CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

Faculty of Tropical AgriSciences

The Influence of Lysine and Methionine on Fattening Parameters and Blood Protein Metabolites of Fallow deer (*Dama dama*)

Master's thesis

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CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

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DIPLOMA THESIS ASSIGNMENT

Lic. Mechie Ann Clar, BSc

Animal and Food Sciences in Tropics and Subtropics

Thesis title

The influence of Lysine and Methionine on fattening parameters and blood protein metabolites of fallow deer (Dama dama)

Objectives of thesis

To assess the effect of amino acids in young fallow deer farmed for meat production. Effects will be assessed from two points of view: productive (carcass traits; growth; body condition) and physiological (blood biochemistry of traits related to protein metabolism).

Methodology

Supplementation of amino acids is a topic of great interest in production of monogastrics, but a quite new research area for ruminants. It has been traditionally assumed that is not necessary to supplement amino acids in ruminants, but recent research have shown that Methionine and Lysine are limiting essential amino acids for them (Kung and Rode, 1996; Merchen and Titgemeyer, 1992; Schwab, 1996). Lysine seems especially interesting for growing ruminants because it is a main component of collagen, precursor of bone tissue; for the same reason, it is also especially important for cervids because of the production of antlers. This is a novel research area, since the only three SCI manuscripts on amino acids in deer nutrition just appeared in the last years.

An experiment of amino acid supplementation will be performed in a herd of farmed fallow yearlings, keeping a control group during the whole experiment. The study will be conducted in a farm located in Mnich, South Bohemia, Czech Republic. Sixty fallow yearlings will be randomly divided into three groups according to its diet: conventional feed regime (pasture+grain) as control group, a second group with supplementation of Lysine and methionine, and a third group only with Lysine.

Supplementation will start in middle summer, and will continue until their culling in autumn and winter. Weight and body condition of the calves will be measured in different moments along the experiment. Blood samples will be collected at the start of the experiment and at culling. Blood biochemistry will be analyzed at the CULS labs for the determination of blood protein metabolites: Total Protein, Albumin, Creatinine, Urea, and Globulins. Carcass traits and post-mortem body condition indexes will be collected during

or after culling. It is expected that supplemented animals will show a better performance in the studied variables.

This study is complementary to previous studies on the topic recently done by the supervisors.

Budget for the experiments and feed supplementation is guaranteed. Budget for blood analyses will be covered by the internal grants of the Faculty.

The timeline of activities will be as follows: Literature review- January to June 2016; Initial data gathering and start of supplementation- June 2016; Data gathering and culling- November 2016 to January 2017; Laboratory Work- January 2017; Data analysis, interpretation, and thesis writing- February to April 2017; Thesis submission- April 2017; Thesis defence- June 2017.

The proposed extent of the thesis

50 pages

Keywords

Body Condition Score; Kidney Fat Index; Total Protein; Albumin; Creatinine; Urea; Globulins; Carcass characteristics.

Recommended information sources

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Expected date of thesis defence

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Prague on 24. 04. 2017

Authorship Declaration:

I hereby declare that this thesis titled "**The Influence of Lysine and Methionine on Fattening Parameters and Blood Protein Metabolites of Fallow deer (*Dama dama*)**" is my own work and all the sources have been quoted and acknowledged by means of complete references.

In Prague on 27th April 2017.

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Signature

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Abstract

This study was conducted to investigate the effects of lysine and methionine supplementation on growth performance, body condition, carcass traits, and blood plasma metabolites of fallow deer during the fattening period, and to determine the influence of two culling seasons on these parameters. Forty five male fallow deer (40.2 ± 2.7 kg) were allocated into three groups with 15 animals each. The deer were pasture-fed and supplemented with barley, mineral premix, silage during winter period, and varying levels of ruminally-protected lysine and methionine: no amino acids (control), 9 g/day of lysine (Lys), and 9 g/day of lysine plus 3 g/day of methionine (Lys+Met). Animals were culled in two separate seasons, late autumn (LA; 6 animals per group), and second group on late winter (LW; 9 animals per group). Average daily gain (ADG) and weight gain showed no significant differences among treatments. However, significant decrease of ADG was observed on LW ($p=0.002$). Body condition score ($p=0.024$), kidney fat index ($p=0.005$), kidney fat ($p=0.001$), and percentage of internal fat ($p<0.001$) increased significantly with Lys+Met supplementation. During LW, kidney fat index ($p=0.004$) and kidney fat ($p=0.001$) were also significantly higher than in LA. Lys+Met supplementation increased ($p=0.002$) dressing percentage, and during LW ($p<0.001$) significant increase was observed. Carcass weight, bone proportion, and blood weight were affected by season of culling ($p<0.05$) but not by treatment. In deer that received Lys and Lys+Met supplementation, creatinine, blood urea nitrogen, and triglycerides concentration were elevated ($p<0.001$). Meanwhile, the increased concentration of creatinine were associated ($r=0.450$; $p=0.019$) with the BCS. In conclusion, supplementation of lysine and methionine to the diets may improve the body condition and metabolism of fallow deer, and the season of culling may influence on the production and physiology of the animals.

Keywords: Amino acids; Body condition; Creatinine; Urea; Carcass traits

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List of Abbreviations Used

ADG	average daily gain
ALB	albumin
BCS	body condition score
BUN	blood urea nitrogen
CREA	creatinine
GLB	globulin
GLMM	Generalized Linear Mixed Model
H	hypothesis
KFI	kidney fat index
LA	late autumn
Lys	lysine
Lys + Met	lysine plus methionine
LW	late winter
NRC	National Research Council
RPAA	ruminally-protected amino acids
TP	total protein
TRIG	triglycerides

1. Introduction

Fallow deer is one of the most common deer species farmed in Europe for meat purposes and significantly contributes to the meat industry. Nowadays, noticeable increases of demands for venison are reported in many parts of the world. With these increasing demands, nutrition of deer is one of the aspects that a deer farmer should pay attention because nutrition plays a great role on animal productivity, reproductive performance and health.

Feeding protein is indispensable for animal growth, reproduction, lactation and maintenance (Kung and Rode, 1996). For non-ruminant, protein and amino acid requirements are established and well defined; however in ruminants, NRC (2007) established the protein requirements, while amino acid requirements are not establish yet. Defining the quality and quantity of amino acid absorbed in ruminant digestion is quite difficult for many reasons, since proteins and amino acids are subjected to microbial degradation in the rumen (Merchen and Titgemeyer, 1992).

Nevertheless, lysine and methionine are recognized as the first limiting amino acids in ruminants (Kung and Rode, 1996; Merchen and Titgemeyer, 1992; Schwab 1996), as it has been observed on several studies on growing Holstein steer calves (Oke et al., 1986), lambs (Storm and Øskov, 1984; Oke et al., 1986), and multiparous Holstein cows (Polan et al., 1991). Lysine is important in protein synthesis such as milk protein, growth, pregnancy, and maintenance (Ordway and Aines, 2010) and methionine is required for growth and development in young ruminants (NRC, 2007). Supplementation of lysine and methionine is well known practice in monogastric animals, however very little information exists in ruminants particularly on deer (Mendoza-Nazar et al., 2012; Huang et al., 2015a, b). Thus, this study was conducted to investigate the effects of lysine and methionine supplementation on growth performance, body condition, carcass traits, and blood plasma metabolites of fallow deer during the fattening period and to determine the possible influence of culling seasons on the fattening parameters and physiology of fallow deer.

2. Literature Review

2.1. Deer farming

Deer farming is a common practice worldwide, in recent times many studies prove the considerable increased of farmed deer population. According to Daszkiewicz et al. (2015) the current global statistics of farmed deer is approximately five million by which New Zealand accounted for over half of the global farmed population. In United States, the total number of deer farms is more than 7,800; with Texas and Pennsylvania have the most numbered farms (Frosch et al., 2008). While Europe has estimated around 410,000 European farmed deer population (Chardonnet et al., 2002). Great Britain, Germany, and Sweden are among the countries having the largest number of farmed deer (Chardonnet et al., 2002; Volpelli, 2002). Czech Republic has around 200 farms carrying between 5,000 and 8,000 deer (Chardonnet et al., 2002). Deer is farmed for variety of purposes such as venison for international market, antler for trophy, and velvet antler for the oriental medicine (Drew, 1992).

Table 1. Chemical composition of venison meat from red deer and fallow deer.

Composition	Farmed fallow deer[a]	Wild fallow deer[b]	Red deer[c]
Dry matter (%)	25.67	25.71	29.20
Total protein (%)	22.46	22.79	24.70
Fat (%)	0.24	0.50	3.30
Ash (%)	1.09	1.10	1.40
Energy (kJ)	385.76	401.07	545
Water/Protein	3.31	3.26	2.87

[a] Daszkiewicz et al., 2009; [b] Daszkiewicz et al., 2015; [c] Drew and Semen, 1987 as cited by Drew, 1992

Fallow deer is one of the most common cervid species farmed in Europe for meat purposes (Hoffman and Wiklund, 2006). It significantly contributes to the meat industry around Europe because of its quality and composition (Table 1). Venison is characterized as a low-fat and low cholesterol meat, very high in protein and iron content, and has good meat quality characteristics such as tenderness, juiciness, taste, and aroma (Drew, 1992; Daszkiewicz et al., 2009; Daszkiewicz et al., 2015). The good

quality carcass of low fat content can be produced from young deer at 15-16 months of age, since fallow deer species mature rapidly and it can be a slaughtered as yearlings (FAO, 1982).

2.2. Fallow deer basic information

Fallow deer are described as among of the world's most attractive deer (Chapman and Chapman, 1975). It is classified as suborder Ruminantia of the order Artiodactyla and family Cervidae (Masters and Murray, 2015). Generally, fallow deer belong to the subfamily of Eurasian deer (Chapman and Chapman, 1975) and comprises of two species, the European or common fallow deer (*Dama dama;* Linnaeus, 1758) and Persian or Mesopotamian fallow deer (*Dama mesopotamica;* Brooke, 1875) (Mittermeier and Wilson, 2011). It originated in the eastern Mediterranean region of southern Europe and Iran, and probably in some parts of North Africa. Common fallow deer was introduced by Romans to mainland Europe and Great Britain (Mittermeier and Wilson, 2011).

Figure 1. Persian fallow deer buck (Photo: Eyal Bartov).

Figure 2. European fallow deer buck (Photo: Manfred Danegger).

The Persian and European fallow deer can be identified by their phenotypic characteristics such size, antler, and tail color. Persian fallow is larger in size than European fallow deer. Both species have palmate type antlers. However, Persian buck has antlers with palms near to its base; in contrast palms are found at the top of the antler of European fallow (Figures 1 and 2). European fallow deer varies from many

colors than Persian fallow. The four main colors commonly found in the European fallow deer are common, menil, white, and black (Chapman and Chapman, 1975).

The fallow deer tail is about 28 cm. Adult females (does) range from 35 kg to 55 kg, while males (bucks) older than 2 years, range from 53 kg to 90 kg, some bucks are over 110 kg. A typical height at the shoulder for a buck is 90 cm, with does about 10 cm shorter. The sizes and weights of fallow deer show considerable variation, based on the age, sex, nutrition, and habitat conditions (Langbein and Chapman, 2003).

Klinkenberg (2014) described the antler of adult male fallow deer as erected and strongly palmate on the upper half, and the main beam is round in cross-section for the first half of its length. However, Langbein and Chapman (2003) defined palm as broad, flattened surface on the distal end of the antler. Full palmation usually occurs in 7 years old bucks or older, while in male fawns, pedicles begin to grow in about 6 or 7 months old. Female fallow deer do not possess antlers.

2.3. Feeding behavior and natural habitat

Fallow deer is highly adaptable species that can survive in a wide range of habitats, from forest, shrub land, grassland, to pastureland and plantations (Masseti and Mertanidou, 2008). They are intermediate feeders and preferential grazers that feed on grass, ground vegetation, forage agricultural crops and pastures (Mittermeier and Wilson, 2011). In some areas where fallow deer has been introduced, populations of this species occur most in woodland. Their habitat utilization changes depend on the seasons, as the availability and nutritional value of different forages varies (Langbein and Chapman, 2003).

Fallow deer is a diurnal animal. They are active all day for feeding, ruminating, resting, and moving (Mittermeier and Wilson, 2011). Feeding activity of fallow deer usually alternates with periods of rumination at around three to four hours interval throughout the day and night. Longer period of time is spent in feeding activities during summer (from June to August); this is recognized as peak of intake during the year. While feed intake is reduced on winter commonly termed as winter inappentance. This is

characterized by reduction of feed intake, lack of any significant growth or weight gain over the winter, and is usually exhibited even if food is made available *ad libitum* (Langbein and Chapman, 2003).

In captive or farmed condition, supplementing pasture grazing is important to meet the target weights, keeping the breeding bucks and does in good condition for rut, pregnancy, and lactation. Supplementary feeding is also common during winter such as hay, silage plus additional concentrates with maximum of 14% crude protein (Masters and Murray, 2015). High water requirements and body water intake is higher during summer seasons. It was observed in the study of Alamer (2011) that water intake increases during summer and decreases on winter. This condition tended the animals to have lower blood volume during winter season.

On the other hand, photoperiod affects voluntary feed intake on deer. In the study of Scott et al. (2013) on fallow deer it was observed that voluntary feed intake is affected by seasonal change and photoperiod. Average daily feed intake started to decrease from autumn to winter and increased was observed on the onset of spring (Figure 3).

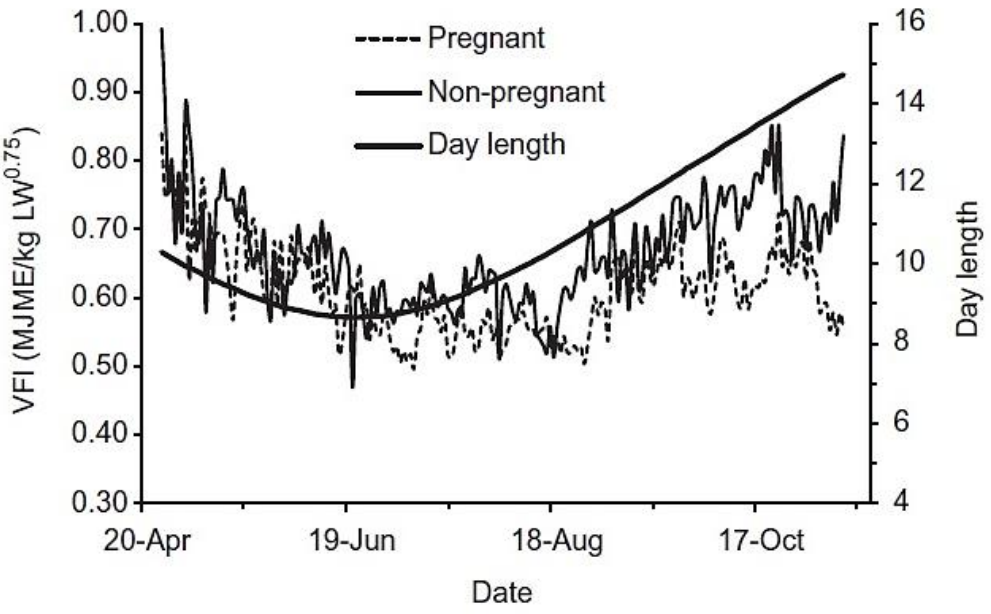


Figure 3. Predicted mean daily voluntary food intake of pregnant and non- pregnant hinds relative to day length (Scott et al., 2013).

