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EFFECT OF FEED RESTRICTION ON BODY COMPOSITION AND METABOLISM OF GOATS OF DIFFERENT GENDERS

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NHAYANDRA CHRISTINA DIAS E SILVA – daughter of Geraldo Dias da Cruz and Sandra Helena da Silva Dias, born in Governador Valadares, Minas Gerais, on July 4 1985. In March 2004, started the Animal Science Undergraduate Course at the Federal University of the Jequitinhonha and Mucuri - UFVJM, Campus of Diamantina, graduating in December 2008. In March 2010, he joined the Graduate Program in Animal Science Course, Master's Degree, Area of Concentration in Nutrition and Animal Nutrition at the Veterinary School Universidade Federal de Minas Gerais (EV/UFMG), Belo Horizonte - MG, under the guidance of Prof. Dr. Iran Borges, defending thesis in February 2012. In March 2012, started the PhD course at the Graduate Program in Animal Science at the Universidade Estadual Paulista "Julio de Mesquita Filho" - Unesp - Jaboticabal Campus, under the guidance of Prof. Dr. Kleber Tomás de Resende. During her doctorate, she did sandwich internship at E (kika) of La Garza- American Institute for Goat Research, Langston University, Oklahoma, USA, under the guidance of Prof. Dr. Arthur L. Goetsch.

“The human science in any way deny the existence of God. When I consider how many and how great things the man understands, research and can perform, then clearly recognize that the human spirit is God's work...”

Galileo Galilei

Dedico...

Aos meus pais,

Sandra Helena da Silva Dias e Geraldo Dias da Cruz, pela confiança e amor incondicional...

Amo vocês!

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SUMMARY

EFEITO DA RESTRIÇÃO NUTRICIONAL SOBRE A COMPOSIÇÃO CORPORAL E METABOLISMO DE CAPRINOS DE DIFERENTES SEXOS

RESUMO - O objetivo deste estudo foi avaliar o efeito da restrição nutricional sobre o metabolismo energético e proteico de cabritos de 15 à 45 kg de peso corporal, sendo que foram utilizados 72 cabritos Saanen: 24 machos inteiros, 24 machos castrados e 24 fêmeas, com peso corporal de 15,76 ± 0,174 kg e idade inicial de 108,4 ± 18,86 dias (Experimento 1) e de 84 cabritos Saanen (26 machos inteiros, 27 machos castrados e 31 fêmeas) com peso corporal de 30,3 ± 0,87 kg (Experimento 2). Um esquema de parcelas subdivididas foi utilizado para avaliar a condição sexual (3 sexos = machos inteiros, machos castrados e fêmeas) e a restrição nutricional (3 níveis de restrição nutricional: 0% [ad libitum], 25% e 50%). Em ambos experimentos, dentro de cada sexo, foram formados seis blocos de três animais e dentro de cada bloco, onde os animais foram distribuídos aleatoriamente em cada nível de ingestão. Assim, a alimentação foi estabelecida dentro de cada bloco com base no consumo dos animais alimentados ad libitum. Os animais de cada grupo foram abatidos quando os animais alimentados ad libitum atingiram 30 kg (Experimento 1) ou 45 kg (Experimento 2). Foram avaliados a retenção de proteína e energia e o perfil metabólico/hormonal no sangue, onde foram analisados a glicose, proteína total, albumina, ureia, creatinina, colesterol, ácido graxos não-esterificados (NEFA), beta-hidroxibutirato (BHB), aspartato aminotransferase (AST), gama glutamil-transferase (GGT), creatinina quinase (CK), triiodotironina (T3), tiroxina (T4), e fator de crescimento semelhante à insulina (IGF-1). As fêmeas apresentaram maior retenção de gordura corporal (% peso de corpo vazio), independentemente do nível de restrição nutricional imposto (P <0,001). Tanto a restrição nutricional quanto o sexo afetou o metabolismo energético e proteico dos animais (P <0,05). Fêmeas de 15 a 30 kg de peso corporal alteraram seu metabolismo glicolítico para manter a deposição de gordura, mesmo quando submetidas à restrição nutricional, enquanto machos alteraram principalmente o seu metabolismo proteico para manter a síntese de proteínas. Machos púberes (de 30 a 45 kg) não foram capazes de manter a síntese de proteínas durante a restrição alimentar enquanto fêmeas e machos castrados mantiveram a deposição de gordura, mesmo quando submetidos a restrição alimentar.

Palavras-chave: metabolismo energético, metabolismo proteico, retenção, sangue

EFFECT OF FEED RESTRICTION ON BODY COMPOSITION AND METABOLISM OF GOATS OF DIFFERENT GENDERS

ABSTRACT - The objective of this study was to evaluate the effect of feed restriction on energy and protein metabolism of 72 Saanen kids: 24 intact males, 24 castrated males, and 24 females with initial BW of 15.76 ± 0.174 kg and initial age of 108.4 ± 18.86 days (Experiment 1) and of 84 Saanen goats (26 intact males, 27 castrated males and 31 females) with initial body weight (BW) of 30.3 ± 0.87 kg (Experiment 2). A split plot design was employed (3 genders = intact males, castrated males, and females; 3 levels of feed restriction = 0% [ad libitum], 25%, and 50%). Groups of 3 goat kids was formed by gender (each goat eating one level of feed restriction); goats of each group were slaughtered when animals fed ad libitum reached 30 kg BW (Experiment 1) and 45 kg (Experiment 2). Blood samples were evaluate glucose, total protein, albumin, urea, creatinine, cholesterol, non-esterified fatty acid, beta-hydroxybutyrate, aspartate aminotransferase, gamma glutamyltransferase, creatine kinase, triiodothyronine, thyroxine, and insulin-like growth factor. Females had greater retention of body fat (% empty BW) regardless the level of feed restriction ($P<0.001$). Both gender and feed restriction affected energetic and proteic metabolism of goats ($P< 0.05$). Females from 15 to 30 kg BW changed their glycolytic metabolism to retain fat deposition even when subjected to feed restriction, while males mainly changed their protein metabolism to retain protein synthesis, and were less affected by feed restriction. Pubertal males (from 30 to 45 kg) were not able to keep protein synthesis during feed restriction and females and castrated males to keep the fat deposition even when they are subjected to feed restriction.

Keywords: blood, energy metabolism, protein metabolism, retention

CHAPTER 1- GENERAL CONSIDERATIONS

1.INTRODUCTION

The world head of goats is estimated at 975 million head, according to the United Nations Food and Agriculture Organization (FAO, 2013), and approximately 8.8 million goats are in Brazil, and 88% of the Brazilian goat herd are located in the Northeast region (IBGE, 2014).

Growth production of goats is accompanied by the interest of many researchers to study various aspects of the proper management of dairy goats, and animal nutrition stands out as one of the most studied areas (HAENLEIN, 2001). Research that addresses the deeper nutrition are key, as in the past little attention was paid to the study of other aspects within this line of research, such as the nutritional challenges facing this species. Among these challenges, we can highlight the feed restriction, either quantitative or qualitative and to understand their effect on the physiology in the animal, can generate large contributions that can reduce unnecessary expenses in a production system.

The effects of the feed restriction on the animal metabolism are still not fully understood, however, it is known that the digestive utilization by the animal changes according to the level of restriction imposed (MERSMANN et al. 1987). It may also vary according to the gender of the animal, resulting in different ways in the use of nutrients (BERESKIN et al., 1990), which may suggest different responses for deposition of nutrients and energy.

According to Butterfield and Berg (1976), the effect of gender has been shown determining differences in growth and deposition rates of different body tissues, which directly affects body composition and therefore nutrient requirement. In addition, according to Payne e Payne (1987), it is possible that animals in different physiological periods, sexual conditions and nutritional levels have different metabolic patterns.

The biochemical blood composition reflects clearly the metabolic status of the animal and therefore can become a great tool to assess tissue damage, disorders in

the functioning of organs, and physiological challenges and metabolic imbalances or nutritional origin can identify potential problems before they come to express drop in production and fertility disorders (KELLY, 1996).

Given this scenario, it is clear that there are still some gaps that need to be researched and clarified, particularly regarding the change in body composition and changes in the blood metabolic profile according to the feed restriction level in the growth phase and different genders. This study has the general objective to evaluate the main differences that continuous feed restriction (moderate or severe) can cause in intact males, females and castrated males goats from 15 to 45 kg of body weight.

The specific objectives of this study is to evaluate the effect of feed restriction levels on body composition (retention) of intact males, females and castrated males; and to study the endocrine, energy and protein metabolism through metabolic indicators (enzymes, hormones and metabolites) in intact males, females and castrated males goats submitted to different levels of feed restriction.

Our hypothesis are that the retention of nutrients and energy are influenced by the level of feed restriction, gender and by the growth phase, wherein different gender respond differently to feed restriction level.

2. WHY SHOULD ONE STUDY THE DIFFERENT STAGES OF GROWTH?

According to Owens (1993), the growth may be defined as the production of new cells, which may be measured as an increase in body weight per unit of time and it can vary from animal to animal and mainly involves adjustments in the organs and tissues of animals, according to physiological needs of the body. According to Grant and Helferich (1991), pre-natal growth is faster and occurs at an exponential rate in all species, however, the growth rate is variable in the animal due to differences in skeletal size, birth weight and gestational age.

Growing animals show proportionally greater deposition of protein and minerals when younger and greater fat deposition with advancing age until the time they reach chemical maturity (FERREIRA et al., 1998). Therefore, body fat is the body component that undergoes greater variation during growth process and its accumulation in the body can directly affect the feed efficiency of the animal. Thus,

periods of growing until puberty are usually characterized by large efficient use of nutrients and energy for the animal. However, it is important to note that this period as well as the animal size at sexual maturity, can vary between breed and gender in the same species.

The body tissues tend to grow and develop in a specific sequence, the nervous tissue is the first to develop, followed by bone tissue, muscle tissue and, finally, adipose tissue (Figure 1).

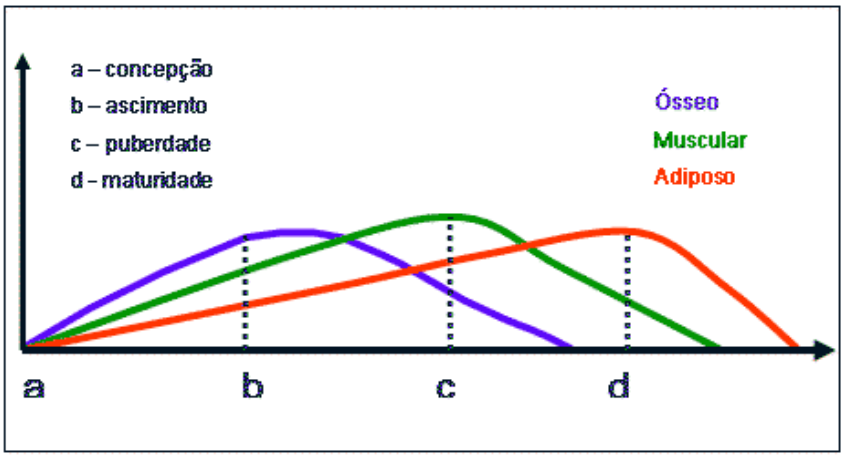

Figure 1. Growth curve of body tissues (Owens 1993 with adaptations).

After birth until puberty, the growth of muscle tissue rate is greater than the rate of bone tissue and adipose, while from puberty to maturity growth of adipose tissue becomes predominant. So, at maturity, it can be said that muscle growth reaches the maximum potential, then weight gain is mainly composed of fat (OWENS et al. 1993) and this profile is related to the animals gender.

The variation in the growth of animal of different genders (intact males, castrated males and females) is through action and concentration of certain hormones that influence muscle, and fat deposition hence body composition and nutritional requirements (SAHLU et al., 2004). Webster (1986) reported that the differences in body composition due to gender, maturity and manipulation of the environment, with temperature or lack or absence of light are main mediated by hormones.

The concentration of androgen hormones mediated by gender directly influences the partition and deposition of nutrients and makes males exhibit greater growth compared to females and castrated males (GOMES, 2008). The greatest concentration of testosterone in males is responsible for promoting the action of Insulin-like growth factor/growth hormone releasing hormone/growth hormone (IGF/ GHRH / GH) axis, resulting in greater concentrations of hormones involved in growth (SILVA et al.,1992).

The effects of GH on growth, cartilage and protein metabolism depend on the interaction between GH and the somatomedins (IGF). GH has an additional role in the growth indirectly by to be hyperglycemic and has anabolic effect, increasing the transport of amino acids into cells, increased protein formation. Thus, for the effective action of IGF / GH in growth, adequate insulin activity is necessary as well as adequate availability of carbohydrates and amino acids (GUYTON, 1982). Furthermore, most circulating GH and IGF in the blood stream stimulate the catabolism of lipids, mobilization and release of free fatty acids from adipose tissue and their increase in body fluids. In the tissues, these hormones increase the conversion of fatty acids into acetyl-CoA, which is used for energy production and thus, favoring the use of fat for energy production, and promoting reduction in plasma cholesterol concentration, by increasing the metabolic rate (GANONG, 1991).

Azzarini (1979), working with different genders sheep, found that gender affects the growth rate and deposition of distinct animal body tissue, and the rate of growth in intact males is 9% greater than in castrated males and 5 % greater than in females.

According to Jacobs et al. (1972), testosterone promotes muscular and skeletal growth, determining leaner and greater muscle content in intact males carcasses. Still, according to Arthaud (1977), intact males show faster weight gain rate, convert lean meat more efficiently and have a satisfactory muscle: bone ratio, with lesser proportions of fat in the carcass, compared to castrated males or female, which directly affects the body composition of the animal. Thus, body composition is closely linked to the growth of the animal and through it one can evaluate the potential of animal growth raised in different production systems and verify changes in the gain composition depending on various factors such as breed, gender, body

weight and composition of the diet (RESENDE, 2004). Knowledge of animal body composition allows us to identify the priority of nutrients, depending on the physiological stage of the animal.

All in all, the most obvious effect of gender in body composition occurs due to adipose tissue deposition. Females tend to have lesser weight at maturity (BERG; BUTTERFIELD, 1976).

Despite advances in research with goats in recent years, many factors related to the use of nutrients have not been sufficiently elucidated, so it is important to consider the differences in digestive physiology and fate of nutrients in metabolism between species, genders and growth phases to adequately represent the utilization of nutrients from feed for goats. Thus, studies involving the isolation of the factors involved in the metabolic and hormonal profile, nutrient use efficiency are necessary to generate data that can contribute to determining the appropriate management according to the physiological characteristics of each animal group.

3.FEED RESTRICTION

Feed restriction can be defined as any quantitative and qualitative limitation, which leads to lesser growth rate than normal (BOIN; TEDESCHI, 1997). Therefore, when the quantity of nutrients given to the animals is restricted, the growth rate is below normal irrespective of the stage of animal growth (OWENS et al., 1993). However, while nutritional deficiency may negatively affect the development of the animal it can also be a tool to improve digestive efficiency and metabolic nutrients when used properly (CHIBA ET AL., 2002), acting as a management strategy.

When animals are without feed or subjected to some nutritional management unable to meet their needs, the same use physiological mechanisms in an attempt to minimize the damage caused (FORBES, 1995). According to Diskin et al. (2003), prolonged nutritional deficiency in ruminants can infer various metabolic and hormonal changes in the body by changing the levels of circulating metabolites and hormones in the blood plasma reflecting on its growth, performance and reproductive life, especially when it comes to females.

During feed restriction, the digestive system adjusts its mass availability of nutrients and this makes the digestion of feed and absorption of nutrients can be affected by changing the enzyme secretion and increasing the absorptive area of the small intestine (Pond et al, 1988), which causes a reduction of amino acid oxidation, increasing the trapping efficiency (SCHEUS et al., 1985).

Also, for nutritional deficiency, the animal's maintenance requirement decreases. This fact is explained by the difference in size of organs, since the energy expended by the viscera corresponds approximately 50% of total energy for maintenance. This is because visceral tissues such as the gastrointestinal tract, and liver have a high protein turnover (SILVA, 2002).

Studies with animals kept under nutritional deficiency showed improvements in absorptive rates primarily by the reduction in requirements, caused by nutritional deficiency (JORGE et al., 1999). Moreover, Doreau et al. (2003) found that nutritionally restricted kept sheep showed no physiologic and structural adaptations can reduce the effects of the restriction, being observed in some cases, reduction in the utilization efficiency of the diet during the restricted period, and thereafter. The authors found that the energy absorption capability in the hepatic portal system has not changed, however the nature of absorption of nutrients changed.

