MS – thesis

Energy and protein nutrition of ewes in late pregnancy

Effect on ewe feed intake, live weight, body condition and plasma metabolites, lamb birth weight and growth rate

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Clarification of contribution

I hereby declare that the experiment this work is based on as well as statistical analysis and writing of the thesis and the two manuscripts is my work under the supervision and assistance of my advisors Jóhannes Sveinbjörnsson and Grétar Hrafn Harðarson.

The experiment took place at the Icelandic Agricultural University's experimental farm, Hestur, in the spring 2008. Daily management during the experimental period was in my hands with the assistance of the staff at the farm when needed. Postpartum the staff took over daily management. Feed and refusal sampling as well as dry matter analysis was performed by me but chemical analysis took place at the laboratory of the Icelandic Agricultural University under the supervision of Tryggvi Eiríksson. Weighing and condition scoring of ewes as well as weighing and ultrasound scanning of lambs was performed by the staff at Hestur. Blood samples were collected by the veterinarians Grétar Hrafn Harðarson and Gunnar Gauti Gunnarsson with my assistance. I prepared the samples for the analysis that was performed in the laboratory of Aarhus University at Foulum, Denmark under the supervision of Torben Larsen .

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Date and place Hallfríður Ósk Ólafsdóttir

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Abstract

Feeding of the pregnant ewe affects its weight and condition and subsequently the ability to supply the lambs with adequate nutrition. Supplementing is commonly used to secure adequate birth weight and growth rate but for efficient use the condition of the ewes as well as total composition of the diet has to be considered thoroughly. In the experiment described here the effect of feeding different concentrates during the last month prepartum on ewe and lambs health and performance were tested on 48 ewes and their progenies. Ewes were assigned to four treatments, each containing equal numbers of single- twin- and triplet bearing ewes. All lambs were reared as twins. Three of the treatment groups (MIX, EN and PRO) were fed, along with *ad libitum* haylage, increasing levels of supplements differing in protein type and content while the fourth group was control group (CTR) and only fed haylage. Ewes were weighed and scored for BCS before and after the experimental period, haylage intake recorded daily and blood samples collected weekly for metabolite analysis. Lambs were weighed at birth, seven days old, approximately seven weeks old and at last at weaning. Supplemented ewes ate significantly less haylage than CTR ewes. Though treatment did not affect weight, BCS nor weight and BCS changes, CTR ewes gained significantly less weight than the PRO ewes and the MIX group ewes had significantly higher BCS in April than other. Single bearing ewes gained less weight and were lighter at parturition than other while triplet bearing ewes had the lowest BCS and lost condition in all supplemented treatments. Treatment affected glucose, BHB, urea, uric acid, AST, ICDH and calcium level significantly, BHB and urea level increased with higher levels of undegradable protein. Glucose, urea, uric acid and ICDH levels were affected by litter size, single bearing ewes having the lowest levels of ICDH and uric acid but the highest levels of glucose and urea. No significant difference was found for birth weight between treatments. The first weeks postpartum lambs reared by CTR ewes had significantly lower growth rate than others but this difference ceased with increasing age. Type of supplements did not affect growth rate significantly and live weight at weaning did not differ significantly between treatments. Lambs reared by ewes that had given birth to twins had significantly higher growth rate the first week postpartum but the difference then ceased and was not found after seven weeks of age.

Keywords: Birth weight, DM intake, late-pregnancy, nutrition, plasma metabolites, protein sheep

Yfirlit

Fóðrun áa á síðasta hluta meðgöngu er mikilvæg fyrir afkomu sauðfjárbúa þar sem hún getur haft áhrif á þunga, holdastig og heilsufar ánna og þar með á hversu vel þær ráða við að veita lömbum þá næringu sem þau þurfa. Í tilrauninni sem hér er lýst voru skoðuð áhrif af fóðrun með mismunandi kjarnfóðurtegundum síðasta mánuð meðgöngu á heilsufar og afurðir áa og lamba þeirra. Tilraunaánum 48 var skipt í fjóra meðferðarhópa sem hver innihélt jafnan fjölda einlemba, tvílemba og þrílemba. Þremur hópanna (MIX, EN, PRO) var gefið, til viðbótar við hey eftir átlyst, vaxandi skammtar af kjarnfóðri sem innihélt mismunandi magn og gerð af próteini. Fjórði hópurinn (CTR) var samanburðarhópur, eingöngu fóðraður á heyi eftir átlyst. Ærnar voru vigtaðar og holdastigaðar í upphafi og enda tilraunar, heyát var mælt og blóðsýni tekin vikulega til mælinga á efnaskiptaafurðum. Lömbin voru vigtuð innan sólarhrings frá burði, aftur viku gömul, u.þ.b. sjö vikna gömul og loks fyrir slátrun. Ærnar í kjarnfóðurhópunum átu marktækt minna hey en CTR ærnar. Þó meðferð hefði ekki marktæk áhrif á þunga, holdastig né breytingar á þessum þáttum frá upphafi til enda tilraunar bættu ær í CTR hópnum marktækt minna við sig í þunga en PRO ærnar og MIX ærnar stiguðust marktækt hærra í holdum en aðrar í lok tilraunar. Einlembur þyngdust minna og voru léttari við burð en tvílembur og þrílembur en þær síðast nefndu höfðu lægst holdastig við burð og töpuðu holdum í öllum kjarnfóðurhópunum. Tilraunameðferð hafði marktæk áhrif á styrk glúkósa, BHB, úrefnis, þvagsýru, AST, ICDH og kalks í blóði. Styrkur BHB og úrefnis í blóði jókst með auknu magni af torleystu próteini í kjarnfóðri. Styrkur glúkósa, úrefnis, þvagsýru og ICDH var einnig háður fjölda fóstra og höfðu einlembur lægstan styrk ICDH og þvagsýru en hæstan af glúkósa og úrefni. Ekki var marktækur munur á fæðingarþunga lambanna eftir meðferð mæðra. Fyrstu vikurnar þyngdust lömb sem gengu undir CTR ánum marktækt minna en önnur en þessi munur minnkaði með tímanum. Ekki var marktækur munur á vaxtarhraða lamba eftir því hvaða kjarnfóður ærnar sem þau gengu undir fengu fyrir burð og meðferð ánna hafði ekki marktæk áhrif á lífþunga lamba að hausti. Lömb sem gengu undir tvílembdum ám uxu marktækt hraðar fyrstu vikuna eftir burð en þessi munur dvínaði svo og sást ekki eftir sjö vikna aldur.

Lykilorð: Átgeta, efnaskiptaafurðir, fóðrun, fæðingarþungi, meðganga, prótein, sauðfé,

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1. Introduction

The main goal of sheep husbandry for meat production is to wean, within reasonable amount of time, healthy lambs that have been able to fill their inherent capacity for growth and development from ewes that are healthy and in acceptable condition to start their next production cycle. The transition time, i.e. the late gestational period that is characterized by rapid foetal growth and preparation of the mammary gland and the first days postpartum when growth potential of the lamb is highest is extremely important in order to achieve that goal. Many studies have been performed during the last decades to investigate the metabolic changes and adaptations occurring in that period in order to supply farmers with information regarding effective feeding of the pregnant and lactating ewe with respect to different environment and economical circumstances.

1.1. Late pregnancy nutrition

Since 80% of the foetal growth takes place in the last trimester of the pregnancy (Robinson, 1983, Robinson et al., 1999) nutritional requirements of the ewe increase to a great extent during this period. During the last six weeks of pregnancy energy requirements of twin bearing ewe are raised by more than 80 % (Sveinbjörnsson & Ólafsson, 1999) and protein requirements by 100% (Ólafsson, 1995). At the same time, pregnancy can reduce eating capacity but increase passage rate through the digestive tract (Gonzalez et al., 1985). Those combined changes in late pregnancy all make it difficult to fill energy requirements of the pregnant and lactating ewe without using significant amounts of concentrates along with high quality roughage (Robinson, 1983).

Nutrition of the pregnant ewe affects the growth of the foetus. Both directly by providing glucose, amino acids and other chemical elements necessary for development and indirectly by influencing the endocrine mechanisms that control the uptake and partitioning of nutrients to the gravid uterus and foetus (Robinson et al., 1999). The nutrition of ewes in this critical prepartum period not only affects the growth of the developing foetus, but also the ability of the ewe to supply the lamb with adequate amount of colostrum and milk postpartum (Robinson, 1983, Treacher, 1983).

1.2. Energy nutrition

Substantial part of carbohydrate digestion in the ruminant occurs in the rumen where dietary carbohydrates are fermented by the rumen microbes. Part of the fermentation products are absorbed and to some extent metabolized in the rumen epithelium, such as the volatile fatty acids (VFA; propionate, acetate and butyrate). Remaining products from the ruminal fermentation are transported with digesta flow to the small intestines where they are, along with the microbes themselves, digested and absorbed. Concentration and proportion of the fermentation products is highly influenced by the diet offered to the animal. As an example of this, higher proportion of straw in the diet results in increased acetate production while increased starch, such as with barley feeding results in elevated level of propionate. Butyrate on the other hand is mainly raised when feeding of highly soluble sugars is increased (Oldham et al., 1977).

The main energetic substrates for foetal development and colostrum/milk production are glucose and amino acids (Banchero et al., 2006, Ingvartsen, 2006). There is only small amount of glucose absorbed from the rumen and therefore glucose requiring tissues rely on gluconeogenesis, mainly in the liver (Annison et al., 2002). Of the fermentation products mentioned above, propionate is the main precursor for gluconeogenesis. Therefore, supplementing ewes with high starch diet such as maize and barley can result in increased foetal weight and colostrum production (Banchero et al., 2007). The use of highly fermentable concentrates (rich in starch) to supply the pregnant or lactating ewe with adequate metabolizable energy (ME) has its limitations. It can induce some metabolic disorders such as rumen acidosis (Imamidoost & Cant, 2005) and reduce degradability of other nutrients fed to the animal due to alterations of the microbial fauna. The severity of these effects depend on the type of concentrates as well as the roughage quality, particularly with regard to fibre type and content (Kaur et al., 2008). Because of that, rapidly fermentable concentrates have to be used systematically, aiming at maximizing their benefit in the ruminant diet while minimizing the disadvantages they can cause (Imamidoost & Cant, 2005). According to Kaur et al. (2008) concentrates can be fed up to a 35% level without affecting fibre digestion and supplementing above that level does not necessarily affect fibre digestion. Fibre type and frequency of feeding are among other factors that have effect in this respect and in fact Kaur et al. (2008)

supplemented up to 45% concentrates without detecting negative effect on rumen pH. Subsequently they concluded that significant negative effect found for dry matter (DM) and fibre digestibility were merely due to type and amount of fibre in the diet than the concentrate level itself.

Effects of different energy levels in late pregnancy differ to some extent between studies. Possible reasons are differences in the genotype of the experimental ewes, their body reserves and overall composition of diet such as combination of energy and protein and concentration of ME (Robinson & McDonald, 1989, Thorsteinsson & Thorgeirsson, 1989).

Carbohydrates from the diet are not the only gluconeogenic precursors. Dietary protein is also to some extent used for glucose production. Furthermore, energetic efficiency is reduced when dietary protein is inadequate (Thorsteinsson et al., 1993) and, as described below, importance of microbial protein for the ewe makes carbohydrate digestion not only important in regard to energy supply but also protein. Therefore, neither energy- nor protein nutrition can be discussed in details without consideration of each other.

1.3. Protein nutrition

Protein available for absorption in the ruminating animal can be divided into two main types. One is the microbial protein, which is the protein derived from activity of the microbial population in rumen that hydrolyses dietary protein into smaller units of peptides, amino acids, organic acids, ammonia and carbon dioxide. These molecules are then reused for production of microbial cells, ammonia being the key intermediate in this process. Insufficient dietary protein supply as well as high level of rumen undegradable protein therefore reduces ammonia concentration in the rumen, hence the growth of the microbial population and subsequently carbohydrate digestion is reduced. Excessive rumen degradable protein along with deficiency of the fermentable organic matter needed to sustain microbial growth however raises ammonia level in the rumen which causes ammonia to be absorbed into the blood and converted to urea in the liver. Even though some of the urea is conserved and reused by returning it to the rumen substantial part of it is excreted from the body and therefore wasted. Because of this activity described above the quantity of protein reaching the small intestines can be greater than the quantity available directly from the food. The microbes are digested and absorbed in the small intestines, as does the other main protein type – the undegradable protein. Undegradable protein is the protein content that escapes the rumen undigested and is not utilized in any way until in the small intestines. A well known source of undegradable protein is fish meal and other products derived from animals as well as some specially treated plant proteins (McDonald et al., 2002).

Since the major protein supply in ruminants is microbial protein, the effect of different levels of nutritional protein is highly related to carbohydrate intake (Annison et al., 2002) as well as digestibility and fermentation rate of the carbohydrates. As an example, immature forages that are very rapidly fermented can yield twice as much microbial protein as diet containing high level of nutrients that are slowly fermented in the rumen (McDonald et al., 2002). Therefore, energy undernutrition is likely to cause deficiency in protein availability, defined as amino acid supply in small intestines (AAT) unless diet is supplemented with undegradable protein (O´Doherty & Crosby, 1997). It also has to be taken into consideration that even though energy intake is adequate for sufficient total production of microbial protein, supply of certain essential amino acids may not be enough in relation to the needs of the foetus (Robinson et al., 1999). Increased passage rate through the rumen that can be achieved with alterations in diet composition or due to changes in physiological factors – such as pregnancy- results in elevated level of protein available in the small intestines (Robinson et al., 1999). This is the consequence of a higher level of dietary protein escaping the rumen undegraded (Gonzalez et al., 1985) as well as raised production of microbial protein due to faster fermentation of carbohydrates (Oldham et al., 1977).

Undegradable protein has for the last decades been considered one of the most important factors in nutrition of the pregnant and lactating ewe (Robinson & McDonald, 1989).
The use of the undegradable protein is generally thought to make it easier for the ewe to use energy from her body reserves for foetal growth and preparation of lactation (Frutos et al., 1998, McNeill et al., 1997, Thorsteinsson et al., 1993). This statement is in agreement with O´Doherty and Crosby (1998) who showed the gap between ME requirements and available

ME from maternal reserves and diet to decrease with elevated level of undegradable protein in the supplements.

It is quite clear that the efficiency of protein supplementing differs to some extent between studies and the outcome is affected by several factors. This can for example be seen in Bell et al. (2000) that reviewed several studies regarding the effect of protein supplement on dairy cows. One of their findings was that positive response to dietary protein on milk yield was strongest when control diet was particularly low in total protein content and/or level of undegradable protein. Furthermore, Robinson & McDonald (1989) and later on Dawson et al. (1999) found strongest effect of undegradable protein when energy content of the diet was limited, probably as a result of amino acids from the degradable dietary protein in those cases being used for gluconeogenesis instead of being portioned to the growing foetus and mammary tissues (Robinson et al., 1999). That makes it necessary to adjust protein supplementing to energy content of the feed as well as the body condition of the ewe, the latter also being known to affect the efficiency of protein supplements (Dawson et al., 1999).

Many studies have shown positive effects of undegradable protein supplements in late pregnancy on udder development and colostrum production (Nottle et al., 1998, O´Doherty & Crosby, 1997, Robinson & McDonald, 1989) as well as the lambs ability to absorb immunoglobulin from the colostrum the first hours postpartum (O´Doherty & Crosby, 1997). According to Bell et al. (2000) the length of experimental treatment seems to be important factor regarding the effect of protein supplementing on milk yield and protein content of milk in dairy cows. Furthermore, positive effect of prepartum protein supplementing mainly appear when lactation diet is low in protein content. Therefore, Bell et al (2000) suggested that high feeding level postpartum could conceal the effect of prepartum nutrition.

Among other benefits, some sources of undegradable protein, such as fish-meal, have especially high concentration of certain essential amino acids. Example of this is cystine that is important in the formation of the lambs birth coat which is one part of the foetus known to be negatively affected by undernutrition (Black, 1983, Robinson et al., 1999). When discussing protein supplementation of ruminants it has to be taken into account that, especially in lactation, some of the amino acids available from feed are used for gluconeogenesis when energy is not adequate. That possibly reduces the effect of protein supplements on protein demanding processes since part of it is used for energy production (O´Doherty & Crosby, 1997).

1.4. Weight and body condition

Using live weight and weight change as an indicator of adequacy of the ewe's nutrition in late pregnancy has its limits, unless the number of foetuses is known and ewes can be compared only with ewes carrying the same litter size (Russel, 1984). It has been suggested that some assessment of body condition is better indicator than live weight on the fatness of the animal (Frutos et al., 1998, Russel, 1984). Body condition scoring (BCS) can be used independently of litter size (Russel, 1984).

The body fat of the ewe is an important source of energy in late pregnancy and early lactation. Ruminants have excellent ability to mobilize fat and protein from their body reserves during undernutrition (Chilliard et al., 2000) and negative energy balance at parturition is in fact quite normal condition (Ingvartsen, 2006). Up to 80% of initial body fat, can be mobilized when needed (Chilliard et al., 2000) and research by Wilson et al. 1988, (cited by Bell et al., 2000) showed up to 34% of casein and 24% of lactose to be derived from body tissue protein. McNeill et al (1997) and Frutos et al. (1998) found up to 7% and 6% respectively of carcass protein to be mobilized the last 30 days of pregnancy, but McNeill (1997) stated that carcass protein was the most important reserve for mobilization of carcass nitrogen. However, it has to be kept in mind that extensive protein deficiency and subsequent mobilization can be detrimental for some parts of normal protein metabolism in the body such as sustaining of the bone matrix and cause bone erosion (Frutos et al., 1998, Robinson et al., 1999). Thorsteinsson and Thorgeirsson (1989) concluded that it is safe to expect twin bearing ewe to give birth to lambs of sufficient weight even when fed 30% below feeding standards in the last month of pregnancy. However, it has to be clear that the validity of those statements depend on the severity of the undernutrition, the duration of feed restriction as well as the initial body fatness of the ewe. The ewe must be in excellent body condition as a result of previous feeding if feeding strategy like this is to be successive (Chilliard et al., 2000, Thorsteinsson &

Thorgeirsson, 1989). Mobilization of body tissues for energy and protein supplying is a process of high energetic cost and furthermore, use of amino acids as energy source is not very efficient. Therefore use of maternal body protein reserves is not a very profitable way for energy utilization. (Thorsteinsson et al., 1993).

Based on this knowledge BCS can be considered a useful estimator of mobilization of energy within the pregnant ewe (Frutos et al., 1998, Russel, 1984) and ewe live weight and BCS together can serve as an estimator of the sufficiency of nutrition in the last weeks prepartum. Russel (1984) stated that under adequate feeding level live weight gain higher than the weight loss from maternal tissues indicates acceptable late pregnancy feeding. Furthermore, as a general rule, 10% and 18% increase in weight of single and twin bearing ewes respectively, as well as 0,5 BCS unit loss would be sign of sufficient nutrition the last eight weeks prepartum.

Both ewe weight and BCS can be affected by supplementation of the ewe in late pregnancy (Frutos et al., 1998, McNeill et al., 1997, Ocak et al., 2005). Ewes that were only fed hay lost more condition than those supplemented during the last seven weeks prepartum in a “whole winter feeding trial” at Hestur experimental farm 1983-1986. Supplement type however neither affected BCS at parturition nor BCS changes the last seven weeks of gestation (Thorsteinsson & Thorgeirsson, 1989). Restrictively fed ewes loose weight earlier than body condition (Nørgaard et al., 2008). When acquiring the optimal BCS it has to be kept in mind that body fatness of the ewe can affect feed intake negatively, for example through changes in the efficiency of energy utilization (Tolkamp et al., 2007) and reduced space available for expansion of the digestive tract with increased fat depots in the abdomen (McDonald et al. 2002).

1.5. Eating capacity

Food quality, for example with regard to fibre content and, for silage, concentrations of fermentary products, is known to affect roughage intake (Roy et al., 1999). Quality of hay is an extremely important factor regarding feed intake (Annett et al., 2008, Thorsteinsson & Thorgeirsson, 1989). Example of this could be seen in a three year (1983-1986) experiment in Hestur experimental farm where difference in hay quality between year resulted in bigger ewe

BCS and lamb birth weight differences between years than between treatments within each year (Thorsteinsson & Thorgeirsson, 1989). Decreased intake with lower energy content of diet has, among other factors, been related to negative effect on VFA uptake in rumen epithelium. As a result VFA can accumulate in the rumen and cause decrease in rumen pH as reviewed by Ingvartsen (2006). Furthermore, low energy diet often has high content of undegradable fibre which reduces rumen passage rate and subsequently eating capacity (Ingvartsen, 2006).

Concentrate supplementing decreases roughage intake, especially with high availability and quality of roughage, a phenomenon known as substitution. Substitution rate is a concept often used in this relationship and refers to how much roughage intake is reduced per unit the supplements is increased (Dove, 2002). It is only with lower forage/hay quality the concentrates fully act as supplementing, i.e. complete addition to the nutrients available from roughage feeding (Dawson et al., 2005, Frutos et al., 1998, Kerslake et al., 2008) which would result in substitution rate of zero.

Physiological factors, such as body fatness can affect feed intake to some extent (Tolkamp et al., 2007). Eating capacity decreases as pregnancy proceeds (Orr & Treacher, 1989, Speijers et al., 2005) and in dairy cattle pregnancy lowers eating capacity by 20-25% (Ingvartsen, 2006).

Effect of litter size on eating capacity is unclear since experimental results are rather inconsistent. Some experiments show no effect of litter size (Sormunen-Cristiana & Jauhiainen, 2001) at least not with single- compared to twin-bearing ewes (Foot & Russel, 1979) while other show great reduction in intake with increased litter size (Orr & Treacher, 1989). Orr & Treacher (1989) linked their results with reduction of free space in their abdominal cavity but the reasons for no response in the study of Sormunen-Cristiana & Jauhiainen (2001) remain unknown. Effects of pregnancy diet on intake is not restricted to pregnancy and early lactation since it can affect voluntary intake until at least 3 months post weaning (Foot & Russel, 1979).

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1.6. Blood parameters

During late gestation several metabolic changes and adaptations take place in the pregnant animals body (Ingvartsen, 2006). These changes are to some extent reflected in concentrations of certain metabolites in plasma which can provide information regarding adequacy of nutrition at each time. The advantage of measuring blood parameters to estimate nutritional status is that it gives more immediate information compared to ewe weight and BCS, lamb birth weight and growth rate, the latter only presenting adequacy of nutrition on a longer term basis (O´Doherty & Crosby, 1998, Russel, 1984).

Glucose is the main energy source for foetal growth and the undernourished ewe is able to make several alterations to make sure that the foetal needs for glucose are fulfilled (Robinson et al., 1999). Elevated glucose need is reflected in the fact that glucose turnover is increased by 45% during late pregnancy and by 119% during lactation (Schlumbohm & Harmeyer, 2004). Positive relationship has been reported between energy intake and plasma glucose that is significantly elevated in the end of pregnancy (O´Doherty & Crosby, 1998). Nevertheless, even though gluconeogenesis is increased in liver and the use of glucose in body tissue (except for mammary) is reduced, glucose level in plasma usually drops postpartum (Ingvartsen, 2006).

As previously discussed, ruminants have excellent ability to use energy from body reserves to meet increasing needs in late gestation. As a result of the lipolysis in adipose tissue that occurs when substrates for foetal growth and preparation of lactation are limited, non-esterified fatty acids (NEFA) are released into the blood circulation (Chilliard et al., 2000) and serve as an important source of energy. Therefore NEFA concentration in plasma can be used as an indicator of adipose tissue mobilization. However, though its usefulness in supplying the growing uterus and udder with energy, NEFA also serve as one of the central elements in the development of the metabolic disorder “fatty liver” and can subsequently be a sign of increased risk of displaced abomasum. NEFA concentration in plasma has been negatively correlated with dry matter intake (DMI), i.e. dip in DMI of late pregnancy cows causes elevated levels of NEFA in plasma (Ingvartsen, 2006).

High level of ketone bodies such as beta-hydroxy-butyrate (BHB) in plasma, along with low to normal level of glucose can be signs of the metabolic disorder ketosis. Ketosis is developed when the ewe unsuccessfully attempts to meet the increasing energy demands of her growing foetus (Harmeyer & Schlumbohm, 2006) and can develop from few circumstances. One example is energy deficiency in feed since the undernutrition stimulates lipid transfer from body reserves (Ingvartsen, 2006) and according to Banchero, et al. (2006), Speijers et al., (2005) and O´Doherty & Crosby (1998) great loss of BCS in late pregnancy is reflected in high BHB concentration in plasma. High butyrate level in diet as well as health problems that for example cause depression in feed intake and consequently lowered glucose level are another risk factors for ketosis (Ingvartsen, 2006). Physiological status of the animal affects plasma BHB level which is significantly higher in late gestation and early lactation compared to the dry period. Furthermore, twin bearing ewes in late pregnancy and lactation have elevated BHB levels compared to single bearing ewes (Harmeyer & Schlumbohm, 2006). Based on the factors listed above it can be suggested that BHB levels are raised as a result of increased ketogenesis in the liver when difference between required and available ME is increased (Ingvartsen, 2006, Russel, 1984).

In sheep, the level of ketone bodies in blood and consequently the risk of developing clinical ketosis or pregnancy toxaemia seem to be highest in late pregnancy. That is inconsistent with cows that are in the highest risk of pregnancy toxaemia in early lactation which is, in sheep as well as cows, the period when the gap between required and available energy is highest and the greatest mobilization of body reserves should occur (Harmeyer & Schlumbohm, 2006). Specific nutrition factors are known to affect BHB level. Soy bean protein supplementation resulted in decreased level of plasma BHB the last two weeks of pregnancy (O´Doherty & Crosby, 1998). So did supplementing concentrates to ewes grazing fields of various sward heights (Kerslake et al., 2008). On the other hand, ewes only fed silage have shown elevated BHB levels even though no signs of pregnancy toxaemia or ketosis were found with lowered energy intake (O´Doherty & Crosby, 1998). Individual difference is however substantial, some animals showing symptoms of pregnancy toxaemia at BHB levels others can achieve without expressing any signs of discomfort (Banchero et al., 2006).

Relationship between ME and BHB seems to be linear up to certain limit, afterwards BHB increases with extra energy (O´Doherty & Crosby, 1998) indicating an optimum level of nutrition. Despite those findings, ketosis in sheep does not seem to be as affected by level of nutrition as in many other species (Everts & Kupier 1983, c.f. Harmeyer & Schlumbohm, 2006). Hyperketonaemia inhibits hepatic glucose production as well as glucose uptake and utilization in peripheral tissues and as a result of that it increases negative energy balance. Ketone bodies are not suitable as fuel for brain function and foetal growth. Therefore risk of pregnancy toxaemia is increased prepartum since the animal cannot use the ketone bodies to meet increasing demands of the foetus.