2.4. Morphology and digestive physiology

All deer are herbivores yet classified according to their morphological feeding type: concentrate selector (CS), intermediate or mixed (IM) and grass/roughage eater (GR). Concentrate selectors are animals that prefer diet sources that are highly digestible, high in starch, proteins and lipids. These are the animals that predominantly feed on woody and non-woody dicotyledonous plants [*e.g.* moose (*Alces alces*), roe deer (*Capreolus capreolus*), and *Muntiacus* spp]. However, GR types of species are those feeding on plant material that is high in fiber. These species are characterized by highly developed fermentation system that allows cellulosic plant materials to be digested. These are the species which feed on graminaceuos plants [*e.g.* sheep (*Ovis aries*), African buffalo (*Syncerus cafer*), and cattle]. The IM types of species consume mixed diets and forages based on season and opportunity (Hofmann and Stewart, 1972; Hofmann, 1989). Most of the intermediate types are opportunistic feeders [*e.g.* red deer (*Cervus elaphus*), fallow deer (*Dama dama*)]. Deer are highly adaptable to climatic conditions: species in seasonal climates build up body fat during spring and summer in preparation for reproductive activity, and for winter when the food is scarce and nutrient quality is low (Masters and Murray, 2015).

Ingestion of feedstuffs varies among ruminants and is influenced by different anatomical structures. In the case of GR species, they have short lips and wide, flat mouth that maximizes the intake of grasses and sedges, while CS species have narrow but deep mouth openings that facilitate the intake of feed materials like twigs and fruits. The size of reticulum-rumen varies according to their feeding type and in response to season and diet. The GR species have larger reticulum-rumen per unit of body weight than CS species. While IM species like fallow deer have relative size of reticulum-rumen adaptive to the season: it is larger during summer when feed resources are abundant and smaller during winter season, when forage is limited (Hofmann, 1989). On the other hand, factors such as season and diet may also affect the rumen microbial population. Microbial population is reduced if food intake is low (*e.g.* winter) and growth rate of microbes in the rumen improves when food intake is high (*e.g.* spring and summer) or when there is an increasing level of forage in the diet (Gillespie and Flanders, 2009).

Omasum size and number of lamina in IM are higher and larger than CS species while largest in GR species. The abomasum or the true stomach of IM and CS species have thicker wall and profuse parietal gland secretions, which is the reason why these species have relatively larger abomasum size than that of GR species. Due to its morphology, it is presumed that CS and IM species have higher production of pepsin-HCl, and hence relatively more dependent on post reticulum-rumen digestion (Hofmann, 1989).

Like in non-ruminants, digestion is carried on abomasum with the same process (Gillespie and Flanders, 2009). The end products of digestion, such as amino acids, fatty acids, and sugars, are absorbed from the small intestine and enter the blood or lymph for distribution and metabolism (Hofmann, 1989).

2.5. Nutrient utilization

Only energy and protein metabolism are described in this section. Other nutrients are not relevant to the study.

2.5.1. Energy

Similar to any other ruminant, deer species like fallow deer, red deer, and roe deer acquire nutrient primarily from microbial fermentation in the rumen and not directly from the ingested feed. Ruminal digestion and metabolism produces products such as carbon dioxide, methane, and volatile fatty acids, mainly acetate, propionate, and butyrate (Gillespie and Flanders, 2009).

The main source of dietary energy in ruminant diets is carbohydrates. This primary nutrient is found in diverse group of compounds from monosaccharides to polysaccharides. Polysaccharides are the main source of energy in most ruminant diets.

In ruminants, carbohydrates are known to provide energy both to the rumen microbial populations and to the host animal. Provision of carbohydrates through fibrous materials like forages also maintains stability of gastro-intestinal tract environment. The structural polysaccharides (*e.g.* cellulose, hemicellulose) add bulk factor to the diets. Non-

structural polysaccharides (*e.g.* starch) escape ruminal fermentation and by enzymatic hydrolysis in the small intestine, this supply energy to the host animal (NRC, 2007).

Regardless of its structure, carbohydrates are always degraded to its basic units, and fermented to volatile fatty acids in the rumen (Annison and Bryden, 1998). Approximately, 75-80% of the intake of starch is digested in the rumen, 35-60% is digested through enzymatic hydrolysis in the small intestine, and about 35-50% of starch is digested in the hindgut fermentation (Harmon et al., 2004).

Dietary lipids also provide a concentrated source of energy. Lipids from feeds are altered in the rumen before they are available for metabolism (NRC, 2007). The ruminal transformation of lipids by microbial lipolytic enzymes release glycerol and free fatty acids. Ruminal microorganisms act on glycerol and may be then metabolized to produce volatile fatty acids (Jenkins, 1993), see Figure 4.

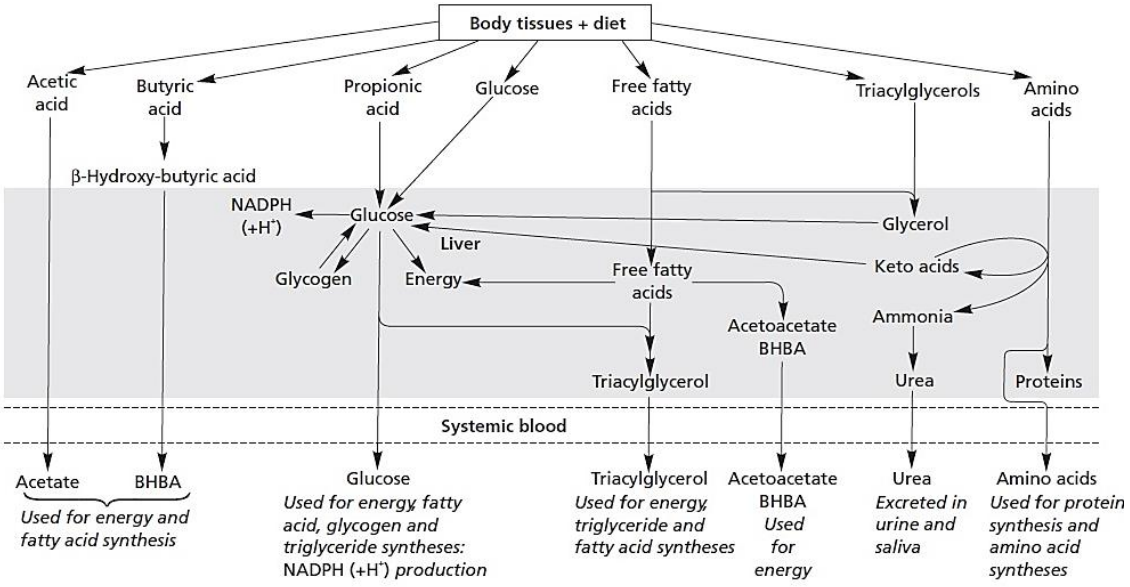


Figure 4. Sources and fates of major body metabolites (McDonald et al., 2011).

2.5.2. Protein

Protein is the largest fraction of nitrogen in the animal body. Crude protein is the basis for structure and function of animals: from contractile functions in muscle fibers, structural functions in connective tissue, up to the specialized functions like production

of hormones, enzymes, antibodies, etc. Protein is also used for energy during production of milk, meat and eggs, during migration and hibernation, and when protein or amino acids are in excess or when other energy sources are deficient (Barboza et al., 2009; Mcdonald et al., 2011).

Protein and nitrogen metabolism in ruminants is a complex process. NRC (2007) published that the primary role of protein in ruminant diets is to maximize the functions of both microbial protein and dietary protein that escape ruminal degradation to the supply of amino acids available for absorption. Recently, metabolizable protein system is identified as the most appropriate method of defining protein requirements for ruminants. This is defined as the true protein derived from dietary and microbial protein combination, which is digested postruminally and absorbed from the intestine in amino acids form (NRC, 2007).

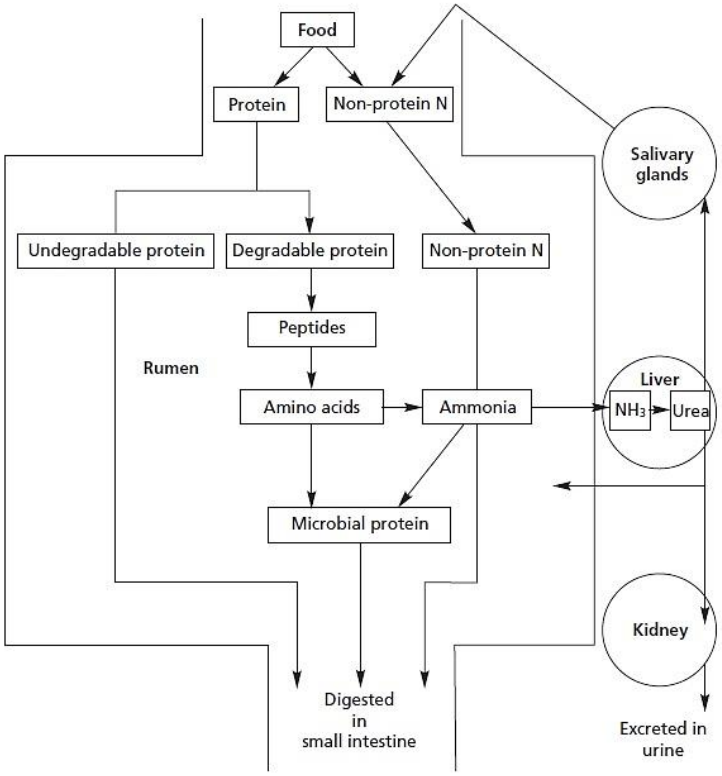


Figure 5. Digestion and metabolism of nitrogenous compounds in the rumen (Mcdonald et al., 2011).

On the other hand, Annison and Bryden (1998) published that rumen is capable to transform large amounts of dietary nutrients except for mineral. Evaluation of essential amino acids is relatively difficult due to the degradation of protein and production of microbial protein. The series of transformations undergone by dietary protein in ruminant is illustrated in Figure 5.

2.6. Amino acids in ruminants

Amino acids are the building blocks of proteins which are necessary for optimal performance of animals in growth, reproduction, lactation, and maintenance (Kung and Rode, 1996). In ruminants, amino acids requirements are not establish yet, however NRC (2007) established the protein requirements in small ruminants, in particular. Predicting the quality and quantity of amino acids absorbed in ruminant digestion is quite difficult since proteins and amino acids are subjected to microbial degradation in the rumen. Absorbed amino acids are originally from microbial protein synthesis and from dietary amino acids that escaped ruminal degradation (Merchen and Titgemeyer, 1992; Kung and Rode, 1996). Furthermore, amino acids that pass through the small intestine of ruminants are highly influenced by the amino acids composition of microbial protein. In the case when microbial protein production is limited, or when amino acid requirement is high, ruminally produced microbial protein may not meet the amino acid requirement of the animal (Merchen and Titgemeyer, 1992). Therefore, ruminally undegraded protein should be provided in the diet to supply sufficient amount of amino acids available for absorption in the intestine, for optimal production (Polan et al., 1991; Kung and Rode, 1996).

2.6.1. Limiting amino acids

All animals require amino acid to extend the protein chain: if one amino acid is absent protein synthesis is terminated. Amino acids can be non-essential when the body is capable of synthesizing these amino acids from another one, or can be essential amino acids when the body is not capable of synthesizing. When essential amino acids are absent during synthetic activity, this will limit the protein synthesis. Thus, the limiting

amino acids are necessary in sufficient amounts in the diet of the animals to meet the total requirements (Häffner et al., 2000).

In ruminants, lysine and methionine are shown as the first limiting amino acid (Polan et al., 1991; Merchen and Titgemeyer, 1992; Kung and Rode, 1996; Schwab, 1996; McDonald et al., 2011). It is observed from several studies on ruminant animals that lysine and methionine are the first limiting amino acid in growing Holstein steer calves (Oke et al., 1986), lambs (Storm and Øskov, 1984; Oke et al., 1986), and multiparous Holstein cows (Polan et al., 1991).

2.6.2. Lysine and methionine

Ordway and Aines (2010) described that lysine is an essential amino acid and a building block of protein, important primarily for the synthesis of protein such as milk protein, growth, pregnancy, and maintenance. Häffner et al. (2000) emphasized that lysine is present in almost all tissues of the animal organism and that this amino acid is particularly important in the development of collagen and in ossification and component of the nucleotides in the nucleus and stimulates cell division.

However, just like lysine, methionine is also an essential amino acid. It also participates as building blocks of proteins and is required for growth and development in young ruminants (NRC, 2007). It has an essential function in metabolism: particularly serve as a precursor of cysteine/cystine and thus also of peptides such as glutathione as an initiator of protein biosynthesis (Häffner et al., 2000).

Supplementation of limiting amino acids is not recommended in a free form (Kung and Rode, 1996); instead it must be provided in a form that will be resistant to microbial degradation in the rumen and be available for absorption in the small intestine (Oke et al., 1986).

Kung and Rode (1996) published that chemical or physical protection is necessary to protect lysine and methionine from microbial degradation, to increase supply of amino acids in the site of absorption. Lysine and methionine are commonly encapsulated with

polymers that are pH sensitive, rendered it to be inactive in the rumen and activated in abomasum where pH is relatively low, thus release of amino acids occur. Polan et al. (1991) reported the efficiency of ruminally-protected amino acids (RPAA) in ruminants: both protection and release of ruminally protected methionine and ruminally protected lysine exceeded 95%.

Several studies reported that a significant increase of methionine and lysine plasma concentrations were observed in the Targhee lambs (Oke et al., 1986) and Holstein cow (Polan et al., 1991) after providing ruminally-protected methionine and ruminally-protected lysine in the diet, indicating that the amino acids were protected and available at the site of absorption.

