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Effect of energy allowance to triplet-bearing ewes in late gestation on ewe performance, lamb viability, and growth

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ABSTRACT

The present study was conducted to evaluate the effects of offering Norwegian white sheep (NKS) ewes a ration of either 85 (85% net energy lactation (NEL)), 100 (100% NEL) or 120% (120% NEL) of recommended NEL requirements from gestation day 96 to lambing on ewe performance, lamb viability and growth. Twenty-seven triplet-bearing ewes were allocated to 1 of 3 dietary treatments based on INRA (Jarrige, 1989) recommendations. Ewes were individually fed and NEL requirements were individually calculated for each ewe. Diets consisted of a restricted portion of grass silage from early harvest and an adjusted level of concentrate. As expected, increasing NEL allowance increased the ewe body weight gain ($=P = 0.0004$) and body condition score (BCS) ($=P = 0.003$) prepartum. The 120% NEL ewes lost more body condition (BC) in lactation compared with the 100% NEL ewes. There was no effect of ewe nutritional treatment on behavioral scores of the lambs (birth assistance, lamb vigour and sucking assistance) or the lamb birth weight. There was a considerable reduction of number of lambs from birth to weaning, due to stillbirths, deaths, and life-support during this period. Early live weight gain and weaning weights of lambs increased nominally with increasing prepartum energy allowance of their mother. However, only lambs in complete triplet litters had significant increases in live weight gain until weaning ($=P = 0.008$). We conclude that it is possible for triple-bearing ewes to meet the increased nutritional demand via intake, and even gain BC, in late gestation when feed quality is high. The high energy intake in ewes during late gestation increased energy mobilization during lactation that seemed to benefit lambs in triplet litters with a growth advance until weaning.

1. Introduction

The Norwegian white sheep (NKS) is a productive and prolific sheep breed for meat and wool production. The average litter size per mated ewe has increased from 1.9 to 2.2 from 2000 to 2017 (Animalia, 2008, 2017). In 2016, 44% of the adult ewes (older than 1-year) gave birth to litters comprising of three or more lambs (Animalia, 2017). An increase in litter size per ewe should offer greater financial gains, but is also associated with high mortality rates (Scales et al., 1986; Everett-Hincks and Dodds, 2008; Holmøy and Waage, 2015), and is consequently a potential welfare concern. For this reason, there is a growing interest in optimal feeding regimens for these prolific ewes given their higher theoretical nutritional requirements in the last trimester of gestation (Jarrige, 1989; NRC, 2007). The growth of approximately 70% of the final birth weight of the fetus occurs in this period (Robinson, 1990), along with colostrogenesis (Castro et al., 2011). The plane of nutrition in late gestation may influence lamb birth weight (Dwyer et al., 2003; Gardner et al., 2007; Meyer et al., 2010), colostrum production (Swanson et al., 2008; Castro et al., 2011) and milk production (McGovern et al., 2015a; Campion et al., 2016), with a potential subsequent effect on weaning rate and weight. Rooke et al. (2015) found, across all studies reviewed regarding gestational undernutrition, that lamb mortality was increased in most studies where birth weight was reduced. In addition, maternal undernutrition can adversely affect lamb neonatal behavior and the mothering ability of the ewe, and hence the establishment of the ewe–lamb bond (Dwyer et al., 2003).