When amino acids in diet exceed what is needed for maintenance and production at each time, and energy is limited, extra amino acids can be used as an energy source. Efficiency of amino acids as an energy source is however limited and disposing of extra nitrogen requires energy (Thorsteinsson et al., 1993). With protein being one of the most expensive nutrients, feeding dietary protein above requirements can be considered highly unfavourable. Excess dietary nitrogen degraded in the rumen is absorbed as ammonia which then accounts for substantial amounts of urea produced, mainly in the liver (Annison et al., 2002). Acquiring the efficiency of protein feeding, detection of plasma urea level can be useful since urea is the animals way to transport excess nitrogen without risking toxic effect (Withers, 1998). Plasma urea increases with undegradable protein supplementing but not, according to O´Doherty & Crosby (1998), with "non protein supplements" even though they give higher total protein level. Plasma urea reflects adequacy of protein availability (dietary and maternal protein) compared to needs (Banchero et al., 2006). Plasma urea as well as uric acid also serves as transporters of the nucleic acids derived from the small intestine digestion of the rumen microbes (Annison et al., 2002).

Uric acid is derived from the breakdown of nucleic acids, especially purines, and its concentration is commonly related to level of microbial growth in the rumen and hence microbes digested and absorbed in the small intestines. Therefore uric acid in plasma can be used to evaluate the amount of microbial protein available for absorption in the small intestines (Dewhurst et al., 2000, Pulido et al., 2010).

Since a great portion of nutrient partitioning and utilization takes place in the liver, concentration of some liver enzymes in plasma can indicate the level of metabolism. As an example glutamate dehydrogenase (GLDH) and aspartate aminotransferase (AST) reflect the rate of utilization of ammonia in the liver indicating active protein catabolism and metabolism and gamma-glutamyl transferase (GGT) plays an important role in peptide formation (McDonald et al., 2002, Milano et al., 2000). Isocitrate-dehydrogenase (ICDH) however takes part in lipogenesis (Suagee et al., 2010) and is one of the most active enzymes in that matter. Based on this, those enzymes levels can be related with fat accumulation in the liver and the subsequent metabolic disorder fatty liver (McDonald et al., 2002, Suagee et al., 2010).

Requirements for calcium are severely extended the last days prepartum and the first days postpartum as a result of high calcium level in colostrum and milk. With sufficient supply of vitamin – D the ewe has good ability to mobilize calcium from her skeleton for use in lactation but yet, calcium level of the diet at this critical time should be elevated. In that respect it has to be kept in mind that reduced feed intake, for example caused by insufficient roughage quality, reduces absorption of calcium from the intestines resulting in lowered level of plasma calcium (Harmeyer & Schlumbohm, 2006). Furthermore, both roughage and supplements are of great diversity respecting calcium supply. Fish meal and silage from dicotyledons are examples of diet having rather high calcium level while many crops and grass species such as timothy, which is common plant in roughage production in Iceland, are low in calcium and many other minerals.

1.7. Lamb birth weight

Many studies have shown effect of insufficient nutrition in pregnancy on growth of the foetus (Frutos et al., 1998, Husted et al., 2008, Husted et al., 2007, Khalaf et al., 1979, Robinson & McDonald, 1989), the effect ranging from gradually slowing down to complete cessation of growth with more sudden and severe undernutrition (Robinson & McDonald, 1989). Some compensation can occur in foetal growth when nutrition is increased after short period of undernutrition but longer periods result in the foetuses lacking the ability to return to normal growth (Robinson & McDonald, 1989). Furthermore, late gestational undernutrition can affect the lambs metabolic control postpartum, up to 19 weeks of age (Husted et al., 2007) and in their adult lives under metabolic stress such as in late pregnancy (Husted et al., 2008).

An example of the effect of gestational undernutrition on birth weight was found in two trials on the Icelandic experimental farm, Hestur, 1977-1982. Supplementing ewes with concentrates fulfilling 20% and 35% of energy requirements and 15% and 30% of protein requirements resulted in 6% and 9% increase in lamb birth weight compared with ewes that were only fed hay (Thorsteinsson & Thorgeirsson, 1989). However, Nørgaard et al (2008) and O´Doherty & Crosby (1997 & 1998) found no significant effect of feed restriction or increasing ME intake on lamb birth weight, even though restricted ewes only were fed 50% of energy requirements in the previously mentioned research and difference in ME intake was considerable in the latter. O´Doherty & Crosby (1997) linked their results with relatively small efficiency of dietary energy for conceptus growth.

Results regarding effect of dietary protein on lamb birth weight are not completely consistent. Annett et al. (2008) found positive effect of undegradable protein on birth weight while both Nørgaard et al. (2008) and O´Doherty & Crosby (1997) failed to detect such difference. O´Doherty & Crosby (1997) suggested that low energy level could have limited the effect the protein supplements would be expected to have. Thorsteinsson & Thorgeirsson (1989) however linked the lack of supplementing effect on lamb birth weight to the excellent body condition the ewes had achieved earlier in pregnancy. Ocak et al. (2005) suggested that increased litter weight from ewes fed excess protein could be related to elevated production of propionate compared to other volatile fatty acids (VFA). The propionate being important source for glucose, its availability to the foetus would be higher with increased protein in diet.

Thorsteinsson & Thorgeirsson (1989) examined the effect of BCS at different times of the year on birth weight of twin lambs and found BCS in late March to have the strongest effect. Furthermore, birth weight increased more with improving BCS in the lower range of the scale. For supplemented ewes increased score above the score of 3,7- 4, that gave the maximal birth weight, resulted in reduced birth weight. Within the only hay fed ewes however, the relationship was closer to linearity, birth weight increasing with every increase in BCS, yet with slightly decreasing effect with increasing BCS. Birth weight is also affected by maternal live weight at parturition, measured at start of lambing. (Scales et al., 1986).

High priority of foetal growth above maternal tissues can be seen where increased level of nutrition in late gestation results in higher birth weight rather than BCS (Thorsteinsson & Thorgeirsson, 1989) and therefore it can be stated that birth weight is influenced by some combination of the condition of the ewe in the latter part of mid pregnancy – as defined by previous feeding - and late pregnancy nutrition (Kerslake et al., 2008, Thorsteinsson & Thorgeirsson, 1989). In addition to this, body condition of the ewe (along with its size and age) also influences the growth of the foetus by taking part in the partitioning of nutrients between the conceptus and other mammary tissues and the maternal body reserves (Robinson et al., 1999).

Researches are in good agreement concerning the positive effect of lamb birth weight on lamb survival, especially around parturition (Gama et al., 1991, Nottle et al., 1998, Robinson & McDonald, 1989). Partially this is an effect of heavier lambs being more developed and fatter at parturition, especially with regard to the amount of the brown adipose tissue that is essential for the thermoregulation and energy supply of lambs during their first hours postpartum (McNeill et al., 1997, Robinson et al., 1999). Bigger body surface relative to body weight in lighter lambs compared to those heavier also increase their risk of hypothermia (Andrews & Mercer, 1985). Moreover, heavier lambs are in less risk of trauma or death because of respiratory- or digestive problems (Gama et al., 1991). That can to some extent be caused by the fact that heavier lambs receive more colostrum than the lighter ones and therefore absorb greater amount of immunoglobulin making them better prepared for infections (Khalaf et al., 1979). Extremes in birth weight however can result in increased lamb mortality, especially due to lambing difficulties (Gama et al., 1991). It has therefore been concluded that lamb survival is highest at some optimal birth weight but decreases both above and below that.

In addition to the effects of ewe nutrition on birth weight found in some studies, litter size (Orr & Treacher, 1989 & 1994, Sormunen-Cristiana & Jauhiainen, 2001, Thorsteinsson & Thorgeirsson, 1989), sex of the lamb (Nørgaard et al., 2008) and age of the dam

(Thorsteinsson & Thorgeirsson, 1989, Warren & Mysterud, 1995) all affect birth weight of the lambs although the last factor is possibly to some extent more related to ewe parity than the age *per se* (Purser & Young, 1959).

1.8. Lamb growth rate

Although condition of the ewe and birth weight of the lamb are to some extent good indicators of nutrition during pregnancy the main goal of the prepartum feeding must be to secure sufficient rearing ability of the ewe i.e. its ability to supply enough milk to meet with the lambs capacity to grow fast (Ocak et al., 2005).

Robinson & McDonald (1989) and Nottle et al. (1998) found that protein supplements fed to ewes in late pregnancy increased colostrum production. Similar results were obtained from Sormunen-Cristiana and Jauhiainen (2001) which found positive effect of elevated energy and protein levels in late pregnancy upon growth rate of lambs during the first six weeks of their life. Furthermore, colostrum production in restrictedly fed ewes was only half of that produced by *ad libitum* fed ewes (Nørgaard et al., 2008). Banchero et al. (2006) found that feeding 70% of requirements was insufficient to sustain optimal colostrum production. Moreover, Banchero et al. (2007) found that supplementing restrictedly fed ewes with energy rich concentrates, supplying extra ME but not undegradable protein resulted in increased colostrum production. That is probably because glucose is the main precursor for lactose synthesis and subsequently milk production and easily fermentable supplements providing high ME content as used in Banchero et al. (2006) are likely to elevate glucose level rapidly.

Dawson et al. (1999) and Annett & Carson (2005) both failed to detect any response for undegradable protein supplements above other supplements on colostrum output. They suggested their results could be affected by the high quality of the forage/silage used in their experiments while Speijers et al. (2005) and Kerslake et al. (2008) linked similar results also with good body condition of the experimental ewes.

In addition, reduced colostrum yield with increased crude protein (CP) found by Ocak et al. (2005) implies that that excessive dietary protein, above certain limit, can negatively affect colostrum production. Importance of this finding however is uncertain since lambs in that research were not significantly affected. Exactly how high the limit is probably depends on the nutritional status of the ewe as well as composition of other diet offered.

Even though Nørgaard et al. (2008) detected as much decrease in colostrum output with feed restriction as described previously, milk production in his restricted experimental ewes had as soon as five days postpartum reached the level found in the *ad libitum* fed ewes. Furthermore, for the whole lactation no significant effect were found on milk production measured as lamb live weight though lambs from restricted ewes were on average little lighter than others. O´Doherty (1997) linked decreased colostrum yield in ewes not fed protein supplement with lower protein availability in the mammary gland.

It can be concluded that variable effect of pregnancy- and early lactation diet on subsequent milk production is because of the high priority of the foetus and mammary gland above maternal body tissue for nutrients. Moreover, the ewe's ability to mobilize maternal tissues to compensate for insufficient late pregnancy nutrition is extremely important in that matter. Undernourished ewes have, as a result of extensive tissue mobilization in the critical period around parturition, less body reserves to rely on postpartum. Negative effect of restricted pregnancy feeding however can be overridden by successful nutrition during lactation (Nørgaard et al., 2008) but that requires greater quality and quantity of diet than for better nourished ewes.

Litter size, i.e. number of lambs reared by one dam, affects growth rate (Sormunen-Cristiana & Jauhiainen, 2001), probably to some extent because of lower birth weight of lambs with increasing litter sizes since Greenwood et al., (1998) found lighter lambs to have lower growth rate the first weeks. This finding is in agreement with Khalaf et al. (1979) that found lambs reared by undernourished ewes to have restricted growth rate. They suggested this would be the result of combination of lower birth weight, hence less ability of the lambs, for feed intake, and decreased colostrum and milk production in the restrictedly fed ewes. Since lamb growth rate is highest the first six to seven weeks of their life, the effects of ewe undernourishment as well as other factors affecting lamb birth weight cease with age, except that difference between sexes increases (Thorgeirsson & Thorsteinsson, 1989). As the growth period proceeds bigger portion of the lambs' nutrition is derived from herbage allowance compared to the mothers' supply of milk. Furthermore, lamb growth rate ceases with advancing age, both these facts resulting in diminishing effect of late pregnancy and early lactation nutrition of the ewe on lamb performance (Guðmundsson & Dýrmundsson, 1989).

2. Aims

Feeding recommendations available to Icelandic farmers are mainly based upon research work that took place decades ago. Sheep husbandry in Iceland has changed a great deal during those years, both with regard to housing and feeding as well as economical circumstances. The main goal of the research work this current project is a part of is to collect data that can become basis for revaluing recommendations regarding late pregnancy feeding of ewes under Icelandic circumstances.

The aim of this particular research was to investigate the effect of combination of roughage and supplements differing in protein type on the health, performance and metabolic balance of the ewes as well as the birth weight and growth rate of their lambs.

Manuscript I presents results regarding effects of feeding ewes in late pregnancy different concentrate types along with *ad libitum* haylage on their eating capacity, weight, body condition and metabolic status

Manuscript II presents results regarding effects of feeding ewes in late pregnancy different concentrate types along with *ad libitum* haylage on lamb birth weight and growth rate from birth to weaning.

Both manuscripts will be submitted to Icelandic Agricultural Sciences.

In this thesis the results presented in the two manuscripts are combined into continuum and attempt made to reveal the connection between the parameters under investigation.

3. Materials and methods

The experiment took place at Hestur, the sheep experimental farm of the Agricultural University of Iceland in the spring 2008.

3.1. Experimental animals, housing and feeding

Forty-eight pregnant ewes of the native Icelandic flock were allocated to one of four dietary treatments (*n=12*) from 30-39 days pre-lambing until lambing. The ewes had been scanned for litter size, each treatment group containing equal numbers of single, twin and triplet bearing ewes. All treatment, before and after the experimental period, was as traditional at the farm. Ewes had been housed since November and fed grass silage or haylage *ad libitum* at all times. Shearing took place in November and again in March. Ewes were mated in December.

Treatment groups were balanced for ewe BCS assessed in February, age, expected lambing date and index for mothering ability, evaluated on the scale 0,1-9,9. For calculation of this index each farms average ewe output is set as the index five and deviation of ewes output from the mean results in their index raising or decreasing to certain level. Table 1 presents treatment means and standard deviations for those factors, as well as means for ewe weight and BCS in late March.

Table 1. Weight, body condition score, age and index for mothering ability of experimental ewes

Treatment	CTR		MIX		EN		PRO	
	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
Body condition score in February	3,67	0,27	3,69	0,24	3,65	0,25	3,64	0,34
Ewe weight in Mars (kg)	82,2	7,7	81,1	7,20	81,80	7,90	79,50	4,60
Body condition score in Mars	3,71	0,23	3,90	0,34	3,79	0,35	3,75	0,32
Ewe age	4,67	1,56	4,67	1,61	4,58	1,68	4,58	1,31
Index for mothering ability	5,10	1,10	5,20	0,70	5,10	0,90	5,10	1,10

Experimental ewes were separated from the flock at April 1st and introduced to the experimental haylage. Each group was divided into two replications (*n=6*), with equal numbers of single, twin and triplet bearing ewes, that were penned and fed separately.

All groups were *ad libitum* fed grass haylage from round bales, the allowance being adjusted daily for each replication, supplying 10% more than the previous day's intake. Daily ration was divided into two portions, one being fed in the morning and the second in the afternoon. All ewes had free access to water and salt block with mineral and trace elements (see appendix I for detailed composition). Otherwise the treatments were as listed below:

- Control group (CTR): Fed only haylage throughout the experiment.
- Mixed supplement group (MIX): Fed increasing ration of a mixture of high protein and high energy concentrates from day 9 of the experiment.
- Energy supplement group (EN): Fed increasing ration of high energy concentrates from day 9 of the experiment.
- Protein supplement group (PRO): Fed increasing ration of high protein concentrates from day 9 of the experiment.

The high energy concentrate consisted of 50% bran, 41,75% maize, 5% molasses, 2% shell calcium, 1% feed salt and 0,25% mineral and vitamin mix (see appendix I for detailed composition). The high protein concentrate however consisted of 69% fish meal, 30% barley, 1% magnesium phosphate and 0,1% E-vitamin. Chemical composition of haylage and concentrates is listed in table 2.

Table 2. Dry matter content and chemical composition of the feed.

	Haylage	High energy concentrate	High protein concentrate
DM (%)	58,5	88	88
Fem (Fe kg DM^{-1})	0,86	0,98	1
Protein (g kg DM^{-1})	170	110	440
AAT (g kg DM^{-1})	84	105	200
PBV (g kg DM^{-1})	24	-35	168
NDF g kg DM^{-1}	514	128	45
Ca g kg DM^{-1}	3	8	55
P g kg DM^{-1}	3	6	32
Mg g kg DM^{-1}	2	2	4
Na g kg DM^{-1}	1	4	6

Concentrates were fed in one single portion in the morning, prior to haylage feeding.
The first days of supplementing, all ewes in supplemented groups were fed the same amount of concentrates. Daily rations were then recalculated and increased regularly until lambing, the

concentrate ration aiming at supplying all concentrate-fed groups with the same amount of total gAAT ewe^{-1} day^{-1} at each time. Daily ration of supplements at each time is shown in table 3.

Table 3. Daily ration of supplements (g DM ewe^{-1} day^{-1}) at each time.

	CTR	MIX	EN	PRO
Experimental week 1	0	*60	60	60
Experimental week 2	0	*100	100	100
Experimental week 3	0	**190	250	131
Experimental week 4 and until lambing	0	***260	343	180

* equal ration of high energy and high protein supplements
** 125 g high energy supplement and 65 g high protein supplement
*** 170 g high energy supplement and 90 g high protein supplement

At parturition ewes were individually penned for around 2 days but moved to groups of increasing size the next days if no problems occurred. For around 8-12 days postpartum all ewes received the same ration, approximately 150 g ewe^{-1} day^{-1} of the high protein supplement. Within the first couple of hours after birth lambs were dosed with 40 mg Clamoxyl to prevent *E.coli* infection (watery mouth disease). Litter sizes were balanced to two lambs per ewe directly after birth, i.e. one lamb was removed from each triplet bearing ewe and one extra lamb, usually triplet or twin from yearling, was added to those that had given birth to singles. Because of this process and the fact that the ewes did not always lamb on the day expected, some of the lambs reared by experimental ewes were not born to ewes from the experiment. One ewe only raised one lamb since no extra lamb was available at parturition. This ewe was therefore excluded from the postpartum data. Within 24 hours from birth the lambs were weighed, ear tagged and sex and colour registered. One lamb was stillborn and two died because of lambing difficulties. Two lambs died within the first 10 days, both reared by the same dam that only reared one lamb afterwards. Approximately two weeks postpartum one lamb was found dead in the pasture and one of the experimental ewes died 10 days postpartum, reason for death in both cases is unknown.

Ewes and lambs were kept indoors but with access to outdoor pen until 8-12 days after lambing, after last “post parturient” blood sampling. Then they were moved to cultivated pasture, yet with free access to haylage as well as 50 g ewe^{-1} day^{-1} of high protein supplement. Approximately one month postpartum all sheep were excluded from the cultivated land and grazed rangeland at Hestur until the end of June. At that time all ewes were gathered and taken to the highlands, except for ewes that had managed to escape to the surrounding farm or lost their lambs. At September 17th the flock was gathered from the highland and grazed on rangeland at Hestur until September 22nd when lambs were weaned and grazed on cultivated pasture until slaughter.

3.2. Measurements and data sampling

The intake of grass silage within each replication was recorded every day from day three, until lambing of the first ewe. Since it was not possible to individually feed the ewes daily intake is calculated as mean intake of each replication – that is: (replication allowance-replications refusals)/number of ewes in replication. Two samples were taken from each round bale and one from each day's refusals (compounded from all replications) and frozen down for later dry matter and chemical analyses.

Blood samples were collected from all ewes every week in one of the replication for each treatment. Sampling started on day three of the experiment and resulted in total of six or seven samples per ewe, depending on the day of lambing. The second last sample was taken as representative of the ewe at lambing and the last representing the changes occurring the first days (5-9) postpartum. Blood samples were collected from jugular vein by venipuncture using 9 ml Lithium Heparin vacuette® (grainer bio-one) vacutainer. Sampling took place in the afternoon on sampling days, prior to haylage feeding. Samples were immediately placed on ice and then centrifuged for 20 minutes. Two times two ml of the plasma was collected into 4 ml tubes and frozen down for later analysis.

Ewe weight and BCS according to the five unit scale described by Russel et al. (1969) was recorded on day 28 of the experiment.

Birth weight was recorded within 24 hours from birth, stillborn lambs and lambs that died at parturition included. Seven days old all lambs reared by the experimental ewes were weighed; however, the lambs from the ewe that died ten days postpartum, and from the two ewes that only reared one lamb were excluded from the statistical analysis, total number of lambs used for the analysis 24, 22, 22 and 22 lambs in treatment groups 1-4 respectively. In end of June, when the lambs were 45-57 (average 49) day old the 76 lambs present at that time (18, 22, 21 and 15 lambs in groups 1-4 respectively) were weighed and used for statistical analysis regarding growth rate from seven days old to end of June. The remaining 14 compared to the one week old weighing were either not present due to escape of their dams or dead, number in each category unknown at that time. The lambs were weighed again at weaning when they were on average 144 days old. Lambs reared by the ewes that were not present in end of June are excluded from statistical analysis of growth rate from end of June to weaning, data of a total of 74 lambs being used for this analysis (18, 20, 21 and 15 lambs in groups 1-4 respectively). For weaning weight and growth rate from birth to weaning the data consisted of 85 lambs (24, 20, 21 and 20 lambs in groups 1-4 respectively), those five missing compared to the one week old weighing having died somewhere in the period from one week old to weaning.

3.3. Chemical analysis

All feed samples, two from each round bale except only one from the last bale were analyzed for dry matter, digestibility, energy (FEm kg DM^{-1}) protein (g $kgDM^{-1}$), AAT (g kg DM^{-1}), PBV (g kg DM^{-1}) and Neutral Detergent Fibre (NDF). Protein was measured using the Kjeldahl method and AAT and PBV values were calculated according to Madsen et al. (1995) NDF was determined using ANKOM technology on Van Soest method (Van Soest et al., 1991) and for dry matter digestibility the modified method of Tilley and Terry was used (Tilley & Terry, 1963). Refusal samples were combined making one sample from each round bale and analyzed by the same methods as feed samples.

For plasma glucose, AST, GGT, urea and uric acid determination standard procedures (Siemens Diagnostics® Clinical Methods for ADVIA 1650) were used and for NEFA the Wako, NEFA C ACS-ACOD assay method. Increase in absorbance at 340 nm due to the production of NADH, at slightly alkaline pH in the presence of ß-OH-butyrate dehydrogenase was used to determine BHB. Sample blank was included and the method involved oxamic acid in the media to inhibit lactate dehydrogenase as proposed by (Harano et al., 1985). Activity of GLDH was quantified in a kinetic, colorimetric assay according to Schmidt & Schmidt, (1995). ICDH was also determined in a kinetic, colorimetric assay using isocitrate as substrate and $NADPH_2$ as response parameter. The autoanalyzer ADVIA 1650® Chemistry System (Siemens Medical Solutions, Tarrytown, NY 10591, USA) was used for all analyzes.

3.4. Statistical analysis

Data was analyzed using the REML method of SAS Enterprise Guide 4.1 and 4.2 (SAS institute, 2004) mixed model analyze. When response variable was individual ewe information such as ewe BCS and weight we used the following model:

$$Y_{ijkl} = \mu + T_i + L_j + A_k + D_l + (T \text{ x } L)_{ij} + \varepsilon_{ijkl}$$

Where Y_{ijkl} is the response variable, μ is the overall mean of the population, T_i is the mean of the experimental treatment (i = 1-4), L_j is the mean effect of litter size (j = 1-3), A_k is the effect of ewe age (k = 3-8) and D_l is the effect of length of the treatment (number of days from onset of the experiment until lambing; l = 30-39), $(T \text{ x } L)_{ij}$ is the interaction between treatment and litter size and ε_{ijkl} represents the unexplained residual elements that are assumed to be independent and normally distributed.

Those effects were always included in the model when estimating individual ewe information though they did not affect response variable significantly in all cases.
When lamb birth weight was the response variable the model was as follows:

$$Y_{ijk} = \mu + T_i + L_j + S_m + A_k + D_l + (T \text{ x } L)_{ij} + \varepsilon_{ijk}$$

Where Y_{ijklm} is the response variable, μ is the overall mean of the population, T_i is the mean of the experimental treatment (i = 1-4), L_j is the mean effect of litter size (j = 1-3), S_m is the mean effect of sex of the lamb (m = 1-2), A_k is the effect of ewe age (k = 3-8) and D_l is the effect of length of the treatment (number of days from onset of the experiment until lambing; l = 30-39), $(T \text{ x } L)_{ij}$ is the interaction between treatment and litter size and ε_{ijkl} represents the unexplained residual elements that are assumed to be independent and normally distributed.

For the data regarding growth rate at any time interval the model was the same as for birth weight except that litter size refers in that case to the number of lambs the ewe rearing each particular lamb gave birth to instead of the birth type of the lamb itself. Furthermore, instead of age of "birth" dam age we used age of the dam that reared the lamb.

For concentration of blood metabolites we used the following models:

$$Y_{ijkl} = \mu + T_i + L_j + S_m + A_k + W_l + ((T \times W)_{il} \text{ or } (L \times W)_{jl)} + \varepsilon_{ijkl}$$

Where Y_{ijkl} is the response variable, μ is the overall mean of the population, T_i is the mean of the experimental treatment (i = 1-4), L_j is the mean effect of litter size (j = 1-3), A_k is the effect of ewe age and (k = 3-8), W_l is the effect of weeks from parturition ((l = -6 -1; week 0 being the second last sampling and representing the status of blood metabolites at parturition). $(T \times W)_{il}$ or $(L \times W)_{jl}$ are the interaction between either treatment and weeks from parturition or litter size and weeks from parturition respectively. ε_{ijkl} represents the unexplained residual elements that are assumed to be independent and normally distributed.

Those effects listed in the formulas above all affected the response variable at some level and therefore they were always included in the model when estimating each factor, even though they did not affect response variable significantly in all cases.

4. Results

4.1. Feed intake

Haylage intake, as presented in figure 1 was greater in the CTR group than the supplemented groups (MIX, EN, PRO) throughout the experiment. However, the difference was not significant until the third week when haylage intake of the control ewes increased while decreasing in those supplemented.