Schwab et al. (2004) published that the estimated required concentrations of lysine and methionine is a 3:1 ratio. This concentration of amino acids resulted to the increase of both milk protein and milk fat in cows.

2.7. Growth indicators

Growth is characterized as an increase of animal's size and weight. The true growth is based on the increase number of cell mass and not an increase of weight due to ingestion of food or water (Warris, 2000). The rate of growth in young animals is higher until it reaches the peak and decreases as animal ages (Figure 6), this is because rates of nutrient metabolism are highest during early growth of the animals since most cells are dividing and growing (Barboza et al., 2009).

According to McDonald et al. (2011) growth and animal nutrition are inherently linked: nutrient requirements of an animal are determined by its growth pattern (Figure 6) and in reverse, altering nutrition can modify an animal's growth pattern. Both for young birds and mammals, supply of proteins are critical to growth and development. The fast increase of protein in lean body mass, linearly increase the demands of dietary protein. Thus, during early life the ratio of dietary protein and energy is higher than the later stage (Barboza et al., 2009).

Growth rate is described by Warris (2000) in two ways: the average growth rate and relative growth rate. The *average growth rate* is derived by dividing the increase in weight over a particular time period by the length of that period (*average growth rate*= $w_2 - w_1/t_2 - t_1$). The *relative growth rate* is calculated as the increase in weight over a period of time divided by the initial weight (*relative growth rate*= $w_2 - w_1/w_1$).

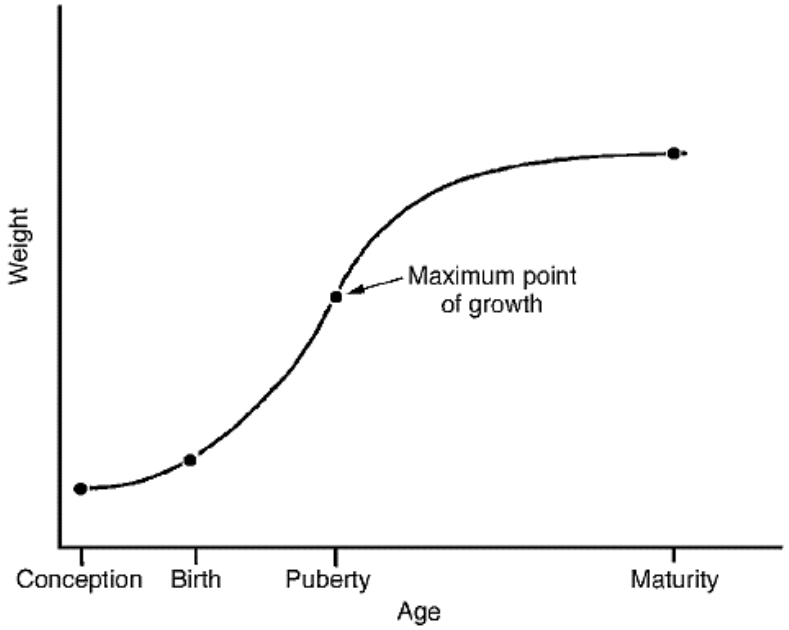


Figure 6. The relationship between the weight of an animal and its age (Warris, 2000).

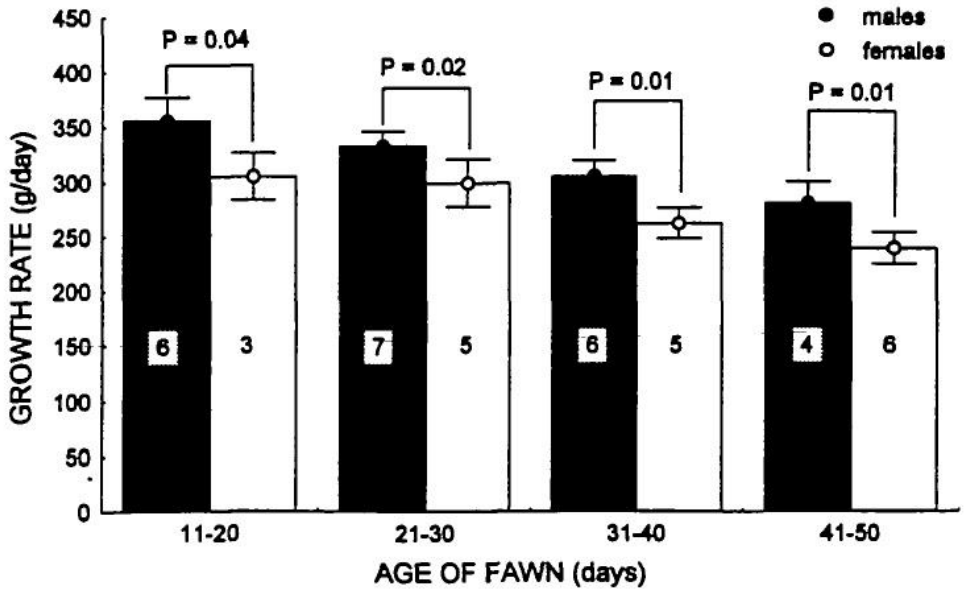


Figure 7. Growth rate of fawns during early growth (Birgersson and Ekvall, 1997).

Birgersson and Ekvall (1997) reported that growth rate was higher during the first two months of fawn's life or from birth to the onset of winter (Figure 7). Male fawns had faster prenatal growth rate than female fawns (191 g/day vs 165 g/day).

2.7.1. Lysine and methionine to growth performance

Average daily gain and feed efficiency were improved after supplementation of ruminally-protected lysine in growing cattle (Xue et al., 2011). Oke et al. (1986) observed higher daily gain and better feed conversion to crossbred Angus steers in diets supplemented with 0.19% ruminally-protected methionine and 0.11% ruminally-protected lysine compared to those animals which had no supplemental amino acids. In the study of Veira et al. (1991) ADG of steers improved 16% and feed:gain ratio was improved after feeding grass silage with ruminally-protected methionine and ruminally-protected lysine supplementation. It is also observed in dairy cattle that feeding ruminally-protected methionine and ruminally-protected lysine had significant response in the body weight of Holstein cows during early and late lactation (Polan et al., 1991). Contrary to the previous studies, a recent study of Torrentera et al. (2017) reported that supplementation of methionine up to 0.128% dietary dry matter did not affect the ADG, but supplementation increased gain efficiency.

A recent study reported by Huang et al. (2015b) showed that ADG of sika deer provided with supplementary methionine improved between 20% and 21% in the early 35-day study period and continue to improve until the late 35-day study period at between 12% and 14%, while feed:gain ratio improved from the early part to the late part of the study period as dietary methionine increased. Similar result on ADG reported by Mendoza-Nazar et al. (2012) after providing ruminally-protected methionine on red deer.

2.8. Body condition indicators

The relationship between fat and non-fat body tissues are the most common way to describe body condition (Caldeira et al., 2007a) and is mostly associated to the nutritional status of the animal (Serrano et al., 2008). Normally when the animal reaches a positive energy balance, surplus energy is stored in the body that results to the

increase of the weight of body tissues and organs. When the supply of nutrients is inadequate, animals will utilize energy reserves in the body in order to satisfy the requirements (Kenyon et al., 2014). Thus, body condition is described and determined by weight, size, appearance, or fatness degree of the animal (Serrano et al., 2008; Kenyon et al., 2014). Body condition is used to assess the performance of reproduction (Tollefson et al., 2010) and growth of animals in the wild (Barboza et al., 2009) and is an acceptable indicator to measure nutritional status in farm animals (Caldeira et al., 2007a). Nutrition during summer and autumn affects the body condition of the animals, the more nutrients available and consumed during these seasons, the more body fat, body mass, and muscle (Tollefson et al., 2010). This enables the animals to deposit fat during summer and mobilize fat during low nutritional condition on winter (Scott et al., 2013).

Measurement of body condition of animals in the wild is classified by Barboza et al. (2009) into two approaches: morphometrical and chemical methods. Morphometry is the method of measuring the form, size, and weight of the body and its constituents. This approach is quick and simple by using visual indicators (*e.g.* plumage color in bird, antler size in deer) or direct measurements (*e.g.* body weight, length, circumference, or fat indices). However, chemical composition measures the total amount of energy and nutrients deposited in the body. Contrary to morphometry, this method requires intensive work. This includes lethal (partitioning body fat mass from lean mass) and non-lethal (*e.g.* internal imaging, electrochemical measures, water dilution) methods of measurements. Table 2 summarizes the two methods.

2.8.1. Body condition score (BCS)

In farmed ungulates, BCS system is a common, practical and easy method to assess body condition (Kenyon et al., 2014). This measurement is performed in dairy cow by judging the animal through visual appearance (Gillespie and Flanders, 2009), or in combination with hands-on palpation of the lumbar region, *e.g.* in beef and lamb (Anon, 2013), ewes (Caldeira et al., 2007a) and farmed deer (Audigé et al., 1998).

In farmed deer, a body condition scoring system (Figure 8) has been developed to assess the body condition with a range of scores from 1 (lean) to 5 (fat) through hands-on palpation of tuber coxae, sacrum, and rump part while the animal is on calm condition (Audigé et al., 1998). As mentioned earlier in this section that BCS influenced nutrition of animals. Hutchison et al. (2012) reported that BCS of fallow deer increased with concentrate feeding.

Table 2. Some common methods of measuring the body condition of wildlife (Barboza et al., 2009).

Method	Measure	Advantages	Disadvantages
Morphometric	Photogrammetry	Repeatable, non-invasive	Non-linear, site dependent, needs validation
	Body mass indices	Repeatable, quick, easily standardized for field	Non-linear, non-specific mass gains (fat vs. eggs or water), needs validation
	Subcutaneous fat and muscle	Repeatable by ultrasound	Limited range in relation to whole body composition
	Internal fat depots (Kidney, omentum)	Quick	Single lethal measure, needs validation
Chemical	Whole body lipid, protein, ash	Validation standard, comprehensive	Single lethal measure, time consuming
	Internal imaging (X-ray tomography)	Repeatable	Site-based machine, expensive
	Electrochemical (BIA[1], TOBEC[2])	Repeatable, quick	Muscle tone and posture effects
	Water dilution	Repeatable, portable	Time for equilibration and sampling

[1]BIA=Bioelectrical impedance analysis
[2]TOBEC=Total body electrical conductivity

2.8.2. Post-mortem assessments

Measurement of body condition is commonly used in ungulates in the wild by post-mortem assessment measuring specific characteristics such as organ weight and fat depth (Serrano et al., 2008). Riney (1955) established the most widely used method and satisfactory indicator of body condition for wild ungulates: the kidney fat index. Kidney

fat index is derived by using the weight of kidney fat tissue around the kidney expressed as percentage of kidney mass without fat. This method is one of the most reliable measurements for body condition (Putman, 2005). Mattiello et al. (2009) reported that KFI is an acceptable indicator of body condition because it is sensitive to change in environmental condition and is dependent to age and sex of the animals. In recent study on sika deer, Kuroiwa et al. (2017) reported that KFI was influenced by seasonal changes from summer to winter condition and had a positive relationship with food intake.

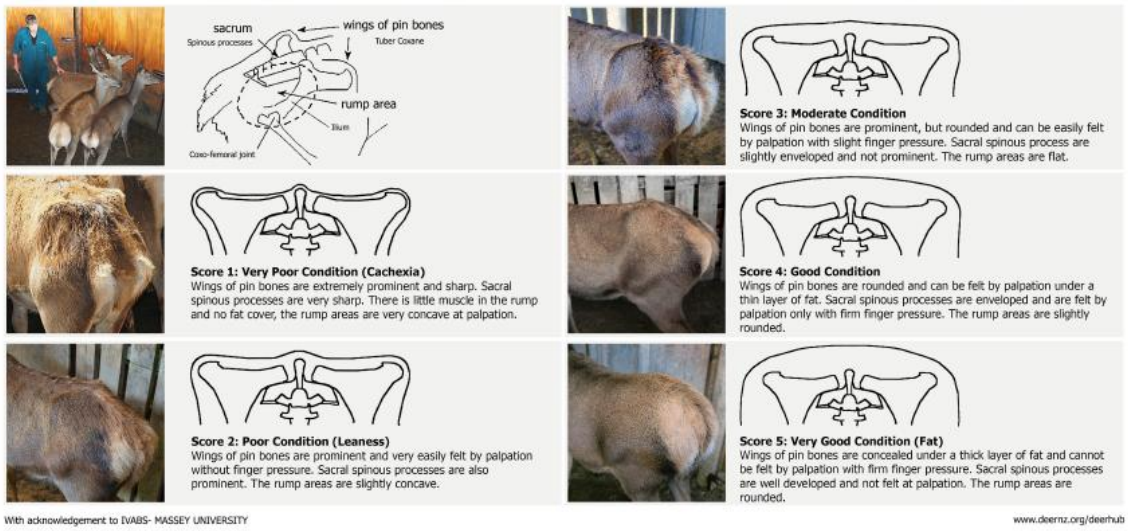


Figure 8. Body condition score chart for deer (Audigé et al., 1998).

Body fat indices were also used in measuring nutritional condition on deer (Mattiello et al., 2009). Tollefson et al. (2010) reported that body fat percentage of the animal started to increase on early autumn until winter (Figure 9) and although no significant influence of nutritional treatments was reported, deer in high-digestible-energy diets had the highest body fat observed.

2.8.3. Relationship of body condition to physical attributes

In a review on sheep it is reported that there is a linear relationship between live weight and BCS, the increase on live weight required an increase BCS by 1.0 unit. This relationship between live weight and BCS varied from sex, age, and breeds. Both gain

in live weight and BCS emanates from energy cost depending on animals physiological condition (Kenyon et al., 2014).