It is known that feed scarcity are inert to many farming systems, either due to climatic conditions or financial problems. In this sense, many studies have been conducted to evaluated the consequences of feed restriction on the animal production, mainly during the period of gestation and lactation (GREENWOOD; BELL 2002; LONG et al., 2009; VELEZ; DONKIN, 2005). For an exemple, many studies about feed deprivation have been performed in Australia due to the environmental conditions of this country (GREENWOOD et al. 2006; HINCH; BRIEN, 2013). However there is still a gap of information about the effects of feed restriction during the growth period, specially for goats.

4.METABOLISM INDICATORS

Metabolic and nutritional studies have been used to establish through blood dosages of certain metabolites, enzymes and hormones, the degree of physiological

suitability of such animals the major metabolic pathways related with energy, protein and minerals, as well as the function of vital organs (GONZALEZ; SCHEFFER, 2003). To date, the metabolic indicators have been widely used to assess the nutritional status of the herds (GONZALÉZ et al., 2000) and the routine use of metabolic profiles in this sense has helped in showing metabolic problems that previously went unnoticed.

Thus, the trend in the coming years is to expand the use of these metabolic indicators to determine the optimal levels of nutrients and studies of adaptive physiological ability of animals, identifying the different metabolic pathways and utilization rates of each nutrient (protein, energy and mineral) specific to sexual condition, life stage and nutritional level (GONZÁLEZ et al., 2000).

However, it is important to emphasize that the main limitation for determining plasmatic hormonal profile is the lack of specific commercial kits on the market for measurement of certain hormone in several species. In these species, one generally uses kits for human, however besides validation for the given species reference data values for comparison of blood parameters for different physiological stages, and so forth should be provided.

In goats, the biochemical characteristics differ from those in other ruminants because their greater ability of physiological adaptation to various conditions (MORAND-FHER, 2005), thus justifying the studies with the goats to the improvement of feeding programs and, consequently, increasing production efficiency of livestock.

Among the blood metabolites commonly used to evaluate the energy status are the beta-hydroxybutyrate (β-HB) and non-esterified fatty acids (NEFA), which are directly related to the mobilization of lipid reserves rate of energy deficit moments (GOMIDE et al., 2004). The β-HB shows considerable increase when the negative balance becomes severe, while under moderate deficit situations NEFA constitute the most significant metabolite to estimate this imbalance, quickly responding to changes in feed consumption (RUSSEL; WRIGHT, 1983) .

Under severe nutritional deficiency situations, animals suffer the intensification of mobilization of body reserves. In these circumstances, the lipid components of body reserves are mobilized and the lipolysis of triglycerides releases NEFAs (which are

usually long-chain fatty acids) and glycerol (Figure 2). Glycerol is taken up by cells and used for glucose production or can be used to re-form triglycerides. Once taken up by hepatocytes, NEFAs are esterified. The esterified fatty acids then have several fates:

1) They can recombine with glycerol to form triglycerides, which are packaged into VLDL. The VLDL are exported from the liver or (if produced in excess) are stored as fat within the hepatocyte (eventually causing lipidosis).

2) They can enter the mitochondria (in a reaction that requires carnitine) and be used for energy production (through the Kreb's cycle) or ketone formation. Within the mitochondria, esterified fatty acids undergo β-oxidation to acetyl CoA. Acetyl CoA combines with oxaloacetate in the Kreb's cycle (tricarboxylic acid cycle) to form citrate. Continued oxidation in this cycle leads to energy (ATP) production.

If oxaloacetate supplies are low (oxaloacetate is used as a substrate for gluconeogenesis in states of negative energy balance and is derived from propionate), all produced acetylCoA, it should condense with oxaloacetate in the Krebs cycle, will have to be converted to ketone bodies, namely the acetoacetate, BHB and acetone which are soluble in the blood and can be excreted in the urine (GONZALEZ; SILVA, 2006).

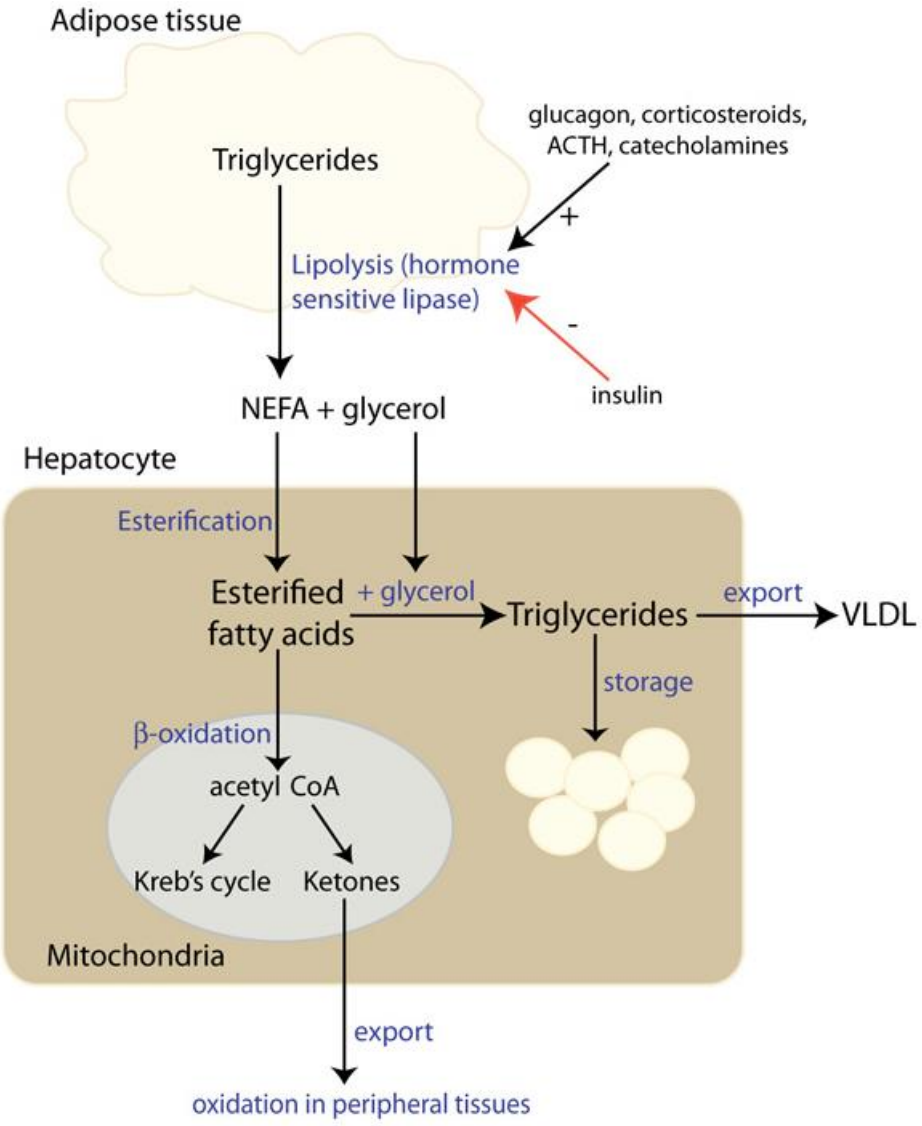

Figure 2. Formation of ketone bodies in the liver (Source: eclinpath).

Among the ketone bodies formed during the mobilization of body reserves (acetoacetate, beta-hydroxybutyrate and acetone), according to the literature (GOMIDE et al., 2004), the B-HB is normally plasma or serum concentrations greater than acetone and acetoacetate as well as being the predominant circulating. Plasma concentrations of B-HB has significant correlation with plasma concentrations of acetoacetate, but this is unstable in samples, while the BHB is relatively stable, which explains its content in blood samples.

The assessment of protein status can be performed by determining the concentrations of total protein, albumin, urea and creatinine in the blood of animals

(PAYNE; PAYNE, 1987) and the reducing the levels of these factors in plasma is strongly related to deficiencies of protein intake in diet (KANEKO et al., 1997). Seasonal or even daily changes in energy: protein ratio in the diet influence urea levels in the blood and their utilization by the animal, demonstrating the protein status of the animal in the short term, while albumin demonstrated long-term and creatinine demonstrated character chronic (WITTWER et al., 1993).

In addition to the metabolites, the study of enzymes is of great importance for the understanding of physiological processes, since they are directly or indirectly related to the main metabolic and nutritional routes having the function of catalyzing (accelerate) the biochemical reactions (GONZÁLEZ; SCHEFFER, 2003).

Aspartate aminotransferase (AST) acts as a catalyst in metabolic processes occurring in abundance in the liver and muscle tissues where high AST values associated with low cholesterol and albumin values reveal disturbances in liver function, suggesting the presence of imbalance in protein and / or energy metabolism (GONZÁLEZ; SILVA, 2006).

Gamma glutamyltransferase (GGT) is linked to the membranes, especially in the epithelia of bile and kidney ducts, serving as hepatic metabolism indicator and renal function (González & Silva, 2006), on the other hand creatine kinase (CK) is primarily related to local activity muscle tissue (skeletal and cardiac), acting in the phosphorylation of creatine as an additional form of energy storage in phosphate bonds; its level is increased when muscle damage from nutritional origin occurs, which is common in cases of severe and chronic malnutrition observed in cases of cachexia.

Another system that plays an essential role in metabolism is the endocrine, maintaining the internal environment in production, development, and storage and utilization of substrates, conducting physiological processes and providing answers in certain metabolic mechanisms (Kaneko et al., 1997), making it possible to identify metabolic imbalances of animals, especially in periods of high nutritional requirement (MORRISON et al., 2002). Among the hormones related to the main metabolic pathways of nutrition and animal growth, are: IGF-1, which is an important growth factor and its level is elevated during periods of intense tissue development (KANEKO et al. 1997).

The triiodothyronine (T3) and thyroxine (T4) are related to the basal metabolism, T3 being the main indicator of thyroid hormone metabolism.

According Toniollo, et al. (1998), thyroid hormones have an important effect on the metabolism and other functions of the animal body.

T3 and T4 modulate all metabolic pathways through changes in oxygen consumption and changes in the metabolism of proteins, lipids, carbohydrates and vitamins (NORMAN; LITWACK, 1997; BOELAERT; FRANKLYN, 2005). They stimulate the synthesis and protein degradation, lipogenesis and lipolysis in adipose tissue, increment the absorption of carbohydrates from the intestine and liver production of glucose by gluconeogenesis stimulate glycogenolysis by insulin secretion and activate substrates that are part glycolysis (ACHMADI; TERASHIMA, 1995; GUYTON; HALL, 2002).

The concentration of metabolites and hormones cited above may vary from species to species, physiological stage, environmental conditions and gender (GONZÁLEZ; SILVA, 2006). As an example, goats generally show lesser blood concentrations of urea, glucose and creatine kinase and increased aspartate aminotransferase and gamma glutamyl transferase activity compared to bovine and ovine (KANEKO et al., 1997). Furthermore, it is expected that goat intact male show greater concentrations of IGFs resulting from testosterone action than compared to castrated male and female when fed ad libitum and under balanced diets (MAHGOUB, et al. 2004).

Given this scenario, it is clear that there are still some gaps that need to be researched and clarified, particularly regarding the change in body composition and changes in the blood metabolic profile, according to the feed restriction level imposed on goats in the growth phase - 15 kg to 45 kg as well as seeking a better understanding of the factors affecting these parameters, justifying the importance of this study.

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CHAPTER 2 - GROWING GOATS OF DIFFERENT GENDERS HAVE DISTINCT METABOLIC RESPONSES TO FEED RESTRICTION

ABSTRACT-We investigated the effect of gender on the hormonal and metabolic changes in prepubertal goats subjected to different levels of feed restriction. Seventy two Saanen kids were used: 24 intact males, 24 castrated males, and 24 females with initial BW of 15.76 ± 0.174 kg and initial age of 108.4 ± 18.86 days. A split plot design was employed (3 genders = intact males, castrated males, and females; 3 levels of feed restriction = 0% [ad libitum], 25%, and 50%). Groups of 3 goat kids was formed by gender (each goat eating one level of feed restriction); goats of each group were slaughtered when animals fed ad libitum reached 30 kg BW. Blood samples were collected every 10 days to evaluate glucose, total protein, albumin, urea, creatinine, cholesterol, non-esterified fatty acid, beta-hydroxybutyrate, aspartate aminotransferase, gamma glutamyltransferase, creatine kinase, triiodothyronine, thyroxine, and insulin-like growth factor. Both gender and feed restriction affected energetic and proteic metabolism of goats ($P< 0.05$). Females changed their glycolytic metabolism to retain fat deposition even when subjected to feed restriction, while males mainly changed their protein metabolism to retain protein synthesis, and were less affected by feed restriction.

Keywords: blood metabolites; castrated; energy; females; males; protein;

1.INTRODUCTION

Metabolism studies allow the evaluation of disturbances in the functioning of organs as well as animal adaptation facing nutritional challenges (GONZÁLEZ et al. 2000).

One of the major nutritional challenges imposed on animals is feed deprivation, either by food shortages in harsh regions, by seasonal variation in feed availability, or due to nutritional management strategies. Previous studies have addressed the changes in the metabolic energy and protein profile of fasted dairy cattle (CHELIKANI et al. 2004) and/or in compensatory growth in cattle and sheep (THERKILDSEN 2005; ATTI; SALEM, 2007). However, there is a lack of information about such changes in long periods of continuous feed restriction in goats.

In a dairy herd, not only females exert economic importance, but also intact males by to be used for breeding and also the castrated males, since the practice of castration is common and it is justified by facilitating the management of males, and can handle females and males together, make the animals more docile and reduce the strong odor caused by the sex glands, characteristic of intact males, which affect the marketing of meat (Braga *et al*. 2003; Simplício *et al.* 2000).

Gender interferes directly in the growth of body tissues of animals, because males tend to produce leaner carcasses due to the effect of testosterone whereas females and castrated males have a greater proportion of fat in the carcass (GUNN 2013). Thus, the practice of feed restriction in goats of different genders results in changes in carcass composition (YAÑEZ et al. 2006). Therefore, it is plausible to assume that animals of different genders use distinct metabolic mechanisms to adapt to periods of feed scarcity, which could result in large changes in blood parameters.

Several studies have addressed the effect of breed and physiological state on metabolism throughout the life of ruminants (TODINI et al. 2007; FERRARETO et al. 2014). However, little is known about the effect of gender and feed restriction on the metabolic and hormonal profile during growth, especially in the prepubescent stage where poorly planned nutritional management, whether

underfeeding or overfeeding, can compromise the productive and reproductive life of the animal.

The objective of this study was to investigate the effect of gender on the blood parameters and energy metabolism and protein in prepubertal goats (from 15 to 30 kg body weight - BW) subjected to levels of feed restriction. The results presented herein may contribute directly in the adoption of nutritional strategies in goat kids.

2. MATERIAL AND METHODS

2.1 Animals and experimental design

Humane animal care and handling procedures were followed, according to the University's Animal Care Committee on the Ethical and Animal Welfare (Comissão de Ética e Bem Estar Animal – CEBEA), under protocol number 008919-08.

During the pre-experimental period goats kids were adapted to the diet, housing, and daily handling, were fed ad libitum, and received treatment against parasitic agents. The goat kids were housed in individual 1.0 m^2 pens equipped with a feeder and water trough.

For this study we used 72 Saanen kids comprising 24 intact males, 24 castrated males, and 24 females with initial BW of 15.76 ± 0.174 kg and age of 108.4 ± 18.86 days.

In this study was used a split plot design in a 3 x 3 factorial arrangement (3 genders = intact males, castrated males, and females; 3 levels of feed restriction = 0% [ad libitum], 25%, and 50%). Eighteen intact males, 18 castrated males, and 18 females were distributed among the feed restriction levels. For each gender were formed six groups of three goat kid each. Each group consisted of a goat kid fed ad libitum (0% feed restriction), a goat kid subjected to 25% feed restriction (pair-fed, receiving 75% of the feed ingested by the goat kid fed ad libitum on the previous day), and a goat kid subjected to 50% feed restriction (pair-fed, receiving 50% of the feed ingested by the goat kid fed ad libitum on the previous day).

To assess fat and protein retention goat kids were slaughtered when the goats that were fed ad libitum reached 30 kg of BW; they were slaughtered along with the other animals of the same group (equal days of experiment). At the beginning of the experiment 6 intact males, 6 castrated males, and 6 females were slaughtered at 16.6 ± 0.997 kg BW and considerate baseline animals to estimate the initial body composition of fat and protein.