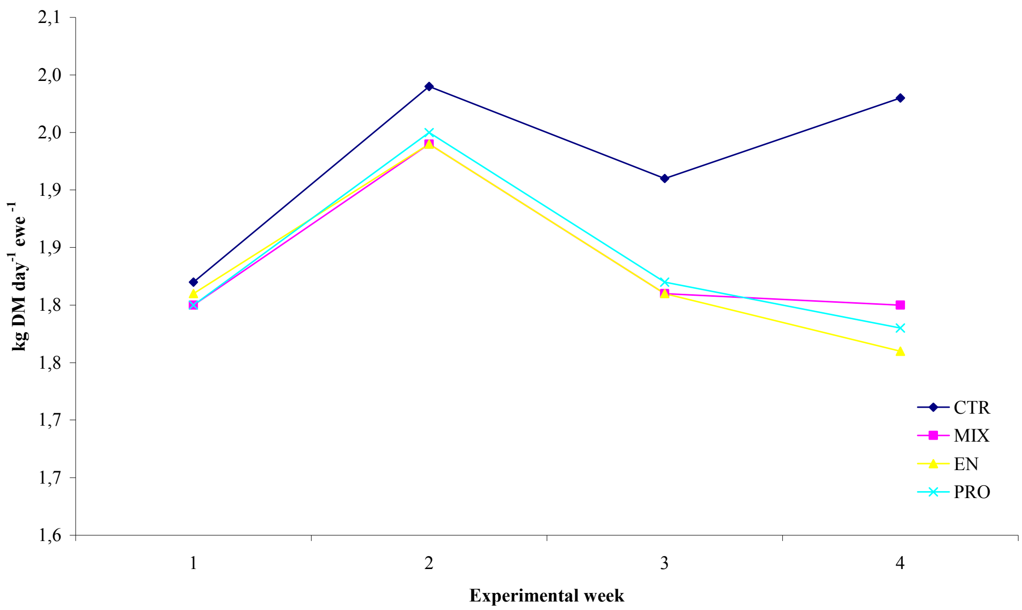

Figure 1. Haylage intake from beginning of experiment until parturition of the first ewe

Pattern of the ewes total intake of energy (FE_m day^{-1} ewe^{-1}) and protein (gAAT day^{-1} ewe^{-1}), including the supplements, can be viewed in figures 2 and 3. For comparison the figures include requirements of twin bearing ewes for energy and protein at each time as described by Sveinbjörnsson & Ólafsson (1999) and Ólafsson (1995). Adjusted means for DM, FE_m and AAT for each treatment and time interval are presented in manuscript I.

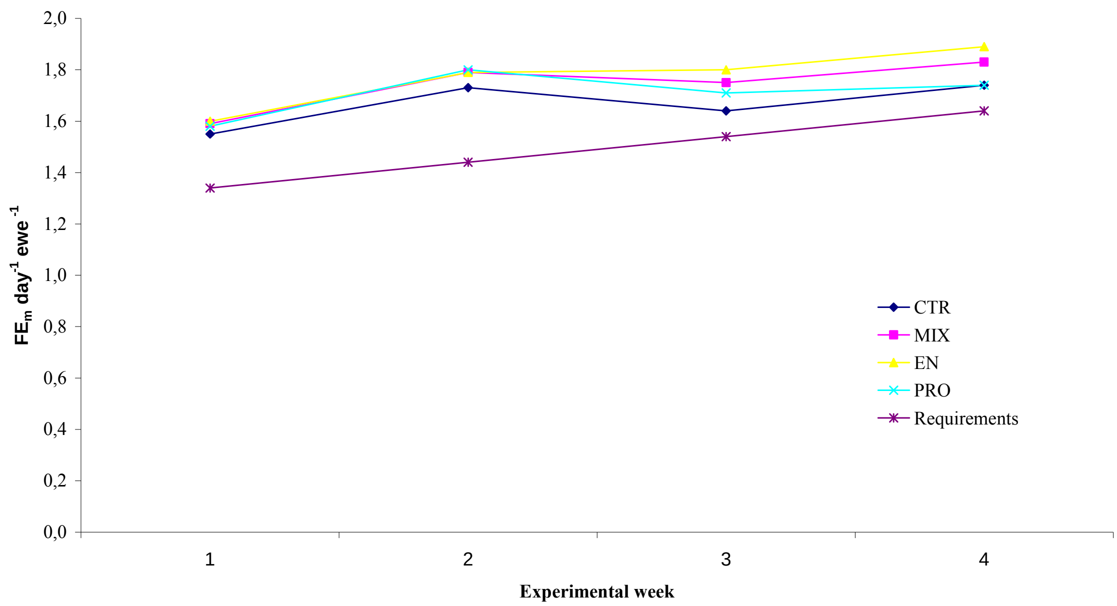

Figure 2. Changes in total energy intake (FE_m day^{-1} ewe^{-1}) from beginning of experiment until parturition of the first ewe

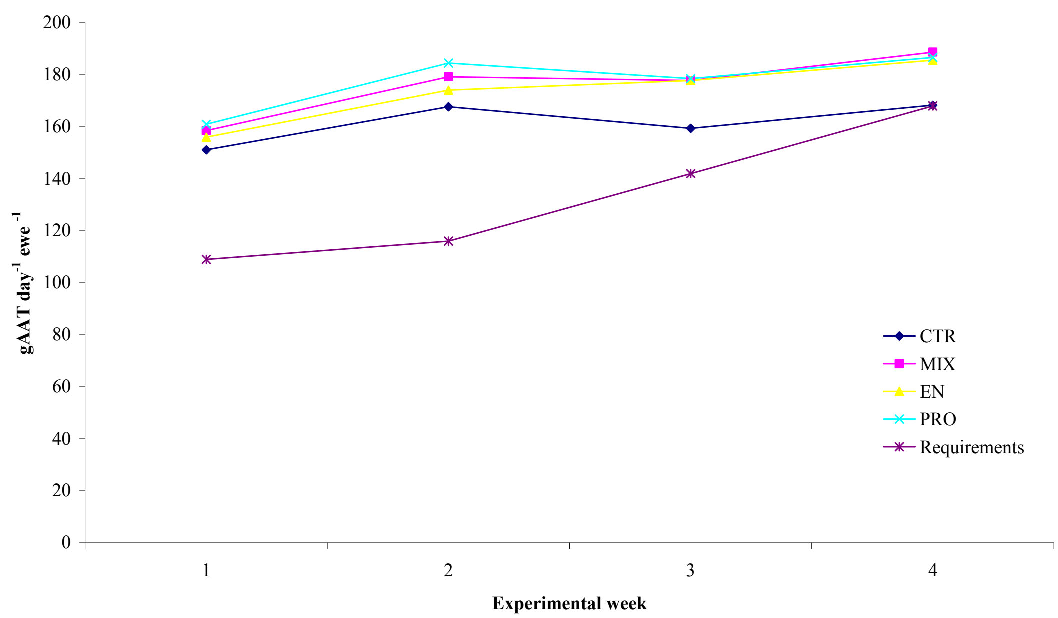

Figure 3. Changes in total protein intake (g AAT day^{-1} ewe^{-1}) from beginning of experiment until parturition of the first ewe

Energy and protein intake is similar in all the supplemented treatments and all treatments show the same pattern from time to time. Both the energy and protein intake of the CTR group is, as expected, lower than in those supplemented but yet, above the requirements of twin bearing ewes.

4.2. Ewe weight and body condition

Ewe weight did not differ significantly between any of the treatments but litter size affected ewe weight significantly, single bearing ewes being lighter than twin and triplet bearing ewes in the end of the experimental period. Weight change of ewes during the experimental period, that is from late March until the parturition of the first ewe in the end of april, is presented in figure 4. Treatment did not affect weight change significantly though PRO ewes were significantly heavier than CTR ewes. As could be expected the single bearing ewes gained less weight than those carrying twins and triplets.

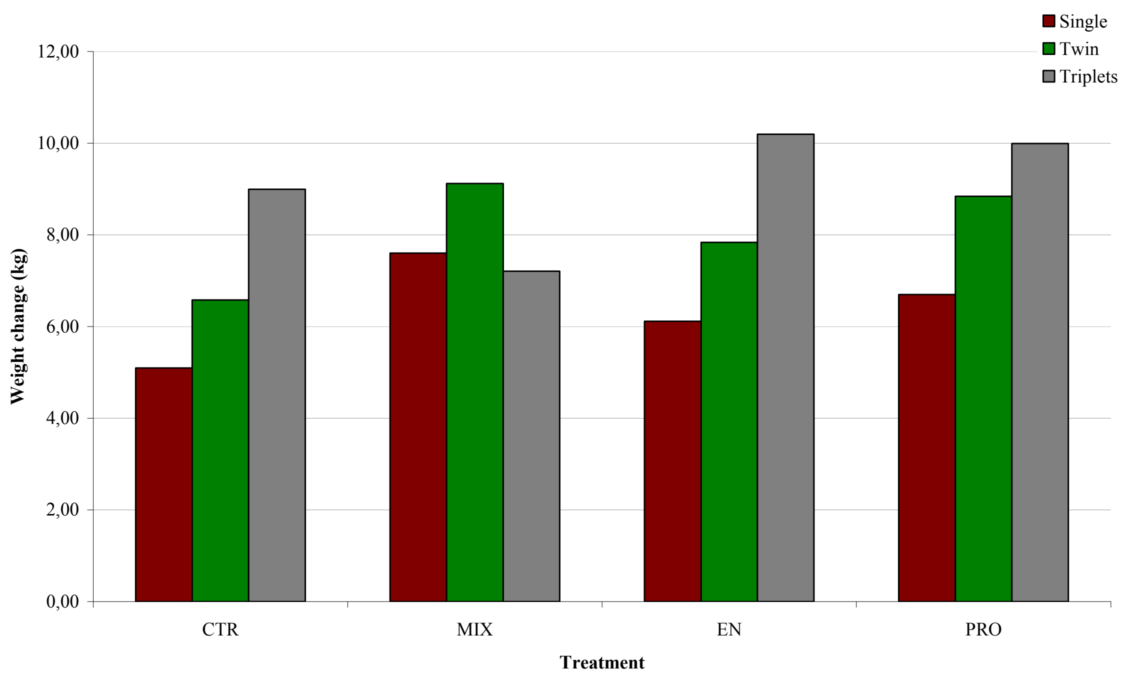

Figure 4. Changes in ewe weight from end of march until parturition of the first ewe in end of april

Triplet bearing ewes gained more weight than those carrying twins in all treatments except the MIX group where twin bearing ewes had the highest weight gain.

BCS was highest in the MIX group though none of the treatment groups differed significantly from each other. In the end of the experiment BCS of triplet bearing ewes was lower than of those carrying singles and twins but the difference between BCS of the latter two was small. Figure 5 presents changes in BCS during the experimental period, i.e. from late March until the parturition of the first experimental ewe in end of April.

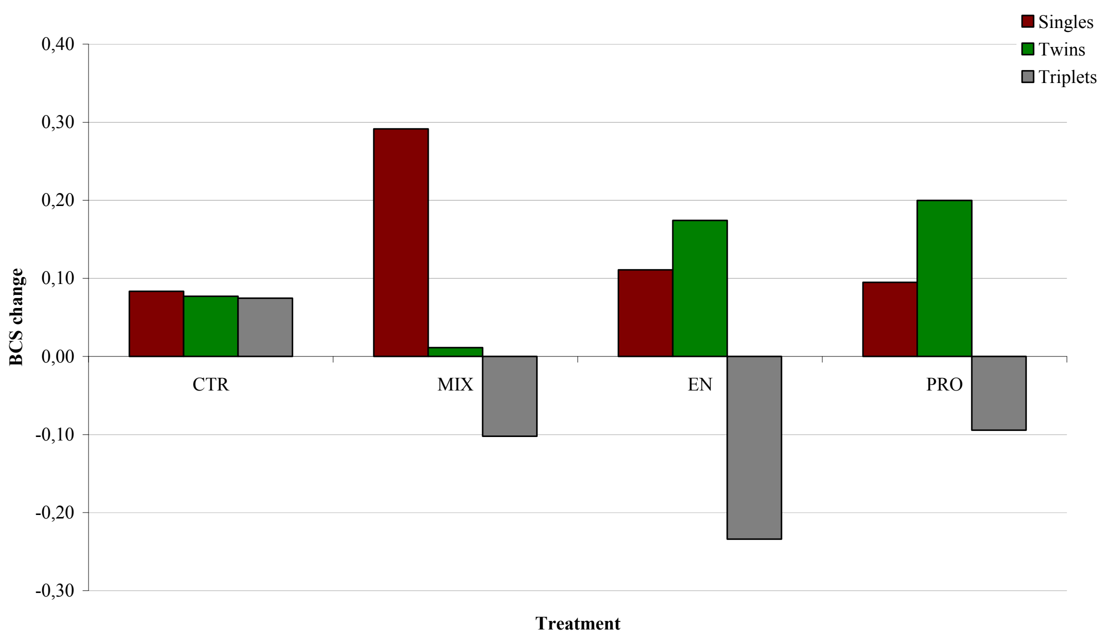

Figure 5. Changes in BCS from end of March until parturition of the first ewe in end of April

Changes in BCS during the experimental period showed more diversity than weight changes. In all the supplemented groups triplet bearing ewes lost condition while in the CTR group all litter sizes gained BCS at a similar level. In the MIX treatment single bearing ewes gained more BCS than those carrying twins while the opposite was found for the EN and PRO group where twin bearing ewes had the highest BCS gain. Adjustued means for changes in ewe weight and BCS during the experiment can be found in manuscript I.

4.3. Blood metabolites

Significance of treatment effects on glucose level was mainly due to elevated levels of glucose in the EN group the last two weeks prepartum. Glucose level increased postpartum in the PRO group while it decreased in the other treatments. However, during most of the experimental period the treatments supplemented with high undegradable protein concentrates (MIX and PRO groups) had lower glucose level than the CTR and EN groups. As presented in figure 6, triplet bearing ewes had lower plasma glucose than other litter sizes at all measurements prepartum. This difference evens out postpartum when glucose levels of single and twin bearing ewes decrease to a greater extent than of those that had given birth to triplets.

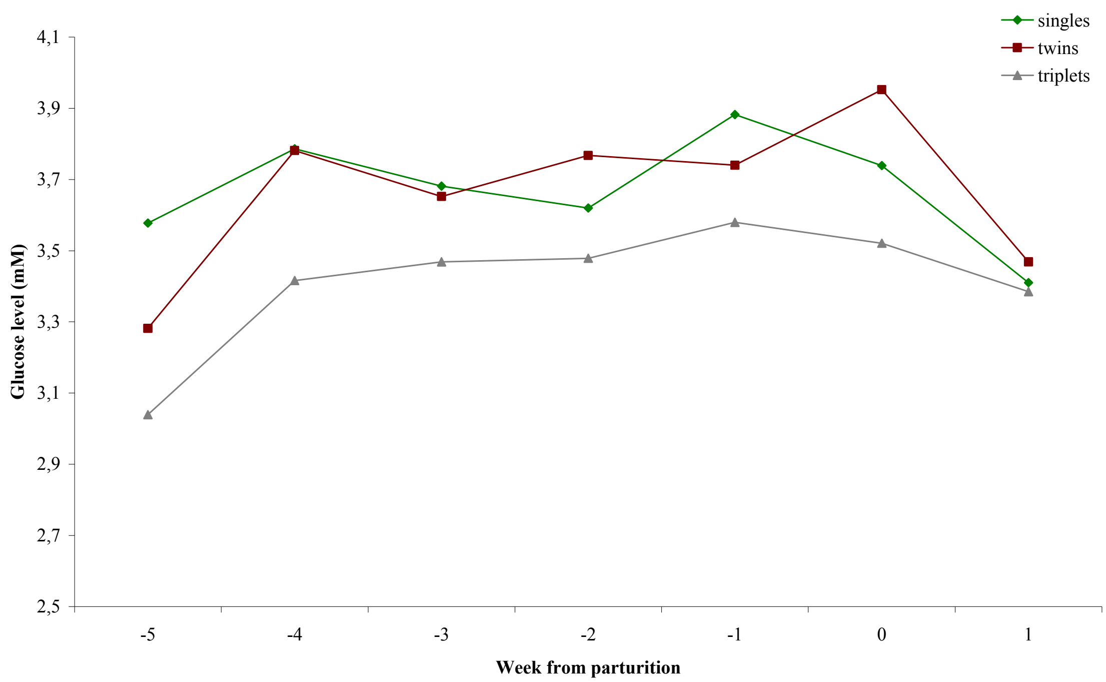

Figure 6. Plasma glucose level from five weeks prepartum to one week postpartum

Even though treatment did not have significant effect on NEFA concentration in plasma, the CTR group had the highest NEFA concentration at all measurements from beginning of supplementation. Nevertheless, as figure 7 presents, changes in NEFA have similar pattern in all groups throughout the experiment and weeks from parturition were in fact the only factor tested that significantly affected NEFA.

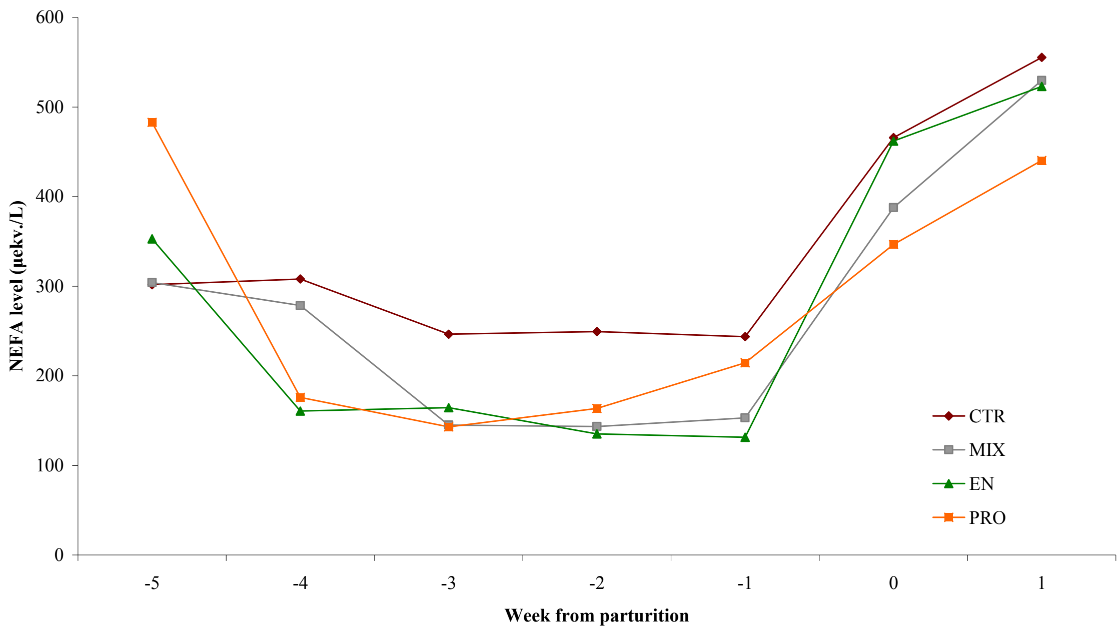

Figure 7. Plasma NEFA level from five weeks prepartum to one week postpartum

Pattern of NEFA changes were also similar between all litter sizes but constantly with lower levels in ewes carrying singles than others.

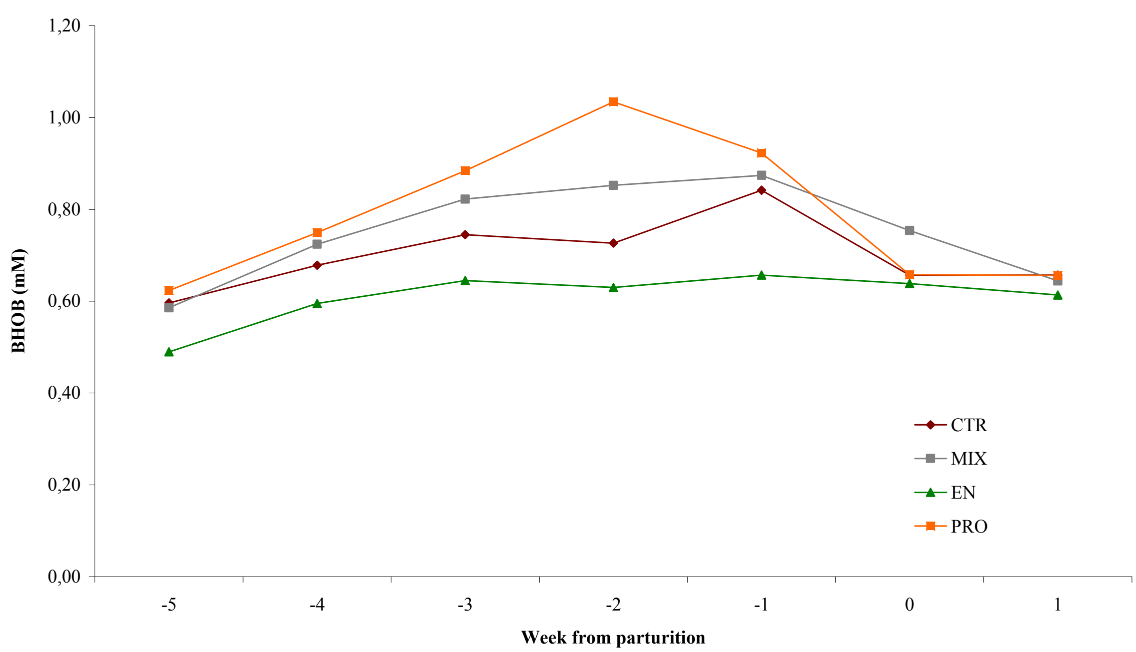

Figure 8. Plasma BHB level from five weeks prepartum to one week postpartum

Like NEFA, BHB level was significantly affected by weeks from parturition and showed similar pattern throughout the experiment within all treatments. Ewe age and treatment also had significant effect on BHB concentration. The PRO group had highest BHB level at all measurements until parturition when it decreased more than others and in the last two measurements it was similar to the levels in the other three groups. Figure 8 presents pattern of BHB changes within treatments.

The BHB difference was not significant between the two undegradable protein supplemented treatments (MIX and PRO) but both differed significantly from, and had throughout the experiment higher levels of BHB than, both CTR and EN groups. Difference was also significant between those two, the EN group having the lowest level and least changes in BHB level from time to time. No difference could be found in BHB levels between litter sizes.

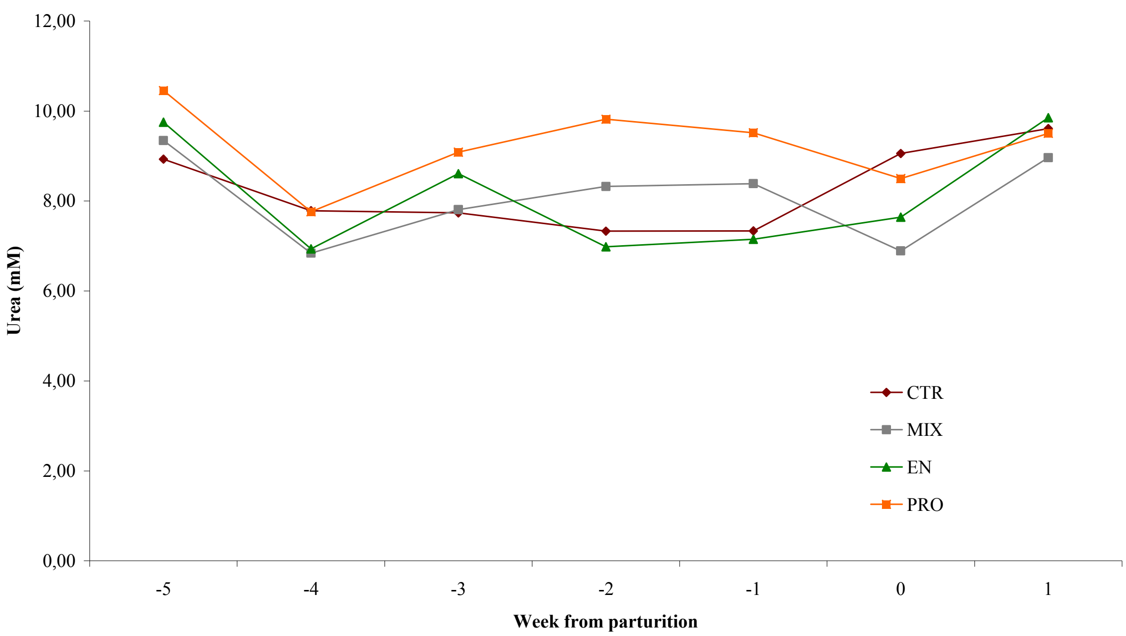

Figure 9. Plasma Urea level from five weeks prepartum to one week postpartum

Urea level in plasma was also significantly affected by weeks from parturition. However, patterns were not as stable within different treatments and litter sizes as for the other metabolites. Treatment affected urea level significantly, the effects being strongest for the PRO group that had, as presented in figure 9, the highest urea level at all measurements until parturition when urea levels of all treatments approached. The urea levels were similar among treatments one week postpartum. Plasma urea level seemed to fluctuate to a great extent within litter sizes, especially in the first three measurements. Nevertheless, litter size effects were significant and urea levels were lowest in the triplet bearing ewes and in most cases highest in those carrying singles - all litter sizes differing significantly from each other.

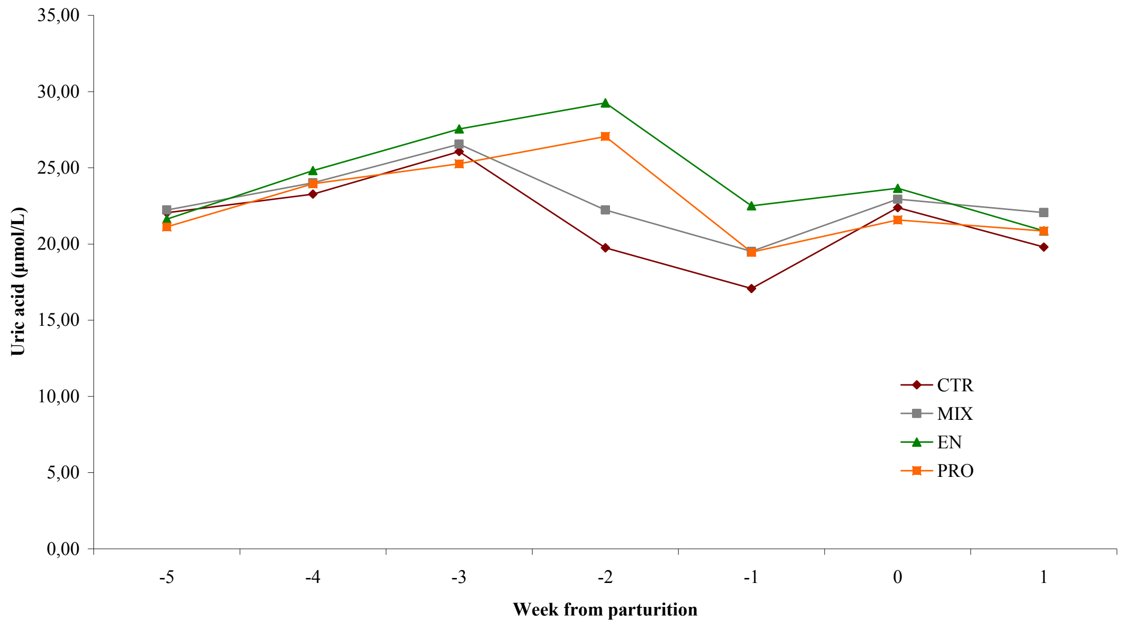

Figure 10. Plasma uric acid level from five weeks prepartum to one week postpartum

Uric acid levels were significantly affected by treatment, litter size and weeks from parturition. However, the only treatment group significantly differing from the others was the EN group that had the highest level from beginning of supplementing. CTR groups level were lower than the others in the last two measurements prepartum but rose to levels similar to the other groups at parturition. As for the treatment difference presented in figure 10, litter size difference is small in the first three measurements but increases in the next two. From two weeks prepartum until end of measurements single bearing ewes had lower levels of uric acid in plasma than those carrying twins and triplets.