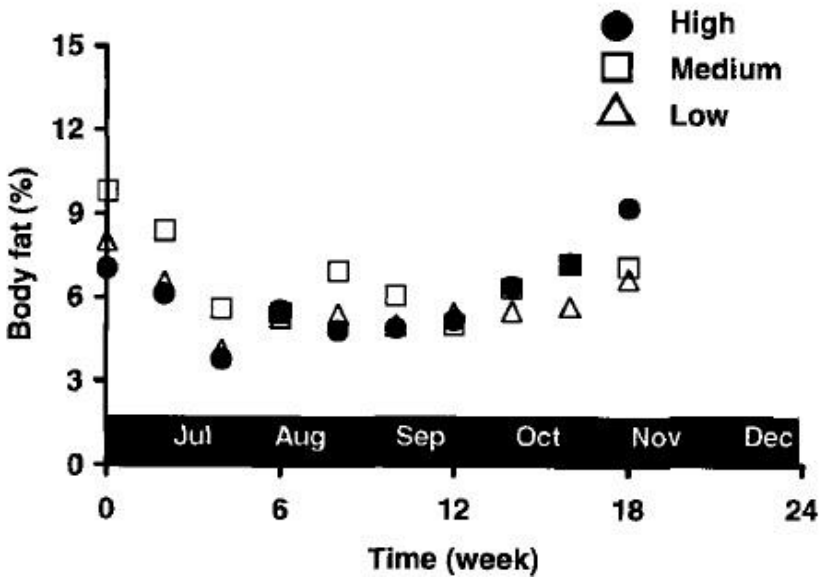


Figure 9. Body fat composition of mule deer fed with different diets: high-, medium-, or low- digestible-energy (Tollefson et al., 2010).

Relationship of BCS and total body fat are also used in describing the body condition. It is reported that BCS was correlated and had a positive relationship to the level of pelvic and heart fat, mesentric, subcutaneous and intramuscular fat, and total body fat (Kenyon et al., 2014).

2.9. Carcass traits

Carcass characteristics of deer and quality of meat are influenced by many factors such as age of animals (Drew, 1992; Volpelli et al., 2003), sex (Daszkiewicz et al., 2009), wild or farmed condition (Daszkiewicz et al., 2015), and nutrition (Volpelli et al., 2002; Wiklund et al., 2003a, b; Philip et al., 2007; Blanco et al., 2012; Kerr et al., 1995).

Volpelli et al. (2002) reported that measurements such as carcass weight and dressing proportion of male fallow deer were higher when supplemented with concentrate (16.1% crude protein) compared to pasture-fed animals. Measurements were also higher in 30 months-old fallow deer than in 18 months-old bucks. Due to the higher dietary energy and proteins available from the supplementary concentrate, deer in this group

had greater muscle development and subsequently fat depositions were higher. Similar performance was observed on reindeer (Wiklund et al., 2003a) and red deer (Wiklund et al., 2003b). Carcass quality on beef cattle under winter feeding was reported by Blanco et al. (2012), carcass weight and dressing percentage were higher from steers fed on supplemented diet.

However, Philip et al. (2007) found no significant differences in the slaughter weight of red deer after feeding varying ratios of concentrate to roughage such 75:25, 50:50, 25:75. Similar result was reported on the slaughter weight of pig after feeding amino acid-supplemented diets (Kerr et al., 1995).

Farmed deer have more fat than wild deer since farmed deer are much larger on equal age than wild deer. In both situation, it is common that deer have more body fat on the early age or on their first winter. However, deer with restricted adipose tissue stored in the body during winter are typically expected to have a very low amount of winter carcass fat content (Drew, 1992).

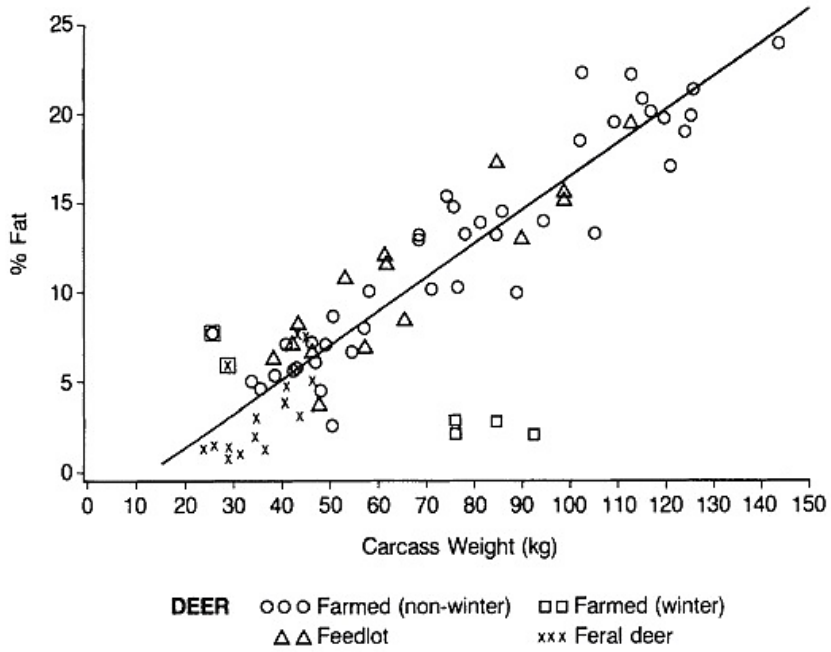


Figure 10. Relationship of carcass weight and fatness in red deer (*Cervus elaphus*) (Drew, 1992).

A study in New Zealand, where farmed and wild carcasses from red deer (*Cervus elaphus*) were analyzed for carcass fatness (Figure 10), reported that differences in fat proportion was primarily correlated with carcass weight, not with environment; and that 1 kilogram of carcass gain was composed of 0.19 kg fat (Drew, 1992).

Drew (1992) pointed out that meat from young deer is extremely lean and deer species like fallow deer, red deer, and New Zealand wapiti younger than 26 months of age composed of separable lean content ranges from 72.7% to 76% of the carcass weight. While separable fats are low in deer less than 2 years of age and very high lean/fat ratio (Table 3).

Table 3. Carcass composition of farmed deer (Drew, 1992).

Species	Age	Weight (kg)	Lean (%)	Fat (%)	Bone (%)	Lean/fat	Lean/bone
Fallow	13-25 mos.	24-40	73.9	9.1	13.6	8.1	5.4
Red	26 mos.	62.6	72.7	7.0	20.3	10.4	3.6
	9 and 10 yr.	129.5	70.9	14.2	14.9	5.0	4.8
Wapiti*	26 mos.	83.0	72.7	4.2	23.5	17.3	3.1
	4 and 5 yr.	115.3	66.8	14.7	18.5	4.5	3.6
Hybrid (wapiti/red)	11 mos.	67.6	76.0	4.7	19.3	16.2	3.9

*New Zealand wapiti

Fallow deer has a good carcass characteristic in terms of muscle and bone proportion. Carcass bone content of fallow deer is lower than other deer species while it has a very high lean/bone ratio (Drew, 1992).

2.10. Metabolic status indicators

Blood parameters are commonly studied in domesticated ruminants such as sheep, goats, and cattle (Kida, 2003; Caldeira et al., 2007a, b; Watanabe, 2010). Many studies on blood biochemistry are focused on dairy cattle thus the first established tool to assess metabolic status and diagnosing metabolic disorders or so-called metabolic profile test (MPT) are functional for dairy animals (Payne and Payne, 1987 as cited by Kida, 2003). Only few studies exists on cervids such as on red deer (Marco and Lavin, 1999), fallow deer (Poljičak-Milas et al., 2004), and sika deer (Huang et al., 2015a, b).

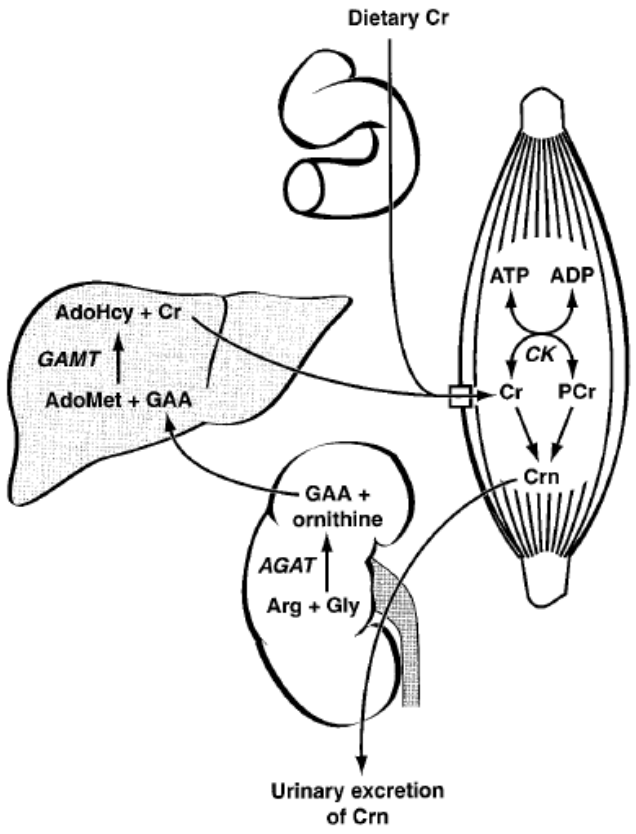
Blood metabolite concentration is associated to diet and nutrition hence this has been used as indicators of protein and fat reserves in the animal (Blowey et al., 1973; Serrano et al., 2008). Blood metabolites are good indicator of protein metabolism for example Albimin (ALB) and Blood Urea Nitrogen (BUN); and metabolites such as glutamic-oxaloacetic, glutamic pyruvic transaminases, cholesterol, phosphorus, triglyceride (TRIG) and globulin (GLB) are used to predict and interpret the variability of animal feeding. (Adachi et al., 1997; Kida, 2003).

Blood parameter reference is affected by factors such as method of capture, age, nutrition, season, and health status of the animals (Marco and Lavin, 1999; Teare, 2013).

2.10.1. Protein-related metabolites

2.10.1.a Creatinine

Creatinine (CREA) is a product of degradation of creatine during muscle metabolism (Figure 11). The production of CREA is less influenced by age, diet or protein catabolism, but slightly affected by the extent of muscle mass. In a normal process, CREA is eliminated through glomerular filtration, tubular secretion, and excretion in the kidneys (Caldiera et al., 2007b; Russell and Roussel, 2007).



AdoMet=S-adenosyl-L-methionine; **AdoHcy**=S-adenosyl-L-homocystein; **CK**=creatine kinase; **Cr**=creatine; **Crn**=creatinine; **AGAT**=L-arginine glycineamidinotransferase; **Arg**=arginine; **GAA**=guanidinoacetic acid; **GAMT**=N-guanidinoacetate methyltransferase; **Gly**=glycine, **PCr**=phosphoryl-creatine

Figure 11. Schematic diagram of creatine and creatinine metabolism (Wyss and Kaddurah-Daouk, 2000).

The biosynthesis of creatine occurs mainly in the kidney, while methylation of guanidinoacetic acid to creatine is completed mainly in the liver. After that, a large concentration of creatine (up to 94%) is found in muscle. Creatinine is then formed in the muscle from creatine phosphate by irreversible dehydration, which then is dispersed out of the cells and excreted by the kidneys into the urine (Wyss and Kaddurah-Daouk, 2000; Caldiera et al., 2007a).

Caldeira et al. (2007a) published that CREA production is influenced positively by factors such as i) amount of creatine which is associated to muscle mass and hence, BCS, and ii) rate of proteolysis and utilization of endogenous nitrogen compounds. The study observed that ewes with lower (BCS 2) and higher (BCS 4) score have higher

levels of CREA due to an increased activity of protein mobilization pathways and a greater muscle mass, respectively. However, ewes with optimal BCS (3) have lower levels of CREA because the low rate of proteolysis and muscle mass are not large enough.

2.10.1.b Urea

Urea is produced in the liver by urea cycle after the production of ammonia, a by-product of protein catabolism. Elimination of urea from the body is through glomerular filtration in the kidneys (Russell and Roussel, 2007; IDEXX, 2014). Production of urea is influenced by diet and hepatic function (Russell and Roussel, 2007) and urea concentration in the kidneys is elevated during water insufficiency or dehydration (Barboza et al., 2009).

This non-toxic form of waste nitrogen can be recycled by ruminants through microbial degradation in the rumen to conserve nitrogen especially when diets are low-nitrogen and high in fiber during winter. This urea recycling turns to minimal losses of nitrogen in the body, and thus nitrogen requirements decrease (Barboza et al., 2009).

Caldeira et al. (2007a) observed that serum urea concentrations increased with overfeeding (BCS 4) and undernutrition (BCS 1.25). The increased levels of urea in overfed ewes resulted from high production of ammonia in the rumen and due to an excess amount of exogenous nitrogen compounds in the intestine. In undernourished ewes the increase of urea is due to utilization of stored body protein to meet the energy requirements. Similar to the study of Russell and Roussel (2007), BUN is elevated (> 20mg/dL) after feeding excessive protein on dairy herds.

In the study of Huang et al. (2015b) plasma amino acids concentration associated with urea cycle were affected by methionine supplementation in sika deer. Tomkins and McMeniman (2006) reported that plasma urea nitrogen concentrations in rusa deer was higher (18.8-24.7 mg/100ml) to the animals fed with higher crude protein diet, while plasma urea nitrogen is low (11.8- 18.7 mg/100ml) in animals fed with low protein diets. Animals fed with low protein diets were found to have higher permanent loss rate

of urea nitrogen. Wiklund et al. (2003a) observed elevated plasma urea concentration in reindeer due to high protein intake. Similar results were reported on sheep, that the concentrations of urea are affected by the diet composed of high crude protein (Allen and Miller, 1976; Aharoni et al., 1991). However, an obvious increase in serum BUN and ALB was observed in Japanese black cattle after the intensified nutrition (Watanabe et al., 2010). Contrary to many reports, Takasu et al. (2005) reported that serum urea nitrogen concentration increased in growth retarded Japanese black cattle due to proteolysis that probably induced increase in BUN. Concentration of BUN indicates chronic shortage of protein (Payne and Payne, 1987 as cited by Kida, 2003).

2.10.1.c Total protein (TP)

Total protein concentration measures all the proteins found in the blood. The two major protein components of diagnostic significance relative to chemistry profile are ALB and GLB. The GLB concentration is determined by subtracting the ALB from the TP. Total protein and ALB are used for metabolic profile test measuring protein metabolism and determining condition such as hepatic and renal function, and degree of hydration (Russell and Roussel, 2007; IDEXX, 2014).

Several studies on deer are available. In sika deer, increased TP in serum concentration was observed when amino acids were added in the diet, and decreased when crude protein level was reduced (Huang et al., 2015a). Poljičak-Milas et al. (2004) observed that concentrations of TP in fallow deer increase with age. However, concentrations of TP, ALB, and GLB were affected by the methods of capture; higher concentrations were detected to red deer captured by physical means (Marco and Lavin, 1999).

2.10.1.d Albumin

Albumin is the largest fraction of the total serum protein. It is synthesized in the liver and plays important function in transporting endogenous and exogenous compounds and primarily necessary for the oncotic pressure in plasma (Russell and Roussel, 2007; IDEXX, 2014).