2.2 Diet and feed intake

The diet (Table 1) was formulated to meet the requirements of growing goat kids according NRC (2003) and was offered twice a day, at 07:00 and 17:00 h.

Feed intake of the animals fed ad libitum was adjusted daily to ensure 10% of orts from the diet offered. The diet offered and orts were weighed, recorded, and sampled daily. Feed intake was considered as the difference between diet offered and orts.

Feed intake of the animals fed ad libitum was adjusted daily to ensure 10% of orts from the diet offered. The diet offered and orts were weighed, recorded, and sampled daily.

Table 1. Chemical composition of feed ingredients and experimental diet

Ingredient	% DM[1]
Dehydrated corn plant	45.4
Soybean meal	22.3
Corn meal	26.6
Soybean oil	1.6
Limestone	1.0
Mineral premix[6]	2.2
Ammonium chloride	0.9
Chemical composition	**g/kg DM**
Dry matter[2]	854
Ash	65
Organic matter	935
Crude protein	204
Ether extract	80
Total carbohydrate	654
NDF[3]	355
ADF[4]	184
Gross energy[5]	18.7

[1]DM = dry matter;
[2]in g/kg as fed;
[3]NDF = neutral detergent fiber;
[4]ADF = acid detergent fiber
[5]in MJ/kg DM
[6] Composition, per kg, as-fed basis: 190 g of Ca; 92 g of Cl; 73 g of P; 62 g of Na; 44 g of Mg; 1.35 g of Zn; 1.06 g of Fe; 0.94 mg of Mn; 0.73 g of F ; 0.34 g of Cu; 18 mg of Se; 16 mg of I; 3 mg of Co.

2.3 Collection and process of blood samples

Blood samples were collected in the morning (before feeding) from all of animals (18 of each gender) periodically every 10 days during the experimental period (70 days). The blood was collected using a 10 mL *vacutainer blood collection* tube with anticoagulant for blood plasma separation and another tube without anticoagulant for serum separation. Blood collection was performed by puncturing the jugular vein with a sterile needle (for specific crops vacuum) and the material collected was placed in an isothermal box with ice blocks for preservation at 10 ºC. Subsequently, the material was centrifuged at 3.000 rpm for 15 min to separate plasma or serum. The supernatant (plasma and serum) was collected and 5 mL was stored in eppendorfs, properly identified, and frozen at -20ºC until further analysis.

2.4 Slaughter procedure and sampling

When animals fed ad libitum reached 31.30 ± 1.63 kg of BW, the respective group of animals fed ad libitum, 25, and 50% feed restriction were slaughtered to estimate the body retention of protein and fat. Animals were slaughtered without prior water or food deprivation and were weighed immediately before slaughter. At slaughter, animals were stunned with a captive bolt pistol followed by severing of the jugular vein and carotid artery. After the removal of organs and viscera, the gastrointestinal tract (GIT) was weighed before and after removal of all content. Empty BW (EBW) was determined subtracting from the BW in before slaughter the contents weight of GIT and bladder. Thereafter, the empty whole body was initially frozen and then cut into small pieces, ground with a large screw grinder through a plate with 0.32 cm holes, and mixed by two additional passes through the grinder. After grinding and homogenization, samples were collected, frozen again, and freeze-dried for 72 h for dry matter (DM) determination and subsequently milled in a ball mill and packed in plastic pots for further analysis.

2.5 Sample analysis

In samples of diet, orts, and body were determined dry matter (DM) content (AOAC 1990, method number 930.15), ash (AOAC 1990, method number 942.05), ether extract (AOAC 1990, method number 920.39), crude protein (CP) by Dumas combustion method (Leco Model FP – LC 528,Leco Corporation) as described by Etheridge *et al*. (1998), and gross energy (GE) using bomb calorimeter (IKA® Calorimeter system C 2000 basic/control).

Blood metabolites (total protein, albumin, urea, creatinine, cholesterol, aspartate aminotransferase [AST], gamma glutamyltransferase [GGT], and creatine kinase [CK]) were analyzed in serum and glucose in plasma using specific commercial kits from Labtest® Diagnóstica S.A., while non-esterified fatty acid (NEFA) and beta-hydroxybutyrate (B-HB) were analyzed in serum using specific commercial kits from Randox®.

Hormonal dosage were performed in plasma using enzyme immunoassay commercial kits from AccuBind® – Monobind Inc. for quantitative dosage strengths of triiodothyronine (T3) and thyroxine (T4). Insulin-like growth factor (IGF – I) was performed in plasma using enzyme immunoassay commercial kits from Enzo® Life Science. The measurements were made with Multiskam MS ELISA reader (Labsystems, Helsinki, Finland), using enzyme linked immuno assay (ELISA) method.

For evaluation of the blood parameters, all commercial kits have been validated for caprine species.

2.6 Calculation and statistics

Fat and protein body retention were obtained by difference between body composition at slaughter and the initial body composition at 16.6 ± 0.997 kg BW estimated using the equations created from baseline animals. Body composition of goat kids at the beginning of the experiment was estimated using regression equations obtained from baseline animals using PROC MIXED procedure. Retention data were analyzed as a split plot design with the group nested to gender using PROC MIXED procedure with the fixed effects of gender (intact males, castrated males, and females) and feed restriction (0%, 25%, and 50%). When means of gender were statistically different they were compared by Fisher's test in the LSMEANS statement. When feed restriction was significant orthogonal polynomial contrasts were performed. Significances levels were set at $P \leq 0.05$.

Protein and fat retention was estimated by equations generated from baseline animals as follows:

$$EBWi = 1.54 \pm 1.43 + 0{,}730 \pm 0.09\ BWi \qquad \text{Eq. [1]}$$

$$DMi = -2088 \pm 957 + 458 \pm 69.6\ EBWi \qquad \text{Eq. [2]}$$

$$FATi = -1438 \pm 915 + 239 \pm 66.8\ EBWi \qquad \text{Eq. [3]}$$

$$CPi = -1663 \pm 903 + 278 \pm 65.9\ EBWi \qquad \text{Eq. [4]}$$

where:

EBWi = initial empty BW (kg).
BWi = BW at the beginning of the experiment (kg).
DMi = dry matter at the beginning of the experiment (g).
FATi = total fat at the beginning of the experiment (g).
CPi = total crude protein at the beginning of the experiment (g).

Feed intake, metabolites, and BW data were analyzed as a split plot design with the group nested to gender as repeated measures over time. Mixed models were solved with the fixed effects of gender (intact males, castrated males, and females), feed restriction (0, 25, and 50% feed restriction) and blood collection (10, 20, 30, 40, 50, 60, and 70 days) and as random effect the group and error, using the SAS MIXED procedure (version 9.2). Various error covariance structures were investigated and the one that best fit the data according to the Bayesian information criterion (BIC) was selected. When means of gender were statistically different they were compared by Fisher's test in the LSMEANS statement. When feed restriction or blood collection was significant orthogonal polynomial contrasts were performed. Significances levels were set at $P \leq 0.05$.

The efficiency of utilization of protein and gross energy was calculated by the ratio of total consumption in kg (protein or energy) and total retained in kg (protein or energy) during all experiment period. Significances levels were set at $P \leq 0.05$.

3.RESULTS

3.1 Body retention

Fat body retention decreased linearly with the increase of feed restriction (*P* = 0.0464). Females and castrated males fed ad libitum showed similar body fat (kg), however under feed restriction females showed greater retention of body fat than intact males and castrated males. Moreover, regardless of feed restriction, females had much more fat deposition than castrated males and intact males in the EBW gain (P< 0.0001; Table 2).

Protein body retention (kg) was not affected by gender, only showing a linear decrease with the increase of feed restriction (*P* < 0.0001). On the other hand, body protein as a percentage of EBW increased linearly with the increase in feed restriction (*P* = 0.0172; Table 2).

There are not difference for efficiency of utilization of CP between genders (P = 0.340). In contrast, the efficiency of energy use was greater in females compared to other genders (P = 0.045). With the increased feed restriction level, so the efficiency of utilization of protein as the energy increased linearly. There was no interaction between the level of feed restriction and gender for the evaluated efficiencies.

Table 2. Nutrient body retention of different gender of Saanen goat kids from 15 to 30 kg of BW subjected to feed restriction

	Restriction (%)													
	0			25			50						Contrast[4]	
Item[1]	Female	Castrated	Intact male	Female	Castrate	Intact male	Female	Castrated	Intact male	SEM[2]			R[3]	R × G[3]
Final BW (kg)	29.8	31.2	32.6	27.2	24.0	27.9	20.1	20.2	20.3	0.66	<0.0001	0.011	0.010	0.008; Q
Final EBW (kg)	25.2	26.1	27.7	22.1	20.0	23.0	16.5	16.8	16.6	0.52	<0.0001	0.018	0.007	0.018; Q
REBW (kg)	12.6	13.2	14.6	9.50	7.08	10.1	3.82	4.09	3.60	0.540	<0.0001	0.057	0.005	0.018; Q
DM (kg)	5.99	5.70	4.75	4.61	3.60	3.47	1.95	1.55	0.889	0.389	<0.0001	0.028	0.731	<0.0001; L
FAT (kg)	3.93	3.79	2.20	2.84	1.67	1.53	1.23	0.709	0.297	0.259	<0.0001	0.0001	0.0464	0.006; L
CP (kg)	1.99	2.27	2.40	1.63	1.63	1.80	0.706	0.887	0.871	0.188	<0.0001	0.387	0.898	<0.0001; L
Fat % REBW	31.4	28.8	15.1	30.6	23.6	14.4	32.8	17.0	4.96	3.23	0.032	<0.0001	0.175	0.0109; L
CP % REBW	15.7	17.3	16.5	17.4	22.5	18.0	18.1	21.2	21.0	2.16	0.042	0.327	0.722	0.0172; L
CP Efficiency[5]	5.06	5.60	4.34	5.10	6.24	3.90	8.57	7.17	7.19	0.929	0.016	0.340	0.787	0.0044; L
GE Efficiency[5]	4.56	6.59	6.67	4.98	8.48	7.31	9.12	11.93	12.1	0.993	<.0001	0.045	0.932	<.0001; L

[1] DM = dry matter; CP=Crude protein; BW=body weight; EBW=empty body weight; REBW= retained empty body weight.
[2] SEM = Mean standard error.
[3] R = feed restriction; G= gender; R × G =interaction between feed restriction and gender.
[4] L = Linear; Q = Quadratic.
[5] CP Efficiency = Total CP intake (kg): Total CP Retained (kg); GE Efficiency = Total GE intake (kg): Total GE retained (kg);

3.2 Body weight

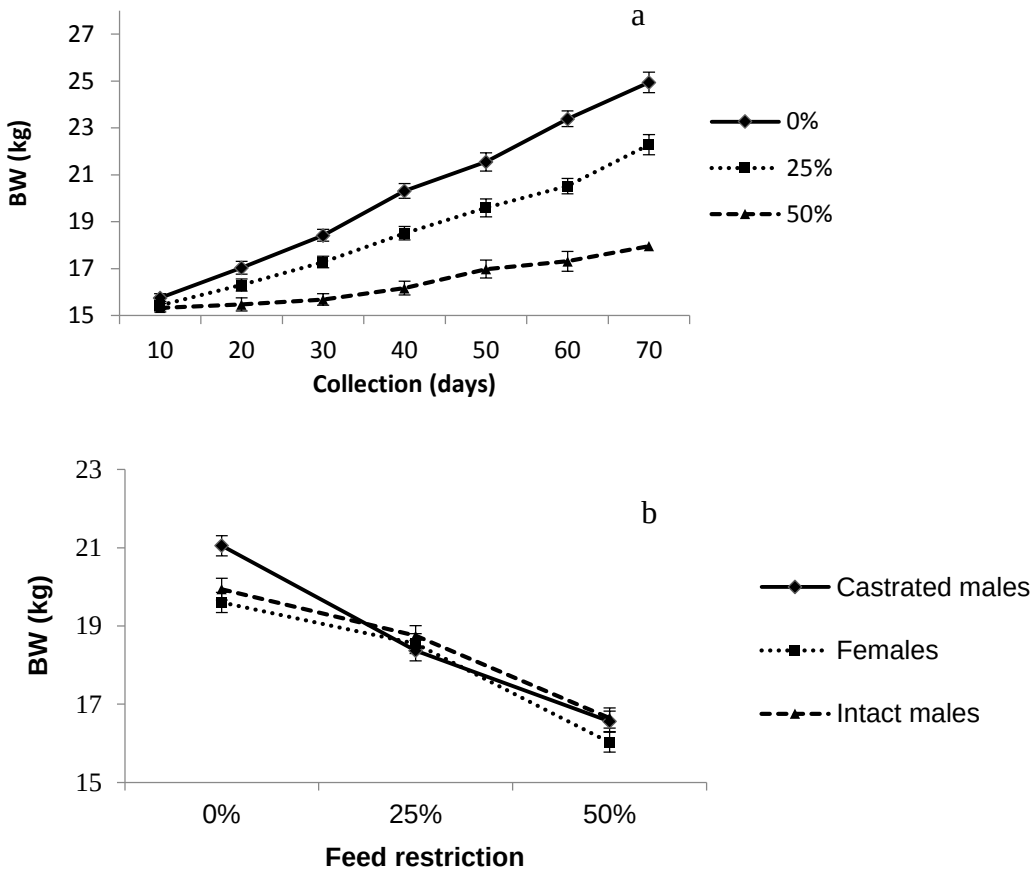

Figure 1. Body weight (BW) of animals fed 0% (ad libitum), 25%, and 50% feed restriction at different periods of blood collection ("a") and BW of castrated males, females, and intact males subjected to 0%, 25%, and 50% feed restriction ("b").

Animals fed ad libitum showed greater BW gain ($P < 0.0001$) followed by the animals subjected to 25% and 50% feed restriction during the course of blood collections. The difference between animals fed ad libitum and those fed with 50% feed restriction increased from 2.73% to 25.17% when the exposure restriction passed from the 10 to the 70 blood collection days , respectively (Figure 1a). Castrated males presented greater BW ($P = 0.0002$) compared to females and intact males when fed ad libitum. However, with 25% and 50% feed restriction, the BW of the animals decreased without difference among genders (Figure 1b).

3.3 Intake and digestibility

Organic matter intake (OMI) increased linearly ($P < 0.0001$) with each blood collection period, with the maximum consumption occurring at the 70 collection days that averaged 582.81 ± 17.16 g/day (Figure 2a).

Crude protein intake (CPI) and gross energy intake (GEI) increased for castrated males and for females throughout the course of days of blood collections. In contrast, intact males decreased CPI and GEI from the 50 collection days onwards with the lowest CPI and GEI at the final blood collection. At this final point, castrated males presented the greatest CPI and females showed intermediate intake (Figure 2b and 2c).