The liver enzymes tested, AST, GGT, GLDH and ICDH, all were significantly affected by weeks from parturition. AST and GLDH had rather constant level postpartum, both within litter sizes and treatment groups, but at parturition levels were greatly elevated. AST level was significantly affected by treatment, levels being higher in the MIX and PRO treatments, but not litter size while the opposite was found for GLDH levels that were higher in single than twin and triplet bearing ewes.

ICDH and GGT levels also increased during the experiment but more gradually from beginning of measurements until parturition than AST and GLDH. GGT level was neither significantly affected by treatment nor litter size though treatment effect were close to significance and CTR group had significantly higher level than those supplemented with undegradable protein (MIX and PRO). Both litter size and treatment affected ICDH level significantly, CTR group being significantly higher than all those supplemented which did not differ from each other. ICDH level in single bearing ewes were in general, in contrast with the other liver ensyme measured, lower than in the other litter sizes though the value approaches twin and triplet bearing ewes postpartum. Adjusted means for the liver enzymes as well as the other blood metabolites are presented in manuscript I.

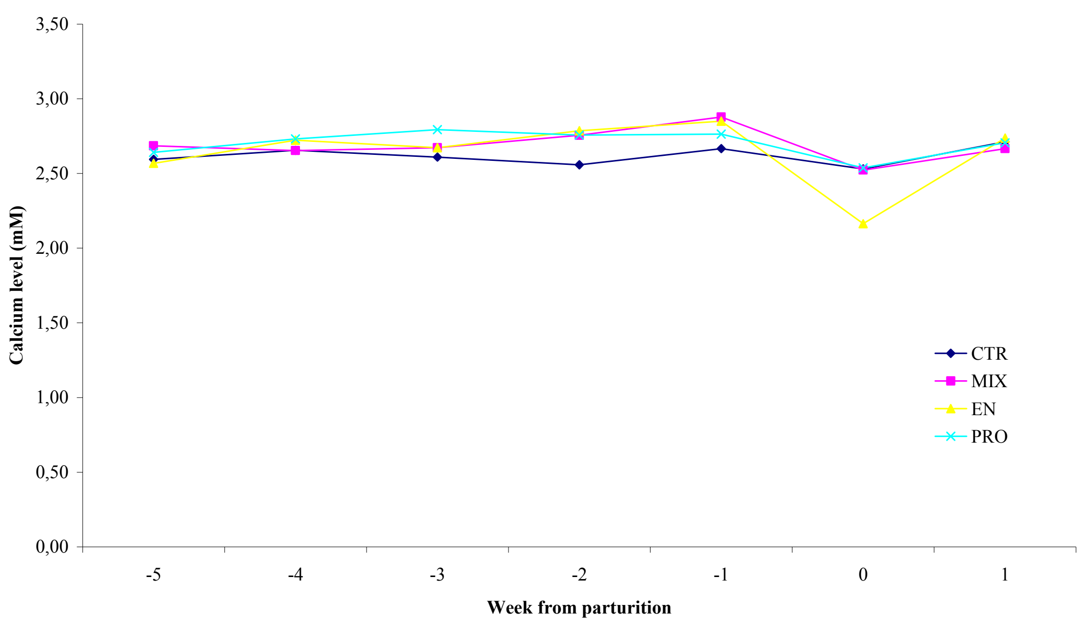

Figure 11. Plasma calcium level from five weeks prepartum to one week postpartum

Level of calcium in plasma was significantly affected by treatment and weeks from parturition. As Figure 11 reveals levels were quite stable within all treatments prepartum but decreased at parturition, the change being greater for the EN treatment than others. However, in the last measurement, representing plasma calcium status approximately one week

postpartum, calcium level of the EN group is raised again to similar level as in the other treatments.
Pattern of changes in calcium level during the experiment were quite similar among the different litter sizes and treatments. Though levels fluctuated to some extent between measurements the main change occured at parturition when levels dropped in all litter sizes. Postpartum levels were raised again in twin and triplet bearing ewes while calcium level continued to drop in those that had given birth to singles.

4.4. Lamb birth weight

Difference in adjusted means between treatments and litter sizes is presented in figure 12. Triplets were lighter than singles and twins though the difference was not significant between twins and triplets in the MIX group.

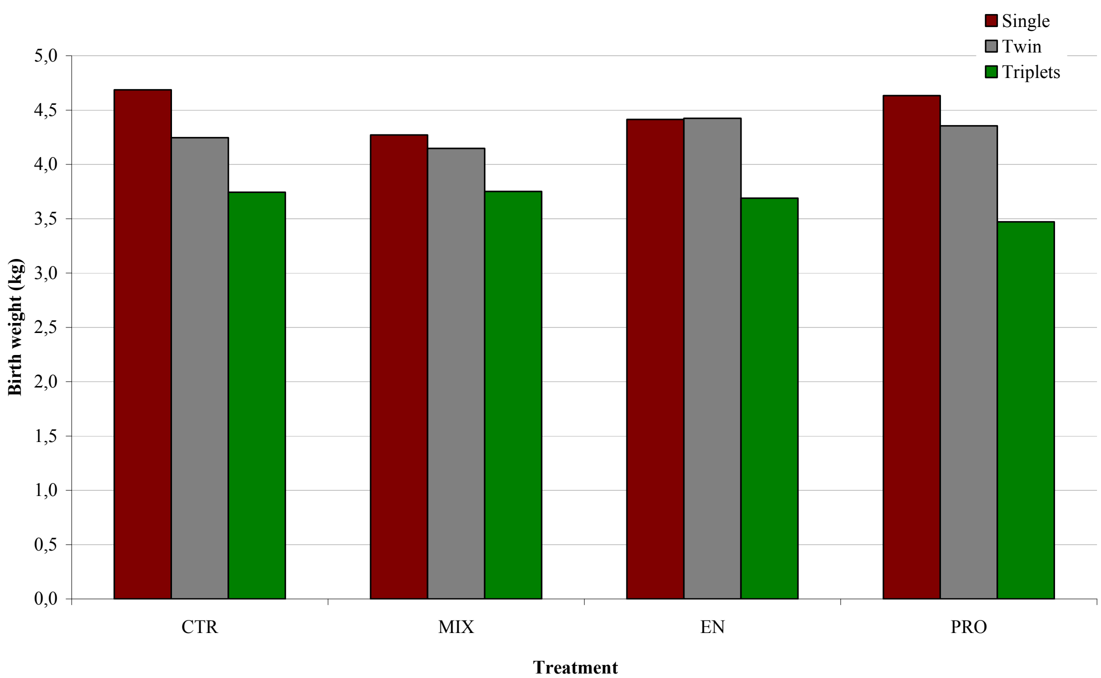

Figure 12. Average birth weight of lambs among treatments and litter sizes

Though singles were in all treatments, except the EN group, heavier than twins, this difference was not significant. Treatment did not affect birth weight significantly though lambs from the CTR group were heavier than lambs from other treatments. Sex of the lambs did not affect birth weight significantly though sex difference approached significance with p-value of 0,07.

4.5. Lamb growth rate

Figure 13 presents growth rate among treatments and in different growth periods. At this point it should be kept in mind that litter sizes in these cases refer to how many lambs ewes had given birth to. Lambs reared by ewes from the CTR group grew significantly slower than others the first seven days but the difference decreased with increasing age and after end of June no difference was found between treatments.

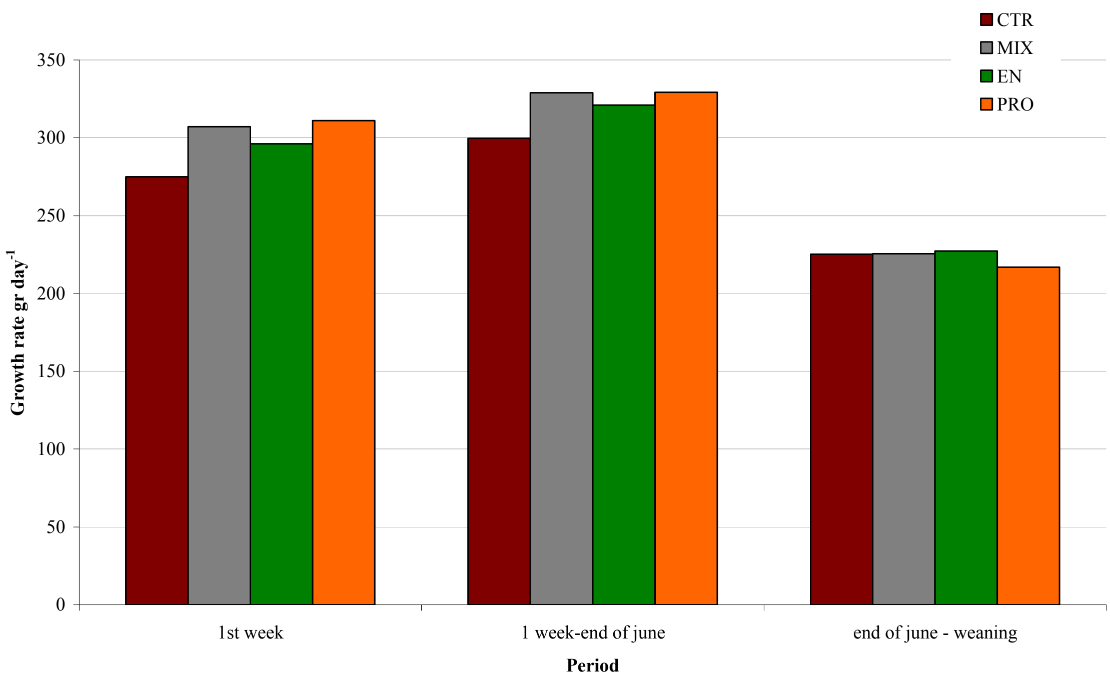

Figure 13. Lamb growth rate in different periods

Litter size affected growth rate significantly in the first week postpartum, lambs reared by twin bearing ewes growing faster than those reared by single- and triplet bearing ewes. This difference decreases with increasing age of lambs, is not significant anymore in the second growth period and not found at all in the period from end of June to weaning.

Weight at weaning did not differ significantly between treatments though CTR group lambs were lighter than others. Furthermore, lambs reared by twin bearing ewes were heavier than those reared by single and triplet bearing ewes. Adjusted means for birth weight, growth rate in different periods and weight at weaning within and between treatments and litter sizes are presented in manuscript II.

5. Discussion

5.1. Dry matter, energy and protein intake

Robinson (1983) stated that energy and protein requirements of the ewe in late pregnancy and early lactation could rarely be met without substantial concentrate feeding. In contrast with this statement the results from the research presented here indicate that with high quality haylage requirements of the twin bearing pregnant ewe can be met, at least until parturition, without supplementing. Good quality of the roughage is likely to be a key factor in this matter as Annett et al. (2008) and Thorsteinsson & Thorgeirsson (1989), among others, consider roughage quality to be of major importance for eating capacity.

DMI was not affected by type of supplement though it is known that combination of feedstuffs in the diet can affect passage rate of the digesta through the rumen. Increased feeding of concentrates in the MIX, EN and PRO groups however decreased intake of haylage significantly compared to the CTR group. These effects are commonly known as substitution effects and are especially notable when quality of the roughage fed is high (Dawson et al., 2005, Frutos et al., 1998, Kerslake et al., 2008) such as in this case. Increased haylage intake of the CTR group in the last experimental week is in contrast with for example Orr & Treacher (1989), Ingvartsen (2006) and Speijers et al. (2005) that all state that DMI is reduced when parturition approaches as was the fact in the supplemented groups.

Since these results indicate that energy and protein requirements of the twin bearing ewe can be met with haylage alone the addition of supplements results in energy- and protein supply well above requirements in the supplemented groups, even though haylage intake is reduced. The protein requirements used here for comparison (Ólafsson, 1995) however are calculated assuming birth weight of singles and twins to be 4 and 3,25 kg respectively. These numbers are based upon experimental work from 1993. Our results as well as data from the experimental farm however reveal substantially higher birth weight of lambs, twins now having similar birth weights as singles did before and lambs from multiple litters today being heavier than the average twin at the same farm 19 years ago. This could indicate an underestimation of the prepartum nutritional requirements used today. Since ewes were not individually fed and single, twin and triplet bearing ewes were penned together this data does not reveal whether individual difference, such as litter size or ewe fatness affected intake to some extent. Possible effect of litter size is unclear since studies are inconsistent regarding that factor (Foot & Russel, 1979, Orr & Treacher, 1989, Sormunen-Cristiana & Jauhiainen, 2001) but assuming equal feed consumption of all ewes and requirements based on the average litter size of lambs 19 years ago only the triplet bearing ewes, both in the CTR as well as the supplemented groups, would have been undernourished but only in the last week prepartum.

5.2. Ewe weight and body condition

In all treatment groups and within all litter sizes ewes gained weight during the last month prepartum, i.e. during the experimental period as can be expected in well nourished ewes in late pregnancy. Non-significant effect of treatment on ewe live weight and weight change is in contrast with both O´Doherty & Crosby (1997) that found increase in live weight gain with protein supplementing as well as Dawson et al. (2005) that found positive linear relationship between increased herbage and concentrate allowance and live weight gain. Kerslake et al. (2008) however found smaller effect of concentrate feeding on live weight when availability of high quality roughage was increased. Similarly, the high quality of the roughage in our experiment could to some extent explain the lack of response to concentrate feeding on live weight gain.

Significance of the positive effect of litter size on live weight gain found here is in agreement with Sormunen-Cristiana & Jauhiainen (2001) who found prepartum weight gain to increase with increased litter size. This is as expected, considering relative weight of mammary tissues carrying and preparing for one, two or three foetuses. Exception from this was found in the MIX group where ewes carrying twins gained more weight than both single and triplet bearing ewes.

Lack of treatment effect on BCS is in agreement with Annett et al. (2008) that failed to detect effect of undegradable protein supplement on BCS. That is, in our case, not surprising at all since Kerslake et al. (2008) even failed to detect effect on BCS when supplemented ewes were fed low quality forage and Husted et al. (2008) also found BCS to increase irrespective of feeding level. Similar BCS gain within all litter sizes in the CTR group compared to the big and somewhat inconsistent difference between litter sizes in the other treatments is a suprise and in disagreement with for example Dawson et al. (2005). Possible reason for this is the effect of protein supplements on the ewes ability to mobilize fat and protein from maternal tissues described for example by Frutos et al. (1998) and McNeill et al. (1997). How the supplementary protein mediates the tissue mobilization seems rather complicated. At some level this could to be caused by the fact that substrate cycles and processes regarding lipolysis are energetically expensive (Chilliard et al., 2000, Cavestany et al., 2009) and extra protein, above what needed to fill protein requirements, can be used for energy supply via gluconeogenesis. This however is only one possible explanation, the process of tissue mobilization and its control is complex and litterature review and research work beyond the framework of this thesis is needed to explain this in details. Greater milk production of the supplemented ewes as described later supports that explanation indicating mobilization of energy towards udder development and preparation for milk production. However, effect of this would also have been expected to be seen on lamb birth weight since less energy would be available for foetal growth in the CTR group but that was not the fact in that case. Furthermore, because of the great feed intake, the need for body tissue mobilization in single and twin bearing ewes should have been minimal, especially in the supplemented groups and the condition gain within those litter sizes supports that.

BCS loss of triplet bearing, supplemented ewes are in agreement with the previously mentioned studies (Chilliard et al., 2000, Frutos et al., 1998, McNeill et al., 1997) regarding effect of supplementing on mobilization of body tissues. As stated before, with equal feed intake among litter sizes, energy and protein requirements of the triplet bearing ewes would have been met until the last week prepartum and therefore severe fat and protein mobilization should hardly have been needed until then. However, based on this research, especially considering results from for example Orr & Treacher (1989), we cannot assume equal feed intake among different litter sizes. Therefore non-supplemented triplet bearing ewes could have been fed below requirements for longer period than the last week of pregnancy.

Based on the high nutrition level in the supplemented ewes, twin bearing ewes could have been expected to gain more condition, especially in comparison with condition gain in the single bearing and CTR ewes, than they did. Higher BCS and BCS change of twin than single bearing ewes in the MIX and PRO groups are surprising considering the bigger gap between intake of nutrients and requirements in those carrying singles which would have been expected to result in greater addition to the maternal body fat and protein deposits in those ewes compared to their twin bearing associates – assuming equal feed intake. In that matter it should be considered that the energy and protein evaluating systems used for assessment of requirements in this experiments have certain limitations. Those limitations are of special consideration when it comes to considering effect of fatness and physiological state of the animal as well as composition and level of intake of the diet, on for example passage rate of digesta in the rumen. Since all the ewes in this experiment were of excellent condition, part of the reason for lack of response of supplements on BCS could be the fact that energetic efficiency is lower in fat ewes than others (Chilliard et al., 2000).

5.3. Concentration of blood metabolites

Since glucose is the most important energy source for foetal growth and later on for colostrum and milk production its concentration in blood indicates the amount of energy available for this activity (O´Doherty & Crosby, 1998). Significant difference between MIX and EN groups on one hand and CTR and PRO on the other hand mainly reflects fluctuation and increased glucose level at some points of the experiment rather than indicating that those groups had elevated glucose level compared to others throughout the experimental period. Interesting treatment effects were found in the PRO group where glucose level was increased postpartum while it decreased in the other treatments, even though similar energy and protein intake compared to needs were observed in all supplemented groups. The patterns for CTR, MIX and EN treatments were in consistency with those previously observed by Husted et al. (2008) and Banchero et al. (2006). Significant difference between PRO group and those fed lower level of undegradable protein is to some extent inconsistant with O´Doherty & Crosby (1998) that found no effect of protein supplement on glucose concentration in plasma. Though glucose concentration changes showed the same pattern as found by Husted et al. (2008) changes were not as great in our case. Husted et al. (2008) found glucose level in plasma to increase by 50% the last three weeks prepartum, peak around lambing and then decrease again. Level of fluctuation in the present experiment are more comparable to Banchero et al. (2006). Positive relationship between energy nutrition and plasma glucose concentration as found by O´Doherty et al. (1998) could in this case have been suppressed by the high level of dietary energy in the CTR group. Lower level of glucose in triplet bearing ewes than those carrying singles and twins can be explained by higher glucose requirements for foetal growth in those ewes, resulting in increased absorbtion, relative to production and consequently lowered level in plasma.

When viewing results regarding glucose concentration in plasma it has to be kept in mind that any changes within the animal make very sudden response in glucose concentration (O´Doherty & Crosby, 1998).

When body of the pregnant ewe fails to produce enough glucose to meet the increasing demands of the growing foetus and developing mammary gland, fat depots are mobilized, via lipolysis of adipose tissue, to increase energy supply. Until the fatty acids are absorbed and utilized as an energy source they circulate in the plasma as NEFA. Therefore concentration of NEFA in plasma can be used to estimate fat mobilization in animals. In this experiment treatment did not affect NEFA level significantly even though the CTR group had, at all sampling dates from beginning of supplementation, higher NEFA concentration than those supplemented. Because of the slightly lower energy and protein intake in the CTR group and therefore possibly more need for fat mobilization from maternal tissues this pattern could be considered logical. However, this finding is inconsistant with the statement previously described that protein addition (in that case raised AAT level) is necessary for the animal to mobilize fat from maternal tissue (Frutos et al., 1998, McNeill et al., 1997). In fact NEFA level was lowest in the PRO group that was fed the highest amount of undegradable protein which does not indicate the extra protein being used for mobilization of maternal tissues – but of course the need for such mobilization should have been negligible in that treatment, except for the triplet bearing ewes.However, again at this point the limitations of the feed evaluation system used regarding difference in characteristics of diet with changes in overall composition and its association with physiological and nutritional status of the animal need to be considered. Furthermore the findings of Dawson et al. (1999) that effects of undegraded protein on fat and protein mobilization, are strongest when dietary energy is limited, should be kept in mind since energy supply did not seem to be a limiting factor in the experiment presented here. Lower NEFA concentration in single- compared to twin and triplet bearing ewes is as expected since single bearing ewes should have had less need for fat mobilization than others. Small difference between twin- and triplet bearing ewes is more surprising since, assuming similar intake, energy and protein requirements of the triplet bearing ewes would hardly have been met the last week. Pattern of NEFA concentration during the experimental period is similar in all treatments and litter sizes. NEFA concentration remains stable or even decreases at first, then it is elevated the last week prepartum, the level continuing to rise postpartum probably as a result of significantly increased energy requirements around parturition. NEFA concentration in plasma is sensitive to stress in the animal during sampling (Speijers et al., 2005) which has to be considered when viewing results regarding NEFA concentration.

BHB is produced as a kind of by-product during utilization of NEFA for energy supply and can accumulate in blood inducing some clinical symptoms such as ketosis and fatty liver. Since BHB usually develops as a result of increased fat mobilization its pattern between treatments and sampling dates would be expected to be somewhat similar to what was found for NEFA. That, however, was not the case. The MIX and PRO goups, i.e. the groups receiving undegradable protein supplements, had significantly higher BHB level than other treatments at all sampling dates. Based on the statement that (undegradable) protein supplement stimulates fat mobilization (Frutos et al., 1998, McNeill et al., 1997) increased BHB level would have been expected in the MIX and PRO groups if the diet would not have met the ewes requirements as well as it did. Those effects should also have been seen for the NEFA level, which they did not as mentioned above. Once again, possible underestimation of requirements, f.ex. as a result of higher birth weight of lambs than was used acquiring requirements (Ólafsson, 1995), higher body condition or other physiological factors of the ewe as well as the limitations of the present feed evaluation system mentioned before should be kept in mind.

The lowest BHB level was found for the EN group at all sampling dates which is as expected since BHB concentration is known to be in opposition with plasma glucose, the EN group having higher glucose level than the other as previously mentioned .Litter size did not affect BHB significantly which does not indicate large mobilization of energy from fat depots of the triplet bearing ewes and is in agreement with non-significant difference between NEFA level in twin and triplet bearing ewes. These results could, to some extent, be affected by the excellent body condition of the experimental ewes as O´Doherty & Crosby (1998) and Speijers et al. (2005) found relationship between dietary energy and plasma BHB to decrease with improving BCS of the ewes. Pattern of BHB changes with time in this study was quite inconsistant with both Husted (2006) that obtained stable BHB levels as pregnancy proceeds but a gradual increase during lactation, and Banchero et al. (2006) who stated that most treatment differences occurs in the beginning of the experimental period but diminishes as parturition approaches. However, conditions in their study with regard to for example experimental length, ewe nutrition and body condition may not have been completely comparable with our study. O´Doherty & Crosby (1998) questioned the use of BHB as an indicator of energy status since severely undernourished ewes in their experiment failed to present response in BHB.

Urea level in plasma is used as an indicator of efficiency of protein nutrition. In this experiment significantly higher urea concentration was found for the PRO group during most of the experimental period. That is to some extent in disagreement with Banchero et al. (2006) and O´Doherty & Crosby (1998) that only detected small effect of undegradable protein supplement on urea level. However, in the latter experiment all treatment groups were undernourished which probably affected the outcome. Results from the experiment presented here indicate waste of the expensive dietary protein in the PRO group above others, even though supplementing rations were calculated to supply the experimental animals with equal ration of supplementary protein available as AAT. Energy intake and some difference in the balance between protein and energy in the digestive tract between the treatments could be affecting the outcome in that matter.

Effect of litter size on urea concentration were significant, triplet bearing ewes having the lowest level as expected due to their higher protein requirements and subsequently better utilization of dietary protein. Values were rather stable from time to time, only minimal fluctuations between samplings, which could indicate resonable changes in feeding through the experiment.

Experimental weeks had significant effect on uric acid level, all treatments having similar pattern of changes. Uric acid level represents the microbial activity in rumen and hence production and availability of microbial protein in the small intestines (Dewhurst et al., 2000). The significantly higher level in the EN group than others throughout the experiment is as expected since supplementing of that group aimed at comparing effect of microbial protein and undegradable protein. CTR group ewes had deeper drop prepartum than others, suggesting decreased rumen activity at that time, possibly due to fall in available energy and nitrogen from diet compared to needs. In the last measurement, one week postpartum, these ewes uric acid level had again reached levels similar to other treatments which could be the result the concentrate feeding starting postpartum.

Significant effect of weeks from parturition on the liver enzymes measured with rising levels as parturition approaches indicates increased metabolic stress during the experiment. That is as expected with significantly raising requirements for energy and protein. Levels for AST and GLDH, the enzyems indicating effective protein metabolism in the liver, are stable until the last week prepartum when they rise suddenly to great extent which seems reasonable since diet met requirements rather well until the great rise at parturition. The lower AST level in the EN group throughout the experiment suggests great efficiency of the use of the microbial protein production indicated by uric acid level. Higher AST level in the CTR than the supplemented groups postpartum is as expected, the latter receiving more energy and protein from diet which results in less pressure on the liver and subsequently less risk of impaired liver function and release of liver enzymes into the blood. Similar pattern was found for GLDH though not significant. Higher level of those enzymes in the treatment receiving least dietary protein is in agreement with the BHB levels that were higher for CTR group that EN as mentioned previously. GLDH and AST levels were also affected by litter size, though not significantly for AST. Triplet bearing ewes had significantly lower level than those carrying singles and twins which is suprising given the insufficient nutrition of the triplet bearing ewes that also is reflected in their decreased BCS and significantly lower glucose level during the experiment.

However, BCS gain in the triplet bearing ewes in the CTR treatment could possibly affect these results in some way.

Level of GGT and ICDH on the other hand rise gradually from beginning of the experiment. No effects were found of litter size on GGT level but singleideraring ewes had lower ICDH level than others which is as expected since they should not have neesed as extensive lipid metabolism as those carrying multible litters. Treatment effect on those metabolites were mainly due to the higher level of CTR ewes most of the experimental time which is not at all surprising based on the high NEFA level found for those ewes and less nutrients availability. However, as mentioned before less intake of protein in this group would have been expected to limit the mobilization of body reserves. Treatment groups receiving undegradable protein (MIX and PRO) had lower levels of GGT and ICDH though not significantly for GGT.