Albumin is used as an indicator of protein intake and dehydration in animals (Adachi et al., 1997; Kida, 2003). The increase of concentrations of ALB is commonly called hyperalbuminemia, caused by dehydration. However, low ALB concentrations are result of insufficient protein intake, breakdown, or absorption, and also found in animals with chronic severe hepatic disease. Albumin and GLB ratio is also used in chemistry profiling (Russell and Roussel, 2007). The increase of ALB levels are influenced by age, nutrition, and metabolic status of the animals (Adachi et al., 1997; Caldeira et al., 2007a, b; Huang et al., 2015a).

Recent study of Huang et al. (2015a) on sika deer reported the effect of methionine on ALB concentrations. Albumin concentration increased as response to the inclusion of methionine in the diet and decreased when crude protein level decreased. On the same study, it was observed that digestibility of dry matter, organic matter, and crude protein improved with methionine or with high crude protein level. Improved crude protein digestibility provides more available protein for absorption, thus possibly explained the increased serum ALB concentration.

Caldeira et al. (2007a) observed differences of ALB concentration in relation to nutritional status of ewes. There was an increase in serum ALB concentration as BCS increased or in overfed animals, and decreased concentration in underfed (BCS 1.25) animals. The increase of serum ALB concentration in the intermediate and overfed animals was due to the amount of protein available for ALB synthesis. However, ALB concentration decreased in cattle with growth retardation because of an energy-negative condition causing protein deficiency (Takasu et al., 2005).

2.10.1.e Globulin

Most of the chemistry profile test includes measured ALB and TP concentrations. Globulin is the remaining protein after subtracting the concentration of ALB from the TP (IDEXX, 2014). Majority of the GLB fraction consists of immunoglobulins that are synthesized by lymphoid cells, and other GLB are synthesized by the liver. The increase of concentration in GLB known as hyperglobulinemia is commonly caused by chronic antigenic stimulation and hepatic disease (Russell and Roussel, 2007).

Caldeira et al. (2007 a, b) observed that serum GLB concentrations decreased in the animals with lower BCS (1 and 2) while concentration is steady and higher on the animals with BCS 3 and 4. The concentration of GLB reflects to the physiological mechanism such as the nutritional status, and level of TP and ALB concentrations. Globulin concentration has the counterbalance function to ALB to support osmotic pressure.

2.10.2. Energy-related metabolite

2.10.2.a Triglycerides

Triglycerides are measured to assess the lipid metabolism. It is synthesized in the liver from carbohydrates as a source of energy and stored in the body (IDEXX, 2014). Serum triglyceride was used as an indicator to monitor the energy metabolism and body condition in goat (Serrano et al., 2008), sheep (Caldeira et al., 2007a, b), and cattle (Otomaru et al., 2016).

The increased rate of triglyceride synthesis in the alimentary tract of ewes with the highest BCS increases the levels of serum triglycerides concentration. Animals with high BCS resulted to the increase of serum triglycerides concentration, and have been associated to the abundance of energy and stored fat (Caldeira et al., 2007a, b). Most of the energy metabolites including triglycerides are greatly affected by age. Higher concentrations were observed in calves than in adult cattle (Otomaru et al., 2016).

3. Aims of thesis

The aims of the study were:

1. To evaluate the growth performance of fallow deer supplemented with lysine and methionine.
2. To determine the effect/s of lysine and methionine supplementation on the nutritional status of fallow deer using body condition indicators.
3. To evaluate the effect/s of lysine and methionine supplementation on carcass traits.
4. To examine the physiological effect of lysine and methionine supplementation through selected blood biochemistry analysis.
5. To determine the influence of culling seasons to the production performance and physiological functions of fallow deer.

Hypotheses of the study:

H1: Lysine and methionine supplementation will increase weight gain and average daily gain of fallow deer.

H2: Supplementation of lysine and methionine will improve body condition and carcass traits of fallow deer.

H3: Lysine and methionine inclusion in the diet will result to higher concentration of protein-related blood metabolites.

H4: Culling season will affect the performance of production and physiological functions of fallow deer.

4. Materials and Methods

4.1. Study area and experimental animals

Forty five (45) farmed fallow deer spikers (Figure 12) of approximately 12 months of age were allocated into three groups according to equal average weight (40.2 ± 2.7 kg). Each group consisted of 15 spikers kept in two hectares of lush paddocks for the whole period of the study. Animals were recorded and identified using ear tag in both sides of the ears.

The study was conducted in a private deer farm in Mnich, South Bohemia Region, Czech Republic, GPS coordinates 49.1671181N, 14.9005053E (Appendix 1), with an altitude of 485 meters above sea level. The experiment was started from June 2016 and terminated in two separate periods, first was on December 2016 and second was on February 2017. Termination of study is described in section 4.5.

Figure 12. The group of experimental spikers (Photo: Author).

4.2. Diet and treatments

Animals were mainly on extensive grazing. The sources of diet were characterized according to the season. On summer and autumn the primary source of diet was grazing

on a good-quality pasture while on winter, where pasture resources were scarce, an appropriate amount of silage was provided to the animals. In both seasons, the animals received a supplementary feeding composed of 0.5 kg of barley, mineral supplement (Premin Slanisko, see Appendix 2) and a corresponding amount of amino acid, according to treatments (Table 4). The nutrient composition of supplementary feed is summarized in Table 5.

Table 4. Amount of lysine and methionine supplementation.

Treatments	Level of RPAA (g/d/animal)
Group 1 (Control)	0.0 Amino acids
Group 2 (Lys)	9.0 Lys (LysiGem™)
Group 3 (Lys+Met)	9.0 Lys + 3.0 Met (Smartamine® M)

RPAA, ruminally-protected amino acids

Amino acids lysine and methionine used in the experiment were RPAA with a pH-sensitive coating which allow the amino acids to absorb in the small intestine of the ruminant. The amount of ruminally-protected Lysine (LysiGem™) and ruminally-protected Methionine (Smartamine® M) was according to dietary treatments, such as no amino acids supplementation (control), 9 gram of lysine per day per animal (Lys), 9 gram of lysine per day per animal plus 3 gram of methionine per day per animal (Lys + Met), respectively.

Table 5. Nutrient composition of supplementary feed.

Composition (%)	Barley	Silage
Dry matter	87.06	51.54
Crude protein	10.75	5.75
Crude fat	3.34	1.06
Crude fiber	8.84	17.62
Ash	2.88	4.73
Nitrogen-free compounds	61.25	22.39
Lignin	4.51	6.55
Acid detergent fiber (ADF)	10.91	21.91
Neutral detergent fiber (NDF)	23.60	30.32

The amount of RPAA supplementation was based on the body weight of the animals using the estimation from the previous study of Mendoza-Nazar et al. (2012) and followed a 3:1 ratio concentrations of lysine and methionine (Schwab et al., 2004). Prior to supplementation, feeding trial of ruminally-protected Lysine and ruminally-protected Methionine were done to test the acceptability of amino acids to the experimental animals.

4.3. Blood sampling

Blood sampling was carried out twice. Initial blood collection was carried out on June 2016 before the start of amino acids supplementation. Final collection was completed during the period of December 2016 and February 2017.

Figure 13. Procedure of initial collection of blood samples (Photo: Author).

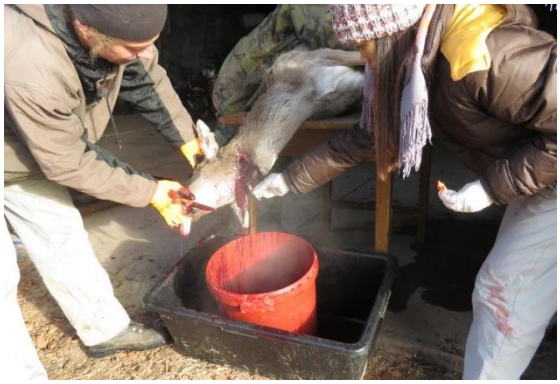


Figure 14. Procedure of final collection of blood samples (Photo: Author).

On the initial blood sample collection, the animals were physically restrained using the restraining chute for fallow deer. A 20 gauge needles were used to drawn the blood samples from *vena jugularis* performed by a specialist (Figure 13). The final blood sample collection was performed during slaughtering. Blood samples were collected using heparinized tubes upon bleeding procedure (Figure 14). In both processes, blood samples were collected and temporarily stored in heparinized tubes (VetTest® Zkumavka Sarstedt Li- Heparin 1.3 ml tube). After collection, heparinized tubes were inverted to ensure that blood was mixed thoroughly and do not coagulate. Blood samples were stored in a cooler box with frozen packs during transportation. On the same day, plasma was extracted from blood samples by allowing the blood samples to

cool in a room temperature. Subsequently, blood samples were centrifuged at 12,000 RCF for 2 minutes and plasma samples were extracted and transferred to untreated microtubes for storage. Extracted plasma samples were stored in -18°C for blood biochemistry analysis. See Appendix 3 for plasma sample preparation.

4.4. Weighing

Live weight and slaughter weight of the animals (Figure 15) were determined using Tru-Test EziWeight tenzometric scale (Tru-Test Group, Auckland, New Zealand) with an accuracy of 0.1 kg. Initial and slaughter/final weighing of the animals were done on the start of the experiment and on slaughtering, respectively.

4.5. Slaughtering

The study was terminated by slaughtering the animals and processing the meat for human consumption. The group of animals were slaughtered in the farm in two separate periods, first group of animals were slaughtered on late autumn whilst the rest were harvested on late winter. A total of 18 animals from each experimental group were slaughtered on December 2016 and 27 were slaughtered on February 2017. The interval between the first and the last slaughtering day was 66 days.

Figure 15. Slaughter weight determination (Photo: Author).

Figure 16. Stunning using captive bolt performed in a restraining chute by farm assistant (Photo: Author).

Prior to bleeding, animals were rendered unconscious by mechanical stunning procedure using captive bolt gun (Figure 16). Slaughter weight and blood weight were determined immediately after bleeding.

4.6. Carcass measurements

After slaughtering, the carcasses were transported from the farm to abattoir for processing. Evisceration was performed and carcasses were chilled for 12 hours at 4° C. Chilled carcasses were weighed along with other carcass measurements before it was butchered. Each carcass was cut into major parts such as shoulder, neck, rump, flank, loin, and tenderloin. All parts were weighed while rump and loin were dissected into lean, fat and bone.

Carcass traits were determined by the proportion of lean meat, bones and tendons, and internal organs relative to the carcass weight. Fat tissues were expressed as gram (g) per kilogram (kg) of slaughter weight.

4.7. Body condition measurements

Body condition was determined through BCS, KFI and body fat depots. Body condition scoring is ideally done with animals standing upright and relaxed in normal posture. To score the animal, the researcher must palpate the tuber coxae, and feel the spinous process over the sacral vertebrae (Audige, et al., 1998).

In this study, body condition score was determined through hands-on palpation while the animal was on relaxed position. In this case, palpation on experimental animal was done immediately after the animal was slaughtered. Body scoring to all the experimental animals was performed by the same person following the body condition score chart (Figure 8) for deer by Audigé et al. (1998) with the interval of 0.25.

On the other hand, while the animals were eviscerated, internal fat such as heart fat, scrotum fat and kidney fat were collected and weighed. The left and right kidneys and the fat attached from the rear of the stomach cavity was removed. Both kidneys were

weighed to the nearest 0.01 g with attached fat and separately, kidney fat collected was also weighed to determine the KFI value. KFI was calculated using (Riney, 1955):

$$KFI,\ \% = \frac{kidney\ fat\ (g)}{kidney\ mass\ without\ fat\ (g)}\ \times 100$$

4.8. Blood biochemical analysis

Frozen blood plasma samples were thawed at room temperature, mixed by inversion, and centrifuged at 12,000 RCF for 2 minutes to ensure homogeneity and remove fibrin particles that may have formed during the storage. Prepared plasma samples were then analyzed for the concentration of blood plasma metabolites such as creatinine, urea, total protein, albumin, globulin, and triglycerides using VetTest® Chemistry Analyzer (IDEXX Laboratories, Westbrook, Maine, USA) with standard commercial kit (IDEXX, Czech Republic).

Analyses were performed at the laboratory of the Department of Animal and Food Science at Czech University of Life Sciences Prague. See Appendix 4 for blood biochemical analysis procedure.

4.9. Data analysis

Data were statistically analyzed using SPSS software version 20.0 (IBM SPSS, 2011). The established calculation of significance level was at α=0.05. The results were expressed as mean (SD) value. All data were initially tested for normality (Shapiro-Wilks test). Non-parametric tests were applied to non-normally distributed data.

One-way ANOVA was used to compare the differences between treatments of final weight, weight gain, average daily gain, carcass traits, body condition parameters, and blood plasma metabolites concentrations. Tukey test was used for comparing the significant difference among treatment means. For comparing the difference of means between two culling periods, independent sample t-test was used. Non-parametric Mann-Whitney U test was applied to the parameters such as weight of bones and

tendons, body condition score, and kidney fat index. Paired-sample t-test was used for comparing the initial and final blood plasma metabolite concentrations, except for triglyceride concentrations which showed a non-normal distribution, and thus, nonparametric Wilcoxon signed rank test was used.

The associations between slaughter weight and body condition were correlated using Pearson correlation coefficient.

Finally, the relationship of treatments and culling season to growth, body condition, carcass traits, and blood plasma metabolites were evaluated using Generalized Linear Mixed Models. Before deciding the main fixed effect factors entering the models, collinearity between variables was tested by calculation of the Variance Inflation Factor. The main fixed effects used in the models were dietary treatments, culling season, initial weight, and the interaction between treatment and culling season. In certain models, BCS, ALB, and GLB were fitted as main fixed effects.

4.10. Reference values

VetTest® Chemistry Analyzer has no reference ranges for cervids. We used the physiological reference intervals (Appendix 5) for *Dama dama* from Teare (2013). For these reference values, samples were obtained from healthy animals, from combined ages and selected regardless of gender.

5. Results

5.1. Growth performance

Mean growth performance among treatments in two culling seasons is shown in Table 6. Initial weight of fallow deer showed no significant differences among groups. No significant differences were observed among treatments in the final weight, weight gain, and ADG in the LA and LW.

Table 6. Mean (SD) values of growth performance of fallow deer (*Dama dama*) supplemented with lysine and methionine during the fattening period and culled on different season: 1st group on late autumn (LA; *n*=18) and 2nd group on late winter (LW; *n*=27).