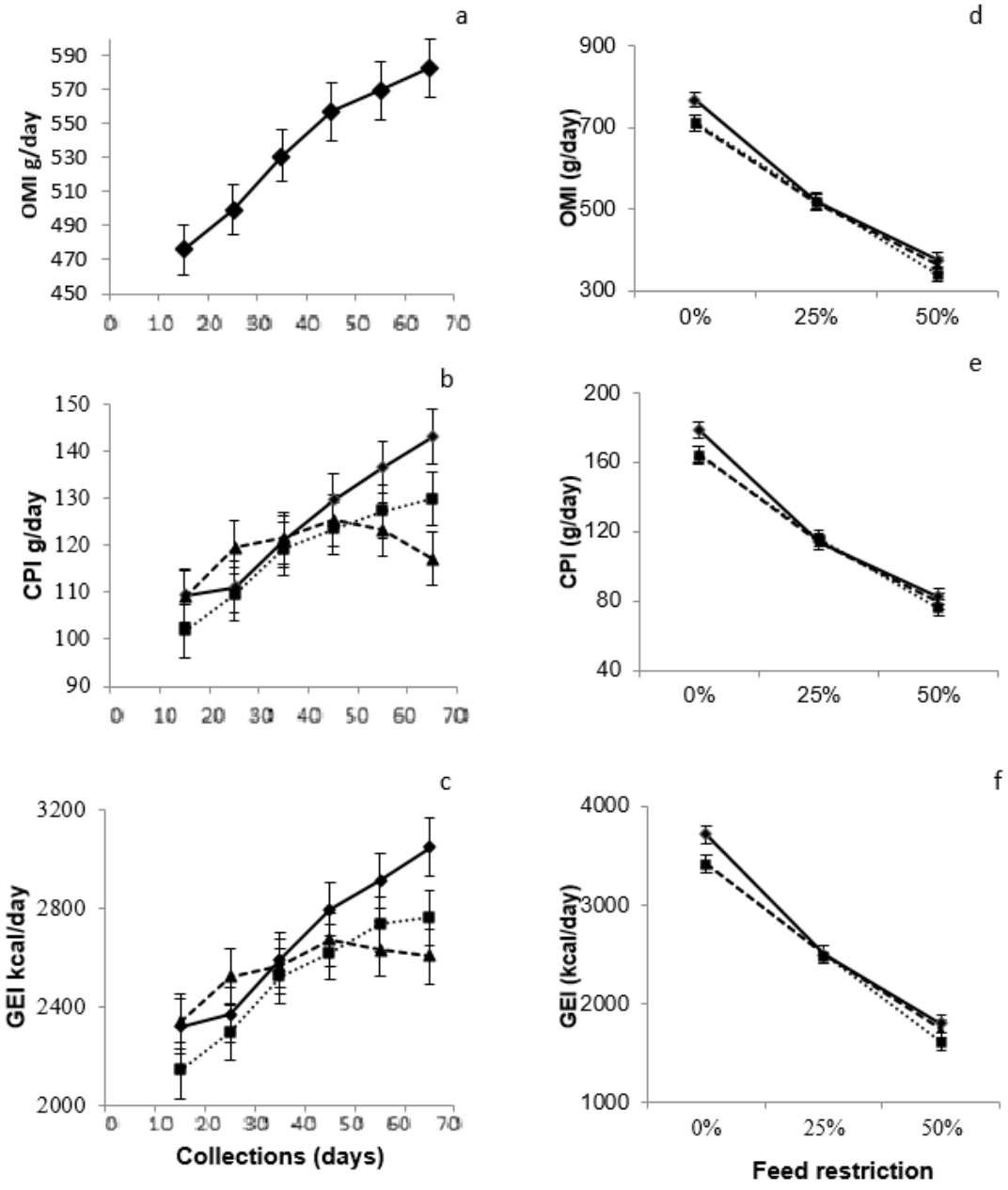

Figure 2. Intake of organic matter (OMI; P < 0.0001); “a”), crude protein (CPI; P = 0.0200; “b”), and gross energy (GEI; P = 0.0495; “c”) of castrated males (—♦—), intact males (---▲---), and females (---■---) at different days of blood collection. OMI (P = 0.0011; “d”), CPI (P = 0.0017; “e”), and GEI (P = 0.0004; “f”) of castrated males (—♦—), intact males (---▲---), and females (---■---) fed 0%, 25%, and 50% feed restriction.

There was an interaction between gender and feed restriction on OMI, GEI, and CPI (P < 0.05; Figure 2d–2f). When castrated males were fed ad libitum, OMI, CPI, and GEI were 7.37%, 8.12%, and 8.01% greater than intact males and females,

respectively. However, when subjected to feed restriction, no difference between the genders was observed in OMI, CPI, and GEI.

The digestibility of NDF increased (P = 0.01) when feed intake was restricted (Table 3). The digestibility of DM and OM were greater for animals subjected to feed restriction of 50% than for the other treatment groups (P = 0.03).

The digestibility of DM and OM increased by 4% and NDF by 6% when the goat kids ad libitum treatment were compared with feed restriction of 50%.

Table 3. Digestibility of dry matter, organic matter and neutral detergent fiber in goat kids of different genders subjected to feed restriction

Digestibility (%)	Gender			SEM[1]	Restriction			SEM[1]	Restriction	Gender	R x G[2]
	Females	Castrated	Intact males		0%	25%	50%				
Dry matter	74.9	76.1	75.5	0.55	74.6	74.4	77.5	0.57	0.001	0.38	0.31
Organic matter	76.3	77.3	76.8	0.53	75.8	75.8	78.5	0.54	0.001	0.43	0.23
Neutral detergent fiber	64.8	67.9	66.8	1.05	65	65.1	69.4	1.04	0.001	0.14	0.25

[1] SEM = Mean standard error;

[2] R × G =interaction between feed restriction and gender;

3.4 Protein and energy metabolism

We observed a linear decrease in blood concentration of glucose as feed restriction increases (from 69.3 ± 1.79 to 63.3 ± 1.76 mg/dL for goats fed ad libitum and subjected to 50% feed restriction, respectively; $P < 0.0001$).

There was interaction between gender and blood collection on plasma glucose concentration. Plasma glucose was similar in intact and castrated males throughout the period of blood collection with an average of 67.4 ± 4.59 mg/dL. In contrast, females showed lesser glucose plasma levels than males between the 10 and 40 blood collections days, but matched the levels found in males from the 50 blood collection days onwards (Figure 3a).

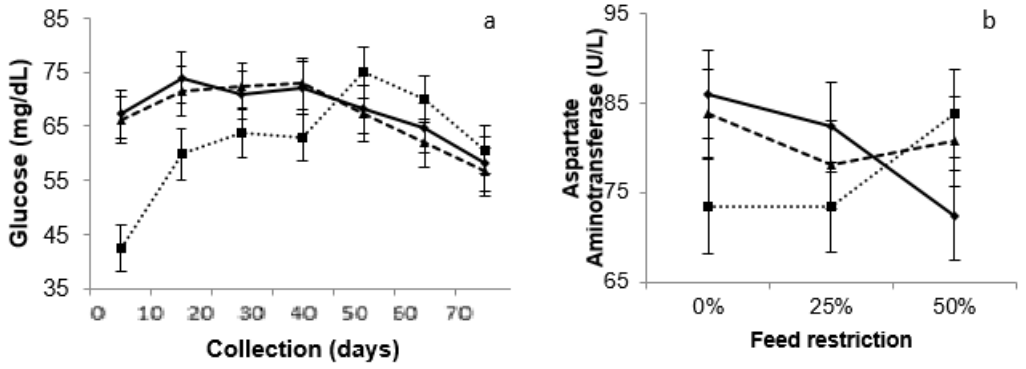

Figure 3. Plasma concentrations of glucose (mg/dL) of castrated males (—♦—), intact males (---▲---), and females (---■---) on the basis of days of blood collection (P = 0.0069; "a") and aspartate aminotransferase activity (U/L) of castrated males (—♦—), intact males (---▲---), and females (---■---) fed 0%, 25%, and 50% feed restriction (P = 0.0251; "b").

For serum AST activity there was an interaction effect between gender and feed restriction (Figure 3b). As the feed restriction level increased, serum AST activity of castrated males decreased (P = 0.0251) in a ratio of 4.3% for those fed with 25% restriction and 15.8% for those fed with 50% restriction compared to castrated males fed ad libitum (Figure 3b). In females, AST activity was greater in those fed with 50% restriction (83.83 ± 4.96 U/L), whereas in intact males levels of this enzyme remained unchanged regardless of the restriction regimen, with mean value of 80.9 ± 4.95 U/L.

Irrespectively of gender, B-HB concentrations changed quadratically for animals subjected to feed restriction ($P < 0.0149$). The greatest concentration was

observed when animals were subjected to the maximum level of feed restriction (0.129 mmol/l) followed by those fed ad libitum (0.103 mmol/L) and restricted by 25% (0.090 mmol/L).

Gender did not influence NEFA and cholesterol blood levels. However, a negative linear effect was verified for both metabolites with the course of blood collection ($P < 0.01$).

Total protein and creatinine seric levels, and CK activity increased with the blood collection trial period (Figure 4a–4c). Creatinine increased with feed restriction, averaging 0.63 mg/dL, 0.66 mg/dL, and 0.68 mg/dL for 0%, 25%, and 50% feed restrictions, respectively ($P < 0.05$; Figure 4d).

Concentration of urea was affected by interaction between gender and feed restriction ($P = 0.0154$; Figure 4e). When fed ad libitum, urea concentration was greatest in females (58.1 mg/dl), followed by castrated males (54.3 mg/dl), and the lowest urea level was observed in intact males (51.2 mg/dl). When goats were fed with 25% feed restriction, a difference between genders was not observed; however, when subjected to 50% feed restriction castrated males showed greater serum urea (54.0 mg/dl).

For GGT activity there was an interaction effect between gender and feed restriction (Figure 4f). The activity of GGT in intact males was greater ($P < 0.0001$) when they were fed ad libitum (51.31 ± 1.58 U/L) and decreased with the increase of feed restriction (47.180 ± 1.56 U/L and 44.368 ± 1.57 U/L for 25% restriction and 50%, respectively). Castrated males fed ad libitum presented lesser GGT activity compared to those fed restricted diets (44.6 ± 1.58 U/L vs. 48.8 ± 1.59 U/L). In contrast, GGT activity in females remained constant (45.66 ± 1.62 U/L) at all levels of restriction imposed (Figure 4f).

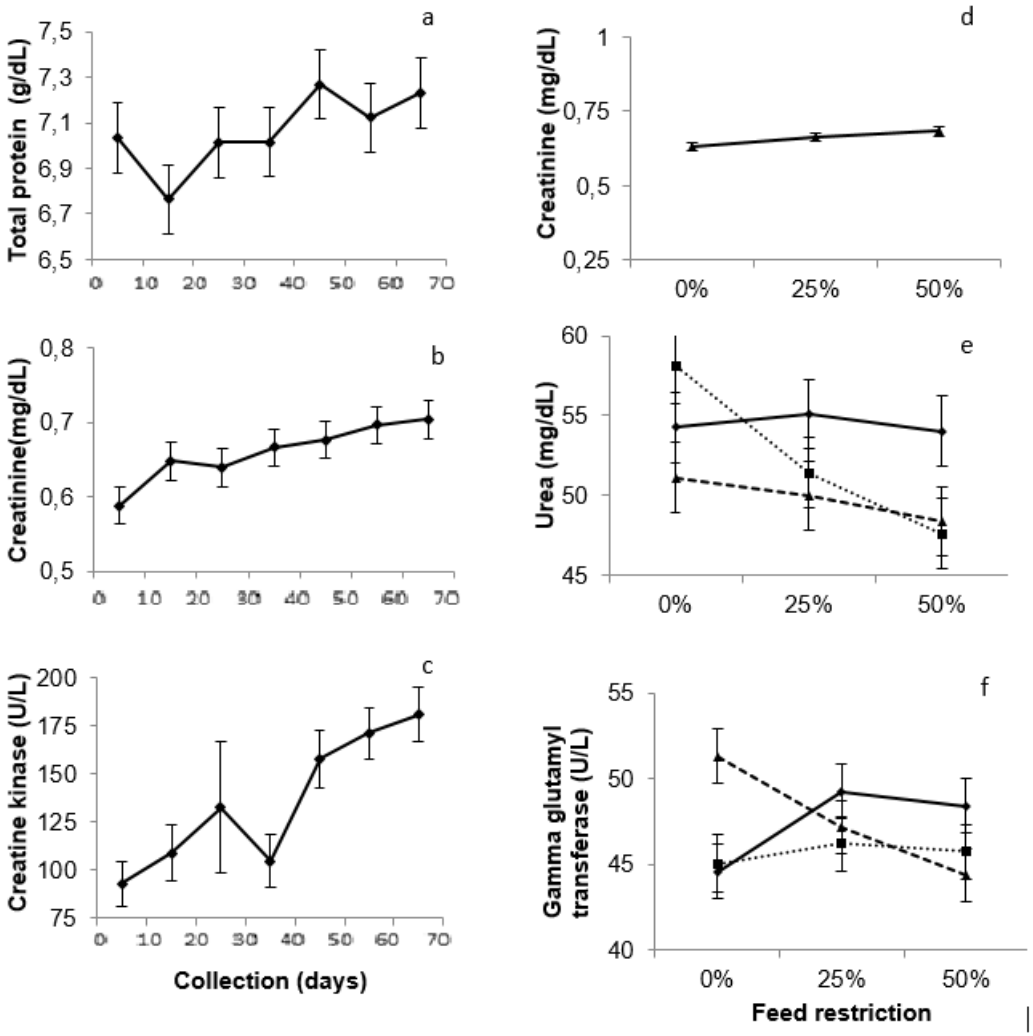

Figure 4. Seric concentration of total protein (g/dL) with positive linear effect (*P* = 0.0441; "a"), creatinine (mg/dL) with positive linear effect (*P* = 0.0007; "b"), and creatine kinase activity (U/L) with positive linear effect (*P* < 0.0001; "c") on the basis of days of blood collection. Urea serum concentration (mg/dl) of castrated males (—♦—), intact males (---▲---), and females (---■---) on the basis of feed restriction ("d"). Creatinine with positive linear effect (*P* = 0.0041; "e") and Gamma glutamyl transferase (*P* < 0.0001; "f") activity (U/L) of castrated males (—♦—), intact males (---▲---), and females (---■---) fed 0%, 25%, and 50% feed restriction.

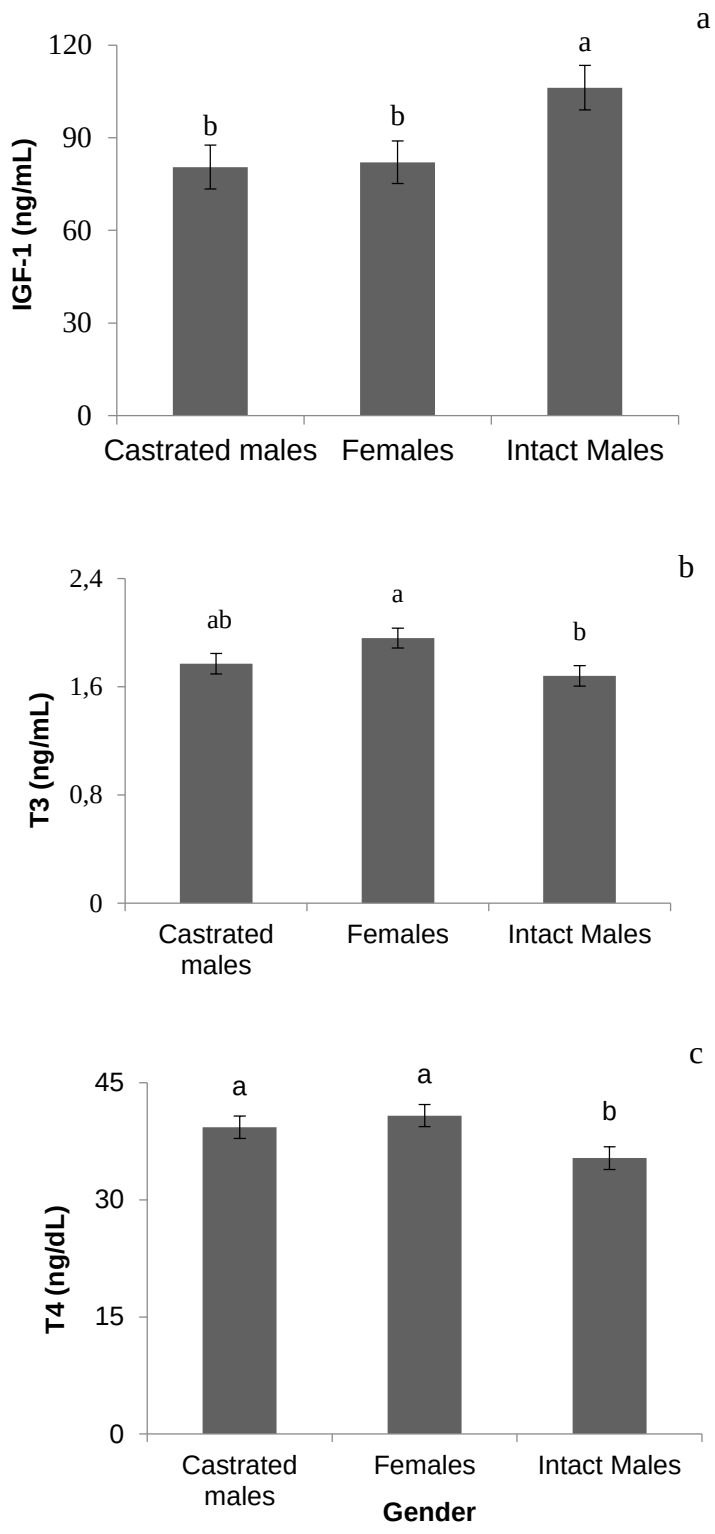

Figure 5. Plasma concentrations of Insulin-like growth factor-I – IGF-1 (*P* = 0.023; “a”), Triiodothyronine – T3 (*P* = 0.0338; “b”) and Thyroxine – T4 (*P* = 0.0285; “c”) in castrated males, females, and intact males.

IGF-1 plasmatic levels decreased at increase rate as feed restriction increased (quadratic; P= 0.0454); on the other hand T3 plasmatic levels decreased linearly with increase of feed restriction (from 2.04 ± 0.07 to 1.57 ± 0.74 ng/mL; $P < 0.0001$).