Pattern of calcium changes during the experiment reflect the highly increasing demands at parturition. Because of the high calcium level of the supplement fed to both MIX and PRO groups, more severe drop in the EN treatment is to some extent as expected. More surprising is that CTR group ewes had similar level as MIX and PRO in spite of no supplementary feeding. Possible reason could be that since those ewes received no additional calcium they were better prepared for using their skeletal reserves, i.e. mild undernutrition of calcium prepartum could have stimulated calcium release from the skeleton. The same reason could count for single bearing ewes taking longer time than others to elevate calcium level postpartum, excess feeding prepartum resulting in the ewes not being ready for calcium release from body reserves.

5.4. Lamb birth weight

As expected, triplets were significantly lighter than lambs of other litter sizes. Lack of difference between singles and twins however is in disagreement with Thorsteinsson & Thorgeirsson (1989), Orr & Treacher (1994) and Sormunen-Cristiana & Jauhiainen (2001) that all found singles to be heavier than twins and triplets lighter than twins. This result is particularly interesting since according to calculated intake of both energy and protein, single bearing ewes were severely overfed which could, according to Thorsteinsson & Thorgeirsson,

(1989) and Annett et al. (2008), have led to some extremes in birth weight. Furthermore, lack of significant difference between treatments and actually higher birth weight of lambs from the CTR group though not significant, are in disagreement with several research linking supplementary diet with increased birth weight (Annett et al., 2008, Thorsteinsson & Thorgeirsson, 1989). Speijers et al. (2005) and Thorsteinsson & Thorgeirsson (1989) suggested that lack of effect obtained with supplementing pregnant ewes could, at least to some extent, be caused by high roughage quality and excellent body condition of experimental ewes, respectively and this could also be the reason in our case. This could also explain the surprisingly low birth weight of singles compared to twins.

The fact that CTR ewes, though giving birth to the heaviest lambs, were slightly lighter than those supplemented, could indicate that some mobilization of maternal reserves took place and higher NEFA concentration in the CTR group supports that suggestion. However, since their feed intake indicates that energy and protein requirements were met and plasma glucose level does not contest that finding and hardly BHB nor liver enzyme concentration neither there should not have been need for mobilization of energy and protein from body tissues in that treatment above others. Once again, however, possible limitations of feed evaluation system as well as underestimation of needs caused by raised birth weight of lambs since the feeding standards were calculated, need to be considered.

5.5. Lamb growth rate

Decreasing effect of treatment and litter size of the dam on lamb growth rate with increasing lamb age found in this experiment are in agreement with Guðmundsson & Dýrmundsson, (1989) that found maternal effect on lamb growth rate to decrease with advancing age due to higher proportion of herbage compared to milk in the diet of lambs as they get older. Lower growth rate of lambs reared by single or triplet bearing ewes compared to those that had given birth to twins could be caused by the fact that all lambs reared by triplet bearing ewes, and half of those reared by single bearing ewes were born as either triplets, or twins from yearlings. Both of those are normally lighter at birth than twins from adult ewes (Thorsteinsson & Thorgeirsson, 1989) and consequently they have lower growth rate in the beginning as stated by for example Sormunen-Cristiana & Jauhiainen (2001), Greenwood et al. (1998) and Khalaf et al. (1979).

Lambs reared by CTR ewes had lower growth rate the first eight weeks but as mentioned above this difference was not present any more in the period from end of June to weaning. Lower growth rate of the CTR lambs the first weeks postpartum is in agreement with for example Robinson & McDonald (1989), Nottle et al. (1998) and Speijers et al. (2005) and suggests positive effect of supplementing ewes prepartum on their ability for colostrum and subsequent milk production.

6. Conclusion

Based on the experimental work presented above the following conclusions can be made:

- Nutrient requirements of twin bearing pregnant ewe, as it is defined today, can be met without concentrate feeding until parturition but it has to be kept in mind that roughage qality probably is a key factor in that matter. BCS gain of single and twin bearing ewes in all treatment groups in this experiment confirms that.
- Feeding high quality roughage supplementation does not seem to be needed for triplet bearing ewes until the last week prepartum. Further research regarding eating capacity of ewes carrying different litter sizes is needed to estimate how long time prepartum supplementation is needed.
- Viewing these results in comparison with older researches makes it reasonable to assume that body condition of the ewes is an important factor in the outcome of studies like this. Therefore it would be interesting to make further research on effects when BCS is of more diversity.
- Supplementing affects the ewes ability to mobilize fat from body reservoirs for energy production when basal diet is not filling energy needs. Possibly due to use of supplementary protein as an glucogenic substrats for the energy requiring process of lipolysis in adipose tissues.
- Prepartum supplementing seems to make ewes better prepaired for lactation as increased growth rate of lambs reared by supplemented ewes presents. However the slightly higher birth weight of lambs from non-supplemented ewes modifies the effect of increased growth rate resulting in non-significant difference between weaning weight of lambs reared by supplemented and non-supplemented ewes. Therefore this research does not indicate economical advantage of supplementating ewes prepartum.
- Better preparation for lactation in the supplemented ewes indicates benefit of feeding concentrates to single bearing ewes that are expected to rear two lambs for some time prepartum. Further research is needed in that matter, such as duration and amount of supplementing as well as economical profitability.
- These results indicate positive effect of prepartum supplementation on the milking ability of the ewes. However, for ewes concuming as much high quality diet as in this

case undegradable protein in the supplements did not have advantage above other types of concentrates.

- Concidering these results possible underestimation of requirements based on increased birth weight of lambs the last decade has to be kept in mind
- Furthermore, possible limitations of nutrition evaluation system such as effect of physiological state of the ewe (BCS, place in the production cycle) as well as particular characteristics of the diet on f.ex. eating capacity and rate of passage through the digestive tract need more interest.

Based on this experiments results in addition to an overview of litterature regarding effect of late pregnancy feeding on lamb birth weigh and growth rate as well as weight, condition and health of the pregnant and lactating ewe it seems clear that, even though several changes in feeding, housing and management of sheep the last decades the following statement, originally published 1989, still describes the most importan things regarding nutrition of the pregnant ewe:

> "It is therefore important to consider the whole winterfeeding strategy all at one, as the feeding level during any particular phase of pregnancy can not be sensibly decided without taking into account the previous nutritional history and body condition as well as the availability and quality of feed for the succeeding period" (Thorsteinsson & Thorgeirsson, 1989)

The results however raise several questions and ideas for further work in this area; work that would clear the practical concerns revealed here, such as:

- How low can roughage level become to maintain acceptable ewe body condition and lamb birth weight and growth rate without supplementing
- Would different results be obtained with different level of supplementing postpartum
- How low level of concentrates fed is enough to stimulate milk production the first weeks postpartum
- How late can supplementing start in order to secure beneficial effect on milk production

- Does lack of supplementing and/or supplement type affect availability and metabolism of the minerals and trace elements pregnant and lactating ewes require to sustain normal productivity and often receive from supplements

And last but not least, what combination of roughage quality and supplement type and level is of the best interest, both for the ewe and lamb physiologically and the farmer economically?

Appendix

Composition of salt block

Minerals:	
Phosphorus (as Dicalcium Phosphate) g kg^{-1}	2,4
Magnesium (as Magnesium oxide) g kg^{-1}	2,1
Sodium (as Sodium Chloride) g kg^{-1}	380
Calcium (as Dicalcium Phosphate) g kg^{-1}	1,5
Trace elements	
Manganese (as manganous oxide) mg kg^{-1}	200
Cobalt (as DMP Cobaltous Carbonate mg kg^{-1}	250
Zinc (as Zinc oxide) mg kg^{-1}	125
Iodine (as Calcium Iodate) mg kg^{-1}	250
Selenium (DMP Sodium Selenite) mg kg^{-1}	25
Vitamins	
Vitamin D3 iu kg^{-1}	40.000

Composition of mineral and vitamin mix in high energy supplements:

Cobalt - Co mg kg^{-1}	2
Copper – Cu mg kg^{-1}	30
Iron – Fe mg kg^{-1}	25
Manganese – Mn mg kg^{-1}	100
Selenium – Se mg kg^{-1}	0,35
Iodine – I mg kg^{-1}	5
Zinc – Zn mg kg^{-1}	100
Vitamin A iu g^{-1}	10
Vitamin D3 iu g^{-1}	2,5
Vitamin E - α tocopherol	50

References

Andrews, J. F., & Mercer, J. B. (1985). *Thermoregulation in the newborn lamb: The first 36 hours.* Paper presented at the Factors affecting the survival of newborn lambs - A seminar in the CEC programme of coordination of agricultural research, Brussel.

Annett, R. W., Carson, A. F., & Dawson, L. E. R. (2008). Effects of digestible undegradable protein (DUP) supply and fish oil supplementation of ewes during late pregnancy on colostrums production and lamb output. *Animal Feed Science and Techonology, 146*, 270-288.

Annison, E. F., Lindsay, D. B., & Nolan, J. V. (2002). Digestion and Metabolism. In M. Freer & H. Dove (Eds.), *Sheep nutrition* (pp. 95-118). Wallingford: CABI Publishing.

Banchero, G. E., Clariget, P., Bencini, R., Lindsay, D. R., Milton, J. T. B., & Martin, G. B. (2006). Endocrine and metabolic factors involved in the effect of nutrition on the production of colostrum in female sheep. *Reprod. Nutr. Dev., 46*, 447-460.

Banchero, G. E., Quintans, G., Vazquez, A., Gigena, F., La Manna, A., Lindsay, D. R., et al. (2007). Effect of supplementation of ewes with barley or maize during the last week of pregnancy on colostrum production. *Animal 1*, 625-630.

Bell, A. W., Burhans, W. S., & Overton , T. R. (2000). Protein nutrition in late pregnancy, maternal protein reserves and lactation performance in dairy cows. *Proceedings of the Nutrition Society, 59*, 119-126.

Black, J. L. (1983). Growth and development of lambs. In W. Haresign (Ed.), *Sheep Production* (pp. 576). London: Butterworths.

Cavestany, D., Kulcsar, M., Crespi, D., Chilliard, Y., La Manna, A., Balogh, O., Keresztes, M., Delavaud, C., Huszenicza, G. & Meikle, A. (2009). Effect of Prepartum Energetic Supplementation on Productive and Reproductive Characteristics, and Metabolic and Hormonal Profiles in Dairy Cows under Grazing Conditions. *Reproduction in Domestic Animals, 44*(4), 663-671.

Chilliard, Y., Ferlay, A., Faulconnier, Y., Bonnet, M., Rouel, J., & Bocquier, F. (2000). Adipose tissue metabolism and its role in adaptions to undernutrition in ruminants. *Proceedings of the Nutrition Society, 59*, 127-134.

Dawson, L. E. R., Carson, A. F., & Kilpatrick, D. J. (1999). The effect of the digestible undegradable protein of concentrates and protein source offered to ewes in late pregnancy on colostrum production and lamb performance. *Animal Feed Science and Techonology, 82*, 21-36.

Dawson, L. E. R., Carson, A. F., Kilpatrick, D. J., & Laidlaw, A. S. (2005). Effect of herbage allowance and concentrate food level offered to ewes in late pregnancy on ewe and lamb performance. *Animal Science, 81*, 413-421.

Dewhurst, R. J., Davies, D. R., & Merry, R. J. (2000). Microbial protein supply from the rumen. *Animal Feed Science and Technology, 85*(1-2), 1-21.

Dove, H. (2002). Principles of Supplementary Feeding in Sheep-grazing Systems. In M. Freer & H. Dove (Eds.), *Sheep nutrition* (pp. 119-142). Wallingford: CABI publishing.

Foot, J. Z., & Russel, A. J. F. (1979). The relationship in ewes between voluntary food intake during pregnancy and forage intake during lactation and after weaning. *Animal Production, 28*, 25-39.

Frutos, P., Buratovich, O., Giráldez, F. J., Mantecón, A. R., & Wright, I. A. (1998). Effects on maternal and foetal traits of feeding supplement to grazing pregnant ewes. *Animal Science, 66*, 667-673.

Gama, L. T., Dickerson, G. E., Young, L. D., & Leymaster, K. A. (1991). Effects of breed, heterosis, age of dam, litter size and birth weight on lamb mortality. *Journal of Animal Science, 69*, 2727-2743.

Gonzalez, J. J., Robinson, J. J., & Fraser, C. (1985). The effect of physiological state on digestion in the ewe and its influence on the quantity of protein reaching the abomasum. *Livestock Production Science, 12*, 59-68.

Greenwood, P. L., Hunt, A. S., Hermanson, J. W., & Bell, A. W. (1998). Effects of birth weight and postnatal nutrition on neonatal sheep: I. Body growth and composition and some aspects of energetic efficiency. *Journal of Animal Science, 76*, 2354-2367.

Guðmundsson, Ó., & Dýrmundsson, Ó. R. (1989). Grazing and lamb growth (Beit og vöxtur lamba). In Ó. R. Dýrmundsson & S. Thorgeirsson (Eds.), *Reproduction, growth and nutrition in sheep. Dr. Halldór Pálsson Memorial publication* (pp. 147-168). Reykjavík: Búnaðarfélag Íslands og Rannsóknarstofnun landbúnaðarins.

Harano, Y., Ohtsuki, M., Ida, M., Kojima, H., Harada, M., Okanishi, T., et al. (1985). Direct automated assay method for serum or ureine levels of ketone bodies. *Clinica Chimica Acta, 151*, 177-183.

Harmeyer, J., & Schlumbohm, C. (2006). Pregnancy impairs ketone body disposal in late gestating ewes: Implications for onset of pregnancy toxaemia. *Research in Veterinary Science 81*, 254-264.

Husted, S. M., Nielsen, M. O., Blache, D., & Ingvartsen, K. L. (2008). Glucose homeostasis and metabolic adaption in the pregnant and lactating sheep are affected by the level of nutrition previously provided during her late fetal life. *Domestic animal endocrinology, 34*, 419-431.

Husted, S. M., Nielsen, M. O., Tygesen, A., Kiani, A., Blache, D., & Ingvartsen, K. L. (2007). Programming of intermediate metabolism in young lambs affected by late gestational maternal undernourishment. *American journal of physiology, endocrinology and metabolism, 293*, 548-557.

Imamidoost, R., & Cant, J. P. (2005). Non-steady state modelling of effects of timing and level of concentrate supplementation on ruminal pH and forage intake in high-producing, grazing ewes. *Journal of Animal Science, 83*, 1102-1115.

Ingvartsen, K. L. (2006). Feeding and management related diseases in the transition cow. Physiological adaptions around calving and strategies to reduce feeding-related diseases. *Animal Feed Science and Techonology, 126*, 175-213.

Kaur, R., Nandra, K. S., Garcia, S. C., Fulkerson, W. J., & Horadagoda, A. (2008). Efficiency of utilisation of different diets with contrasting forages and concentrate when fed to sheep in a discontinuous feeding pattern. *Livestock Science, 119*, 77-86.

Kerslake, J. I., Kenyon, P. R., Morris, S. T., Stafford, K. J., & Morel, P. C. H. (2008). Effect of concentrate supplement and sward height on twin bearing ewe body condition and the performance of their offspring. *Australian journal of experimental agriculture, 48*, 988-994.

Khalaf, A. M., Doxey, D. L., Baxter, J. T., Black, W. J. M., FitzSimons, J., & Ferguson, J. A. (1979). Late pregnancy ewe feeding and lamb performance in early life. 2. Factors associated with perinatal lamb mortality. *Animal Production, 29*, 401-410.

Madsen, J., Hvelplund, T., Weisbjerg, M., Bertilson, J., Olson, I., Spörndly, R., et al. (1995). The AAT/PBV system for ruminants. A revision. . *Norwegian Journal of Agricultural Science. Suppl No 19, 37*.

McDonald, P., Edwards, R. A., Greenhalgh, J. F. D., & Morgan, C. A. (2002). *Animal Nutrition* (sixth ed.). London: Pearson Education Limited.

McNeill, D. M., Slepetis, R., Erhardt, R. A., Smith, D. A., & Bell, A. W. (1997). Protein requirements of sheep in late pregnancy: Partioning between gravid uterus and maternal tissues. *Journal of Animal Science, 75*, 809-816.

Milano, G. D., Hotston-Moore, A., & Lobley, G. E. (2000). Influence of hepatic ammonia removal on ureagenesis, amino acid utilization and energy metabolism in the ovine liver. [Article]. *British journal of nutrition, 83*(3), 307-315.

Nottle, M. B., Kleemann, D. O., Hockin, V. M., Grosser, T. I., & Seamark, R. F. (1998). Development of a nutritional strategy for increasing lamb survival in merino ewes mated in late spring/early summer. . *Animal Reproduction Science, 52*, 213-219.

Nørgaard, J. V., Nielsen, M. O., Theil, P. K., Sørensen, M. T., Safayi, S., & Sejrsen, K. (2008). Development of mammary glands of fat sheep submitted to restricted feeding during late pregnancy. *Small Ruminant Research, 76*, 155-165.

O´Doherty, J. V., & Crosby, T. F. (1997). The effect of diet in late pregnancy on colostrum production and immunoglobulin absorption in sheep. *Animal Science, 64*, 87-96.

O´Doherty, J. V., & Crosby, T. F. (1998). Blood metabolite concentrations in late pregnant ewes as indicators of nutritional status. *Animal Science, 66*, 675-683.

Ocak, N., Cam, M. A., & Kuran, M. (2005). The effect of high dietary protein levels during late gestation on colostrum yield an lamb survival rate in singleton bearing ewes. *Small Ruminant Research, 56*, 89-94.

Oldham, J. D., Buttery, P. J., Swan, H., & Lewis, D. (1977). Interactions between dietary carbohydrate and nitrogen and digestion in sheep. *Journal of agricultural science, Cambridge, 89*, 467-479.

Orr, R. J., & Treacher, T. T. (1989). The effect of concentrate level on the intake of grass silages by ewes in late pregnancy. *Animal Production, 48*, 109-120.

Orr, R. J., & Treacher, T. T. (1994). The effect of concentrate level on the intakes of silages or hays by ewes in the 1st month of lactation. *Animal Production, 58*, 109-116.

Ólafsson, B. L. (1995). AAT-PBV próteinkerfið fyrir jórturdýr [AAT-PBV protein system for ruminants]. *Ráðunautafundur 1995*, 46-60. [In Icelandic].

Pulido, R. G., Munoz, R., Jara, C., Balocchi, O. A., Smulders, J. P., Wittwer, F., et al. (2010). The effect of pasture allowance and concentrate supplementation type on milk production performance and dry matter intake of autumn-calving dairy cows in early lactation. *Livestock Science, 132*, 119-125.

Purser, A. F., & Young, G. B. (1959). Lamb survival in two hill flocks. *Animal Production, 1*, 85-91.

Robinson, J. J. (1983). Nutrition of the pregnant ewe. In W. Haresign (Ed.), *Sheep Production* (pp. 576). London: Butterworths.

Robinson, J. J., & McDonald, I. (1989). Ewe nutrition, foetal growth and development (Um fóðrun áa og vöxt og þroska fósturs). In Ó. Dýrmundsson & S. Þorgeirsson (Eds.), *Reproduction, growth and nutrition in sheep. Dr. Halldór Pálsson Memorial Publication.*

Robinson, J. J., Sinclair, K. D., & McEvoy, T. G. (1999). Nutritional effects on foetal growth. *Animal Science, 68*, 315-331.

Roy, A., Laforest, J. P., Castonguay, F., & Brisson, G. J. (1999). Effects of maturity of silage and protein content of concentrates on milk production of ewes rearing twin or triplet lambs. *Canadian journal of animal science, 79*, 499-508.
Russel, A. J. F. (1984). Means of assessing the adequacy of nutrition of pregnant ewes. *Livestock Production Science, 11*, 429-436.
Russel, A. J. F., Doney, J. M., & Gunn, R. G. (1969). Subjective assessment of body fat in live sheep. *Journal of agricultural science, Cambridge, 72*, 451-454.
SAS Institute (2004). *SAS/STAT User's Guide, Version 9.* SAS Inst. Inc., Cary, NC
Scales, G. H., Burton, R. N., & Moss, R. A. (1986). Lamb mortality, birth weight and nutrition in late pregnancy. *New Zealand Journal of Agricultural Research, 29*, 75-82.
Schlumbohm, C., & Harmeyer, J. (2004). Hyperketonemia impairs glucose metabolism in pregnant and nonpregnant ewes. *Journal of Dairy Science, 87*, 350-358.
Schmidt, E., & Schmidt, F. W. (1995). Glutamate dehydrogenase (EU 1.4.1.3). In H. U. Bergmeyer (Ed.), *Methods of Enzymatic Analysis 3th edition*. Weinheim, Germany.
Sormunen-Cristiana, R., & Jauhiainen, L. (2001). Comparison of hay and silage for pregnant and lactating Finnish Landrace ewes. *Small Ruminant Research, 39*, 47-57.
Speijers, M. H. M., Fraser, M. D., Haresign, W., Theobald, V. J., & Moorby, J. M. (2005). Effects of ensiled forage legumes on performance of twin bearing ewes and their progeny. . *Animal Science, 81*, 271-282.
Suagee, J. K., Corl, B. A., Crisman, M. V., Wearn, J. G., McCutcheon, L. J., & Geor, R. J. (2010). De novo fatty acid synthesis and NADPH generation in equine adipose and liver tissue. *Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology, 155*(3), 322-326.
Sveinbjörnsson, J., & Ólafsson, B. L. (1999). Orkuþarfir sauðfjár og nautgripa í vexti með hliðsjón af mjólkurfóðureiningakerfi [Energy requirements of sheep and growing cattle with respect to milk feed unit system]. *Ráðunautafundur 1999*, 127 - 204.
Thorgeirsson, S., & Thorsteinsson, S. S. (1989). Growth, development and carcass characteristics (vöxtur, þroski og kjötgæði). In Ó. R. Dýrmundsson & S. Thorgeirsson (Eds.), *Reproduction, growth and nutrition in sheep. Dr. Halldór Pálsson Memorial publication* (pp. 169-204). Reykjavík: Búnaðarfélag Íslands og Rannsóknarstofnun landbúnaðarins.
Thorsteinsson, S. S., Sigurðsson, I. G., & Jónsson, S. (1993). Prótein í fóðri tvílembna eftir burð [Protein in the postpartum diet of twin bearing ewes]. *Ráðunautafundur 1993*. [In Icelandic].
Thorsteinsson, S. S., & Thorgeirsson, S. (1989). Winterfeeding, housing and management (Vetrarfóðrun og hirðing fjár). In Ó. R. Dýrmundsson & S. Thorgeirsson (Eds.), *Reproduction, growth and nutrition in sheep. Dr Halldór Pálsson, Memorial Publication*. Reykjavík: Búnaðarfélag Íslands og Rannsóknarstofnun landbúnaðarins.
Tilley, J. M. A., & Terry, R. A. (1963). A two-stage technique for the *in vitro* digestion of forage crops. . *Journal of British Grassland Society, 18*, 104-111.
Tolkamp, B. J., Yearsley, J. M., Gordon, I. J., Illius, A. W., Speakman, J. R., & Kyriazakis, I. (2007). Predicting the effects of body fatness on food intake and performance of sheep. *British journal of nutrition, 97*, 1206-1215.
Treacher, T. T. (1983). Nutrient requirements for lactation in the ewe. In W. Haresign (Ed.), *Sheep Production* (pp. 576). London: Butterworths.

Van Soest, P. J., Robertson, J. B., & Lewis, B. A. (1991). Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition. *Journal of Dairy Science, 74*, 3583-3597.

Warren, J. T., & Mysterud, I. (1995). Mortality of domestic sheep in free ranging flocks in southeastern Norway. *Journal of Animal Science, 73*, 1012-1018.

Withers, P. C. (1998). Urea: Diverse functions of a 'waste' product. [Article]. *Clinical and Experimental Pharmacology and Physiology, 25*(9), 722-727.

Energy and protein in the diet of ewes in late pregnancy: Effect on ewe feed intake, life weight, body condition and concentration of plasma metabolites

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ABSTRACT

Effects of feeding different types of concentrates to ewes during late pregnancy on feed intake, body weight, body condition score (BCS) and concentration of blood metabolites were tested on 48 ewes during the last month prepartum. Ewes were assigned to four treatments, each containing equal numbers of single- twin- and triplet bearing ewes. Three groups (MIX, EN and PRO) were fed, along with *ad libitum* haylage, supplements differing in protein type and content, but the fourth group (CTR) was only fed haylage. Ewes were weighed and condition scored before and after the experimental period, haylage intake measured and blood samples collected weekly for analysis of metabolites. Supplemented ewes ate significantly less haylage than CTR ewes. Ewe weight and BCS as well as weight and BCS change did not differ significantly between treatments. Single bearing ewes gained less weight and were lighter at parturition than other while triplet bearing ewes had the lowest BCS and lost condition in all supplemented treatments. Treatment affected glucose, BHB, urea, ureic acid, AST, ICDH and calcium levels significantly, BHB and urea level increased with higher levels of undegradable protein. Glucose, urea, ureic acid and ICDH levels were also affected by litter size, single bearing ewes having lower levels for ICDH, urea and ureic acid but the highest glucose levels.

Keywords: DM intake, late-pregnancy, metabolites, nutrition, protein, sheep

Yfirlit

Áhrif af fóðrun með mismunandi kjarnfóðurtegundum á átgetu, lífþunga, holdastig og styrkleika efnaskiptaafurða í blóði voru prófuð á 48 ám á síðasta mánuði meðgöngu. Ánum var skipt í fjóra hópa sem hver innihélt jafnan fjölda einlemba, tvílemba og þrílemba. Þremur hópanna (MIX, EN og PRO) var gefið, til viðbótar við hey eftir átlyst, kjarnfóður sem innihélt prótein af ólíkum uppruna. Fjórði hópurinn (CTR) var samanburðarhópur sem var eingöngu fóðraður á heyi eftir átlyst. Ærnar voru vigtaðar og holdastigaðar í upphafi og lok tilraunar, heyát mælt daglega og blóðsýni tekin vikulega til mælinga á efnaskiptaafurðum. Ærnar í kjarnfóðurhópunum átu marktækt minna hey en CTR ærnar. Meðferð hafði ekki marktæk áhrif á þunga, holdastig og breytingar á þessum þáttum frá upphafi til enda tilraunarinnar. Einlembur þyngdust minna og voru léttari við burð en tvílembur og þrílembur en þær síðast nefndu höfðu lægst holdastig við burð og töpuðu holdum í öllum kjarnfóðurhópunum. Fóðrunarmeðferð hafði marktæk áhrif á styrk glúkósa, BHB, úrefnis, þvagsýru, AST og ICDH og kalks í blóði. Styrkur BHB og úrefnis í blóði jókst með auknu magni af torleystu próteini í kjarnfóðri. Styrkur glúkósa, úrefnis, þvagsýru og ICDH var einnig háður fjölda fóstra og höfðu einlembur lægstan styrk ICDH, úrefnis og þvagsýru en hæstan af glúkósa.