Parameters	Season	Treatments			p- value[A]
		Control	Lys	Lys+ Met	
Initial wt. (kg)	Summer	39.6 (2.6)	40.2 (2.6)	41.0 (2.9)	0.331
Final wt. (kg)	LA	45.4 (3.8)	45.9 (1.8)	47.4 (3.2)	0.530
	LW	43.6 (3.3)	44.9 (4.6)	44.8 (4.1)	0.762
Weight gain (kg)	LA	5.50 (3.1)	5.82 (1.2)	4.84 (2.0)	0.745
	LW	4.31 (3.7)	4.66 (2.8)	4.81 (2.4)	0.937
ADG (g)	LA	34.1 (19.1)	36.1 (7.6)	30.1 (12.2)	0.745
	LW	19.0 (16.2)	20.5 (12.3)	21.3 (10.9)	0.932

Control=pasture with barley and mineral supplementation; *Lys*=9 g/d/deer lysine; *Lys*+*Met*= 9 g/d/deer lysine and 3 g/d/deer methionine
ADG=average daily gain
p-value is significantly different at p≤ 0.05
[A]One-way ANOVA

However, significant differences were found in the ADG (t=3.329; df=43; p=0.002) between seasons (Figure 17). Average daily gain of the animals culled in the LA was significantly higher than in the LW (33.4±13.2 *vs*. 20.3±12.8). No significant differences were found in weight gain between culling seasons (p>0.05).

These results were similar when data were evaluated through Generalized Linear Mixed Models (Table 7): treatment had no significant effects on growth parameters while ADG significantly decreased in the LW (GLMM: β=-13.2±4.0; t=-3.329; p=0.002).

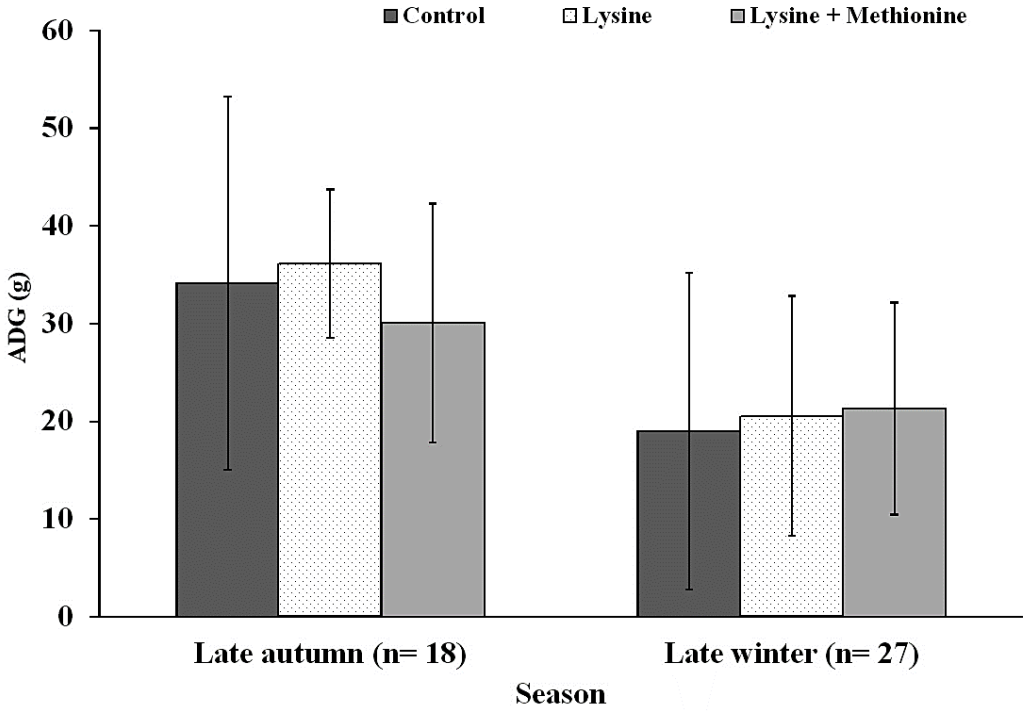


Figure 17. Effect of culling season on ADG. Data are presented as mean ± SD.

Table 7. Generalized Linear Mixed Models for body condition.

Measurement	Model Term	β	t	p-value
Final weight (kg)	Intercept	6.94±6.0	1.154	0.255
	Initial weight	0.950±0.15	6.373	<0.001***
ADG (g)	Intercept	33.4±3.1	10.926	<0.001***
	Late winter	-13.2±4.0	-3.329	0.002**

ADG=average daily gain
p-value is significantly different at **p< 0.01, ***p< 0.001

5.2. Body condition

Mean values of body condition parameters of treatment groups are shown in Table 8. Comparison of mean values between culling season are shown in Table 9. Effects of treatments and season using the model are shown in Table 10.

5.2.1. Body condition score

Table 8. Mean (SD) values of body condition of fallow deer (*Dama dama*) supplemented with lysine and methionine during the fattening period and culled on different season: 1st group on late autumn (LA; *n*=18) and 2nd group on late winter (LW; *n*=27).

Parameters	Season	Treatment			p- value[A]
		Control	Lys	Lys + Met	
BCS	LA	3.25 (0.27)	3.50 (0.32)	3.50 (0.32)	0.286
	LW	2.89 (0.33)[b]	3.06 (0.37)[ab]	3.33 (0.38)[a]	0.046*
KFI (%)	LA	90 (64)	119 (53)	166 (54)	0.102
	LW	140 (61)	216 (102)	215 (63)	0.076[†]
Kidney wt. (g)	LA	104.1 (5.9)	108.1 (8.0)	113.0 (10.0)	0.188
	LW	99.4 (8.6)	104.3 (4.8)	107.7 (7.1)	0.061[†]
Kidney fat (%)	LA	0.209 (0.136)[b]	0.287 (0.128)[ab]	0.401 (0.096)[a]	0.047*
	LW	0.332 (0.144)[b]	0.509 (0.204)[ab]	0.537 (0.141)[a]	0.030*
Internal fat (%)	LA	0.511 (0.246)[b]	0.688 (0.246)[ab]	0.969 (0.218)[a]	0.014*
	LW	0.552 (0.184)	0.752 (0.259)	0.781 (0.175)	0.059[†]

Control=pasture with barley and mineral supplementation; *Lys*=9 g/d/deer lysine; *Lys*+*Met*= 9 g/d/deer lysine and 3 g/d/deer methionine
BCS=body condition score; *KFI*=kidney fat index; *Internal fat*=kidney fat+scrotum fat+heart fat
p-value is significantly different at [†]≤0.1, *p≤ 0.05
[a,b]Means with different superscripts differ at p<0.05
[A]One-way ANOVA

Body condition score in the LW was significantly different among treatment groups ($F_{2,24}$=3.50; p=0.046; Table 8). The experimental group Lys + Met showed higher values among treatments. Moreover, BCS of fallow deer showed significant differences between two culling periods (U=131; z=-2.748, p=0.006; Table 9). Body condition score of animals were higher during LA than in LW.

In the model (Table 10), treatment group Lys + Met (β=0.340±0.145; t=2.354; p=0.024) showed positive effect to BCS, while animals culled in LW (β=-0.589±0.119; t=4.931; p<0.001) showed decreased BCS.

5.2.2. Kidney fat index

Kidney fat index showed no significant differences between control and experimental group both in the LA and LW (Table 8). However, significant differences were found when the mean value of KFI between two culling seasons were compared (U=135; z=-2.502; p=0.012; Table 9). The animals in later winter had significantly higher KFI than the animals in LA.

Table 9. Mean (SD) values of body condition parameters during the fattening period of fallow deer (*Dama dama*) culled on different season: 1st group on late autumn (LA; *n*=18) and 2nd group on late winter (LW; *n*=27).

Parameters	Culling season		*p*-value
	LA	LW	
BCS[B]	3.42 (0.31)	3.10 (0.39)	0.006**
KFI (%)[B]	125 (63)	190 (83)	0.012*
Kidney wt. (g) [A]	108.4 (8.4)	103.8 (7.6)	0.063†
Kidney fat (%)[A]	0.299 (0.140)	0.459 (0.184)	0.003**
Internal fat (%)[A]	0.722 (0.295)	0.695 (0.226)	0.729

BCS=body condition score; *KFI*=kidney fat index; *Internal fat*=kidney fat+scrotum fat+heart fat
p-value is significantly different at †≤0.1, *p<0.05, **p<0.01
[A] Parametric test (Independent Sample T-Test)
[B] Non-parametric test (Mann-Whitney U Test)

In the GLMM, the experimental group Lys + Met and LW culling season had both positive effect on KFI of the animals (Table 10). KFI was higher in Lys + Met group (β=75.3±25.4; t=2.966; p=0.005), likewise in LW culling season (β=65.4±21.2; t=3.091; p=0.004).

5.2.3. Kidney weight

Weight of kidney had no significant differences between control and experimental group both in the LA and LW. No significant difference was found even when the mean values of kidney weight between two slaughter seasons were compared. In the GLMM, the weight of kidneys were positively affected by the treatment Lys + Met (β=6.77±2.47; t=2.740; p=0.009).

Table 10. Generalized Linear Mixed Models for body condition.

Measurement	Model term	β	t	p-value
BCS	Intercept	1.88 ± 0.917	2.045	0.047*
	Treatment-Lys	0.208±0.141	1.472	0.149
	Treatment-Lys+Met	0.340±0.145	2.354	0.024*
	Initial weight	0.041±0.023	1.801	0.079†
	Late winter	-0.589±0.119	-4.931	<0.001***
KFI%	Intercept	80.5±22.0	3.659	0.001***
	Treatment-Lys	57.5±25.4	2.266	0.290
	Treatment-Lys+Met	75.3±25.4	2.966	0.005**
	Late winter	65.4±21.2	3.091	0.004**
Kidney wt. (g)	Intercept	56.00±15.66	3.579	0.001***
	Treatment-Lys	3.78±2.42	1.565	0.126
	Treatment-Lys+Met	6.77±2.47	2.740	0.009**
	Late winter	-3.40±2.04	-1.667	0.103
	Initial weight	1.20±0.39	3.091	0.004**
Kidney fat (%)	Intercept	0.187±0.047	3.982	<0.001***
	Treatment-Lys	0.137±0.054	2.540	0.015*
	Treatment-Lys+Met	0.200±0.054	3.688	0.001***
	Late winter	0.160±0.045	3.559	0.001***
Internal fat (%)	Intercept	0.536 ±0.057	9.408	<0.001***
	Treatment-Lys	0.191 ±0.081	2.370	0.022*
	Treatment-Lys+Met	0.320 ±0.081	3.981	<0.001***

Lys=9 g/d/deer lysine; *Lys+Met*= 9 g/d/deer lysine and 3 g/d/deer methionine
BCS=body condition score; *KFI*=kidney fat index; *Internal fat*=kidney fat+scrotum fat+heart fat
p-value is significantly different at †≤0.1, *p< 0.05, **p< 0.01, ***p< 0.001

5.2.4 Kidney fat and internal fat

Significant differences were observed in kidney fat among treatments in both culling seasons, in LA ($F_{2,15}$=3.790; p=0.047) and LW ($F_{2,24}$=4.057; p=0.030), see Table 8. In both seasons, Lys + Met group was significantly higher than other treatments. Mean value of kidney fat was also significant when compared between culling seasons (t=-

3.140; df=43; p=0.003). Animals culled in LW had higher kidney fat percentage than the animals in LA (Table 9).

Mean internal fat was found significant between control and experimental groups in the LA season ($F_{2,15}$=5.700; p=0.014; see Table 8). Higher internal fat percentage was observed in Lys + Met group (p<0.05). No significant differences in internal fat was observed between two culling season.

In the GLMMs (Table 10), both kidney fat and internal fat were linearly affected by treatments. Kidney fat percentage was significantly higher by Lys group (β=0.137±0.054; t=2.540; p=0.015) and Lys + Met group (β=0.200±0.054; t=3.688; p=0.001). At the same time, kidney fat was higher at LW (β=0.160±0.045; t=3.559; p=0.001). Similarly, Lys group (β=0.191±0.081; t=2.370; p=0.022) and Lys + Met group (β=0.320±0.081; t=3.981; p<0.001) had a positive effect to the internal fat percentage.

Table 11. Pearson correlation coefficients between body condition parameters and slaughter weight of fallow deer (*Dama dama*) culled on different season: 1st group on late autumn (W_{LA}) and 2nd group on late winter (W_{LW}) period.

Parameters	W_{LA} (*n*= 18)		W_{Lw} (*n*=27)	
	r	p-value	*r*	p-value
BCS	0.490	0.039*	0.638	< 0.001***
KFI (%)	0.606	0.008**	0.717	< 0.001***
Kidney wt. (g)	0.425	0.079	0.503	0.008**
Kidney Fat (%)	0.591	0.010**	0.647	< 0.001***
Internal Fat (%)	0.538	0.021*	0.691	< 0.001***

BCS=body condition score; *KFI*=kidney fat index; *Internal fat*=kidney fat+scrotum fat+heart fat
Correlation is significant at *p < 0.05, **p ≤ 0.01 ***, p < 0.001

5.2.5. Relationship of slaughter weight and body condition indicators

Slaughter weight at two culling seasons had a positive relationship with body condition indicators (Appendix 6). In LA culling, slaughter weight was correlated with BCS (r=0.490; p<0.05), KFI (r=0.606; p<0.01), kidney fat (r=0.591; p=0.01), and internal fat (r=0.538; p<0.05). Slaughter weight was also correlated in the LW with BCS (r=0.638;

p<0.001), KFI (r=0.717; p<0.001), kidney weight (r=0.503; p<0.01), kidney fat (r=0.647; p<0.001), and internal fat (r=0.691; p<0.001). Higher correlation coefficients between slaughter weight and body condition were observed during LW culling season (Table 11).

Table 12. Mean (SD) values of carcass traits of fallow deer (*Dama dama*) supplemented with lysine and methionine during the fattening period and culled on different season: 1st group on late autumn (LA; *n*=18) and 2nd group on LW (LW; *n*=27).