Plasmatic concentration of IGF-1 was similar between castrated males and females (81.3 ng/ml), and was lesser than the concentrations found in intact males (106.20 ng / ml; Figure 5a).

Gender also influenced plasmatic T3 and T4 levels (Figure 5b and 5c). Intact males showed lesser concentrations of T3 and T4 compared to females (1.7 ng/ml vs. 1.9 ng/ml for T3 and 35.4 ± 1.45 vs. 40.8 ± 1.41 for T4).

It was found that T3 plasmatic levels were affected by blood collection period, showing a linear decrease from the 1st through the 5th blood collection ($P < 0.0001$).

4.DISCUSSION

In this study it was observed that both gender and feed restriction influenced energy and protein metabolism. Females changed their entire glycolytic metabolism to retain the deposition of adipose tissue, even when subjected to feed restriction. In contrast, males (intact and castrated) mainly changed their proteic metabolism to lessen the effect of feed restriction on protein synthesis.

All animals (irrespective of gender) decreased their growth rate as feed restriction increased. This can be confirmed by decrease of serum level of IGF-1, T3, glucose, and body protein and fat retention. The levels of NEFA and cholesterol indicated that animals did not mobilize body reserves even under 50% feed restriction.

Under feed restriction the females changed their physiological mechanisms to ensure the minimum amount of body fat required, which can vary from 20 to 25% during puberty (PARDINI, 2001). This observation is attributed to changes in glycolytic metabolism that females used to offset the lesser energy input caused by the continuous feed restriction. In fact, when the restriction became maximum (50% feed restriction) to the point of compromising fat deposition, greater enzymatic activity of AST was observed. Among the mechanisms used by mammals, there is a more complex and efficient mechanism it uses the malate aspartate to ATP synthesis,

which takes place mostly in the liver. As oxaloacetate cannot cross the inner mitochondrial membrane to be transferred to the cytosol, it is then converted to aspartate, and this route is catalyzed by AST (NELSON; COX, 2011). Thus possibly intense liver metabolism, has caused the greatest concentration of AST into the bloodstream. According Resende et al. (2008), the fat deposition is an energetically costly process. Unlike females, castrated males showed a decrease in AST activity, suggesting that the deposition of fat is not a priority for this gender when subjected to feed shortages.

Under feed restriction intact males decreased their energetic metabolism, indicated by the lesser serum concentration of thyroid hormones T3 and T4 (Nelson and Cox 2011). On the other hand, intact males maintained their priority for protein synthesis as a result of greater plasma concentration of IGF-1, which probably occurred due to testosterone action, which accords with previous studies in sheep and cattle (MATEESCU; THONNEY 2005; MOREIRA et al., 2010).

The reduction in levels of testosterone induced by castration caused a decrease in serum levels of IGF-1 and increased T3 levels, which put castrated males in a metabolic situation very close to that of females, especially as regards the deposition of fat in the body. However, although it is expected that castrated males have body fat deposition closer to that of females, this fact was not observed when the animals were subjected to feed restriction. While females maintained greater body fat growth when subject to the restriction, castrated males did not activate the same metabolic mechanisms for the deposition of fat. Indeed, the enzymatic activity of AST in castrated males was lesser than that of females with 50% feed restriction.

Castrated males fed ad libitum had greater DMI, protein, and energy, and consequently had greater BW compared to intact males and females; these data are in accordance with findings reported by Bello and Adama (2012) and Muhikambele *et al.* (1994). A possible explanation could be the action of leptin which is involved in the homeostatic control of body energy, acting in the regulation of appetite for food (BAN-TOKUDA *et al.* 2008). As castrated males retained less fat as a proportion of EBW gain compared to females (fed ad libitum), it is possible that over the experimental period castrated males produced less leptin and consequently maintained the high consumption of nutrients associated with an intense energy

metabolism due to their serum T3 levels. Rather, as females had greater fat deposition and probably their consumption was depressed by the regulatory action of leptin, while this, the lowest consumption of males may be related to lesser levels of T3 acting on the central nervous system regulating food intake (COLL et al. 2007).

Our results for creatinine, CK, and protein retention show that regardless of gender the animals had similar muscle growth and rising rates for each level of feed restriction. However, under feed restriction each gender has different priorities and responds with distinct physiological adjustments.

The greatest serum urea concentration of females when fed ad libitum compared to intact or castrated males suggests that there was more recycling of urea in females for protein synthesis. This strategy was possibly adopted to ensure the physiological status of females for the sake of reproduction. In contrast, we observed a reduction of serum urea with increasing level of feed restriction, showing that under deprivation of nutrients the metabolism involved in fat synthesis overcame protein metabolism, unlike what occurred in males. Supporting this view, the enzymatic activity of GGT was constant at the levels of feed restriction imposed, showing that protein synthesis not is preferred in females. This can be explained by the fact that GGT is an enzyme which contributes to protein synthesis, being responsible for degradation of glutathione, which becomes a source of amino acid cysteine for protein synthesis (TATEISHI et al. 1974). The greatest concentration of T3 in females possibly is one of the factors responsible for altering protein synthesis under conditions of feed restriction. CASSAR-MALEK et al. (1999) demonstrated that T3 is a regulatory hormone of satellite cells (CS) that is able to reduce the proliferation of these cells in a dose-dependent manner. These cells have large mitogenic activity and contribute to postnatal muscle growth and maintenance of adult skeletal muscle (FOSCHINI et al. 2004). During growth the CS are activated and their proliferation may be merged with existing or neighboring CS to generate new muscle fibers.

Under ad libitum feeding conditions the primary means of maintaining the protein growth of castrated males was from dietary protein. When subjected to feed restriction the participation of endogenous urea recycling increased the supply of protein to meet the requirements for growth. This hypothesis is supported by the greater feed intake observed in castrated males when fed ad libitum as well as by the

increase in GGT activity associated with increased serum levels of urea when fed a restricted diet. Males have a greater ruminal volume than females, which facilitates the retention of larger amounts of feed in the rumen, improving the recyclability of urea (BARBOZA; BOWYER 2000). This increase in the recycling of urea allows males to minimize their dietary protein requirements for the synthesis of microbial protein (LONG et al. 2009). Although increased recycling of urea is reported for males in general (NRC, 1996), in our study we found differences in the way that intact and castrated males use this resource to meet their metabolic requirements for protein.

Possibly other metabolic pathways were activated in feed restriction to help keep protein growth. One such pathway may have involved the growth hormone (GH) which usually occurs in greater concentrations in intact males and castrated males than in females (PLOUZEK; TRENKLE, 1991). Indeed, GH is directly related to cell division and formation of skeletal muscle protein synthesis (HOSSNER, 2005).

Taken together, our data indicate that under feed restriction the mechanism used by the females to compensate for dietary restriction could have been changes in glycolytic metabolism to produce ATP required for the deposition of fat. Feed restriction in males (intact and castrated) retains the recycling of urea probably by the action of GH (acting jointly with testosterone), favoring protein metabolism. So even with nutritional deficiency, both females and males can develop the functions of deposition of fat (females) and greater growth (males), targeting their productive and reproductive capacity. However, more studies are needed to clarify exactly what causes the different reactions of each gender to ensure similar protein growth when subjected to feed restriction.

To our knowledge this is the first study showing how goats of different genders physiologically act upon the challenge of feed restriction. Furthermore the study indicates that the analyzes of AST, GGT, T3, and urea can be a good parameter to evaluate the energy and protein metabolic status of goats during feed restriction. Our findings may be relevant in the adoption of nutritional management strategies for goats.

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CHAPTER 3 – PUBERTAL GOATS OF DIFFERENT GENDERS CHANGE THE METABOLISM WHEN SUBJECTED TO FEED RESTRICTION

ABSTRACT - The objective of this study was to evaluate the effect of feed restriction on energy metabolism of 84 Saanen goats (26 intact males, 27 castrated males and 31 females) with initial body weight (BW) of 30.3 ± 0.87 kg. At the beginning of the experiment 8 intact males, 9 castrated males and 13 females were slaughtered at 30 kg of BW to estimate initial body composition. The remaining goats were assigned to 3 levels of feed restriction (ad libitum, 25% and 50% feed restriction), with 6 goats per gender and feeding level. Animal sets (1 goat per gender-feeding level) were slaughtered when BW of goat fed ad libitum reached 45 kg. During this experimental period, we evaluated concentrations of glucose, total protein, albumin, urea, creatinine, cholesterol, non-esterified fatty acids (NEFA), beta-hydroxybutyrate (B-HB), aspartate aminotransferase (AST), gamma glutamyltransferase (GGT) and creatine kinase (CK), triiodotironina (T3), tiroxina (T4) and insulin-like growth factor 1 (IGF-1). Females had greater retention of body fat (% empty BW) regardless the level of feed restriction ($P<0.001$). Irrespective of gender, plasma glucose level decreased as feed restriction increased ($P<0.0001$). Levels of NEFA and B-BH increased with increasing feed restriction, regardless of gender ($P<0.0001$). Intact males subjected to 50% of feed restriction had lowest concentration of IGF-1 ($P = 0.018$). Regardless gender, the concentration of T3 decreased as feed restriction increased ($P<0.0001$), with concentrations of 1.60, 1.54 and 1.53 mg/mL for animals fed ad libitum, 25 and 50% of feed restriction, respectively. Gender and feed restriction did not influence the serum levels of albumin, total protein, cholesterol, AST and GGT in the blood. ($P <0.05$). Pubertal males were not able to keep protein synthesis during feed restriction and females and castrated males to keep the fat deposition even when they are subjected to feed restriction.

Keywords: dairy goats**,** females, males, metabolic profile, retention

1. INTRODUCTION

According to FAO (2013), the majority of the world's goat herd is in Asia and Africa, being just the China holds 148.4 million heads, which represents 20% of world effective. In Brazil, according to IBGE (2014), 88% of the herd of goats are allocated in the Northeast region of the country. Several African countries as well as Asia, there is a moderate or regular amount of water reserves, but suffer in the same way with the lack of water (UNESCO, 2012), and this can affect the animal production by the decrease of food production.

Thus, one of the biggest challenges that the goat herd may face in relation to where their production stands out is the lack of food. This lack of food may be by the economically infeasible or environmental problems, given that the largest proportion of countries facing economic difficulties are located in the aforementioned continents (UNESCO, 2012). Similarly, the northeast region of Brazil, is an underdeveloped region, and that suffers to the lack of rain, which directly affects the food availability (REBOUÇAS, 1997; SANTOS, 2013).

The growth phase in ruminants is a process that involves adjustments in the organs and tissues of animals according to the physiological needs of the body to then start the reproductive process (OWENS et al., 1993). The rearing period until puberty, are usually characterized by high efficiency in the use of nutritional resources on the part of the animal body (OWENS et al. 1993). However, adequate nutritional supply is necessary to support this efficiency in order to be able to meet the animal's demand of nutrients and energy.

Another factor that must be taken into consideration in the production of goats, is the gender of the animal, which imposes differences in development between intact males, castrated males and females, through action and concentration certain hormones that affect a greater deposition of muscle or fat and, consequently, body composition and nutritional requirements (SAHLU et al., 2004).

Several studies have shown that feed restriction may interfere with the growth and weight gain in ruminants (OWENS et al. 1993). However, there are few studies about ruminants that show how the metabolism in different stages of growth may be affected by feed restriction, and the most studied species is the bovine (HORNICK et

al. 1998; CHELIKANI et al. 2004). In addition, to our knowledge, there is a lack of information on the effect of feed restriction on energy and protein metabolism during specific stages of growth, especially for goats from different genders.

Taking into account the exposed above, this study aims to evaluate the metabolic changes and understand how they happen in goats of different sexual conditions (females, castrated males and intact males) with 30 to 45 kg body weight in the final growth phase when they are subjected to different levels of feed restriction. In this study, our hypothesis is that the protein and energy metabolism in different levels of feed restriction can be influenced by gender.

2. MATERIALS AND METHODS

2.2 Animals, experimental design and diet

The experiment was conducted at the Goat facility of Univ Estadual Paulista/Jaboticabal São Paulo, Brazil.

Humane animal care and handling procedures were followed, according to the University's Animal Care Committee on the Ethical and Animal welfare (Comissão de Ética e Bem Estar Animal - CEBEA), under protocol number 004972-09.

For this study we used 84 Saanen kids, 26 intact males, 27 castrated males and 31 females with initial BW 30.32 ± 0.87 kg and age in average of 147 days. It was used a split block design in a 3 × 3 factorial arrangement, thus 3 genders (intact males, castrate males, and females) with 3 treatments defined by 3 levels of feed restriction = 0% [ad libitum], 25% and 50%).

The intake of animals fed *ad libitum*, was adjusted to allow 20% of daily leftovers, while animals subjected to the other two levels of feed restriction (25 and 50%) would have their intake based on the amount consumed by animals fed *ad libitum*.

The animals were fed twice a day (07:00 and 16:00 h), and the experimental diet (Table 1) was formulated to meet growing goats' requirements at 45 kg BW according to NRC (2007).

Table 1. Chemical composition of feed ingredients and experimental diet.

Ingredient	% DM[a]
Corn hay	46.2
Soybean meal	15.2
Corn meal	30.9
Soybean oil	1.9
Limestone	1.0
Mineral mixture[d]	4.8
Chemical composition	**g/kg DM**
Dry matter	863
Organic matter	918
Crude protein	147
Ether extract	49
NDF[b]	278
ADF[c]	138
Gross energy (MJ/kg DM)	21.3

[a] DM, 105°C dry matter, g/kg as fed
[b]NDF, neutral detergent fiber
[c]ADF, acid detergent fiber
[d]Composition, per kg, as-fed basis: 190 g of Ca; 92 g of Cl; 73 g of P; 62 g of Na; 44 g of Mg; 1.35 g of Zn; 1.06 g of Fe; 0.94 mg of Mn; 0.73 g of F ; 0.34 g of Cu; 18 mg of Se; 16 mg of I; 3 mg of Co.

To estimate the body fat and protein retention, on the first experimental day, 30 randomly selected goats (8 intact males, 9 castrated males and 13 females) were slaughtered to use as baseline group. The remaining 18 intact males, 18 castrated males and 18 females were distributed at the feed restriction levels. For each gender were formed six groups with three animals each. Each group consisted of an animal fed ad libitum (0% of feed restriction), other animal was subjected to 25% of feed restriction (pair-fed, receiving only 75% of the feed ingested by the animal fed ad libitum on the previous day) and the third animal of the group was subjected to 50% of feed restriction (pair-fed, receiving only 50% of the feed ingested by the animal fed ad libitum on the previous day). When the animals fed ad libitum of each group reached 45 kg of BW they were slaughtered, as well as the others animals of the same group (equal days of experiment).

2.2. Collection and process of blood samples

The blood samples were collected from all of animals (18 of each gender) periodically every 14 days during the experiment in the morning (before feeding), totaling 98 blood collections days for metabolites and hormones.

The blood was collected using a 10 mL *vacutainer blood collection* tube with anticoagulant for blood plasma separation and another tube without anticoagulant for serum separation.

The blood collection was performed by puncturing the jugular vein with a sterile needle (for specific crops vacuum) and the material collected was placed in an isothermal box with batteries ice for preservation in 10º C. Subsequently, the material was centrifuged at 3.000 rpm for 15 min to separate plasma or serum. The supernatant (plasma and serum) was collected and 5 mL was stored in eppendorfs properly identified, and frozen until further analysis.

2.3. Slaughter procedure and sampling

When animals fed ad libitum reach 45 kg of BW, the respective group of animals fed ad libitum, 25 and 50% of feed restriction were slaughtered. Animals were slaughtered without prior water or food deprivation and were weighed immediately before slaughter. At slaughter, the animals were first stunned with a captive bolt pistol followed by severing the jugular vein and carotid artery. Blood and organs were collected and weighed. The digestive tract was weighed before and after it was emptied and flushed with water.

The EBW was computed as BW at slaughter minus the weight of the contents of the digestive tract, bladder, and biliary vesicle. The empty whole body was initially frozen at –12°C and then cut into small pieces, ground with a large screw grinder (Grinder CAF 114DS inox NR 12; C.A.F. Máquinas, Rio Claro, São Paulo, Brazil) through a plate with 0.32-cm holes, and mixed by 2 additional passes through the grinder. After grinding and homogenization, samples were collected, frozen again, and freeze-dried for DM determination and subsequently milled in a ball mill and packed in plastic pots at -20ºC for further analysis.