Lykilorð: Efnaskiptaafurðir, Fóðrun, Heyát, Meðganga, Prótein, Sauðfé,

INTRODUCTION

Energy and protein requirements of twin bearing ewes are raised by over 80% and 100% respectively during the last six weeks prepartum (Ólafsson 1995, Sveinbjörnsson & Ólafsson 1999). At the same time availability of nutrients is often decreased due to lowered eating capacity. This creates a great challenge for the pregnant ewe. It has generally been believed that this challenge is rarely met unless the ewe is fed significant amount of concentrate in addition to high quality roughage (Robinson 1983).

The main energetic substrates for foetal development and colostrum production are amino acids and glucose (Banchero et al. 2006, Ingvartsen 2006). Since very little glucose is absorbed directly from the rumen, nutrition of the pregnant ewe aims at providing enough glucogenic substrates for adequate glucose production (Annison et al. 2002). The growing foetus and developing mammary tissues require great amount of amino acids. In addition to the amino acids serving as glucogenic substrates dietary protein has been considered important for the ewes ability to mobilize tissues from her body. This mobilization is needed to fill the gap between the requirements of the growing conceptus and available nutrients from the diet. In that matter undegradable protein has been considered especially useful since research have shown greater response for increased level of undegradable protein than microbial protein (Frutos et al. 1998, McNeill et al. 1997, O´Doherty & Crosby, 1998). However, some researchers have found that effect to be highly linked to condition of the ewe as well as other qualities of the diet offered (Bell et al. 2000, Dawson et al.1999, Nottle et al. 1998).

Concentrate feeding, if not practised systematically and relative to other diet as well as condition of the ewe, can cause metabolic disorders such as rumen acidocis and fatty liver (Imamidoost & Cant 2005) as well as reduced degradability of the other nutrients fed to the ewe (Kaur et al. 2008). Ewe weight and BCS can be used as an indicator of the ewes nutritional status and thereupon her ability to fill nutrient requirements by mobilizing body reserves (Chilliard et al. 2000, Russel 1984, Thorsteinsson & Thorgeirsson 1989).

As indicators of nutritional efficiency, ewe weight and BCS have the disadvantage that it only presents adequacy of nutrition on a longer term basis. (O´Doherty & Crosby 1998, Russel 1984). More immediate information can be gained by measuring concentration of some metabolites in plasma since several metabolitical adaptions take place in the body of the pregnant animal (Ingvartsen 2006). In addition to revealing the metabolical status of the animal, these metabolites can indicate if the animal is in risk of developing metabolic disorders.

The aim of the experiment described below was to test the effect of different concentrate types, especially with regard to type of dietary protein, fed to ewes in late pregnancy on ewe weight and BCS as well as feed intake and concentration of metabolites in plasma. A subsequent paper (Ólafsdóttir et al. 2012b) describes the effect of prepartum supplementing on lamb birth weight and growth rate.

MATERIALS AND METHODS

Experimental animal housing and feeding

The research took place at Hestur, the experimental sheep farm of the Icelandic agricultural university. Forty-eight pregnant ewes of the native Icelandic flock were allocated to one of four dietary treatments (*n=12*) from 30-39 days pre-lambing until lambing, each treatment group containing equal numbers of single, twin and triplet bearing ewes. All treatment, before and after the experimental period, was as traditional at the farm.

Treatment groups were balanced for ewe BCS in February, age, expected lambing date and index for mothering ability, evaluated on the scale 0.1-9.9. For calculation of this index each farms average ewe output is set as the index five and deviation of ewes output from the mean results in their index raising or decreasing to certain level. Each group was divided into two replications (*n=6*) that were penned and fed separately.

All groups were *ad libitum* fed grass haylage from round bales and had free access to water and salt block with mineral and trace elements. Otherwise the treatments were as listed below:

- Control group (CTR): Fed only haylage throughout the experiment.
- Mixed supplement group (MIX): Fed increasing ration of a mixture of high protein and high energy concentrates from day 9 of the experiment.
- Energy supplement group (EN): Fed increasing ration of high energy concentrates from day 9 of the experiment.
- Protein supplement group (PRO): Fed increasing ration of high protein concentrates from day 9 of the experiment.

The high energy concentrate consisted of 50% wheat-bran, 41.75% maize, 5% molasses, 2% shell calcium, 1% feedsalt and 0.25% mineral mix. The high protein concentrate however consisted of 69% fish meal, 30% barley, 1% magnesium phosphate and 0.1% E-vitamin. Chemical composition of haylage and concentrates is listed in Table 1.

Concentrates were fed in one single portion in the morning. Daily rations increased regularly until lambing, rations aiming at supplying all concentrate-fed groups with the same amount of total g AAT day^{-1} ewe^{-1} at each time. Daily ration of supplements at each time is presented in Table 2.

Litter sizes were balanced to two lambs per ewe directly after birth. Within 24 hours lambs were weighed, ear tagged and sex and colour registered. Feeding and all treatment and measurements of ewes and lambs postpartum are described in a companion paper (Ólafsdóttir et al. 2012b).

Measurement, data sampling and chemical analysis

For each treatment, haylage intake was recorded every day from day three, until lambing of the first ewe. Daily intake is calculated as mean intake of each replication – that is: (replication allowance-replication refusals)/number of ewes in replication). Samples were taken daily from both haylage and refusals and frozen down for later dry matter and chemical analyses. All feed and refusal samples were analyzed for dry matter, digestibility, energy (FE_m kg DM^{-1}) crude protein (g kg DM^{-1}), AAT (g kg DM^{-1}), PBV (g kg DM^{-1}) and Neutral Detergent Fibre (NDF, g kg DM^{-1}). Crude protein was measured using the Kjeldahl method, and AAT and PBV values were calculated according to Madsen et al. (1995). NDF was determined using ANKOM technology on Van Soest method (Van Soest et al. 1991) and for dry matter digestibility modified method of Tilley and Terry (Tilley & Terry 1963).

Ewe weight and BCS according to the five unit scale described by Russel et al.(1969) was recorded on day one and 28 of the experiment.

Blood samples were collected every week from half of the ewes from each treatment, six or seven samples per ewe depending on the day of lambing. The second last sample was taken as representative of the ewe at lambing and the last representing the changes occurring the first days (5-9) postpartum. Blood samples were collected from jugular vein by venipuncture using 9 ml Lithium Heparin vacuette® (grainer bio-one) vacutainer. Samples were immediately placed on ice and then centrifuged for 20 minutes. Plasma was collected into 4 ml tubes and frozen down for later analysis.

For plasma glucose, AST, GGT, urea and uric acid determination standard procedures (Siemens Diagnostics® Clinical Methods for ADVIA 1650) were used and for NEFA the Wako, NEFA C ACS-ACOD assay method. Increase in absorbance at 340 nm due to the production of NADH, at slightly alkaline pH in the presence of ß-OH-butyrate dehydrogenase was used to determine BHB. Sample blank was included and the method involved oxamic acid in the media to inhibit lactate dehydrogenase as proposed by Harano et al. (1985). Activity of GLDH was quantified in a kinetic, colorimetric assay according to Schmidt & Schmidt (1995). ICDH was also determined in a kinetic, colorimetric assay using isocitrate as substrate and $NADPH_2$ as response parameter. The autoanalyzer ADVIA 1650® Chemistry System (Siemens Medical Solutions, Tarrytown, NY 10591, USA) was used for all analyzes.

Statistical analysis

Data was analyzed using the REML method of SAS Enterprise Guide 4.1 and 4.2 (SAS institute, 2004) mixed model analyze. When response variable was individual ewe information such as ewe BCS and weight we used the following model:

$$Y_{ijkl} = \mu + T_i + L_j + A_k + D_l + (T \text{ x } L)_{ij} + \varepsilon_{ijkl}$$

Where Y_{ijkl} is the response variable, μ is the overall mean of the population, T_i is the mean of the experimental treatment (i = 1-4), L_j is the mean effect of litter size (j = 1-3), A_k is the effect of ewe age (k = 3-8) and D_l is the effect of length of the treatment (number of days from onset of the experiment until lambing; l = 30-39), $(T \text{ x } L)_{ij}$ is the interaction between treatment and litter size and ε_{ijkl} represents the unexplained residual elements that are assumed to be independent and normally distributed. Those effects were always included in the model when estimating individual ewe information though they did not affect response variable significantly in all cases.

For concentration of blood metabolites we used the following models:

$$Y_{ijkl} = \mu + T_i + L_j + S_m + A_k + W_l + ((T \text{ x } W)_{il} \text{ or } (L \text{ x } W)_{jl)} + \varepsilon_{ijkl}$$

Where Y_{ijkl} is the response variable, μ is the overall mean of the population, T_i is the mean of the experimental treatment (i = 1-4), L_j is the mean effect of litter size (j = 1-3), A_k is the effect of ewe age (k = 3-8) and W_l is the effect of weeks from parturition (l = -6 -1; week 0 being the second last sampling and representing the status of blood metabolites at parturition).

$(T \times W)_{il}$ or $(L \times W)_{jl}$ are the interaction between either treatment and weeks from parturition or litter size and weeks from parturition respectively. ε_{ijkl} represents the unexplained residual elements that are assumed to be independent and normally distributed.

Those effects listed in the formulas above all affected the response variable at some level and therefore they were always included in the model when estimating each factor, even though they did not affect response variable significantly in all cases.

RESULTS

Dry matter, energy and protein intake

Table 3 presents intake of DM, energy (FE_m day^{-1} ewe^{-1}) and protein (g AAT day^{-1} ewe^{-1}) from haylage exclusively. Haylage intake was similar among the supplemented treatments while the CTR group had the highest intake from the second week of the experiment. However, the difference did not become significant until the third week when intake of supplemented ewes decreased to a greater extent than that of the CTR ewes that in fact increased their intake again the last week of measurements.

Table 4 presents total DM, energy (FE_m) and protein (gAAT) intake of treatment groups, including concentrates, week by week.

Least square means for treatments as presented in tables 3 and 4 were adjusted for experimental week, number of days on experimental diet and interaction between treatment and experimental week. P-values for these factors are presented below the tables.

Ewe weight and body condition

Average ewe weight at the beginning of the experiment was 82 kg and BCS 3.8. The only factor significantly affecting ewe weight after the four weeks of experimental treatment was age ($p < 0.05$) while litter size had the greatest effect on weight change during the experiment. Neither treatment, litter size, ewe age, length of experimental treatment nor interaction between treatment and litter size affected BCS in late april significantly though BCS changes were affected by both litter size and length of experimental treatment.

Ewe weight in end of April did not differ significantly between treatments but single bearing ewes were significantly lighter than twin- and triplet bearing ewes. BCS was higher in the MIX group but the difference was not significant between any of the treatments. BCS of triplet bearing ewes was significantly lower than of those bearing singles or twins, the latter two showing very little difference. Weight and BCS changes during the experimental period are presented in Tables 5 and 6. Means regarding BCS and ewe weight, both for treatment and litter size, are adjusted for ewe age, length of experimental treatment and interaction between treatment and litter size (ADJ mean).

Lowest weight change was found for the CTR group; its weight change being more than one kg less than the group where weight change was the second lowest and significantly less than the PRO group which had the highest weight gain but did not differ significantly from the MIX and EN treatments. Changes in BCS during the experiment were smallest in the EN group although group differences in this respect were not significant. Triplet bearing ewes lost condition during this period except from those in the CTR group where all litter sizes gained condition at the same level.

Plasma concentrations of metabolites

Table 7 presents adjusted LSD means for all metabolites analyzed by litter sizes but adjusted LSD means for plasma metabolites by treatments and experimental weeks can be seen in Tables 8-17.

Treatment affected glucose level significantly but the main difference was found for the EN group. That was particularly the case in the last two weeks before parturition when glucose levels in the EN group was raised while it remained rather constant in the other groups throughout the experimental period. Postpartum glucose level was elevated in the PRO group while it either decreased or remained constant in the other groups. Means of all metabolite concentration, both for treatment and experimental week are adjusted for ewe age, weeks from parturition and interaction between treatment and weeks from parturition (ADJ mean).

Table 7 reveals that triplet bearing ewes had significantly lower glucose levels than others. This difference however was only present prepartum, the difference evened out at parturition when glucose level of the single and twin bearing ewes dropped.

Weeks from parturition were the only factor significantly affecting plasma NEFA level. Even though treatment in general did not have significant effect on NEFA, the CTR group level was significantly higher than the other groups, the difference being present from starting of supplementing. NEFA levels in the supplemented groups were similar throughout the experimental period, remaining rather constant until the week before parturition when all treatment groups had elevated plasma concentrations. NEFA levels in single bearing ewes were lower than in those carrying twins and triplets but the same pattern was found as in group means; levels remained similar until parturition approached, when it was strongly elevated.

Plasma BHB levels were significantly affected by ewe age, treatment and weeks from parturition. The BHB level differed significantly between all groups except between the MIX and PRO groups. Furthermore, those two treatments had higher levels than found in the other groups but the EN group had BHB levels significantly lower than all the others. In general BHB levels had the same trend within all treatments and litter sizes during the experiment; increased until about week before parturition when it started to decrease gradually throughout the experimental period.

Treatment, litter size and weeks from parturition all affected plasma urea level significantly. Strongest treatment effects were found for the PRO group that had higher urea levels from beginning of supplementing. The EN group had the lowest level, though not significantly different from CTR and MIX group. In all treatment groups urea level decreased when supplementing started and then remained stable until parturition when it increased again. Triplet bearing ewes had most of the time lower plasma urea levels than those carrying singles and twins and all litter sizes differed significantly from the others.

Uric acid level in plasma was significantly affected by treatment, litter size and weeks from parturition. The only treatment group that differed significantly from the others was the EN group that had higher levels from beginning of supplementing throughout the experiment. The CTR group had most of the time the lowest values for uric acid though the difference was not significant.

The liver enzymes (AST, GGT, GLDH, ICDH) levels are all significantly affected by weeks from parturition. Those effects are highly significant and showing the same trend within all treatments and litter sizes for both AST and GLDH which remained stable prepartum but increased rapidly at parturition. GGT and ICDH also increased during the experiment but more gradually from the start of the measurements.

AST was significantly affected by treatment, the MIX and PRO groups being higher than the other two at all times, though only significantly higher than the EN group. Levels of the PRO group were especially high in the second last measurement which would represent the status at parturition. Litter size did not affect AST level significantly though single bearing ewes had significantly higher levels than those carrying triplets.

Ewe age had highly significant effect on GGT levels in plasma but neither treatment nor litter size did, although treatment effect were close to significance with p-value of 0.06. However, GGT levels of the CTR group were significantly higher than MIX and PRO groups. The EN group did not differ significantly from any of the others, even though it had slightly higher level than the PRO group. The MIX and PRO groups also differed from the others in that their levels increased after birth from levels below the CTR and EN groups up to levels similar or above. No difference was found for litter size except that GGT levels of triplet bearing ewes increased more postpartum than single- and twin bearing ewe levels.

GLDH was significantly affected by litter size which all differed significantly from each other. Single bearing ewes had the highest levels but showed the same trend along the experimental time as ewes carrying twins and triplets, except for increased level in experimental week two. Twin and triplet bearing ewes had similar GLDH level during the experimental period and the significance of the difference between them was most likely due to a smaller increase of GLDH level in the triplet bearing ewes postpartum. The three supplemented groups had lower levels of GLDH than the CTR group the whole experimental period, except for the measurement representing parturition when all levels were similar. The CTR group differed significantly from the MIX and EN groups but not PRO.

Weeks from parturition, treatment and litter size all affected ICDH level significantly. As for the GLDH the supplemented treatments had lower level of ICDH than the CTR group except in two measurements when levels of the MIX and PRO groups were elevated above the others. The difference between the CTR group and the supplemented was significant but none of the supplemented differed from one another. Except from the last measurement plasma ICDH level was lower in single bearing ewes than in those carrying twins or triplets. This is in contrast with the other liver enzymes measured where single bearing ewes usually had higher plasma levels, if any difference. However, postpartum ICDH level in the triplet bearing ewes increased while it decreased in the single and twin bearing ewes resulting in quite similar ICDH levels in the end of the period and non-significant difference between single- and triplet bearing ewes.

Calcium level in plasma was significantly affected by both treatment and weeks from parturition. Ewe age effect also approached significance with p-value of 0.078.

Plasma calcium levels remained stable until parturition. Even though differences were significant between the CTR group and the other treatments very little group difference can actually be seen. CTR group levels were slightly lower in the middle of the prepartum period and levels of the EN group ewes were decreased around parturition but, as in the other groups, increased quickly again postpartum. No clear effect were found of litter size on plasma calcium level. Pattern of calcium level changes between litter sizes were not as constant as between treatments but in general showed the same trend; little difference from week to week prepartum, dropped around parturition and rose again postpartum – except for single bearing ewes where plasma calcium continued to decrease.

DISCUSSION

Dry matter, energy and protein intake

In contrast with previously published research (Robinson 1983) these results indicate that with high quality roughage, ewes in late pregnancy are able to consume enough energy and protein to meet the calculated requirements of a twin bearing ewe, at least until parturition. As suggested by Annett & Carson (2005) and Thorsteinsson & Thorgeirsson (1989) high quality of the roughage used, in this case 0.86 FE_m and 83.9 gAAT (170 g crude protein) per kg DM, is probably a key factor in that matter, allowing high level of DM intake throughout the experimental period. However, those calculated requirements (Ólafsson 1995, Sveinbjörnsson & Ólafsson 1999) can be questioned due to increased birth weight of lambs the last decades. At the time requirements were last estimated birth weight of singles and twins was similar or lower than it is of triplets and twins, respectively, on the same farm today (Thorsteinsson & Thorgeirsson 1989 Thorsteinsson et. al 1993), indicating underestimation of requirements. The increasing level of DM intake of the CTR ewes throughout the experiment is in contrast with Orr & Treacher (1989) and Speijers et al.(2005) that found feed intake to decrease as parturition approached. Since individual feeding was not possible this time it can not be assessed whether litter size or other individual difference affected eating capacity in late pregnancy as stated by Orr & Treacher (1989). Therefore it remains unknown whether ewes of all litter sizes were able to meet their requirements with the roughage exclusively but assuming equal DMI with all litter sizes and requirements as they have been defined until now only triplet bearing ewes would have been fed below requirements.

Decreased roughage intake found with increased supplementing –a phenomenon known as substitution- is probably to some extent result of the high quality roughage offered. It is particularly when feeding rather low quality roughage that concentrate feeding serves completely supplementary to other diet offered (Dawson et al. 2005, Frutos et al. 1998, Kerslake et al. 2008).

Ewe weight and body condition

All treatment groups and litter sizes gained weight during the last month prepartum as expected due to the rapid growth of the conceptus. Lack of treatment effects on ewe weight and weight changes the last month prepartum however is in disagreement with both Dawson et al.(2005) and O´Doherty & Crosby (1997) that found increased protein supplementing and herbage allowance to induce live weight gain. As for the feed intake, high quality of the roughage offered could be the reason as roughage allowance was the same for all treatments. Kerslake et al. (2008) found less effect of supplementing on live weight and weight gain when roughage quality was raised. Greater weight of the gravid uterus and related tissues in ewes carrying multiple litters explains significant difference between litter sizes and is in agreement with Sormunen-Cristiana & Jauhiainen (2001).

Since both Annett et al. (2008), Husted et al. (2008) and Kerslake et al. (2008) failed to connect BCS and prepartum BCS changes to feeding level, lack of treatment effect in our case is not surprising. Though the CTR group was the one most likely to be undernourished it was the only treatment where neither single, twin or triplet bearing ewes lost condition. That supports the finding of for example Frutos et al. (1998), McNeill et al. (1997) and Thorsteinsson et al. (1993) that supplementary protein induces mobilization of body tissue. Possible reason, among many others, for these effects of supplementary protein could be that gluconeogenesis from amino acids provides energy necessary for lipo- and proteolysis (Dawson et al. 2005, Chilliard et al. 2000, Cavestany et al, 2009). Condition loss of the triplet bearing ewes in the supplemented groups was as expected and supports that assumption (Frutos et al. 1998, McNeill et al. 1997), the gap between requirements and intake, as mentioned in the previous section, probably being greater for them than others. However, due to the great feed intake and high quality of diet the supplemented ewes, except for the triplet bearing, should not have needed extensive fat mobilization. Further research are required regarding eating capacity of ewes carrying different litter sizes as well as the effect of different physiological status, BCS and feed quality on, for example, passage rate of digesta and uptake and utilization of nutrients.

Blood metabolites

As suggested by O´Doherty & Crosby (1998), high quality of diet as well as great eating capacity is likely to have decreased the positive effect of high energy concentrate on plasma glucose and resulted in lack of difference between MIX and EN groups compared to CTR. Plasma glucose responses rapidly to changes in intake of glucogenic substrates as well as changes in glucose requirements. Therefore lower levels of glucose in the plasma of triplet bearing ewes were expected due to their greater needs and possibly lower feeding level.

According to Frutos et al. (1998), McNeill et al. (1997) and Chilliard et al. (2000), a greater supply of gluconeogenic substrates in the supplemented ewes, though not reflected in glucose level, would have been expected to increase their ability for fat mobilization. However, based on the requirements of twin bearing ewes as they are defined in Ólafsson (1995) and Sveinbjörnsson & Ólafsson (1999) and the roughage intake measured here, little mobilization should have been needed since even non-supplemented ewes consumed enough to fill energy and protein requirements (Dawson et al. 1999). Lack of changes in BCS of single and twin bearing ewes support that. Effect of litter size on NEFA concentration were as expected with single bearing ewes, having the least need to rely on body reserves, having the lowest level at almost all times. Though plasma level of NEFA is known to indicate magnitude of mobilization of body reserves, changes in BCS of the supplemented triplet bearing ewes are not reflected in NEFA level. In this matter it has to be kept in mind that NEFA levels in plasma are sensitive to stress in the experimental animal during sampling (Speijers et al. 2005) Though results regarding NEFA level were somewhat surprising, a greatly rising level around parturition at the same time as energy requirements are greatly elevated, were as expected.

BHB difference between treatments and sampling dates is not as similar to the pattern found for NEFA as could be expected with the two metabolites both indicating fat mobilization. Higher BHB level in the MIX and PRO treatments than others are in agreement with older research work that found protein supplements to stimulate fat mobilization (Chilliard et al. 2000, Frutos et al. 1998, McNeill et al. 1997). Lowest level of BHB in the EN group however is suprising, those ewes receiving the same amount of AAT as the other supplemented groups. That could indicate some advantage of undegradable protein supplements above microbial protein as a fuel for body tissue mobilization. As mentioned for the NEFA level, effect of supplementing on BHB level is somewhat surprising because of the high intake and quality of diet of all treatments which would be expected to minimize the need for body tissue mobilization. Little effect of litter size does not indicate large fat mobilization in the triplet bearing ewes, in spite of their loss of BCS during the experiment. Good condition of the experimental ewes can affect the results found for BHB as O´Doherty & Crosby (1998) and Speijers et al. (2005) found that with increased BCS of ewes, relationship between dietary energy and plasma BHB concentration was decreased.

Animal condition, among other factors, could explain some of the difference between our results and those from Banchero et al. (2006) and O´Doherty & Crosby (1998) that failed to detect effects of protein supplements on plasma urea. Assuming that urea concentration in plasma gives information on the efficiency of protein nutrition our results indicate quite extensive waste of the expensive undegradable protein supplemented to ewes in the PRO group. It is however interesting that urea level in the PRO group was that much higher than in the MIX and EN groups since all supplement rations aimed at supplying the ewes with the same amount of dietary protein, measured as gAAT ewe^{-1} day^{-1} the difference only consisting in the origin of the protein. This discrepancy could be caused by difference in energy intake between treatments as well as the balance between energy and protein in the digestive tract. Because of higher protein requirements of triplet bearing ewes, significantly lower level of plasma urea in those was expected and indicates higher utilization and better efficiency of dietary protein.

Level of the liver enzymes tested, reaching maximum around parturition, reveals the increase in metabolic stress when requirements rise in late gestation and early lactation. Since energy and protein requirements in the MIX and PRO treatments should have been met just as well as in the EN group it is surprising that AST level around parturition was significantly higher in the MIX and PRO than in the EN treatment. It is possible, at least for the PRO treatment, that this is some effect of the excess ammonia the liver needs to convert as indicated by the elevated urea level in that treatment compared to others. For the MIX group this however can barely be the explanation since urea level in that treatment does not indicate increased urea production above others. Postpartum AST and GLDH level was highest in the CTR group indicating increased stress in the liver of non-supplemented ewes and subsequent release of liver enzymes to the blood. That is in accordance with the slightly highest NEFA level in the CTR ewes which suggests mobilization of body fat. Lower levels of GGT and ICDH in the MIX and PRO group are in disagreement with results for BHB that indicated stronger effect of undegradable than microbial protein supplement on body tissue mobilization. That however confirms other results from this research that reveal limited effect of supplying ewes with undegradable protein above other supplements on fat mobilization. That kind of limitations have in other research work been linked to good condition of the ewes as well as high quality diet fed which is likely to be the reason in our case. Furthermore, it should be kept in mind that moderate fat mobilization is not likely to cause strong response in the plasma metabolites viewed in this project.

Calcium level in plasma reflects the sudden increases in requirements occurring at onset of lactation. High calcium level in the PRO and MIX supplements results in plasma calcium level of ewes receiving those supplements falling less around parturition than in the EN treatment. That reveals the importance of choosing supplements with regard to other nutrients than just energy and protein.

CONCLUSION

As nutrient requirements of pregnant ewes is defined today requirements of single and twin bearing ewes can be met without concentrate feeding until parturition and triplet bearing ewes do not need supplementing until the last week prepartum. Roughage quality as well as previous condition of the ewes probably are key factors in that matter and further research is needed regarding the effects of supplements when roughage quality and BCS are of greater diversity. Supplementing seems to affect the ewes ability to mobilize fat from body reserves for energy production when basal diet is not filling energy needs, possibly due to use of supplementary protein as an glucogenic substrate for the energy requiring process of lipolysis in adipose tissues. Concentration of different plasma metabolites however are rather inconsistent in that matter but high energy level in all treatments could affect those results.

