior growth performance compared to those on *Lupinus luteus*, indicating differential effects of lupin species on growth. The variations observed across the studies can be attributed to several factors, including differences in lupin species, diet composition, and processing methods. Lupin species vary in their composition of ANFs such as alkaloids, lectins, and tannins, which can have an impact on animal health and performance (Reinhard et al. 2006). For example, *Lupinus albus* is known to contain alkaloids, while *Lupinus luteus* may contain lectins. The concentration of these ANFs can differ not only between lupin species but also within varieties of the same species, as observed in Stanek et al. (2015) where different *Lupinus angustifolius* varieties were used. The discrepancies witnessed in the composition of (ANFs) across various lupin species may offer valuable insights into the contrasting growth performance outcomes documented in this study and other relevant research. More specifically, disparities in the concentrations of targeted suspected ANFs, such as alkaloids, lectins, and tannins, could potentially influence the variations observed in animal reactions to lupin-derived diets. Consequently, the quantification and acknowledgment of these ANF contents in forthcoming investigations are imperative for comprehending their implications on growth performance and enhancing dietary formulations to achieve optimal animal well-being and productivity.

Furthermore, differences in diet composition and processing methods can further affect the availability and impact of ANFs in lupin-based diets. Processing techniques such as flaking or ensiling may reduce ANF levels, thus mitigating their negative effects on growth performance (Gefrom et al. 2013). Additionally, variations in animal species and physiological differences among cattle breeds may contribute to different responses to lupin supplementation (El Otman et al. 2013). Therefore, despite the use of different meals in these trials, the findings highlight the importance of considering how lupin species, feed mix, processing techniques, and animal physiology interact to determine the outcomes of growth performance. Further research is needed to fully comprehend these complex relationships and customise lupin-based diets for various livestock species and production methods, encompassing prepatation methods (and their effects on ANFs), inclusion rates, and digestibility as important next steps.

Despite the lack of significant alterations in feeding behaviour across dietary groups in the present study, differences in palatability and feed intake between lupin-supplemented diets and the control diet might have been initially expected. Lupin-based diets, particularly those containing *Lupinus albus* and *Lupinus luteus*, have the potential to exhibit different palatability profiles compared to the control diet containing rapeseed. Lupins are known for their bitter taste due to the presence of alkaloids and other ANFs, which might be perceived differently by the animals and thus affect their acceptance and consumption levels (Reinhard et al. 2006). *Lupinus albus*, in particular, is notorious for its bitterness, which could deter animals from consuming it in large quantities (Olkowski et al. 2001). Additionally, differences in the processing methods of lupin meals, such as grinding or pelleting, could influence their palatability and subsequent intake. For example, finely ground lupin meals may have a more pronounced bitter taste compared to coarser meals, affecting their acceptance by the animals (Olkowski et al., 2001).. Moreover, variations in the inclusion levels of lupin meals in the diets could impact overall diet palatability. High levels of lupin inclusion might intensify the bitterness, potentially reducing palatability and feed intake. Conversely, lower inclusion levels might minimize the bitter taste, thereby enhancing palatability and acceptance by the animals (Olkowski et al. 2001). The lack of significant differences in feeding behaviour across dietary groups could also be attributed to the adaptability of the animals to varying taste profiles. While lupin-based diets may have initially presented novel flavours to the animals, their adaptation period to these diets might have led to habituation, thereby diminishing any initial aversion or preference.

The physiological effects of lupin supplementation on reproductive development in cattle are complex and influenced by the presence of various antinutrient factors inherent in lupins. Lupins, such as *Lupinus albus* and *Lupinus luteus*, contain compounds such as alkaloids, phytic acid, and protease inhibitors, which can elicit diverse responses in animals (Reinhard et al. 2006). Of particular concern are alkaloids, specifically quinolizidine alkaloids found in Lupinus species, which have been linked to reproductive toxicity in livestock (Olkowski et al. 2001). These alkaloids have the potential to disrupt hormone signaling pathways, leading to adverse effects on reproductive organ development and function. In addition to alkaloids, lupins also contain secondary metabolites such as flavonoids, saponins, and tannins, which possess various biological activities (Borreani et al. 2009). For example, flavonoids have antioxidant properties and can modulate inflammatory responses, potentially influencing reproductive processes in animals. Saponins and tannins, on the other hand, may have hormone-mimicking effects or interfere with nutrient absorption, further complicating their impact on reproductive health (Borreani et al. 2009).