2.4. Sample Analysis

Feed ingredients and orts were dried in a forced air oven at 60 to 65°C for 72 h and ground through a 1-mm screen using a Willey mill (Arthur H. Thomas Co., Philadelphia, PA). They were analyzed to determine the extract ether content (based on weight loss of the dry sample upon extraction with petroleum ether in a Soxhlet extraction apparatus for 6 h (Association of Official Analytical Chemists - AOAC), 1990; method number 930.15)), protein (N analysis performed by Dumas combustion using LECO FP-528LC, LECO Corp., St. Joseph, MI; Etheridge et al., 1998), ash (complete combustion in a muffle furnace at 600°C for 6 h; AOAC 1990; method number 924.05), neutral detergent fiber (NDF) with amylase and without sulfite (Van Soest et al., 1991), acid detergent fiber (ADF - Goering et al., 1970), and gross energy (GE) using a bomb calorimeter (IKA Works, Inc., Wilmington, NC).

In the samples of body was determined the DM content (AOAC, 1990, method number 930.15), ash (AOAC, 1990, method number 942.05), ether extract (AOAC, 1990, method number 920.39), CP by the Dumas combustion method (Leco Model FP - LC 528,Leco Corporation) as described by Etheridge et al. (1998), and GE using bomb calorimeter (IKA Works, Inc., Wilmington, NC).

In the blood samples it was determined the following metabolites: total protein, albumin, urea, creatinine, cholesterol, non-esterified fatty acids (NEFA), beta-hydroxybutyrate (B-HB), aspartate aminotransferase (AST), gamma glutamyltransferase (GGT) and creatine kinase (CK) were analyzed in serum and glucose was analyzed in plasma using specific commercial kits.

Hormonal assays were performed in plasma using enzyme immunoassay kits for quantitative dosage strengths of triiodothyronine (T3), thyroxine (T4) and IGF - I. The measurements were made using the apparatus Multiskam MS Labsystems® ® Version 8.0 using the enzyme linked immuno assay (ELISA) method.

For evaluation of the blood parameters, all commercial kits have been validated for caprine species.

2.5. Calculation and Statistics

The body retention of nutrients was obtained by the difference between the body composition at slaughter in the 45 kg of BW, and the initial body composition at 30 kg of BW that was estimated using the equations created from the baseline animals. The body composition of the animals at beginning of the experiment was estimated using regression equations obtained from the baseline animals using the MIXED procedure of SAS (version 9.2, SAS Institute Inc., Cary, NC). The EBW was estimated using the equation generated from baseline animals (Eq. [1] and [2]). The intercept of the equations to estimate the EBW was similar between females and castrate males, and different for intact males ($P < 0.05$). The initial DM, fat and CP was similar between the genders and was estimated considering as DM 37.5 % of EBW, FAT 18.6% of EBW and CP 17.8% of EBW.

EBWi (females and castrated males) = 18.04 ± 3.78 + 0.2126 ± 0.123 BWi Eq. [1]

EBWi (intact males) = 19.19 ± 4.03 + 0.2126 ± 0.123 BWi Eq. [2]

Where:

EBWi = Initial empty body weight at the beginning of the experiment (kg),

BWi = body weight at the beginning of the experiment (kg).

The retention data was analyzed as a split plot design with the group to nested gender using MIXED procedure of SAS with the fixed effects of gender (intact males, castrated males and females) and feed restriction (0%, 25% and 50%). When the means of gender were statistically different they were compared by Fisher's test in the LSMEANS statement of MIXED procedure. When the feed restriction was significant orthogonal polynomial contrasts were performed. Significances levels were set at $P \leq 0.05$.

The efficiency of utilization of protein and gross energy was calculated by the ratio of total consumption in kg (protein or energy) and total retained in kg (protein or energy) during all experiment period. Significances levels were set at $P \leq 0.05$.

The intake, metabolites and BW data were analyzed as a split plot design with the group nested to gender as repeated measures over time. Mixed models were solved with the fixed effects of gender (intact males, castrated males and females), feed restriction (0, 25 and 50 % of feed restriction and days of blood collection (14, 28, 42, 56, 70, 84 and 98) and as random effect the group and error, using the MIXED procedure of SAS (version 9.2). Various error covariance structures were investigated and the one that best fit the data according to the Bayesian information criterion (BIC) was selected. When the means of gender were statistically different they were compared by Fisher's test in the LSMEANS statement. When the feed restriction or blood collection was significant orthogonal polynomial contrasts were performed. Significances levels were set at $P \leq 0.05$.

3.RESULTS

3.1. Body retention

The retention in kg of dry matter (Table 2) decreased linearly with the increase of feed restriction (P < 0.0001), being greater for females and lesser in intact males.

There was interaction between gender and feed restriction on retention of body fat (kg), wich the same decreased quadratically for animals of different gender with increasing feed restriction (P = 0.0305). Females even submitted feed restriction, showed greater retention of body fat than castrated and intact males, both for kg as for EBW gain.

The retention of body protein (kg) was similar between females and males castrated and greater than in intact males (P = 0.0209). With the increased feed restriction level, there was a linear decrease of the protein retention in the body. Further, the amount of proteins in the body EBW percentage decreased linearly with increasing feed restriction (P <0.0001; Table 2).

Table 2. Nutrient body retention of Saanen kids of different genders from 30 to 45 kg of body weight subjected to feed restriction

	Feed Restriction (%)													Contrast	
	0			25			50								
	Female	Castrate	Intact male	Female	Castrate	Intact male	Female	Castrate	Intact male	SEM[1]	Restriction	Gender	R × G[2]	R	R × G
DM[3] (kg)	10.5	8.82	6.92	5.67	5.25	2.98	2.50	1.31	0.759	0.455	<0.0001	<0.0001	0.127	<0.0001; L	
FAT (kg)	8.38	5.45	3.23	4.19	3.14	0.391	1.51	0.344	-0.827	0.426	<0.0001	<0.0001	0.0305		0.049; Q
CP (kg)	2.89	2.07	1.53	0.692	1.00	0.240	-0.506	-0.091	-0.722	0.340	<0.0001	0.0209	0.418	<0.0001; L	
Fat %EBW	44.2	28.4	17.6	35.1	25.7	2.43	25.8	5.81	-6.48	3.68	<0.0001	<0.0001	0.244	<0.0001; L	
CP%EBW	15.0	10.4	8.39	5.81	6.43	2.26	-13.1	-4.45	-10.6	3.19	<0.0001	0.282	0.408	<0.0001; L	
CP Efficiency[4]	7.08	11.70	13.02	14.01	1.00	24.70	-4.73	0.99	-22.77	9.80	0.018	0.995	0.206	0.0178; L	
GEEfficiency[4]	7.17	8.38	9.98	13.89	11.84	17.76	24.81	47.94	21.14	2.66	<.0001	0.0296	<.0001		<.001; L

1 SEM = Mean standard error;
2 R = feed restriction; G= gender; R × G =interaction between feed restriction and gender.
3 DM= dry matter; CP=Crude protein; L=linear; Q=quadratic; EBW=empty body weight;
4 CP Efficiency = Total CP intake (kg): Total CP Retained (kg); GE Efficiency = Total GE intake (kg): Total GE retained (kg);

3.2 Body weight

In our study, females had lesser BW gain (kg) during the experimental period when compared to intact males and castrated males, regardless of feed restriction level. There were no differences in BW between males and castrated evaluated with restriction levels (P = 0.0408), however the BW of all animals decreased linearly with increasing feed restriction (Figure 1a).

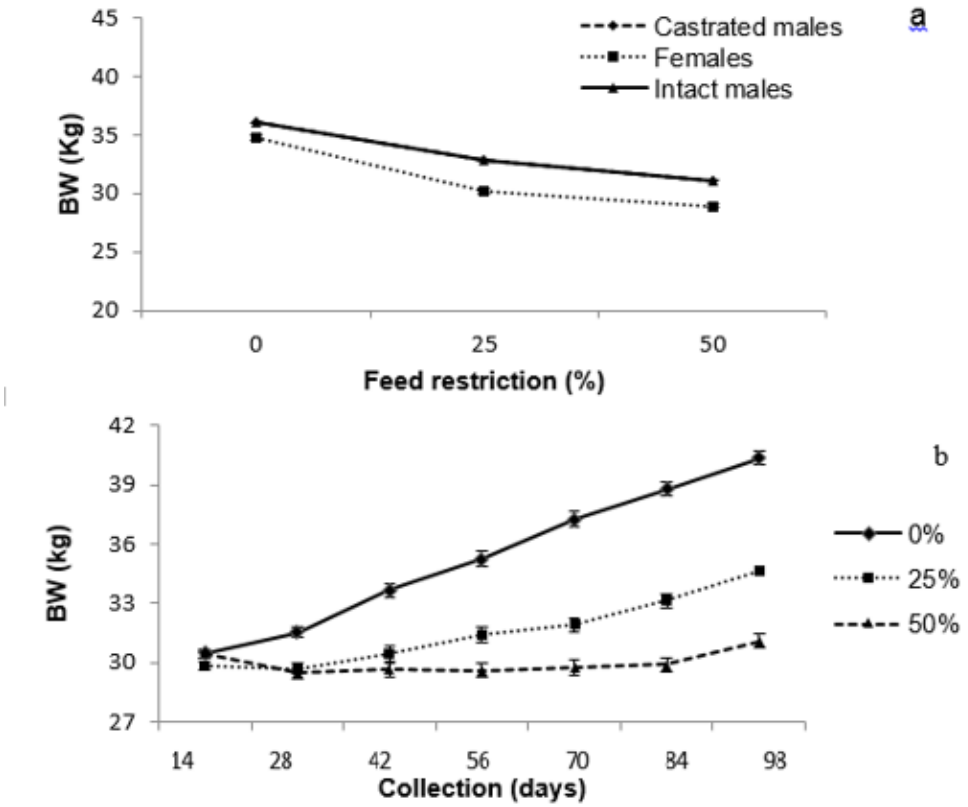

Figure 1. Body weight (BW) of castrated males, females and intact males subjected 0%, 25% and 50% feed restriction ("a") and BW of kids fed 0% (ad libitum), 25% and 50% feed restriction of blood collection days ("b").

Kids fed ad libitum showed greater BW gain (P < 0.001), followed by the animals submitted to 25% food restriction and 50% during periods of blood collection (Figure 1b).

3.3 Intake and digestibility

As expected, the organic matter intake (OMI) of animals decreased linearly (P < 0.0001) with increasing level of feed restriction (Figure 2).

Females had lesser OMI (688 g / day) than intact males (745.8 g / day) and castrated males (730.1 g / day), which were similar to each other (P <0.001; Figure 2).

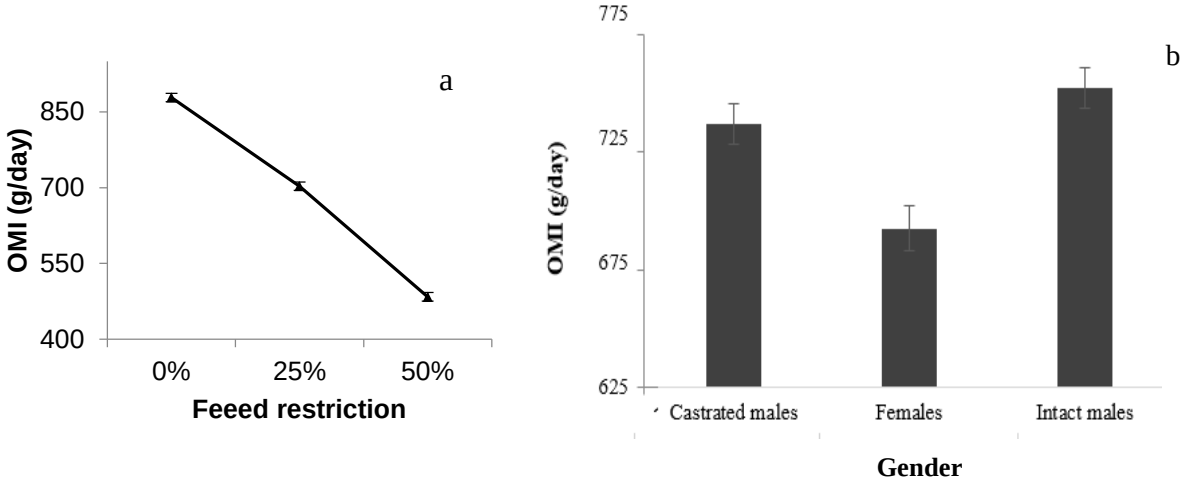

Figure 2. Intake of organic matter (OMI; P < 0.0001); “a”) of kids fed 0%, 25% and 50% of feed restriction. OMI (P = 0.001; “b”) of castrated males, females and intact males kids.

In the course of blood collection days, OMI increased until three weeks, reaching the maximum consumption of 765.5 g/day. In the 56 blood collection days onwards, consumption decreased abruptly, presenting lesser in the 84 blood collection days (678.31 g/day; P <0.001; Figure 3).

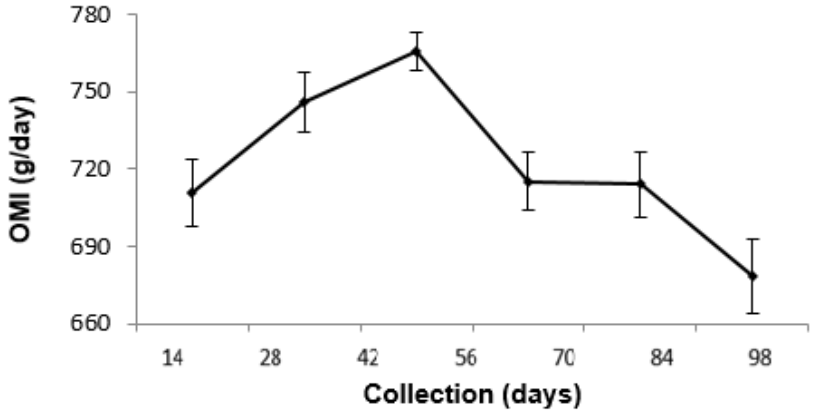

Figure 3. Intake of organic matter (OMI; P < 0.0001) on the basis of days of blood collections.

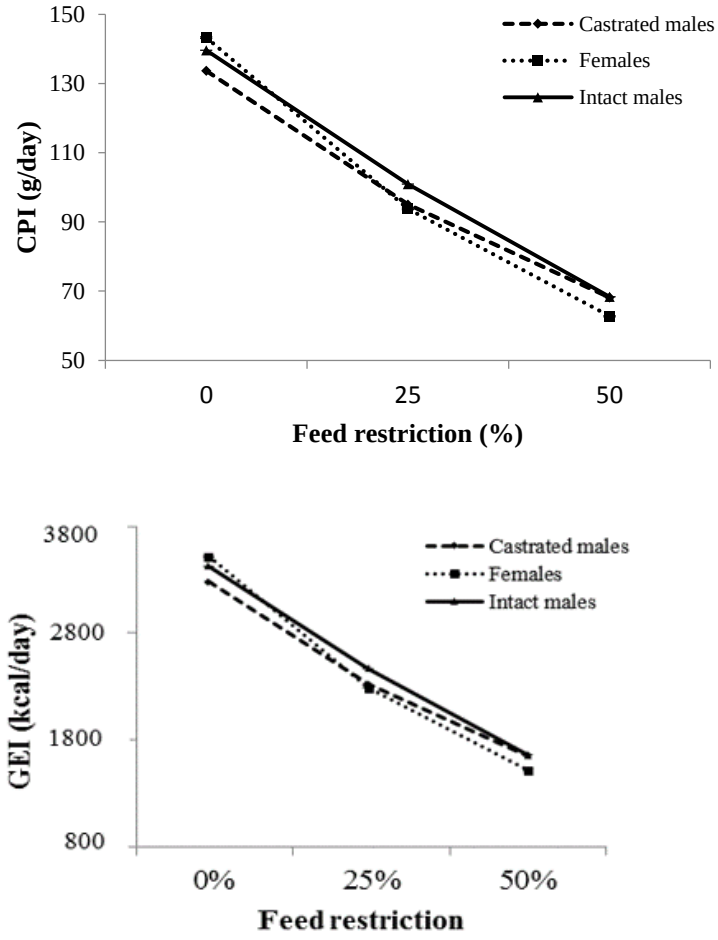

Figure 4. Intake of crude protein (CPI; P = 0.0408); “a”) and Gross Energy (GEI; P = 0.0445; “b”) of castrated males, intact males and females fed 0%, 25% and 50% of feed restriction.