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REFERENCES

Annett RW & Carson AF 2005. The effect of digestible undegradable protein (DUP) content of concentrates on colostrum production and lamb performance of triplet-bearing ewes on grass-based diets during late pregnancy. *Animal Science 80*, 101-110.
Annett RW, Carson AF & Dawson LER 2008. Effects of digestible undegradable protein (DUP) supply and fish oil supplementation of ewes during late pregnancy on colostrums production and lamb output. *Animal Feed Science and Techonology, 146*, 270-288.
Annison EF, Lindsay DB & Nolan JV 2002. Digestion and Metabolism. In M. Freer & H. Dove (Eds.), *Sheep nutrition* (pp. 95-118). Wallingford: CABI Publishing.
Banchero GE, Clariget P, Bencini R, Lindsay DR, Milton JTB & Martin GB 2006. Endocrine and metabolic factors involved in the effect of nutrition on the production of colostrum in female sheep. *Reprod. Nutr. Dev., 46*, 447-460.
Bell AW, Burhans WS & Overton TR 2000. Protein nutrition in late pregnancy, maternal protein reserves and lactation performance in dairy cows. *Proceedings of the Nutrition Society, 59*, 119-126.
Cavestany D, Kulcsar M, Crespi D, Chilliard Y, La Manna A, Balogh O, Keresztes M, Delavaud C, Huszenicza G & Meikle A 2009. Effect of Prepartum Energetic Supplementation on Productive and Reproductive Characteristics, and Metabolic and Hormonal Profiles in Dairy Cows under Grazing Conditions. *Reproduction in Domestic Animals, 44(4),* 663-671.
Chilliard Y, FerlayA, Faulconnier Y, Bonnet M, Rouel J & Bocquier F (2000). Adipose tissue metabolism and its role in adaptions to undernutrition in ruminants. *Proceedings of the Nutrition Society, 59*, 127-134.
Dawson LER, Carson AF & Kilpatrick DJ 1999. The effect of the digestible undegradable protein of concentrates and protein source offered to ewes in late pregnancy on colostrum production and lamb performance. *Animal Feed Science and Techonology, 82*, 21-36.
Dawson LER, Carson AF, Kilpatrick DJ & Laidlaw AS 2005. Effect of herbage allowance and concentrate food level offered to ewes in late pregnancy on ewe and lamb performance. *Animal Science, 81*, 413-421.
Frutos P, Buratovich O, Giráldez FJ, Mantecón AR & Wright IA 1998. Effects on maternal and foetal traits of feeding supplement to grazing pregnant ewes. *Animal Science, 66*, 667-673.
Harano Y, Ohtsuki M, Ida M, Kojima H, Harada M, Okanishi, T, Kashiwagi A, Ochi Y, Uno S & Sihigeta Y 1985. Direct automated assay method for serum or ureine levels of ketone bodies. *Clinica Chimica Acta, 151*, 177-183.
Husted SM, Nielsen MO, Blache D & Ingvartsen KL 2008. Glucose homeostasis and metabolic adaption in the pregnant and lactating sheep are affected by the level of nutrition previously provided during her late fetal life. *Domestic animal endocrinology, 34*, 419-431.
Imamidoost R & Cant JP 2005. Non-steady state modelling of effects of timing and level of concentrate supplementation on ruminal pH and forage intake in high-producing, grazing ewes. *Journal of Animal Science, 83*, 1102-1115.
Ingvartsen KL 2006. Feeding and management related diseases in the transition cow. Physiological adaptions around calving and strategies to reduce feeding-related diseases. *Animal Feed Science and Techonology, 126*, 175-213.
Kaur R, Nandra KS, Garcia SC, Fulkerson WJ & Horadagoda A 2008. Efficiency of utilisation of different diets with contrasting forages and concentrate when fed to sheep in a discontinuous feeding pattern. *Livestock Science, 119*, 77-86.

Kerslake JI, Kenyon PR, Morris ST, Stafford KJ & Morel PCH 2008. Effect of concentrate supplement and sward height on twin bearing ewe body condition and the performance of their offspring. *Australian journal of experimental agriculture, 48*, 988-994.
Madsen J, Hvelplund T, Weisbjerg MR, Bertilson J, Olson I, Spörndly R, Harstad OM, Volden H, Tuori M, Varvikko,T, Huhtanen P & Olafsson BL 1995. The AAT/PBV system for ruminants. A revision. *Norwegian Journal of Agricultural Science.* Suppl. No 19, 37
McNeill DM, Slepetis R, Erhardt RA, Smith DA & Bell AW 1997. Protein requirements of sheep in late pregnancy: Partioning between gravid uterus and maternal tissues. *Journal of Animal Science, 75*, 809-816.
Nottle MB, Kleemann DO, Hockin VM, Grosser TI & Seamark RF 1998. Development of a nutritional strategy for increasing lamb survival in merino ewes mated in late spring/early summer. . *Animal Reproduction Science, 52*, 213-219.
O´Doherty JV & Crosby TF 1997. The effect of diet in late pregnancy on colostrum production and immunoglobulin absorption in sheep. *Animal Science, 64*, 87-96.
O´Doherty JV & Crosby TF 1998. Blood metabolite concentrations in late pregnant ewes as indicators of nutritional status. *Animal Science, 66*, 675-683.
Orr RJ & Treacher TT 1989. The effect of concentrate level on the intake of grass silages by ewes in late pregnancy. *Animal Production, 48*, 109-120.
Ólafsdóttir HÓ, Sveinbjörnsson J & Harðarson GH 2012b. Energy and protein in the diet of ewes in late pregnancy: Effect on lamb birth weight and growth rate. *Submitted to Icelandic Agricultural Sciences*
Ólafsson BL 1995. AAT-PBV próteinkerfið fyrir jórturdýr [AAT-PBV protein system for ruminants]. *Ráðunautafundur 1995*, 46-60. [In Icelandic].
Robinson JJ 1983. Nutrition of the pregnant ewe. In W. Haresign (Ed.), *Sheep Production* (pp. 576). London: Butterworths.
Russel AJF 1984. Means of assessing the adequacy of nutrition of pregnant ewes. *Livestock Production Science, 11*, 429-436.
Russel AJF, Doney JM & Gunn RG 1969. Subjective assessment of body fat in live sheep. *Journal of agricultural science, Cambridge, 72*, 451-454.
SAS Institute 2004. *SAS/STAT User's Guide, Version 9.* SAS Inst. Inc., Cary, NC
Schmidt E & Schmidt FW 1995. Glutamate dehydrogenase (EU 1.4.1.3). In H. U. Bergmeyer (Ed.), *Methods of Enzymatic Analysis 3th edition.* Weinheim, Germany.
Sormunen-Cristiana R & Jauhiainen L 2001. Comparison of hay and silage for pregnant and lactating Finnish Landrace ewes. *Small Ruminant Research, 39*, 47-57.
Speijers MHM, Fraser MD, Haresign W, Theobald VJ & Moorby JM 2005. Effects of ensiled forage legumes on performance of twin bearing ewes and their progeny. . *Animal Science, 81*, 271-282.
Sveinbjörnsson J & Ólafsson BL 1999. Orkuþarfir sauðfjár og nautgripa í vexti með hliðsján af mjólkurfóðureiningakerfi [Energy requirements of sheep and growing cattle with respect to milk feed unit system]. *Ráðunautafundur 1999*. [In Icelandic].
Thorsteinsson SS & Thorgeirsson S 1989. Winterfeeding, housing and management (Vetrarfóðrun og hirðing fjár). In Ó. R. Dýrmundsson & S. Thorgeirsson (Eds.), *Reproduction, growth and nutrition in sheep. Dr Halldór Pálsson, Memorial Publication*. Reykjavík: Búnaðarfélag Íslands og Rannsóknarstofnun landbúnaðarins.
Thorsteinsson SS, Sigurðsson IG & Jónsson S 1993. Prótein í fóðri tvílembna eftir burð [Protein in the postpartum diet of twin bearing ewes]. *Ráðunautafundur 1993*. [In Icelandic].

Tilley JMA & Terry RA 1963. A two-stage technique for the *in vitro* digestion of forage crops. . *Journal of British Grassland Society, 18*, 104-111.
Van Soest PJ, Robertson JB & Lewis BA 1991. Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition. *Journal of Dairy Science, 74*, 3583-3597.

Table 1. Dry matter content and chemical analysis of the experimental feedstuff.

	Haylage	High energy concentrate	High Protein concentrate
DM %	58.5	88	88
FEm, kg DM^{-1}	0.86	0.98	1
Protein, g kg DM^{-1}	170	110	440
AAT, g kg DM^{-1}	84	105	200
PBV, g kg DM^{-1}	24	-35	168
NDF, g kg DM^{-1}	514	128	45
Ca, g kg DM^{-1}	3	8	55
P g kg DM^{-1}	3	6	32
Mg g kg DM^{-1}	2	2	4
Na g kg DM^{-1}	1	4	6

Table 2. Daily ration of supplements (g DM day^{-1} ewe^{-1}) at each time

	CTR	MIX	EN	PRO
Experimental week 1	0	*60	60	60
Experimental week 2	0	*100	100	100
Experimental week 3	0	**190	250	131
Experimental week 4 and until lambing	0	***260	343	180

* equal ration of high energy and high protein supplements

** 125 g high energy supplement and 65 g high protein supplement

*** 170 g high energy supplement and 90 g high protein supplement

Table 3. Total haylage intake (kg DM, FE_m and gAAT day^{-1} ewe^{-1}) by treatment groups and weeks.

	Week 1			Week 2			Week 3			Week 4		
	Kg DM	FE_m	gAAT	Kg DM	FE_m	gAAT	Kg DM	FE_m	gAAT	Kg DM	FE_m	gAAT
CTR	1,82	1.55	151.1	1.99	1.73	167.7	1.91	1.64	159.4	1.98	1.74	168.3
MIX	1.80	1.53	149.3	1.94	1.69	163.0	1.81	1.56	151.7	1.80	1.58	152.8
EN	1.81	1.54	149.7	1.94	1.69	163.6	1.81	1.57	151.5	1.76	1.55	149.6
PRO	1.80	1.53	149.0	1.95	1.70	164.5	1.82	1.57	152.3	1.78	1.56	150.7

p-values for fixed effects	Kg DM	FE_m	gAAT
Treatment	< 0.0001	< 0.0001	< 0.0001
Experimental week	< 0.0001	< 0.0001	< 0.0001
Length of experimental treatment	0.0451	0.0003	0.0259
Treatment * experimental week	< 0.0001	< 0.0001	< 0.0001

Table 4. Total intake (kg DM, FE_m and gAAT day⁻1) by treatment groups and weeks.

	Week 1			Week 2			Week 3			Week 4		
	Kg DM	FE_m	gAAT	Kg DM	FE_m	gAAT	Kg DM	FE_m	gAAT	kg DM	FE_m	gAAT
CTR	1.82	1.55	151.1	1.99	1.73	167.7	1.91	1.64	159.4	1.99	1.74	168.3
MIX	1.86	1.59	158.5	2.04	1.79	179.2	2.00	1.75	177.8	2.06	1.83	188.7
EN	1.87	1.60	156.0	2.04	1.79	174.1	2.06	1.81	177.8	2.11	1.89	185.6
PRO	1.86	1.59	161.0	2.05	1.80	184.5	1.95	1.71	178.5	1.96	1.74	186.7

p-values for fixed effects	Kg DM	FEm	gAAT
Treatment	<0.0001	<0.0001	<0.0001
Experimental week	<0.0001	<0.0001	<0.0001
Length of experimental treatment	0.0451	0.0003	0.0259
Treatment * experimental week	<0.0001	<0.0001	<0.0001

Table 5. Changes in ewe weight during experiment

Treatment	Single	Twin	Triplets	ADJ mean
CTR	5.10^{a}	6.58^{ab}	8.99^{bc}	6.89^{A}
MIX	7.60^{abc}	9.12^{bc}	7.21^{abc}	7.98^{AB}
EN	6.11^{ab}	7.83^{abc}	10.19^{c}	8.05^{AB}
PRO	6.70^{ab}	8.84^{bc}	9.99^{c}	8.51^{B}
ADJ mean	6.38^{A}	8.09^{B}	9.10^{B}	

Different letters in superscript represent significant difference

p-values for fixed effects	
Treatment	0.4133
Litter size	0.0111
Ewe age	0.9965
Length of experimental treatment	0.1515
treatment*litter size	0.4348

Table 6 Changes in BCS during experiment

Treatment	Singles	Twins	Triplets	ADJ mean
CTR	0.08^{bcd}	0.08^{bcd}	0.07^{bcd}	0.08^{NS}
MIX	0.29^{d}	0.01^{abc}	-0.10^{ab}	0.07^{NS}
EN	0.11^{bcd}	0.17^{cd}	-0.23^{a}	0.02^{NS}
PRO	0.09^{bcd}	0.20^{cd}	-0.09^{ab}	0.07^{NS}
ADJ mean	0.15^{A}	0.12^{A}	-0.09^{B}	

Different letters in superscript represent significant difference

p-values for fixed effects	
Treatment	0.8367
Litter size	0.0013
Ewe age	0.0563
Length of experimental treatment	0.0143
treatment*litter size	0.1025

Table 7. Adjusted mean for blood metabolites by litter sizes

	Single	twin	triplets
Glucose (mM)	3.67^{A}	3.74^{A}	3.42^{B}
NEFA (μekv L^{-1})	245^{A}	322^{B}	317^{B}
BHB (mM)	0.71^{A}	0.70^{A}	0.73^{A}
Urea (mM)	9.22^{A}	8.60^{B}	7.44^{C}
Uric acid (μekv.L^{-1})	21.63^{A}	23.71^{B}	22.97^{B}
AST (u L^{-1})	160^{A}	152^{AB}	148^{B}
GLDH (u L^{-1})	37.5^{A}	25.4^{B}	17.1^{C}
GGT (u L^{-1})	53.9^{A}	54.8^{A}	55.7^{A}
ICDH (u L^{-1})	20.6^{A}	27.7^{B}	23.3^{A}

Calcium (mM)	2.71^{A}	2.67^{B}	2.67^{AB}

Different letters in superscript within rows represent significant difference

Table 8. Plasma glucose levels by treatments and experimental weeks (mM)

Treatment	-5	-4	-3	-2	-1	0	1	ADJ mean
CTR	3.42^{a}	3.69^{a}	4.05^{b}	3.60^{a}	3.59^{a}	3.56^{a}	3.42^{a}	3.62^{B}
MIX	3.38^{a}	3.66^{a}	3.53^{a}	3.56^{a}	3.76^{a}	3.81^{a}	3.46^{a}	3.59^{B}
EN	3.37^{a}	3.73^{a}	3.69^{a}	3.97^{b}	4.26^{b}	3.99^{b}	3.45^{a}	3.78^{C}
PRO	3.24^{a}	3.57^{a}	3.53^{a}	3.35^{a}	3.32^{a}	3.60^{a}	3.85^{a}	3.42^{A}
ADJ mean	3.35	3.66	3.70	3.62	3.73	3.74	3.42	

Different letters in superscript represent significant difference

p-values for fixed effects

Ewe age	0.9914
Treatment	0.0143
Litter size	0.0029
Weeks from parturition	0.0575
Treatment * week from parturition	0.7108

Table 9. Plasma NEFA levels by treatments and experimental weeks (µekv./L).

Treatment	-5	-4	-3	-2	-1	0	1	ADJ mean
CTR	301.77^{ab}	308.10^{ab}	246.56^{ab}	249.49^{ab}	243.78^{ab}	465.79^{b}	555.51^{b}	338.71^{B}
MIX	304.07^{ab}	278.48^{ab}	145.16^{a}	143.42^{a}	153.07^{a}	387.75^{b}	529.73^{b}	277.38^{A}
EN	352.88^{ab}	160.57^{a}	164.38^{a}	135.25^{a}	131.45^{a}	461.99^{b}	523.10^{b}	275.66^{A}
PRO	482.96^{b}	176.03^{a}	143.09^{a}	163.68^{a}	214.53^{ab}	346.57^{ab}	440.39^{b}	281.04^{A}
ADJ mean	360.42	230.79	174.80	172.96	185.71	415.52	512.19	

Different letters in superscript represent significant difference

p-values for fixed effects

Ewe age	0.3357
Treatment	0.3479
Litter size	0.0568
Weeks from parturition	< 0.0001
Treatment * weeks from parturition	0.9528

Table 10. Plasma BHB level by treatments and experimental weeks (mM).

Treatment	-5	-4	-3	-2	-1	0	1	ADJ mean
CTR	0.60^{ab}	0.68^{bc}	0.75^{bc}	0.73^{bc}	0.84^{c}	0.66^{b}	0.66^{b}	0.70^{B}
MIX	0.59^{ab}	0.72^{bc}	0.82^{c}	0.85^{c}	0.87^{c}	0.75^{bc}	0.64^{ab}	0.75^{C}
EN	0.49^{a}	0.60^{ab}	0.65^{ab}	0.63^{ab}	0.66^{b}	0.64^{ab}	0.61^{ab}	0.61^{A}
PRO	0.62^{ab}	0.75^{bc}	0.88^{c}	1.03^{d}	0.92^{cd}	0.66^{b}	0.66^{b}	0.79^{C}
ADJ mean	0.57	0.69	0.77	0.81	0.82	0.68	0.64	

Different letters in superscript represent significant difference

p-values for fixed effect

Ewe age	0.0092
Treatment	< 0.0001

Litter size	0.4714
Weeks from parturition	< 0.0001
Treatment * Weeks from parturition	0.1263

Table 11. Plasma urea level by treatments and experimental weeks (mM).

Treatment	-5	-4	-3	-2	-1	0	1	ADJ mean
CTR	8.93^{ab}	7.78^{ab}	7.73^{ab}	7.33^{ab}	7.34^{ab}	9.06^{b}	9.61^{b}	8.25^{A}
MIX	9.34^{b}	6.84^{a}	7.81^{ab}	8.32^{ab}	8.38^{ab}	6.89^{a}	8.96^{b}	8.08^{A}
EN	9.75^{b}	6.94^{a}	8.61^{ab}	6.98^{a}	7.15^{ab}	7.64^{ab}	9.85^{b}	8.13^{A}
PRO	10.45^{b}	7.76^{ab}	9.09^{b}	9.82^{b}	9.52^{b}	8.50^{ab}	9.50^{b}	9.23^{B}
ADJ mean	9.62	7.33	8.31	8.11	8.10	8.02	9.48	

Different letters in superscript represent significant difference

p-values for fixed effect

Ewe age	0.7602
Treatment	0.0078
Litter size	<0.0001
Weeks from parturition	0.0001
Treatment * weeks from parturition	0.4591

Table 12. Plasma Uric acid level by treatments and experimental weeks (µekv./L).

Treatment	-5	-4	-3	-2	-1	0	1	ADJ mean
CTR	22.04^{a}	23.27^{bc}	26.07^{bc}	19.75^{a}	17.08^{a}	22.38^{bc}	19.80^{ab}	21.49^{A}
MIX	22.23^{bc}	24.02^{bc}	26.55^{c}	22.23^{b}	19.52^{ab}	22.93^{bc}	22.07^{b}	22.79^{B}
EN	21.63^{ab}	24.82^{bc}	27.55^{c}	29.25^{c}	22.50^{bc}	23.65^{bc}	20.87^{ab}	24.32^{C}
PRO	21.12^{ab}	23.95^{bc}	25.27^{bc}	27.05^{c}	19.47^{ab}	21.58^{ab}	20.85^{ab}	22.61^{AB}
ADJ mean	21.51	24.01	26.36	24.57	19.64	22.64	20.90	

Different letters in superscript represent significant difference

P-values for fixed effect

Ewe age	0.9849
Treatment	0.0099
Litter size	0.0092
Weeks from parturition	<0.0001
Treatment * weeks from parturition	0.201

Table 13. Plasma AST level by treatments and experimental weeks (u/L).

Treatment	-5	-4	-3	-2	-1	0	1	ADJ mean
CTR	138.91^{ab}	147.09^{ab}	138.70^{ab}	136.32^{ab}	136.95^{ab}	167.92^{b}	195.95^{b}	151.69^{B}
MIX	151.16^{ab}	144.38^{ab}	142.41^{ab}	144.93^{ab}	153.55^{ab}	171.98^{b}	193.31^{b}	157.39^{BC}
EN	124.19^{a}	127.60^{a}	127.3^{2a}	122.67^{a}	122.12^{a}	166.90^{b}	191.12^{b}	140.27^{A}
PRO	147.09^{ab}	149.13^{ab}	151.61^{ab}	144.24^{ab}	148.91^{ab}	225.36^{c}	190.18^{b}	165.22^{C}
ADJ mean	140.34	142.05	140.01	137.04	140.38	183.04	192.64	

Different letters in superscript represent significant difference

P-values for fixed effects

Ewe age	0.8439
Treatment	0.0089
Litter size	0.1651
Weeks from parturition	<0.0001
Treatment * weeks from parturition	0.851

Table 14. Plasma GGT level by treatments and experimental weeks (u/L).

Treatment	-5	-4	-3	-2	-1	0	1	ADJ mean
CTR	46.29^{ab}	53.87^{ab}	55.04^{ab}	56.21^{ab}	64.87^{b}	63.21^{b}	67.54^{bc}	58.15^{C}
MIX	44.13^{a}	47.52^{ab}	49.36^{ab}	52.19^{ab}	53.69^{ab}	52.69^{ab}	74.86^{c}	53.49^{AB}
EN	46.15^{ab}	53.21^{ab}	54.54^{ab}	55.54^{ab}	60.71^{b}	60.71^{b}	63.21^{b}	56.29^{BC}
PRO	43.98^{a}	49.53^{ab}	52.70^{ab}	51.36^{ab}	53.20^{ab}	50.86^{ab}	65.70^{b}	51.26^{A}
ADJ mean	45.14	51.03	52.91	53.82	58.12	56.87	65.70	

Different letters in superscript represent significant difference

p-values for fixed effects

Ewe age	0.0001
Treatment	0.0607
Litter size	0.7702
Weeks from parturition	<0.0001
Treatment * weeks from parturition	0.09032

Table 15. Plasma GLDH level by treatments and experimental weeks (u/L).

Treatment	-5	-4	-3	-2	-1	0	1	ADJ mean
CTR	20.10^{ab}	41.96^{ab}	24.21^{ab}	23.32^{ab}	23.71^{ab}	10.94^{a}	100.26^{c}	31.93^{B}
MIX	20.45^{ab}	19.88^{ab}	12.30^{a}	11.82^{a}	15.18^{a}	14.49^{a}	50.28^{b}	20.62^{A}
EN	12.38^{a}	17.81^{ab}	13.64^{a}	10.14^{a}	14.29^{a}	10.46^{a}	83.76^{c}	23.21^{A}
PRO	21.55^{ab}	25.97^{ab}	23.29^{a}	13.98a	20.92^{a}	13.61^{a}	82.84^{bc}	28.88^{AB}
ADJ mean	18.62	26.40	18.36	14.82	18.52	12.38	79.28	

Different letters in superscript represent significant difference

P-values for fixed effects

Ewe age	0.3587
Treatment	0.1467
Litter size	0.0023
Weeks from parturition	<0.0001
Treatment * weeks from parturition	0.9735

Table 16. Plasma ICDH level by treatments and experimental weeks (u/L)

Treatment	-5	-4	-3	-2	-1	0	1	ADJ mean
CTR	23.84^{ab}	25.65^{ab}	28.06^{b}	23.41^{ab}	27.32^{ab}	33.05^{b}	36.54^{b}	28.27^{B}
MIX	18.73^{ab}	22.11^{ab}	23.63^{ab}	24.77^{ab}	21.94^{ab}	24.48^{ab}	17.92^{ab}	21.94^{A}
EN	13.45^{a}	24.26^{b}	25.58^{ab}	21.82^{ab}	22.78^{ab}	29.92^{b}	31.46^{b}	24.18^{A}
PRO	16.00^{ab}	18.54^{b}	19.82^{ab}	20.12^{ab}	19.03^{ab}	36.73^{b}	20.05^{a}	24.47^{A}
ADJ mean	18.01	22.64	24.27	22.53	22.77	31.05	26.49	

Different letters in superscript represent significant difference

P-value for fixed effect

Ewe age	0.9902
Treatment	0.0471
Litter size	0.0086
Weeks from parturition	0.0236
Treatment * weeks from parturition	0.7921

Table 17. Plasma Calcium level by treatments and experimental weeks (mM)

treatment	-5	-4	-3	-2	-1	0	1	ADJ mean
CTR	2.59^{a}	2.65^{a}	2.61^{a}	2.56^{a}	2.67^{a}	2.53^{a}	2.71^{a}	2.62^{A}

MIX	2.69^{a}	2.65^{a}	2.67^{a}	2.76^{ab}	2.88^{b}	2.52^{a}	2.67^{a}	2.69^{B}
EN	2.57^{a}	2.72^{a}	2.67^{a}	2.79^{ab}	2.85^{ab}	2.16^{a}	2.74^{ab}	2.72^{B}
PRO	2.64^{a}	2.73^{ab}	2.79^{ab}	2.76^{ab}	2.76^{ab}	2.54^{a}	2.70^{a}	2.70^{B}
ADJ mean	2.62	2.69	2.69	2.71	2.79	2.58	2.70	

Different letters in superscript represent significant difference

P-values for fixed effect

Ewe age	0.0777
Treatment	0.0059
Litter size	0.2244
Weeks from parturition	<0.0001
Treatment * weeks from parturition	0.1842

Energy and protein in the diet of ewes in late pregnancy: Effect on lamb birth weight and growth rate

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ABSTRACT

Effect of feeding different types of concentrates during late pregnancy on lamb birth weight and growth rate were tested on 48 ewes the last month prepartum. Ewes were assigned to four treatments, each containing equal numbers of single- twin- and triplet bearing ewes. Three of the treatment groups (MIX, EN and PRO) were fed, along with *ad libitum* haylage, increasing levels of supplements differing in protein type and content. The fourth group, control group (CTR), was only fed haylage. All lambs were reared as twins. Lambs were weighed at birth, seven days old, approximately eight weeks old and at weaning. No significant birth weight differences were found among treatment groups. The first week postpartum lambs reared by non-supplemented ewes had significantly lower growth rate than others but this difference ceased with increasing age. Growth rate was not significantly different between the supplemented groups. Lambs reared by ewes that had given birth to twins had significantly higher growth rate the first week postpartum than lambs reared by ewes that had given birth to singles or triplets.