Parameters	Season	Treatments			p-value[A]
		Control	Lys	Lys + Met	
Slaughter wt. (kg)	LA	43.7 (3.7)	44.6 (1.6)	45.5 (3.0)	0.572
	LW	41.6 (3.3)	43.0 (4.3)	42.8 (3.9)	0.708
Dressing percentage	LA	50.0 (2.2)[b]	49.7 (1.8)[b]	54.0 (1.2)[a]	0.001***
	LW	55.3 (2.1)[b]	58.0 (2.1)[a]	56.7 (1.6)[ab]	0.026*
Carcass wt. (kg)	LA	22.8 (2.9)	22.9 (1.4)	25.6 (2.0)	0.065[†]
	LW	24.2 (2.7)	26.0 (3.2)	25.4 (2.7)	0.387
Lean (%)	LA	50.6 (1.7)	51.1 (0.7)	51.3 (0.6)	0.522
	LW	50.9 (1.0)	50.1 (0.4)	50.9 (1.0)	0.060[†]
Fat (g/kg)	LA	9.3 (4.1)	10.9 (2.9)	12.3 (4.3)	0.410
	LW	14.8 (6.2)	12.5 (8.3)	14.3 (5.6)	0.749
Bones (%)	LA	9.52 (0.54)	9.35 (0.59)	8.99 (0.27)	0.188
	LW	9.08 (0.44)[a]	6.39 (1.12)[b]	6.56 (0.34)[b]	<0.001***
Organs (%)	LA	3.13 (0.24)	3.15 (0.37)	3.07 (0.27)	0.889
	LW	3.03 (0.30)	2.77 (0.23)	2.80 (0.17)	0.434
Blood wt. (kg)	LA	1.73 (0.17)[a]	1.37 (0.23)[b]	1.88 (0.27)[a]	0.005**
	LW	2.07 (0.17)	1.91 (0.35)	2.08 (0.39)	0.475

Control=pasture with barley and mineral supplementation; *Lys*=9 g/d/deer lysine; *Lys+Met*= 9 g/d/deer lysine and 3 g/d/deer methionine
p-value is significantly different at [†]≤0.01, *p< 0.05, **p< 0.01, ***p≤ 0.001
[a,b]Means with different superscripts differ at p<0.05
[A]One-way ANOVA

5.3. Carcass traits

Animals culled in LA were not significantly different in the slaughter weight, carcass weight, lean proportion, fat proportion, bones and tendons, and internal organs among

treatments; except for blood weight ($F_{2,15}$=7.813; p=0.005; Table 12). Control group and Lys + Met group had higher amount of blood on LA.

Similar results were found in animals culled on LW: slaughter weight, carcass weight, lean proportion, fat proportion, internal organs, and blood weight had no significant differences among treatments, except for the percent bone and tendons ($F_{2,24}$=39.247; p<0.001; Table 12). Percent of bone and tendons were significantly higher in control group than in experimental group.

Table 13. Generalized Linear Mixed Models for carcass traits.

Parameters	Model Term	β	t	p-value
Slaughter weight (kg)	Intercept	9.104±5.936	1.534	0.133
	Initial weight	0.868±0.145	6.006	<0.001***
	Late winter	-1.286±0.783	-1.641	0.108
Dressing percentage	Intercept	42.317±4.456	9.497	<0.001***
	Treatment-Lys	-0.262±1.058	-0.247	0.806
	Treatment-Lys+Met	3.581±1.096	3.269	0.002**
	Late winter	5.449±0.968	5.631	<0.001***
	Initial weight	0.191±0.110	1.738	0.090†
Blood wt. (kg)	Intercept	-0.328±0.618	-0.530	0.599
	Treatment-Lys	-0.270±0.095	-2.836	0.007**
	Treatment-Lys+Met	-0.010±0.097	-0.102	0.919
	Late winter	0.412±0.080	5.123	<0.001***
	Initial weight	0.051±0.015	3.336	0.002**
Carcass wt. (kg)	Intercept	-3.474±4.977	-0.698	0.489
	Late winter	2.073±0.657	3.156	0.003**
	Initial weight	0.668±0.121	5.510	<0.001***
Bones and tendons (kg)	Intercept	-377.7±388	-0.973	0.336
	Late winter	833±51	16.248	<0.001***
	Initial weight	46.7±9.5	4.937	<0.001***

Lys=9 g/d/deer lysine; *Lys+Met*= 9 g/d/deer lysine and 3 g/d/deer methionine
p-value is significantly different at †≤0.01, **p< 0.01, ***p≤ 0.001

However, dressing percentage was found significantly different among treatments in both culling seasons. In LA, dressing percentage ($F_{2,24}$=39.247; p<0.001) was significantly different among treatments, where Lys + Met group was found higher than other treatments. In LW, dressing percentage ($F_{2,24}$=39.247; p<0.001) was found higher in Lys group among treatments (Table 12).

The GLMMs (Table 13) showed that dressing percentage was affected by treatments and culling season, while blood weight, carcass weight and bone and tendons were only affected by culling season. A linear increase of dressing percentage was found in Lys + Met group (β=3.581±1.096; t=3.269; p=0.002) and higher dressing percentage found in the animals culled during LW season (β=5.449±0.968; t=5.631; p<0.001). On the other hand, carcass weight (β=2.073±0.657; t=3.156; p=0.003), bone and tendons (β=833±51; t=16.248; p<0.001), and blood weight (β=0.412±0.080; t=5.123; p<0.001) had a positive increase in the animals culled in the LW season.

5.4. Blood plasma metabolites

5.4.1. Protein-related blood metabolites

Mean values of protein-related blood plasma metabolites between treatments in LA and LW culling season are shown in Table 14. The GLMMs for protein-related blood plasma metabolites are shown in Table 15.

Total protein, ALB, and GLB concentration showed no significant difference among treatments in both culling seasons.

Significant differences were observed in CREA and BUN among treatments in both seasons. In LA, CREA concentration ($F_{2,15}$=8.679; p=0.003) in Lys + Met group was significantly higher among treatments. On the same season, BUN ($F_{2,15}$=8.210; p=0.004) had significant difference between control and experimental group. Blood urea nitrogen concentration was higher in the group of Lys + Met than in the other treatments. However, BUN concentrations ($F_{2,24}$=4.593; p=0.020) in the LW were found

significantly different among treatments. Blood urea nitrogen concentration was found higher in Lys + Met group among treatments.

Table 14. Mean (SD) values of protein-related metabolites concentration of fallow deer (*Dama dama*) supplemented with lysine and methionine during the fattening period and culled on different season: 1st group on late autumn (LA; *n*=18) and 2nd group on late winter (LW; *n*=27).

Parameters	Season	Treatment			p- value[A]
		Control	Lys	Lys + Met	
CREA (μmol/L)	Initial	106.5 (6.8)[a]	93.1 (6.8)[b]	87.8 (10.8)[b]	<0.001***
	LA	103.8 (9.5)[b]	102.3 (8.1)[b]	121.7 (9.1)[a]	0.003**
	LW	111.7 (6.2)	113.8 (10.8)	121.7 (15.9)	0.180
BUN (mmol/L)	Initial	7.79 (0.74)	7.64 (0.69)	7.49 (0.72)	0.526
	LA	8.07 (0.50)[a]	6.25 (0.50)[b]	8.03 (1.37)[a]	0.004**
	LW	5.37 (0.81)[b]	6.04 (0.83)[ab]	6.36 (0.39)[a]	0.020*
TP (g/L)	SI	66.9 (2.1)	66.9 (2.7)	65.1 (2.8)	0.077[†]
	LA	64.0 (3.6)	66.0 (4.1)	62.5 (2.3)	0.235
	LW	65.8 (3.5)	63.3 (4.4)	64.2 (2.3)	0.335
ALB (g/L)	Initial	28.1 (0.8)	27.8 (1.2)	28.4 (1.4)	0.369
	LA	26.2 (1.7)	26.8 (1.7)	26.0 (0.9)	0.605
	LW	27.3 (1.9)	27.6 (1.5)	27.8 (1.4)	0.842
GLB (g/L)	Initial	39.0 (1.9)[a]	39.1 (2.3)[a]	36.7 (2.4)[b]	0.006**
	LA	38.0 (4.0)	39.2 (2.8)	36.3 (3.3)	0.338
	LW	38.3 (2.5)	35.9 (3.6)	36.7 (2.3)	0.190

Control=pasture with barley and mineral supplementation; *Lys*=9 g/d/deer lysine; *Lys*+*Met*= 9 g/d/deer lysine and 3 g/d/deer methionine
CREA, creatinine; *BUN*, blood urea nitrogen; *TP*, total protein; *ALB*, albumin; *GLB*, globulin
p-value is significantly different at [†]≤0.01, *p< 0.05, **p< 0.01, ***p< 0.001
[a,b]Means with different superscripts differ at p<0.05
[A]One-way ANOVA

The GLMMs (Table 15) showed the effects of treatments and culling season on blood metabolites, and also showed the relationship of certain blood metabolites to another.

Creatinine concentration had a significant increase by the supplementation of Lys (β=11.5±3.6; t=3.190; p=0.002) and Lys + Met (β=25.2±3.7; t=6.890; p<0.001).

Creatinine concentration was not affected by culling season but significant decrease of concentration was observed with decreasing BCS (β=-7.39±3.14; t=-2.353; p=0.021).

Table 15. Generalized Linear Mixed Model for protein-related blood metabolites.

Metabolites	Model term	β	t	p-value
CREA (μmol/L)	Intercept	121±10	12.026	<0.001***
	Treatment-Lys	11.5±3.6	3.190	0.002**
	Treatment-Lys+Met	25.2±3.7	6.890	<0.001***
	BCS	-7.39±3.14	-2.353	0.021*
BUN (mmol/L)	Intercept	7.74±0.16	48.377	<0.001***
	Treatment-Lys	-1.39±0.34	-4.074	<0.001***
	Treatment-Lys+Met	-0.486±0.34	-1.429	0.157
	Late winter	-0.373±0.20	-1.884	0.063†
TP (g/L)	Intercept	66.1±0.36	183.782	<0.001
	Treatment-Lys	-1.72±0.97	-1.760	0.082†
	Treatment-Lys+Met	-2.58±0.97	-2.650	0.010**
ALB (g/L)	Intercept	22.1±1.4	15.993	<0.001
	Treatment-Lys	-0.643±0.390	-1.651	0.102
	Treatment-Lys+Met	-1.10±0.40	-2.768	0.007**
	Late winter	1.23±0.31	3.940	<0.001***
	BCS	1.57±0.40	3.883	<0.001***
GLB (g/L)	Intercept	1.14±1.07	1.062	0.291
	TP (g/L)	0.992±0.017	58.731	<0.001***
	ALB (g/L)	-1.020±0.037	-27.741	<0.001***

Lys=9 g/d/deer lysine; *Lys+Met*= 9 g/d/deer lysine and 3 g/d/deer methionine
CREA=creatinine; *BUN*=blood urea nitrogen; *TP*=total protein; *ALB*=albumin; *GLB*=globulin; *BCS*=body condition score
p-value is significantly different at † ≤0.01, *p< 0.05, **p< 0.01, ***p< 0.001

However, BUN had significant decrease in Lys group (β=-1.39±0.34; t=-4.074; p<0.001) and concentration decreased significantly in the animals culled in LW (β=-0.373±0.20; t=-1.884; p=0.063).

Total protein was affected negatively by the supplementation of amino acid. Concentration of TP decreased significantly in Lys + Met group (β=-2.58±0.97; t=-2.650; p=0.010) and marginally decreased in Lys group (β=-1.72±0.97; t=-1.760; p=0.082).

Similarly, ALB concentration had a significant decrease in Lys + Met group (β=1.10±0.40; t=-2.768; p=0.007). Late winter (β=1.23±0.31; t=3.940; p<0.001) culled animals had higher ALB concentration than in the LA.

Concentration of GLB is dependent on the concentration of TP and ALB. The model showed a significant increase of GLB concentration as TP (β=0.992±0.017; t=58.731; p<0.001) increases while decreases as ALB (β=-1.020±0.037; t=-27.741; p<0.001) decreases.

5.4.2 Energy-related blood metabolite

Table 16. Mean (SD) values of blood plasma triglycerides (TRIG) concentration in fallow deer (*Dama dama*) supplemented with lysine and methionine during the fattening period and culled on different season: 1st group on late autumn (LA; *n*=18) and 2nd group on late winter (LW; *n*=27).

Parameters	Season	Treatment			p- value[A]
		Control	Lys	Lys + Met	
TRIG	Initial	0.0393 (0.0697)	0.0020 (0.0077)	0.0047 (0.0181)	0.136
(mmol/L)	LA	0.1200 (0.0888)	0.1100 (0.0672)	0.0450 (0.0704)	0.264
	LW	0.0100 (0.0141)	0.1244 (0.1748)	0.0689 (0.0901)	0.491

Control=pasture with barley and mineral supplementation; *Lys*=9 g/d/deer lysine; *Lys+Met*= 9 g/d/deer lysine and 3 g/d/deer methionine
p-value is significantly different at p< 0.05
[A]One-way Anova

Triglyceride concentration showed no significant differences between treatments in LA and LW culling season (Table 16). However, mean comparison between the initial and final values in LA (t=64; z=2.756; p=0.006) and LW (t=86; z=2.104; p=0.035) differ significantly (Appendix 7). Triglyceride concentrations were significantly higher during culling period (after treatment) than the initial period (before treatment) both in LA

(0.0194±0.0568 *vs*. 0.0917±0.0792) and in LW (0.0126±0.0346 *vs*. 0.0678±0.1193) seasons.

In the GLMM (Table 17), TRIG concentrations were affected by dietary treatments but not by culling season. Triglyceride concentration increased significantly in Lys group (β=0.101±0.028; t=3.664; p<0.001).

Table 17. Generalized Linear Mixed Model for blood plasma triglycerides (TRIG).

Metabolites	Model term	B	t	p-value
TRIG (mmol/L)	Intercept	0.018±0.006	2.732	0.008**
	Treatment-Lys	0.101±0.028	3.664	<0.001***
	Treatment-Lys+Met	0.042±0.028	1.514	0.134

Lys=9 g/d/deULer lysine; *Lys+Met*= 9 g/d/deer lysine and 3 g/d/deer methionine
p-value is significantly different at **p< 0.01, ***p< 0.001

6. Discussion

6.1. Growth performance

Final weight, body weight gain, and ADG of fallow deer are not affected by lysine and methionine supplementation. These results may be due to the level of concentration of lysine and methionine in the diet. The inclusion levels of lysine and methionine were estimated based on the previous studies on red deer (Mendoza-Nazar et al., 2012) and on dairy cattle (Schwab et al., 2004) because nutritional requirements for lysine and methionine are not established yet for fallow deer (NRC, 2007). This result is similar to the study of Huang et al. (2015a) where ADG of sika deer is lower in the diet (13% crude protein) with 3g/kg lysine and 2g/kg methionine inclusion than the ADG of the diet (16% crude protein) without amino acids supplementation. In steer calves, supplementation of methionine up to 0.128% dietary dry matter did not affect the ADG (Torrentera et al., 2017). Contrary results were reported on growing cattle (Xue et al., 2011), steers (Oke et al., 1986; Veira et al. 1991), Holstein cows (Polan et al., 1991) where daily gain, feed conversion, weight gain were improved after supplementation of RPAA. In this study, the body weight of experimental fallow deer at two culling period ranges from 45.4 kg - 47.4 kg which is higher than the weight range of fallow deer in the study of Drew (1992).