There was interaction between feed restriction and gender on crude protein intake (CPI, P = 0.0235) and gross energy intake (GEI, P = 0.0312). For CPI and GEI, the intake for all genders were the same, however, with increasing levels of feed restriction, the CPI and GEI decreased linearly, and in the 25% of feed restriction intact males had intake 6% greater than females and castrated males, which had similar intake. During feed restriction of 50%, females consumed 9% less than castrated males and intact males, which in turn were equal to each other (Figure 4).

The digestibility of DM, CP and GE were greater in animals subjected to feed restriction of 50%. Females showed digestibility of DM greater than the average of males in the order of 11.7% (Table 3).

Table 3. Digestibility of Saanen kids of different genders from 30 to 45 kg of body weight subjected to feed restriction

Digestibility (%)	Feed restriction (%)									SEM[4]	Restriction	Gender	R x G[5]
	0			25			50						
	Females	Castrated	Intact males	Females	Castrated	Intact males	Females	Castrated	Intact males				
DM[1]	67.6	67.6	68.2	75.7	65.5	69.6	83	71.6	79.1	5.09	0.001	0.007	0.18
CP[2]	66.1	64.4	63.4	72.9	66.3	76.8	81.6	75.6	65.5	6.24	0.0003	0.01	0.3
NDF[3]	49.1	45.9	51.3	60.9	47.9	56.2	73.6	57.9	67.2	7.88	**	0.007	0.61

[1]DM = Dry matter; [2] CP = Crude protein; [3] Neutral detergent fiber;

[4] SEM = Mean standard error;

[5] R × G =interaction between feed restriction and gender;

** P < 0.000

3.4. Blood metabolites

Plasma glucose levels were greater in castrated males (P = 0.0067) than males and females during the course of blood collection days, except in the 98 days of blood collection, where females had greater plasma concentrations of glucose (Figure 5). Males and females showed similar plasma glucose concentrations during blood collection days, with the exception of the 14 days of blood collection, which females had lesser plasma glucose (55.42 mg/dL).

The glucose levels decreased linearly (P < 0.0001) with the feed restriction (Figure 6) corresponding to a reduction of 1.30% from 0 to 25% feed restriction and 4.98% from 25 to 50% feed restriction.

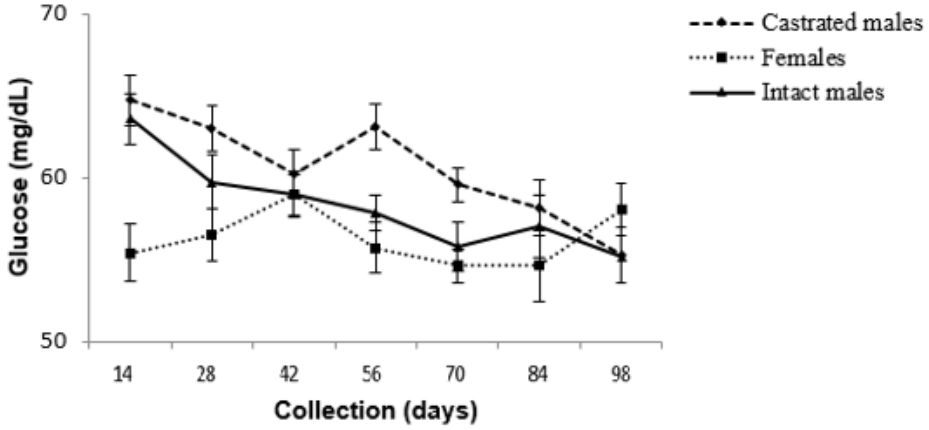

Figure 5. Plasma concentrations of glucose (mg/dL) of castrated males, intact males and females on the basis of days of blood collections (P = 0.0067).

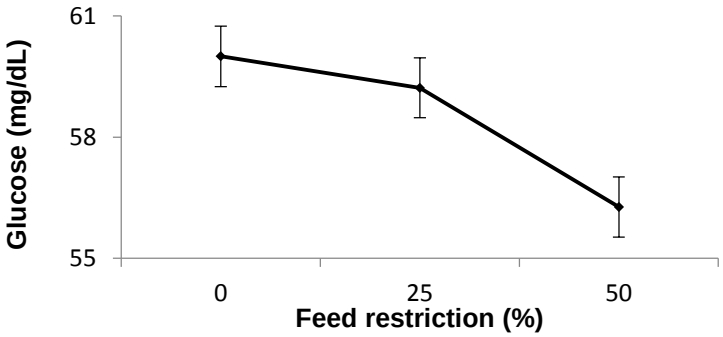

Figure 6. Plasma concentration of glucose (mg/dL; P < 0.0001; “a”) of kids fed 0%, 25% and 50% of feed restriction.

Serum NEFA increased linearly with increasing of feed restriction (P< 0.0001), while B-BH concentrations had quadratic increase with increasing of feed restriction (P = 0.021; Figure 7).

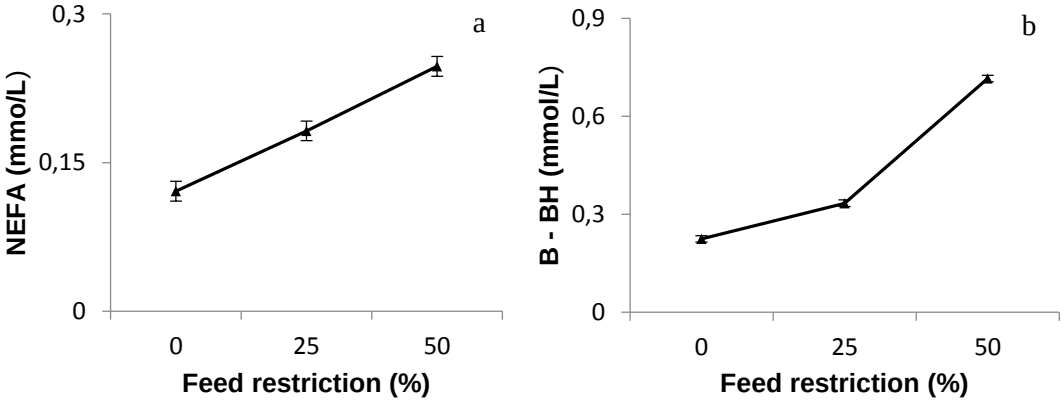

Figure 7**.** Serum concentration of non-esterified fatty acid (NEFA; P<0.0001); “a”) and B-hydroxybutyrate (B-HB; P = 0.021; “b”) kids fed 0%, 25% and 50% of feed restriction.

Gender and feed restriction did not influence the serum levels of albumin, total protein, cholesterol, AST and GGT in the blood. (P < 0.05).

Creatinine concentrations and activity of creatine kinase in the blood of goat kids increased linearly with blood collection days (Figure 8). From 14 to 98 blood collections days, there was increase of 43.41% in the levels of creatinine (P = 0.032) and 42.40% in serum creatine kinase levels (P < 0.0001).

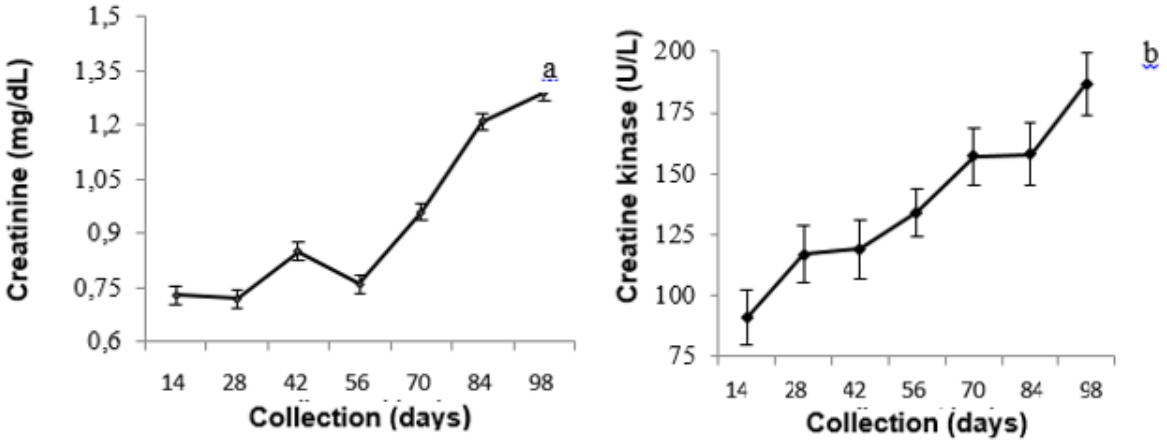

Figure 8. Seric concentration of creatinine (mg/dL; P = 0.032; “a”) and creatine kinase activity (U/L; P < 0.0001; “b”) on the basis of days of blood collections.

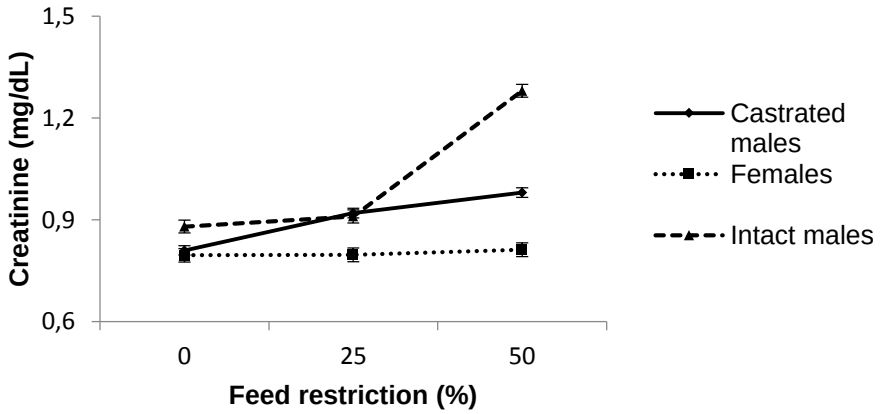

Figure 9. Serum concentrations of creatinine (mg/dL) in castrated males, females and intact males on the basis of feed restriction (P=0.0144).

The creatinine concentration was affected by the interaction between gender and feed restriction (P = 0.0144; Figure 9). For the kids fed ad libitum, the creatinine concentration was greater in intact males (0.880 mg / dL) and similar between castrated males and females (0.802 mg / dL). When animals were restricted to 25% feed restriction, no difference was observed for creatinine levels in castrated males and intact males, which in turn were greater than in females. When submitted to 50% feed restriction, intact males had greater serum creatinine levels (1.28 mg / dL), followed by castrated males (0.98 mg / dL) and females (0.81 mg / dL), respectively.

There was interaction effect between gender and feed restriction on serum urea (P = 0.020; Figure 10). Females fed ad libitum had lesser blood concentrations of urea than intact and castrated males. When submitted to feed restriction, females increased serum concentrations of blood urea with increased of feed restriction, whereas intact males decreased blood urea with the increase of feed restriction. At 25 and 50% of feed restriction, castrated males and females showed similar blood urea concentrations with each other.

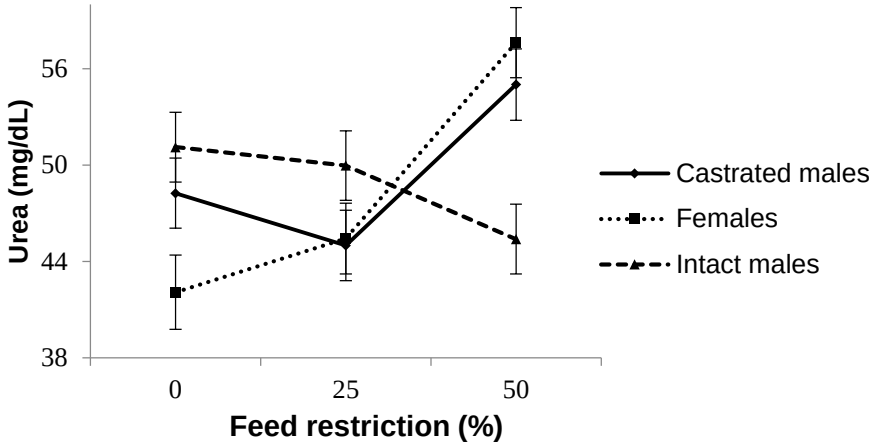

Figure 10. Serum concentrations of urea (mg/dL) in castrated males, females and intact males on the basis of feed restriction (P = 0.020).

Figure 11 shows the effect of interaction between gender and feed restriction level for creatine kinase activity (P = 0.087). Intact males had greater activity of the enzyme creatine kinase that castrated males and females, regardless of feed restriction level. The creatine kinase activity was similar in females and castrated males fed ad libitum, however when subjected to 25% and 50% of feed restriction, castrated male increased this enzyme activity, whereas female kept constant the activity throughout the period.

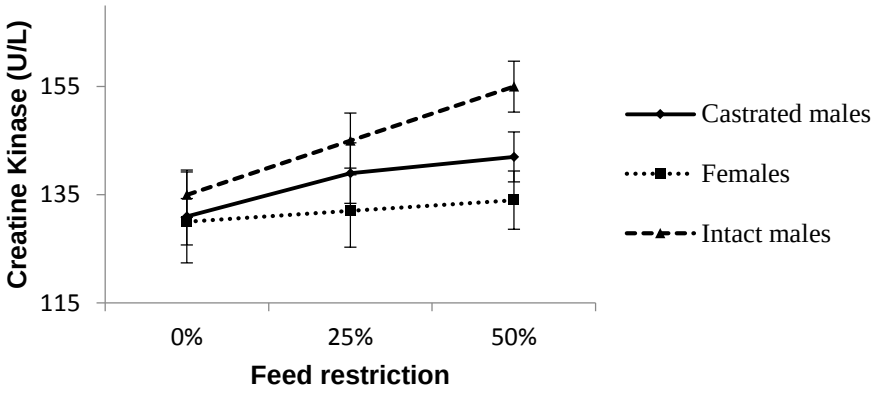

Figure 11. Serum concentrations of creatine kinase (U/L) in castrated males, females and intact males on the basis of feed restriction (P = 0.020).

3.5. Hormone

Intact males fed ad libitum and 25% of feed restriction showed greater plasma concentrations of IGF-1 than castrated males and females, respectively (P = 0.018; Figure 12). However, when the three genders were fed with 50% restriction, IGF-1 concentrations were similar, with average 85.01 ng / mL. As increased feed restriction level, plasma IGF-I decreased for all genders.

The plasma IGF-1 concentrations decreased between the first and third collection (P = 0.0059). In the blood collection days from 42 to 84, IGF-1 concentrations remained constant, but it was observed decrease in the concentration of IGF-1 from 84 to 98 days of blood collection. (Figure 13).

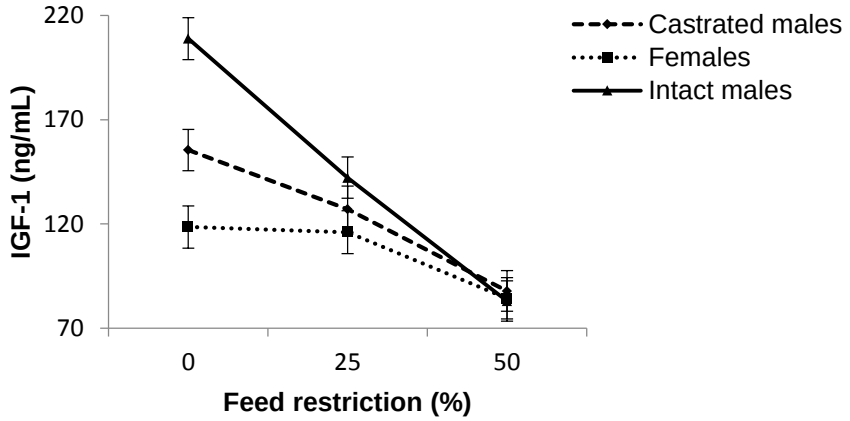

Figure 12. Plasma concentrations of Insulin-like growth factor-I (IGF-I) in castrated males, females and intact males (P = 0.018).