Keywords: birth weight, growth rate, nutrition, protein, late-pregnancy, sheep

Yfirlit

Áhrif af fóðrun með mismunandi kjarnfóðurtegundum á fæðingarþunga og vaxtarhraða lamba voru prófuð á 48 ám síðasta mánuð meðgöngu. Ánum var skipt í fjóra meðferðarhópa sem hver innihélt jafnan fjölda einlemba, tvílemba og þrílemba. Þremur hópanna (MIX, EN og PRO) var gefið, til viðbótar við hey eftir átlyst, vaxandi skammtar af kjarnfóðri sem innihélt mismunandi magn og gerð af próteini. Fjórði hópurinn (CTR) var samanburðarhópur sem var eingöngu fóðraður á heyi eftir átlyst. Lömbin voru vigtuð innan sólarhrings frá burði, viku gömul, við fjallrekstur (u.þ.b. átta vikna gömul) og loks fyrir slátrun. Ekki var marktækur munur á fæðingarþunga lambanna eftir meðferð mæðra. Fyrstu vikuna þyngdust lömb sem gengu undir CTR ánum marktækt minna en önnur en þessi munur minnkaði með vaxandi aldri. Ekki var marktækur munur á vaxtarhraða lamba eftir því hvaða kjarnfóður ærnar sem þau gengu undir fengu fyrir burð. Lömb sem gengu undir tvílembdum ám uxu marktækt hraðar fyrstu vikuna eftir burð en þessi munur dvínaði svo og sást ekki eftir átta vikna aldur.
Lykilorð: sauðfé, fæðingarþungi, prótein, fóðrun, meðganga

INTRODUCTION

It is well known that ewe nutrition in late pregnancy can affect birth weight of lambs and several studies can be mentioned that support that statement (Frutos et al. 1998, Husted et al. 2007 & 2008, Khalaf et al. 1979, Robinson & McDonald 1989). Even though dietary protein has generally been considered especially important for the adequacy of nutrition of the pregnant ewe (Robinson & McDonald 1989), results regarding effect of dietary protein on lamb birth weight are quite inconsistent. Annett et al. (2008) found positive effect of undegradable protein on birth weight while both Nørgaard et al. (2008) and O´Doherty & Crosby (1997) failed to detect such difference. Along with effect of late gestation feeding level the body condition of the mother also affects lamb birth weight (Thorsteinsson &

Thorgeirsson 1989). The fact that ruminants have excellent ability to mobilize and use nutrients stored in body tissues (Chilliard et al. 2000) as well as the high priority of foetal growth for nutrients (Thorsteinsson & Thorgeirsson 1989) allows us to state that birth weight is influenced by some combination of the condition of the ewe in the latter part of mid pregnancy – as defined by previous feeding - and late pregnancy nutrition (Kerslake et al. 2008).

Lamb survival, especially the first hours postpartum, is affected by birth weight (Gama et al. 1991, Nottle et al. 1998, Robinson & McDonald 1989). The main reasons for that is probably more effective thermoregulation and energy supply (Andrews & Mercer 1985, McNeill et al. 1997, Robinson et al. 1999) and greater resistance to infections (Gama et al. 1991, Khalaf et al. 1979) with increasing birth weight. The latter effects can also be caused by a greater amount of colostrum available to and consumed by the heavier lambs, probably also borne to better nourished ewes (Khalaf et al. 1979).

Though keeping ewes in acceptable condition and securing normal birth weight of lambs is important factor in the sheep husbandry, the main goal of the prepartum feeding must be to secure sufficient rearing ability of the ewe in order to supply enough milk to meet with the lamb's capacity to grow fast (Ocak et al. 2005). As for the birth weight undegradable protein has been considered important factor in the nutrition of the lactating ewe and several research have revealed effects of protein supplements on colostrum production (Banchero et al. 2006, Nottle et al. 1998, Nørgaard et al. 2008, Robinson & McDonald 1989, Sormunen-Cristiana & Jauhiainen 2001). However, other research, such as Annett & Carson (2005), Dawson et al. (1999), Kerslake et al. (2008) and Speijers et al. (2005) have failed to detect such effect but in these cases the authors have linked their results with the excellent body condition of their experimental ewes as well as high quality of the roughage offered in their experiments. Though adequate colostrum output is a good indicator of the adequacy of prepartum feeding and promising for the upcoming lactation it is not an indefectible parameter in that matter. With adequate lactation diet milk production of ewes, though underfed prepartum to a level known to negatively affect colostrum output, has as soon as five days postpartum reached the same level as in *ad libitum* fed ewes (Nørgaard et al. 2008). Furthermore, Nørgaard et al. (2008) failed to detect effect of prepartum feed restriction on the complete lactation output measured as lamb weight at weaning.

With advancing age a greater proportion of the lambs nutrition is derived from herbage compared with the mothers supply of milk. Furthermore, lamb growth rate ceases with advancing age, both these facts resulting in diminishing effect of late pregnancy and early lactation nutrition of the ewe on lamb performance (Guðmundsson & Dýrmundsson 1989).

In the experiment described below effect of concentrate types, especially with regard to type of dietary protein, on lamb birth weight and growth rate were tested. A companion paper (Ólafsdóttir, et al. 2012a) presents results regarding effect on the ewe (eating capacity, live weight, body condition and plasma metabolites).

MATERIALS AND METHODS

Experimental animal housing and feeding

The research took place at Hestur, the experimental farm of the Icelandic agricultural university. Forty-eight pregnant ewes of the native Icelandic flock were allocated to one of four dietary treatments ($n=12$) from 30-39 days pre-lambing until lambing, each treatment group containing equal numbers of single, twin and triplet bearing ewes. All treatment, before and after the experimental period, was as traditional at the farm.

Treatment groups were balanced for ewe BCS in February, age, expected lambing date and index for mothering ability, evaluated on the scale 0.1-9.9. For calculation of this index each farms average ewe output is set as the index five and deviation of ewes output from the mean results in their index raising or decreasing to certain level. Each group was divided into two replications ($n=6$) that were penned and fed separately.

All groups were *ad libitum* fed grass haylage from round bales and had free access to water and salt block with mineral and trace elements. Otherwise the treatments were as listed below

- Control group (CTR): Fed only haylage throughout the experiment.
- Mixed supplement group (MIX): Fed increasing ration of a mixture of high protein and high energy concentrates from day 9 of the experiment.
- Energy supplement group (EN): Fed increasing ration of high energy concentrates from day 9 of the experiment.
- Protein supplement group (PRO): Fed increasing ration of high protein concentrates from day 9 of the experiment.

The high energy concentrate consisted of 50% wheat-bran, 41.75% maize, 5% molasses, 2% shell calcium, 1% feedsalt and 0.25% mineral mix. The high protein concentrate consisted of 69% fish meal, 30% barley, 1% magnesium phosphate and 0.1% E-vitamin. Chemical composition of haylage and concentrates is listed in table 1.

Concentrates were fed in one single portion in the morning. Daily rations increased regularly until lambing, rations aiming at supplying all concentrate-fed groups with the same amount of total g AAT ewe^{-1} day^{-1}at each time. Daily ration of supplements at each time is presented in table 2.

Litter sizes were balanced to two lambs per ewe directly after birth, i.e. one lamb was removed from each triplet bearing ewe and one extra lamb, usually triplet or twin from yearling, was added to those that had given birth to singles. Because of this process and the fact that the ewes did not always lamb on the day expected, some of the lambs reared by experimental ewes were not born to ewes from the experiment. One ewe only raised one lamb since no extra lamb was available at parturition and was therefore excluded from the postpartum data. One lamb was stillborn and two died because of lambing difficulties. Two lambs died within the first 10 days, both reared by the same dam that only reared one lamb afterwards. Approximately two weeks postpartum one lamb was found dead in the pasture and one of the experimental ewes died 10 days postpartum, reason for death in both cases unknown.

At parturition ewes were individually penned for around 2 days but moved to groups of increasing size the next days if no problems occurred. Within 24 hours lambs were weighed, ear tagged and sex and colour registered. For around 8-12 days postpartum ewes and lambs were kept indoors but with access to outdoor pen and all ewes received the same ration, approximately 150 g ewe^{-1} day^{-1}, of the high protein supplement.

Then they were moved to cultivated pasture, yet with free access to haylage as well as 50 g ewe^{-1} day^{-1} of the high protein supplement. Approximately one month postpartum all sheep were excluded from the cultivated land and grazed rangeland at Hestur until end of June. At that time all ewes available were taken to the highland. At September 17th the flock was gathered from the highland and grazed on rangeland at Hestur until September 22nd when lambs were weaned and grazed on cultivated pasture until slaughter.

Measurements and data sampling

Birth weight was recorded within 24 hours from birth, stillborn lambs and lambs that died at parturition included. On the seventh day postpartum all lambs reared by the experimental ewes were weighed; however, the lambs from the ewe that died ten days postpartum and the two ewes that only reared one lamb were excluded from the statistical analysis. Total number of lambs used for the analysis therefore was 24, 22, 22 and 22 lambs in treatment groups CTR, MIX, EN and PRO respectively. In end of June when the lambs were 45-57 (average 49) day old the 76 lambs present (18, 22, 21 and 15 lambs in the CTR, MIX, EN and PRO groups respectively) were weighed and again at weaning when lambs were on average 144 days old, total of 85 lambs. All lambs that survived from birth to weaning and were not known to have had any problems that could have affected their growth rate were included in the analysis regarding average growth rate from birth to weaning, (24, 20, 21 and 20 lambs in CTR, MIX, EN and PRO groups respectively). Lambs that were not present in end of June are excluded from statistical analysis of growth rate from end of June to weaning resulting in 74 lambs being used for this analysis (18, 20, 21 and 15 lambs in the CTR, MIX, EN and PRO respectively).

Statistical analysis

Data was analyzed using the REML method of SAS Enterprise Guide 4.1 and 4.2 (SAS institute 2004) mixed model analyze. When lamb birth weight was the response variable the model was as follows:

$$Y_{ijk} = \mu + T_i + L_j + S_m + A_k + D_l + (T \times L)_{ij} + \varepsilon_{ijk}$$

Where Y_{ijklm} is the response variable, μ is the overall mean of the population, T_i is the mean of the experimental treatment (i = 1-4), L_j is the mean effect of litter size (j = 1-3), S_m is the mean effect of sex of the lamb (m = 1-2), A_k is the effect of ewe age (k = 3-8) and D_l is the effect of length of the treatment (number of days from onset of the experiment until lambing;

l= 30-39), $(T \times L)_{ij}$ is the interaction between treatment and litter size and ε_{ijkl} represents the unexplained residual elements that are assumed to be independent and normally distributed.

For the data regarding growth rate at any time interval the model was the same as for birth weight except that litter size refers in that case to the number of lambs the ewe rearing each particular lamb gave birth to instead of the birth type of the lamb itself. Furthermore, instead of age of "birth" dam we used age of the dam that reared the lamb.

RESULTS

Treatment did not have significant effect on birth weight neither for singles, twins or triplets. Though not significant, lambs from the CTR group had on average higher birth weight than those born to ewes from the supplemented groups. Triplets were significantly lighter than singles in all treatment groups and twins in the EN and PRO groups.

The first seven days postpartum lamb growth rate was significantly affected by litter size, and age of the dam. It has to be kept in mind that since litter sizes were balanced at birth and all ewes reared two lambs as described in materials and methods, all postpartum effects of litter sizes refer to the litter size the dam rearing the lamb gave birth to, not whether the lambs themselves are born as singles, twins or triplets.

Growth rate of lambs during their first week was significantly higher for lambs reared by twin bearing ewes than those reared by single- or triplet bearing ewes. Furthermore, lambs reared by the CTR ewes grew significantly slower the first seven weeks.

In this period, the effect found in the first week postpartum of the litter size ewes had given birth to on lamb growth rate, was still apparent but not significant anymore. In the last period, from 7 weeks old to weaning, treatment effect are not present any more and in fact the only effect significantly affecting growth rate at that time was sex of the lamb.

When the whole growth period is viewed as a continuum sex of the lamb is the only effect tested that significantly affected growth rate. Growth rate of lambs reared by ewes from CTR and PRO groups however was somewhat lower than of those reared by the MIX and EN group ewes, though not significant.

Lambs reared by ewes that had given birth to triplets had the highest average growth rate but those reared by the single bearing ewes had the lowest.

DISCUSSION

Lamb birth weight

Lower birth weight of triplets than singles and twins was as expected and in agreement with Sormunen-Cristiana & Jauhiainen (2001) and Thorsteinsson & Thorgeirsson (1989) but non-significant difference between singles and twins were more surprising. That is particularly interesting due to the high feeding level above requirements for the single bearing ewes which would have been expected to result in extremes in birth weight (Annett et al. 2008, Thorsteinsson & Thorgeirsson 1989) and subsequent lambing difficulties (Gama et al. 1991). The CTR ewes giving birth to the heaviest lambs is inconsistent with several authors positively linking prepartum nutrition and birth weight (Annett et al. 2008, Thorsteinsson & Thorgeirsson 1989) though comparable to O´Doherty & Crosby (1997 & 1998) that found no effect of considerably reduced ME intake on birth weight and suggested their results to be linked with relatively small efficiency of dietary energy for growth of the conceptus.

The good condition of our experimental ewes as well as high haylage quality is likely to be important factor regarding this "lack of effect" as stated by (Speijers et al. 2005, Thorsteinsson & Thorgeirsson 1989), the latter research revealing reduction in birth weight of supplemented ewes with BCS higher than 3.7-4.

Higher birth weight of lambs from CTR group, even though their dams were slightly lighter than others in end of pregnancy as well as elevated NEFA level could indicate some mobilization of maternal reserves. That is however surprising since calculated energy and protein intake should have met these ewes requirements as they are defined now. This is discussed in our companion paper (Ólafsdóttir et al. 2012a). Non significant effects of treatment on birth weight should have minimized the effect on subsequent survival and performance of the lambs. However, some of the lambs reared by the experimental ewes were triplets or twins from yearling and therefore possibly smaller at birth.

Lamb growth rate

Lower growth rate the first week postpartum of the lambs reared by CTR ewes indicates positive effect of supplementing ewes prepartum on colostrum and subsequently milk production and is in agreement with several older research (Nottle et al. 1998, Robinson & McDonald 1989, Speijers et al. 2005) that have revealed effect of prepartum feeding up to six weeks postpartum. Many of those researches aimed at estimating effect of feeding concentrates rich in undegradable protein compared with only haylage feeding. According to Banchero et al. (2007) high level of undegradable protein in supplements did not have advantage above concentrates rich in ME but without undegradable protein which is in agreement with our results that showed no advantage of undegradable protein supplements above other. In that matter it has to be kept in mind that high energy content of diet as used in this research results in elevated levels of microbial protein reaching the small intestines, serving the animal in the same way as undegradable protein. Elevated level of uric acid in plasma indicates for example increased supply of microbial protein in the EN ewes (Ólafsdóttir et al. 2012a).

All lambs reared by triplet bearing ewes were triplets and half of those reared by single bearing ewes were either triplets or twins from yearlings and consequently in most cases lighter than others (Sormunen-Cristiana & Jauhiainen 2001, Thorsteinsson & Thorgeirsson 1989). This is a possible reason for the lower growth rate of lambs reared by single and triplet bearing ewes the first week postpartum as for example Sormunen-Cristiana & Jauhiainen (2001), Greenwood et al. (1998) and Khalaf et al. (1979) found growth rate to be positively related to birth weight.

Maternal effects on growth rate decrease with advancing age as herbage becomes higher portion of the lambs diet compared to the milk (Guðmundsson & Dýrmundsson 1989).

An example of this can be seen in our results where effect of both treatment and number of lambs ewes gave birth to decreased with time from parturition and were not apparent in the period fromafter seven weeks of age until weaning.

CONCLUSION

Extreme feeding level increases birth weight up to some level but not above that, indicating that the biggest extremes in that matter are not due to feeding level of ewes, at least not exclusively. Subsequently lambing difficulties are not necessarily increased with raised feeding level. Concentrate feeding of ewes makes them better prepared for milk production as can be seen by higher growth rate of the lambs reared by supplemented ewes. Reasonable level and duration of the supplementing remains to be defined and requires further studies. Number of lambs ewes gave birth to also seems to affect growth rate. It is not clear whether that difference is caused by twin bearing ewes being somehow better prepared to nurse two lambs, or the fact that lambs reared by ewes giving birth to singles and triplets had on average lower birth weight than those born as twins and raised by their own twin bearing mother. In opposition with some older research, concentrate type, especially with regard to protein type (i.e. bypass vs. microbial protein), did not seem to affect birth weight, or milking ability of the ewes. But high quality of the roughage fed as well as good condition of ewes has to be considered when viewing those results.

REFERENCES

Andrews JF & Mercer JB 1985. *Thermoregulation in the newborn lamb: The first 36 hours.* Paper presented at the Factors affecting the survival of newborn lambs - A seminar in the CEC programme of coordination of agricultural research, Brussel.

Annett RW & Carson AF 2005. The effect of digestible undegradable protein (DUP) content of concentrates on colostrum production and lamb performance of triplet-bearing ewes on grass-based diets during late pregnancy. *Animal Science, 80*, *101-110.*

Annett RW, Carson AF & Dawson LER 2008. Effects of digestible undegradable protein (DUP) supply and fish oil supplementation of ewes during late pregnancy on colostrums production and lamb output. *Animal Feed Science and Techonology, 146*, *270-288*.

Banchero GE, Clariget P, Bencini R, Lindsay DR, Milton JTB & Martin GB 2006. Endocrine and metabolic factors involved in the effect of nutrition on the production of colostrum in female sheep. *Reprod. Nutr. Dev., 46*, *447-460.*

Chilliard Y, Ferlay A, Faulconnier Y, Bonnet M, Rouel J & Bocquier F 2000. Adipose tissue metabolism and its role in adaptions to undernutrition in ruminants. *Proceedings of the Nutrition Society, 59*, *127-134*.

Dawson LER, Carson AF & Kilpatrick DJ. 1999. The effect of the digestible undegradable protein of concentrates and protein source offered to ewes in late pregnancy on colostrum production and lamb performance. *Animal Feed Science and Techonology, 82*, *21-36.*

Frutos P, Buratovich O, Giráldez FJ, Mantecón AR & Wright IA 1998. Effects on maternal and foetal traits of feeding supplement to grazing pregnant ewes. *Animal Science, 66*, *667-673*.

Gama LT, Dickerson GE, Young LD & Leymaster KA 1991. Effects of breed, heterosis, age of dam, litter size and birth weight on lamb mortality. *Journal of Animal Science, 69*, *2727-2743*.

Greenwood PL, Hunt AS, Hermanson JW & Bell AW 1998. Effects of birth weight and postnatal nutrition on neonatal sheep: I. Body growth and composition and some aspects of energetic efficiency. *Journal of Animal Science, 76*, *2354-2367.*

Guðmundsson Ó & Dýrmundsson ÓR 1989. Grazing and lamb growth (Beit og vöxtur lamba). In Ó. R. Dýrmundsson & S. Thorgeirsson (Eds.), *Reproduction, growth and nutrition in sheep. Dr. Halldór Pálsson Memorial publication* (*pp. 147-168*). Reykjavík: Búnaðarfélag Íslands og Rannsóknarstofnun landbúnaðarins.

Husted SM, Nielsen MO, Blache D & Ingvartsen KL 2008. Glucose homeostasis and metabolic adaption in the pregnant and lactating sheep are affected by the level of nutrition previously provided during her late fetal life. *Domestic animal endocrinology, 34, 419-431.*

Husted SM, Nielsen MO, Tygesen A, Kiani A, Blache D & Ingvartsen KL 2007. Programming of intermediate metabolism in young lambs affected by late gestational maternal

undernourishment. *American journal of physiology, endocrinology and metabolism, 293, 548-557.*
Kerslake JI, Kenyon PR, Morris ST, Stafford KJ & Morel PCH. 2008. Effect of concentrate supplement and sward height on twin bearing ewe body condition and the performance of their offspring. *Australian journal of experimental agriculture, 48, 988-994.*
Khalaf AM, Doxey DL, Baxter JT, Black WJM, FitzSimons J & Ferguson JA 1979. Late pregnancy ewe feeding and lamb performance in early life. 2. Factors associated with perinatal lamb mortality. *Animal Production, 29, 401-410.*
McNeill DM, Slepetis R, Erhardt RA, Smith DA & Bell AW 1997. Protein requirements of sheep in late pregnancy: Partioning between gravid uterus and maternal tissues. *Journal of Animal Science, 75, 809-816.*
Nottle MB, Kleemann DO, Hockin VM, Grosser TI & Seamark RF 1998. Development of a nutritional strategy for increasing lamb survival in merino ewes mated in late spring/early summer. . *Animal Reproduction Science, 52, 213-219.*
Nørgaard JV, Nielsen MO, Theil PK, Sørensen MT, Safayi S & Sejrsen K 2008. Development of mammary glands of fat sheep submitted to restricted feeding during late pregnancy. *Small Ruminant Research, 76, 155-165.*
O´Doherty JV & Crosby TF 1997. The effect of diet in late pregnancy on colostrum production and immunoglobulin absorption in sheep. *Animal Science, 64, 87-96.*
O´Doherty JV & Crosby TF 1998. Blood metabolite concentrations in late pregnant ewes as indicators of nutritional status. *Animal Science, 66*, 675-683.
Ocak N, Cam MA & Kuran M 2005. The effect of high dietary protein levels during late gestation on colostrum yield an lamb survival rate in singleton bearing ewes. *Small Ruminant Research, 56, 89-94.*
Ólafsdóttir HÓ, Sveinbjörnsson J & Harðarson GH 2012a. Energy and protein in the diet of ewes in late pregnancy: Effect on ewe feed intake, life weight, body condition and concentration of plasma metabolites. *Submitted to Icelandic Agricultural Sciences*
Robinson JJ & McDonald I 1989. Ewe nutrition, foetal growth and development (Um fóðrun áa og vöxt og þroska fósturs). In Ó. Dýrmundsson & S. Þorgeirsson (Eds.), *Reproduction, growth and nutrition in sheep. Dr. Halldór Pálsson Memorial Publication.*
Robinson JJ, Sinclair KD & McEvoy TG 1999. Nutritional effects on foetal growth. *Animal Science, 68, 315-331.*
SAS Institute 2004. *SAS/STAT User's Guide, Version 9*. SAS Inst. Inc., Cary, NC
Sormunen-Cristiana R & Jauhiainen L. 2001. Comparison of hay and silage for pregnant and lactating Finnish Landrace ewes. *Small Ruminant Research, 39, 47-57.*
Speijers MHM, Fraser MD, Haresign W, Theobald VJ & Moorby JM 2005. Effects of ensiled forage legumes on performance of twin bearing ewes and their progeny. . *Animal Science, 81, 271-282.*
Thorsteinsson SS & Thorgeirsson S 1989. Winterfeeding, housing and management (Vetrarfóðrun og hirðing fjár). In Ó. R. Dýrmundsson & S. Thorgeirsson (Eds.), *Reproduction, growth and nutrition in sheep. Dr Halldór Pálsson, Memorial Publication*. Reykjavík: Búnaðarfélag Íslands og Rannsóknarstofnun landbúnaðarins.

Table 1. Dry matter content and chemical analysis of feed

	Haylage	High energy concentrate	High Protein concentrate
DM (%)	58.5	88	88
Fem (Fem kg DM^{-1})	0.86	0.98	1
Protein (g kg DM^{-1})	170	110	440
AAT (g kg DM^{-1})	84	105	200
PBV (g kg DM^{-1})	24	-35	168
NDF (g kg DM^{-1}	514	128	45
Ca g kg DM^{-1}	3	8	55
P g kg DM^{-1}	3	6	32
Mg g kg DM^{-1}	2	2	4
Na g kg DM^{-1}	1	4	6

Table 2. Daily ration of supplements (g DM day^{-1}) at each time

	CTR	MIX	EN	PRO
Experimental week 1	0	*60	60	60
Experimental week 2	0	*100	100	100
Experimental week 3	0	**190	250	131
Experimental week 4 and until lambing	0	***260	343	180

* equal ration of high energy and high protein supplements
** 125 g high energy supplement and 65 g high protein supplement
*** 170 g high energy supplement and 90 g high protein supplement

Table 3. Birth weight of lambs (kg)

Treatment	Singles	Twins	Triplets	ADJ mean
CTR	4.69^{c}	4.25^{c}	3.74^{ab}	4.23^{A}
MIX	4.27^{c}	4.15^{bc}	3.75^{ab}	4.06^{A}
EN	4.41^{c}	4.42^{c}	3.69^{ab}	4.18^{A}
PRO	4.63^{c}	4.36^{c}	3.47^{a}	4.15^{A}
ADJ mean	4.50^{A}	4.29^{A}	3.66^{A}	

Different letters in superscript represent significant difference

p-values for fixed effects	
Litter size	<0.0001
Sex	0.0682
Age of dam	0.1366
treatment	0.7726
Days on experimental treatment	0.0125
treatment * litter size	0.6687

Table 4. Growth rate of lambs in their first week (g day^{-1})

Treatment	Singles	Twins	Triplets	ADJ mean
CTR	255.6^{ab}	318.3^{b}	250.5^{a}	274.8^{A}
MIX	319.3^{b}	323.5^{b}	278.2^{ab}	307.0^{B}
EN	276.7^{ab}	350.1^{b}	291.3^{ab}	306.0^{B}
PRO	309.8^{ab}	347.5^{b}	282.7^{ab}	313.3^{B}

ADJ mean	290.4^{A}	334.9^{B}	275.7^{A}

Different letters in superscript represent significant difference

p-value for fixed effects

litter size	0.0023
sex	0.1793
age of dam	0.0256
treatment	0.1716
days on treatment	0.2851
treatment * litter size	0.8727

Table 5. Growth rate of lambs from 2-7 weeks of age (g day^{-1})

Treatment	Singles	Twins	Triplets	ADJ mean
CTR	310.3^{ab}	285.2^{a}	303.2^{a}	299.6^{A}
MIX	344.0^{ab}	329.6^{ab}	318.7^{ab}	330.8^{B}
EN	301.8^{a}	326.2^{ab}	369.3^{b}	332.4^{B}
PRO	344.3^{ab}	329.5^{ab}	308.9^{a}	327.6^{B}
ADJ mean	325.1^{A}	317.6$^{.A}$	325.0^{A}	

Number with different superscript differ significantly

p-value for fixed effect

litter size	0.1829
sex	0.9032
age of dam	0.4212
treatment	0.8382
Days on treatment	0.3381
treatment * liter size	0.1893

Table 6. Growth rate of lambs from seven week of age to weaning (g day^{-1})

Treatment	Singles	Twins	Triplets	ADJ mean
CTR	227.8^{a}	232.2^{a}	216.6^{a}	225.6^{A}
MIX	217.6^{a}	212.4^{a}	245.9^{a}	225.3^{A}
EN	225.2^{a}	229.9^{a}	231.3^{a}	228.8^{A}
PRO	202.8^{a}	222.9^{a}	222.3^{a}	216.0^{A}
ADJ mean	218.4^{A}	224.3^{A}	229.0^{A}	

Number with different superscript differ significantly

p-value for fixed effect

litter size	0.5996
sex	0.0105
age of dam	0.7205
treatment	0.7379
days on treatment	0.4024
treatment * litter size	0.5034

Table 7 Growth rate of lambs from birth to weaning (g day $^{-1}$)

Treatment	Singles	Twins	Triplets	ADJ mean
CTR	245.4^{a}	252.8^{a}	254.4^{a}	250.9^{A}

MIX	256,5 [a]	256.9 [a]	267.9 [a]	260.5 [A]
EN	249.2 [a]	265.6 [a]	264.8 [a]	259.9 [A]
PRO	250.8 [a]	255.5 [a]	256.3 [a]	254.2 [A]
ADJ mean	250.5 [A]	257.7 [A]	260.9 [A]	

Number with different superscript differ significantly

p-value for fixed effect	
litter size	0.4185
Sex	0.0018
age of dam	0.8746
treatment	0.6273
days on treatment	0.4913
treatment * litter size	0.9908