Decreased ADG during LW can be attributed to age (Birgersson and Ekvall, 1997), growth stage of animal (Barboza et al., 2009), and seasonal adaptation (Scott et al., 2013). Age of experimental fallow deer culled on LW was approximately 20 months of age to which growth rate is expected to decrease (Birgersson and Ekvall, 1997). Growth rate decreases as the animal exceeds the peak of growth since nutrient metabolism is highest only during early growth of the animals (Barboza et al., 2009). Recent similar study was reported by Huang et al. (2015b) where ADG of sika deer provided with supplementary methionine improved between 20% and 21% in the early 35-day study period and ADG continue to improve until in the late 35-day study period at between 12% and 14%.

First group of culled animals in LA had higher ADG since these animals are still in good growing condition while the animals in the second group of culling in the LW has already reach the point where growth has started to diminish and nutrient metabolism has started to decline. Season has also influence to the growth of the animal due to some adaptions of cervids. Voluntary feed intake of the animals normally decreases during LW. Scott et al. (2013) reported that daily feed intake of deer hinds decreased by 0.146±0.060 MJME/kg live weight$^{0.75}$ from winter to spring.

6.2. Body condition

Body condition of the studied animals is significantly influenced by the supplementation of lysine and methionine both measuring the body condition of live animals (BCS) and the post-mortem evaluation (KFI, kidney fat, kidney mass and internal fat).

The results show that lysine and methionine supplementation increases the BCS of the animals in both seasons of production. Optimal BCS (3.33±0.38) is observed in the animals receiving the combination of lysine and methionine in the diet. This improved BCS observed presumably demonstrate that amino acids supplementation was metabolized properly and good enough to increase muscle and fat deposition in the body. Similar findings were found in fallow deer (Hutchison et al., 2012) and in sheep (Caldeira et al., 2007b), where BCS of animals increase by supplementary feeding. These findings confirm that BCS of the animals could be associated to the nutritional intake (Mattiello et al., 2009; Scott et al., 2013).

BCS had a significant decrease during LW, this notable changes is maybe due to the seasonal adaption of the animals to low nutritional condition, since during this season nutritional intake of the animal decreases (Scott et al., 2013).

Body condition score had a positive relationship to the slaughter weight of the animals, higher correlation coefficients were observed in LW animals. The increase of BCS when slaughter weight increases is probably because BCS is determined by measuring the rump muscle and fat deposition of live animals (Audigé et al., 1998) and slaughter

weight was mainly determined by weighing the whole animals after bleeding, thus this include muscle and body fat. This result is related to the report of Kenyon et al. (2014) that live weight and BCS has a linear relationship and the relationship between live weight and BCS varied from sex, age, and breeds. Furthermore, both gain in live weight and BCS emanates from energy cost depending on animals physiological condition.

Kidney fat index is considered as the most reliable indicator of body condition (Putman, 2005) since fat surrounding kidneys are among of the last fat reserves consumed during fat mobilization (Riney, 1955). In the model, KFI was significantly higher when animals received the combination of amino acids lysine and methionine. This result shows that amino acids are metabolized as a source of energy (Barboza et al., 2009) during the period that animals are expected to grow. In our study, the experimental supplementation started from summer up to the LW season, thus the animals needed sufficient amount of energy and nutrient both for growing and building body fat reserves for surviving winter condition (Tollefson et al., 2010). Higher KFI in the LW observed in this study means that the animals are still in good body condition even after adverse weather conditions. The same result was observed during winter in the experiment of Kuroiwa et al. (2017) in sika deer.

Internal fat depots are also a good indicator to measure body condition or nutritional status in farm animals (Caldeira et al., 2007a). In this study, internal fat depots including kidney fat, scrotum fat, and heart fat are significantly higher when the animals are supplemented with amino acids. Percentage of internal fats is even higher when combination of lysine and methionine is used. High internal fat depots demonstrate that the animals have a good body condition. This further explains that supplementation of lysine and methionine is influencing the nutritional status of fallow deer. Tollefson et al. (2010) reported that nutrition during summer and autumn influence the body condition of the animals since the more nutrients available and consumed during these seasons, the more body fat reserved, body mass and muscle developed. The increase of internal fat depots in the animals culled in the LW further indicates that fallow deer in our study are in good nutritional condition even during and after winter season. Comparable results in mule deer were reported by Tollefson et al. (2010) where body fat percent of the animal started to increase on early autumn until winter and although no significant

influence of nutritional treatments reported, deer in high-digestible-energy diets had the highest body fat observed.

Body condition of the animals appeared to be positively influenced by lysine and methionine supplementation in the diet. Protein or amino acids are metabolized and used as energy source during the period of fattening, hence little or no influence observed in growth. According to Barboza et al. (2009) protein is use for energy when protein or amino acid are in excess or when other energy sources are deficient. The improved BCS, higher KFI value, and internal fat depots are evidence that lysine and methionine increased the body condition or nutritional status of fallow deer.

6.3. Carcass traits

Slaughter weight was not affected by the supplementation of lysine and methionine and season of culling. This result corresponds to the study of Phillip et al. (2007) in red deer and Kerr et al. (1995) in pig. Slaughter weight was not considered as parameter in several studies reported on carcass quality of deer (Volpelli et al., 2002; Wiklund et al., 2003a, b; Hutchison et al., 2012).

Dressing percentage is influenced by dietary lysine and methionine in both season of culling. In LA, the dressing percentage of fallow deer is higher when animals received the combination of lysine and methionine in the diet, however, animals in the LW had a better dressing percentage in dietary lysine. These findings are associated to the growth and metabolism of animals. Animals culled in LA are still on growing stage and so metabolism is still high as explained by Barboza et al. (2009). Thus, animals that received more available nutrient during this stage performed better. The reason of the increased dressing percentage in the LW is unclear, but the possibility that animals during this season continue to metabolize the dietary treatment and probably converts amino acids to energy for body fat reserved and body mass during the supplementation period. This finding is similar to the study of Blanco et al. (2012) on winter feeding on beef cattle where dressing percentage is higher when fed with supplemented diet. Dressing percentage of fallow deer (Volpelli et al., 2002), reindeer (Wiklund et al.,

2003a) and red deer (Wiklund et al., 2003b) were reported higher when fed with commercial concentrate than pasture-fed.

Carcass weight was not highly affected by amino acid supplementation, however, a significant increase of carcass weight on LW was observed. This increase is due to the high dressing percentage of the slaughtered animals. Volpelli et al. (2002) obtained a carcass weight of 25.4 kg, which is slightly higher to the average carcass weight of our study (24.5 kg).

The carcass compositions such as lean, fat, bones, internal organs, and blood weight were not significantly affected by amino acids supplementation except for the notable decrease of bone proportion in the animals culled in the LW period. This decrease is related to the slaughter weight on LW. The observed decrease of blood weight on LA is presumably due to seasonal adaption of the animals and is associated to the less water intake during the adverse weather condition that resulted to lower blood volume (Alamer, 2011).

6.4. Blood plasma metabolites

Our study used the physiological reference intervals for fallow deer (*Dama dama)* from Teare (2013). Reference values may be affected by factors such as method of capture, age, nutrition, season, and health status of the animals (Marco and Lavin, 1999; Teare, 2013). The metabolites concentration in our study corresponds to the reference intervals from Teare (2013).

Concentration of protein-related blood metabolites such as CREA and BUN are elevated with lysine and methionine supplementation. However, TP, ALB, and GLB are not influenced by lysine and methionine supplementation.

Creatinine concentration was higher when animals received lysine and methionine. The increase of CREA concentration in blood plasma is influenced by amino acid intake and is associated to the body condition score of the animals in the group. This corresponds to the report of Russell and Roussel (2007) that CREA production is affected with the

extent of the muscle mass in the body. In our study, the positive correlation of CREA to body condition (Appendix 8) confirmed that CREA is associated to body condition and is influenced by lysine and methionine supplementation. This result is similar to the study of Caldeira et al. (2007a) on sheep. Furthermore, CREA concentration obtained with lysine and methionine supplementation (121.7 μmol/L) corresponds to the reference interval of 53-174 μmol/L of Teare (2013).

The highest BUN concentration in control group (LA: 8.07 mmol/L) and lysine and methionine group (LA: 8.03 mmol/L, LW: 6.36 mmol/L) corresponds to the normal reference value 8.40 mmol/L (Teare, 2013). Blood urea nitrogen indicates high protein metabolism (Russell and Roussel, 2007), or chronic shortage of protein (Payne and Payne, 1987 cited by Kida, 2003).

Increase of BUN concentration in the animals with lysine and methionine in the diet is clearly associated to amino acid intake. This finding agree to the report of Russell and Roussel (2007) that production of urea is influenced by diet when BUN increased after feeding excessive protein to dairy cattle. Similar studies reported on sika deer (Huang et al., 2015b), rusa deer (Tomkins and McMeniman, 2006), reindeer (Wiklund et al., 2003a), sheep (Allen and Miller, 1976; Aharoni et al., 1991), and Japanese black cattle (Watanabe et al., 2010) that BUN concentration is influenced by feeding high protein diet. Increasing levels of urea in the blood is due to the high production of ammonia in the rumen and high exogenous nitrogen in the intestine (Caldeira et al., 2007a). Moreover, the elevated levels of urea on the control group are maybe due to utilization of stored body protein (Caldeira et al., 2007a). As Takasu et al. (2005) reported that the increase of BUN on underfed animals could also be due to proteolysis.

Decreased concentration of TP in the experimental group is unclear. Huang et al. (2015a) reported that decreased concentration was observed when crude protein levels in the diet were reduced. Poljičak-Milas et al. (2004) observed that concentrations of TP in fallow deer increase with aged. However, Marco and Lavin (1999) published that TP, ALB, and GLB concentration are affected by the methods of capture.

The value of TP concentration in the experimental group lysine (LA: 66.0 g/L; LW: 63.3 g/L) and lysine plus methionine (LA: 62.5 g/L; LW: 64.2 g/L) are within the reference interval of 49-78 g/L and normal average value of 64 g/L (Teare, 2013).

Decreased concentration of blood plasma ALB in experimental group lysine and methionine is associated to the decreased concentration of TP since ALB is the largest fraction of the TP (IDEXX, 2014). Albumin is also associated to BCS of the animals. In this study, the increase of ALB as BCS is similar to the result of Caldeira et al. (2007a) in sheep. The value of ALB concentration in the experimental group lysine plus methionine (LA: 26.0 g/L; LW: 27.8 g/L) are within the normal range (23-48 g/L) of the reference value (Teare, 2013).

Concentration of plasma GLB increases as TP increases and the GLB value is also affected with the value of ALB. This is because GLB is the remaining protein after subtracting the concentration of ALB from the TP (IDEXX, 2014). The concentration of GLB reflects physiological mechanisms such as the nutritional status, and level of TP and ALB concentrations. Globulin concentration has the counterbalance function to ALB to support osmotic pressure (Caldeira et al., 2007 a, b). In our study, the lowest (36.3 g/L) concentration value was found in lysine and methionine group and the highest (39.2 g/L) concentration was in lysine group. The concentration value in the study are normal and within the reference interval (10-45 g/L) of GLB concentration.

Plasma TRIG concentration was influenced by lysine supplementation. The highest concentrations are found in the animals receiving lysine. This result can be associated to body condition and age of fallow deer. Higher BCS, KFI, and fat depots were found within the experimental group and although the difference was not significant, higher concentration of TRIG was observed in the animals culled in the LW. These results correspond to the report that the concentration of TRIG are associated to body condition (Serrano et al., 2008) and to age (Otomaru et al., 2016) of the animals. Triglyceride is used as an indicator to monitor the energy metabolism and body condition (Serrano et al., 2008). In our study, the TRIG concentration in lysine group (LA: 0.1100 mmol/L; LW: 0.1244 mmol/L) are within the reference intervals (0.00-0.65 mmol/L) of Teare (2013) and are found in normal median value.

7. Conclusions

It has been shown that ADG and weight gain were not influenced by the levels of amino acid supplementation. Thus, the first hypothesis of the study was rejected. Although amino acids supplementation are proven essential and effective for optimal performance of the animal's growth, in our study, it was found out that supplementation of lysine and methionine needed a good timing of supplementation and proper inclusion levels.

The carcass traits of fallow deer were slightly affected by lysine and methionine since carcass traits were associated to growth. The reliable increase of dressing percentage from LA up to LW, when fallow deer received lysine and methionine supplementation proved that supplementation of amino acids influenced the growth and metabolism of the experimental animals, and resulted to a higher dressing percentage than without amino acid supplementation.

Lysine and methionine supplementation revealed interesting results on body condition. The supplementation of amino acids had high influence on body condition parameters such as BCS, KFI, kidney fat, kidney mass, and internal fats of the animals which proved what was hypothesized. The increased body condition parameters indicated that fallow deer supplemented with amino acid had a good nutritional status than in control group. These findings suggest that supplementation of amino acids has advantage for deer production especially in preventing mortality of young animals due to winter condition.

Blood plasma metabolites further showed the effect of lysine and methionine supplementation to the extent of metabolic levels. The increased concentration of protein-related blood metabolites such as creatinine and blood urea nitrogen and the energy-related metabolites (TRIG) validates that lysine and methionine influenced the physiological status and metabolism of fallow deer. Thus, what we hypothesized was accepted. These results provide a better understanding on how amino acids interrelate to different functions such as growth, body condition, and metabolism.

The season of culling influenced growth, carcass traits and body condition of fallow deer. The decrease of ADG and slaughter weight in the LW reflected that growth and metabolism slow down during this season. The improved body condition during LW showed that fallow deer had a good nutritional status that was influenced by supplementation. These effects of culling seasons to the production parameters and physiological functions of fallow deer confirm our hypothesis.

In general, supplementation of lysine and methionine to fallow deer had low influence on production parameters including growth performance and carcass traits. The effects of amino acids supplementation was higher on the physiological function on how amino acids interact to the body condition and blood plasma metabolites of fallow deer. Season of fattening and culling of fallow deer had also influence on the production and physiology of the animals. Further research should focused on supplementation on early growth of fallow deer, and on the adequate amount of supplementation, that remain challenging due to the absence of standard nutritional requirements for amino acids on deer, and on ecological and economic importance of supplementation.

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