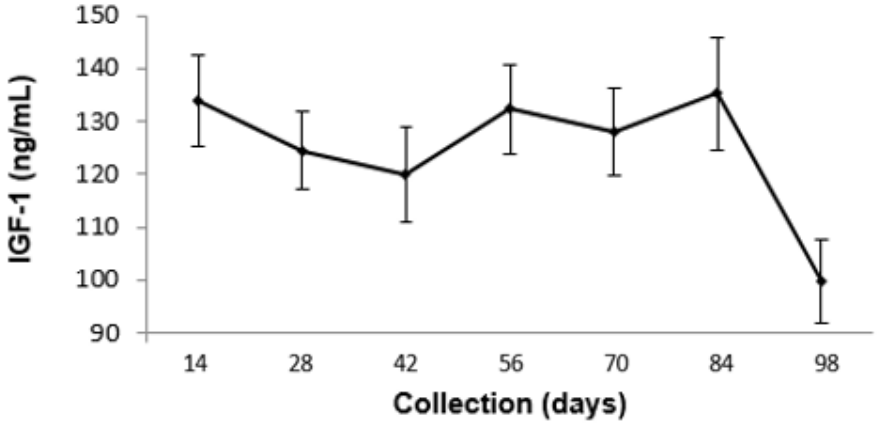

Figure 13. Plasma concentration of Insulin-like growth factor-I (IGF –1; Cubic effect, P = 0.0059) on the basis of days of blood collections.

The plasma T3 concentrations were similar in females and castrated males (1.57 ng / mL) and lesser in intact males (1.43 ng / ml, P = 0.0011; Figure 14). Furthermore, the concentration of T3 in the blood showed a linear decrease with the increase of feed restriction (P<0.0001; Figure 15).

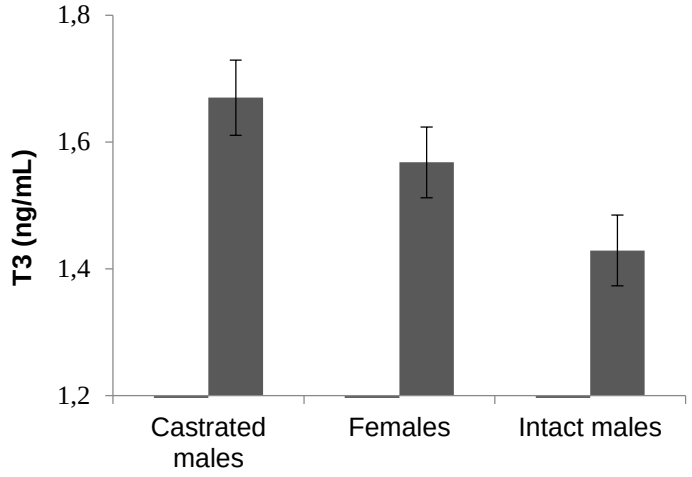

Figure 14. Plasma concentrations of Triiodothyronine – T3 (P = 0.0011) in castrated males, females and intact males.

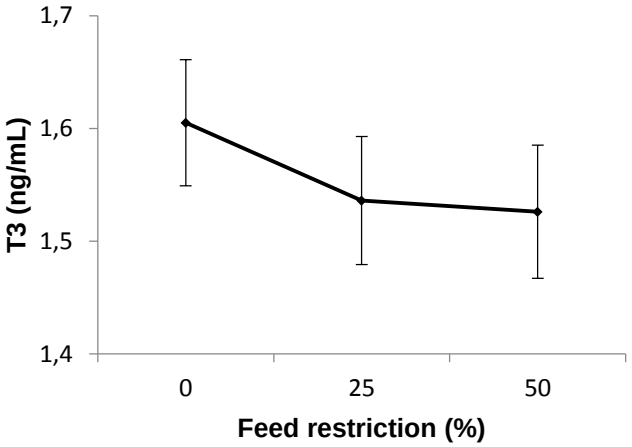

Figure 15**.** Plasma concentration of Triiodothyronine – T3 (Linear effect, P<0.0001) of kids fed 0%, 25% and 50% of feed restriction.

Plasma T3 levels were affected by blood collection period, showing linear reduction from collection 1 to collection 7 (P = 0.0002; Figure 16).

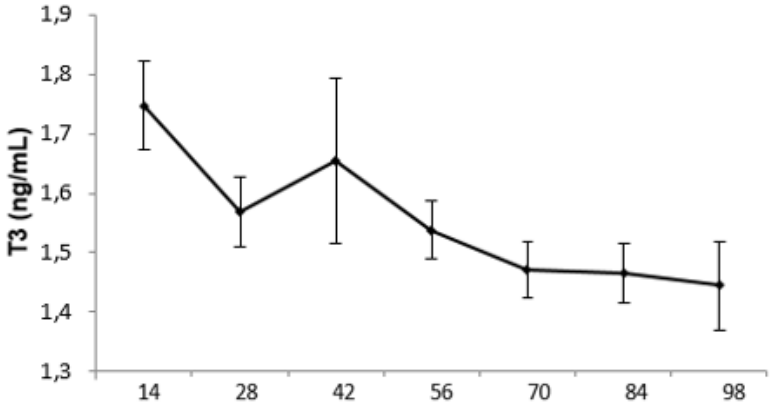

Figure 16. Plasma concentration of Triiodothyronine – T3 (Linear effect, P<0.0002) on the basis of days of blood collections.

Intact males presented lesser T4 concentrations than females and castrated males regardless of feed restriction level. Females and castrated showed similar T4 levels (P=0.0158; Figure 17).

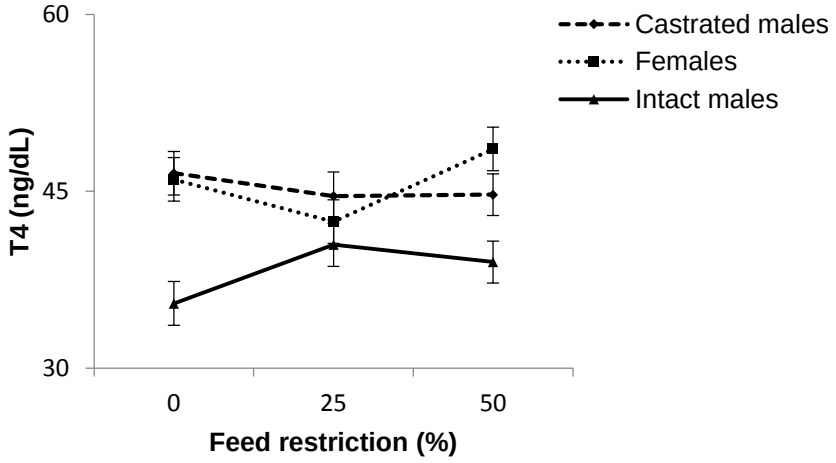

Figure 17. Plasma concentrations of thyroxine – T4 (ng/dL) in castrated males, females and intact males on the basis of feed restriction (P = 0.0158).

4.DISCUSSION

Our study shows that feed restriction and gender affected body composition and energy metabolism and protein in goats from 30 to 45 kg BW. In the growth phase studied, the feed restriction of 50% damaged the development of adipose and

muscle tissue, especially for intact males, causing mobilization of energy and protein degradation to meet the requirements for maintenance.

Intact males deposit less body fat and more muscle tissue when compared to females and castrated males, because the constancy in the release of androgens is greater (HAFEZ, 2004). According Wallimann et al. (1992), when there is some energetic limitation or high nutritional requirement and the diet is not enough to meet all nutritional requirements, males tend to use muscle degradation as an alternative to energy production from phosphocreatine. The consequence that, is to promote rapid resynthesis of ATP when the utilization rate exceeds the capacity of generating of ATP in other metabolic pathways, because among energy systems, phosphocreatine hydrolysis is responsible for the greater generation of ATP when the metabolism increases (WALLIMANN et al. 1992). According to our results, this can be confirmed by the greater concentration of serum creatinine and increased creatine kinase activity in intact males than other genders, followed by lesser retention protein during the trial period.

Conversely, we observed that even with feed restriction, females were able to keep the energy metabolism with less glycolytic contribution, since that the lesser intake of glucose caused by feed restriction was not sufficient to initiate the process of intense adipose tissue mobilization, although the adipose tissue deposition was less pronounced. This fact is possibly attributed to changes in energy metabolism in which females use to compensate for lesser energy input caused by the continued restriction to prioritize stock body fat, which besides serving as a store of energy during pregnancy and lactation. During puberty, the energy supply is essential so that there is not permanent changes in the endocrine system and adipose tissue from females, which this type of disorder can inhibit lactogenesis and/or homeorresis control during lactation (CAPUCO et al, 1995). Moreover, fat deposition can be used for synthesis of hormones derivatives from cholesterol during puberty (NELSON; COX, 2011).

Furthermore, because the greater retention of fat in females, it was possible that there was a greater effect of leptin on inhibiting feed intake in female. According Coll et al. (2007), the leptin transmits to the hypothalamus information concerning the amount of energy stored in adipose tissue, suppressing appetite and affect energy

expenditure, which can be confirmed by the lesser intake of OMI by females than intact males and castrated observed in this study.

Unlike females who had enough body reserves to be used, our study shows that intact males were the ones who suffered most from the feed restriction. Males require more energy to have a more intense protein synthesis and deposition than females (PURCHAS, 1991). Metabolically, the energy cost for producing one protein molecule is greater than 1 for the production of lipid molecule (COX; NELSON, 2011), and in this way a prolonged feed restriction situation adversely affects protein synthesis, and therefore its deposition. Under the influence of testosterone, IGF has a fundamental role in stimulating protein synthesis (MOREIRA et al, 2010). However, the less plasma levels of IGF - 1 and less T3 and T4 concentrations, found in the animals when fed at 50% of feed restriction, probably decreased protein anabolism in these animals.

According to Owens (1993), when the quantity of nutrient to be given to the animals is restricted, the growth rate is below normal. As expected, with increasing feed restriction, the goat kids changed the growth rate, regardless of gender, showing growth rates lesser than normal, which can be confirmed by lesser plasma concentrations of IGF-1, T3 and retention of DM, protein and fat in the body.

The intensity of negative energy balance in ruminants is related NEFA mobilization intensity and consequently the BHB production (LI et al. 2012). The concentration of NEFA and BHB is a reflection of the mobilization magnitude of body reserves and starts to rise a few days after the decrease of dry matter intake, aiming to provide energy to meet the nutritional requirements (GRUMMER, 1995; LEBLANC, 2010). The observed NEFA levels and BHB in our results indicate that there was adipose tissue mobilization to support gluconeogenesis. This can be clearly observed in males who showed negative retention of fat to 50% restriction. In addition, although females have had positive retention, NEFA indicated that occurred fat synthesis and degradation simultaneously in females.

According Vagnoni (1997), all ruminant in healthy state, regardless of gender, has the constant excretion of creatinine for a period of 24 h, however, some authors have found increased creatinine excretion with increased crude protein content of the diet in cows (MOSCARDINI, et al. 1998; VAGNONI; BRODERICK, 1997). Creatinine

is formed in muscle, by the irreversible and non-enzymatic removal of water from creatine phosphate, which is derived from the amino acid metabolism and to be excreted via the urine, but first the plasma must be cleared by the kidneys (LEHNINGER,1995). In our study, even females subjected to more severe feed restriction of 50% and having a negative protein retention, the serum creatinine concentration in females was kept constant in all feed restriction levels. Possibly the phosphocreatine phosphorylation was not intense enough to be able to change the values of creatinine and creatine kinase activity because of lesser intensity of protein catabolism in females than males.

In addition, the greatest concentration of creatinine and creatine kinase activity throughout the experimental period can be related to the growth of muscle tissue in the animals. Given that during puberty, mass gain rates are greater, mainly due to the deposition of muscle and gain rates decrease as approaching maturity (OWENS et al., 1993; LAWRENCE; FOWLER, 2002).

According to the results, we can infer that castrated males as well as females may be less efficient in the use of nitrogen in this growth phase for protein synthesis, showing that protein synthesis is not a priority, mainly during moderate and severe feed restriction. It is possible to note that castrated males were who less metabolized muscle tissue during the experimental period and even with the high concentration of urea in the blood were not able to suppress the degradation of muscle phosphocreatine. There are two main strategies to increase the utilization efficiency of N in ruminants, which can explain the results of blood urea concentration found in castrated males: first is to reduce the amount of N that is converted to urea in the liver by reducing the absorption of ruminal ammonia or decrease in amino acid catabolism. The second would increase the amount of urea produced by the liver that returns the rumen to be incorporated into microbial protein or with anabolic purpose when the protein metabolism is intense (LAPIERRE; LOBLEY, 2001).

Unlike, we observed that when intact males were submitted to 50% feed restriction, serum urea concentration decreased abruptly, probably because the nitrogen demand to be high for to meet the protein demand and at the same time to insufficient, possibly all urea that reached the blood, was used immediately for protein synthesis, not having enough time to accumulate in the bloodstream. On the

other hand, it may also be possible that the urea is being recycled for further microbial protein production so as to ensure the supply of amino acids for protein synthesis. According Visek (1984), the use of urea in ruminants can come via exogenous by dietary and endogenous derived from biosynthesis in the liver in the urea cycle, and the urea formed endogenously can be eliminated via urine, return to the rumen by saliva (recycling) or diffusion by ruminal epithelium then be used. The production of urea in the liver represents a continuous supply of urea to support microbial fermentation in the rumen, and are also dependent on the physiological state of the animal (LAPIERRE & LOBLEY, 2001). Thus, the increase in microbial efficiency and urea return to gastrointestinal tract through recycling, to be the biggest challenge for nutritionists, since this strategy is great benefit in microbial N incorporation efficiency by increasing the amount of microbial protein reaches the abomasum for anabolic purposes. Therefore, it is necessary to know what the actual mechanism involved in the increased transport efficiency of urea to the rumen, since that is known that the output of urea is strongly stimulated by plasma concentration (LAPIERRE; LOBLEY, 2001).

Our data indicate that under feed restriction, the mechanism used by females and castrated to compensate feed restriction may have been changes in energy metabolism, which shows that females and castrated pubescent have considerable flexibility to fat deposition by the feed restriction on long term.

Furthermore, intact males during feed restriction are not able to maintain protein synthesis, and an alternative to supply all energy demand is the muscle degradation to generate ATP and thus be used for their vital functions. According Cunningham (2004), the gluconeogenesis in ruminants occurs usually when the majority of the propionate from the fermentation reaching the hepatic portal vein after rumenal absorption, is converted to glucose, after being converted to propionyl CoA, methylmalonyl CoA, Succinyl CoA and finally into the Krebs Cycle like succinate. Otherwise, during fasting or prolonged restriction, another way that ruminants use for the maintenance of blood glucose is the mobilization of the muscle proteins in order to obtain glucogenic amino acids. Just like propionate, which turns into a precursor of oxaloacetate, the glucogenic amino acids, highlighting alanine and glutamine, are

degraded and their carbon skeletons, pyruvate and α- ketoglutarate, respectively, are channeled into gluconeogenesis (LEHNINGER, 1995).

Therefore, goat kids in growth in the weight maturity were influenced by gender and feed restriction. Pubertal males were not able to keep protein synthesis during feed restriction and females and castrated males to keep the fat deposition even when they are subjected to feed restriction. However, more studies are needed to clarify exactly what causes the different reactions of each gender when subjected to feed restriction.

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CHAPTER 4 – IMPLICATIONS

Given the importance of the proposed study and based on results we obtained in both experiments, it is undeniable that this work can to contribute in a unique way for the world scientific community and especially to farmers as a whole.

It is common sense that climate conditions are increasingly getting worse over the years, as the financial crisis in many countries, which can results in shortage of feed, preventing the production of animal products quality and / or economically unfeasible in a production system, since more than 70% of the costs of any animal production system is due to nutrition.

This study shows that during the feed restriction, intact males, castrated males and females react in different ways and are able to use different metabolic responses to meet their need of energy and thus keep the adipose tissue synthesis and muscle tissue for long periods restriction. However, depending on the stage of growth of the animal, moderate to severe feed restriction may be able to cause damage to the development of the reproductive system of the animal, such as birth defects, impaired synthesis of androgens hormones, including others.

As shown in this study, the imposition of feed restriction is less aggravating for growing goats from 15 to 30 kg, since they are able to bypass the demand for energy to supply the synthesis and deposition of nutrients. The animals in this growth phase are still on phase of greater growth efficiency than animals from 30 to 45 kg. Animals in the later growth phase are closer to maturity weight, which can be an aspect that implies in greater difficult to use nutrients. Thus, energy and protein restriction for goats during puberty can negatively affect their development and can lead to great economic losses in a production system.

Furthermore, to know the consequences caused by the utilization of certain feed restriction levels is essential to the decision of management strategies. For example, a moderate feed restriction (around 25%) can be economically viable according to the priority of farmers, since not bring significant losses in production and can result in economic and environmental gain, due to the lower amount of methane emissions, contributing so to sustainability.

This study can contribute to the purpose of determining reference values of metabolites and hormones studied for growing goats, since the existing reference values for comparison in the literature in some species including goats, does not distinguish the physiological stage and gender of the animal.