The observed differences in prostate weight and sperm quality in this study may be influenced by several factors associated with lupin supplementation. Prostate weight is closely connected to hormonal regulation, especially androgens like testosterone, which play pivotal roles in reproductive physiology (Olkowski et al., 2001). Changes in hormonal balance, possibly induced by phytoestrogens present in lupin supplementation, could affect prostate development and size. Additionally, alterations in seminal vesicle fluid composition, as indicated by variations in ALP, Na, and Cu concentrations among the diet groups, may also contribute to differences in reproductive parameters (Table 4). Cattle fed the white lupin meal exhibited higher ALP and Cu concentrations, while both lupin-based diets showed elevated Na levels compared to the control group. These changes in seminal vesicle fluid content could potentially influence sperm quality and reproductive function, highlighting the intricate interplay between diet composition and reproductive physiology in cattle.

Lupinus albus contain phytoestrogens, which are compounds derived from plants that can affect hormonal signaling pathways (El Otman et al. 2013). Therefore, including *Lupinus albus* in the diet may have changed the hormonal environment in the animals, resulting in varying effects on prostate development and size. Likewise, the differences observed in sperm quality could be attributed to the unique nutritional composition of lupin meals, particularly their protein content and amino acid profile (Arfaoui et al. 2021). These nutrients play crucial roles in supporting sperm production and function. On the otherhand, lupins contain antioxidants such as polyphenols and flavonoids, which can reduce oxidative stress and safeguard sperm from damage (Stanek et al. 2020). The availability of these nutrients and bioactive compounds in lupin meals may differ from other protein sources, potentially impacting sperm quality through their influence on metabolic pathways and cellular processes. However, these differences are boarding on significance, and further consideration of the effects of these lupin species on these reproductive parameters, especially under extended feeding periods longer than used in the present study, as would be expected in breeding animals.

While studies have highlighted the reproductive toxicity of lupin alkaloids in species such as goats and sheep (Borreani et al. 2009), their effects in cattle are still not well understood. However, it is plausible that these compounds, along with other antinutrient factors present in lupins, could have similar effects on reproductive development in cattle. Therefore, further research is needed to clarify the specific mechanisms underlying the influence of lupin supplementation on reproductive physiology in cattle and to develop strategies for mitigating potential adverse effects in livestock production systems. Different Lupinus species may exhibit varying profiles of antinutrient factors and secondary metabolites, leading to divergent impacts on reproductive parameters. For example *Lupinus albus* contain different levels or types of alkaloids compared to *Lupinus luteus*, resulting in differential effects on prostate size and sperm quality.

6. Conclusions and recommendations

This study aimed to examine the potential for two lupin species (*Lupinus albus* and *Lupinus luteus*) to replace traditional protein sources in the diets of cattle. Specifically, the effects of supplementing these lupin meals on the growth performance and reproductive parameters of Fleckvieh bulls were considered, to address the limited available literature on supplementing these sources to cattle, particularly growing bulls. The findings revealed that complete substitution of dietary protein sources by *Lupinus albus* and *Lupinus luteus* meal resulted in inferior growth performance compared to the control diet, which consisted of rapeseed meal. Moreover, differences in prostate weight, sperm quality, and seminal vesicle fluid composition were noted, indicating potential implications of lupin supplementation on reproductive functioning and health in cattle. Whilst some differences are minor, they warrant further investigation under extended feeding periods.

Moving forward, it is recommended to conduct further research to elucidate the underlying mechanisms behind the observed effects of lupin supplementation on cattle physiology. It is crucial to explore the optimal inclusion levels of lupin meals in cattle diets and develop strategies to mitigate the potential adverse effects of antinutrient factors Specifically, comprehensive studies should investigate the specific impact of these lupin species, their inclusion levels, different dietary compositions, and processing techniques on growth performance and reproductive health in cattle. These efforts are necessary for refining dietary recommendations to efficiently and safely maximising potential the benefits of using these lupin supplementations in cattle production. Collaborative endeavors that involve multidisciplinary approaches, including nutritional science, animal physiology, and feed technology, will play a pivotal role in advancing our understanding and application of lupin-based diets to promote sustainable and efficient livestock production practices.

7. References

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