The net energy (NE) system of INRA (Jarrige, 1989) has been used as a guideline for nutritional requirements in Norway, and the recommendations for prolific ewes in late gestation (weeks 6 to 0 before

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parturition) are below the true requirements for maintenance of the ewe and the conceptus (growth and maintenance of the fetus and placenta). This recommendation is based on the consideration that ewes with multiple fetuses are unable to increase their feed intake capacity, due to the uterus physical encroachment of the rumen (Forbes, 1986), and therefore must rely on mobilization of body lipids (Jarrige, 1989). The newer recommendations from the National Research Council (NRC), Washington (NRC 2007), however, recommend between 5 and 40% higher energy allowances during the last 6 weeks of gestation for adult ewes, with the greatest difference for heavy ewes with 3 fetuses, 6 to 5 weeks prepartum. Eknæs et al. (2009) and Nadeau et al. (2016) have shown that multiple-bearing ewes in late gestation are able to ingest feed above the requirements set by Jarrige (1989) when offered highly digestible grass silage, despite the increasing volume of the uterus. Those studies, however, offered different feed rations in late pregnancy as well as in early lactation, and few of the ewes were triplet bearing. Therefore, it was needed to study the performance of triplet bearing adult ewes and their lambs until weaning, as an effect of different intakes in late gestation, only.

Compared to lambs born as twins, triplets have a lower birth weight and lower weaning weight (Gardner et al., 2007). Also, lambs born as triplets but reared as twins have been found to have lower weaning weight than lambs born and reared as twins (Morris and Kenyon, 2004).

Our hypothesis was that improving triplet-bearing ewe nutrition during late pregnancy would improve the performance of lambs reared as triplets. Therefore, the objective of this experiment was to study the effect of three different levels of NE allocations during late gestation to mature, triplet-bearing ewes on the ewes' and lambs' performances.

2. Materials and methods

Laws and regulations controlling experiments with live animals by the Norwegian University of Life Sciences Animal Care and Use Committee and the Norwegian Animal Research Authority were implemented in the experiment (Norwegian Ministry of Agriculture and Food, 2015).

2.1. Animals, experimental design and dietary treatments

The experiment was carried out with ewes in their second to eight parity of the Norwegian White Sheep breed which lambed between April 3rd and May 6th' 2016. Initially, gimmers (1.5-year-old ewes) were enrolled given they had delivered at least one lamb the previous year and adult ewes, if they had delivered at least two lambs the previous year. Totally, 67 ewes with mean body weight (BW) of 80.2 ± 10.3 kg by early October fulfilled the inclusion criteria and were allocated to group feeding. Ewes were mated between November 11 and December 9 at BW 84.4 ± 11.5. Fetal numbers were determined by ultrasonography on day 38–60 of gestation. The mean fetal number was 2.66. Only ewes carrying three fetuses were retained in the study (n = 27, 17 adult ewes and 10 gimmers, and mean BW of 82.7 ± 8.1 at mating). They were placed in individual pens for the experiment. Until gestation day 96, the ewes were fed at maintenance level on an individual weight basis. At gestation day 96, the 27 ewes were distributed into three groups, each of 9 ewes, that were balanced according to expected lambing date, BW, body condition score (BCS) and age. Thereafter, the three groups were randomly allocated to dietary energy treatments based on INRA (Jarrige, 1989) requirements: (1) 85%; (2) 100% and (3) 120% of predicted net energy lactation (NEL) requirement. For all treatments, energy allowance was adjusted to individual BW recorded at gestation day 96, and also revised according to BW at gestation day 104 and 135. Individual requirements were revised before the last two weeks of pregnancy to allow for the increasing fetal energy demands. Ewes were shorn on approximately gestation day 105.

2.2. Feeds and feeding

The grass silages offered in the experiment were made from the primary growth at the Animal Production Experimental Centre at the University of Life Sciences, Ås, Norway (60 °N, 11 °E, elevation 93 m.a.s.l.). The sward consisted of 80% timothy (*Phleum pretense*) and some meadow fescue (*Festuca pratensis*) and red clover (*Trifolium pretense*). An early harvest was taken before the boot stage of timothy on June 4th, after wilting overnight, and a late harvest at the boot stage on June 16th, after wilting for about 2 h. The grass was baled using Orkel hiQ smartbaler (Orkel AS, Fannrem, Norway) with 20 fixed knives, and added about 4.8 Liters/ton GrasAAT Pluss (580 g formic acid, 120 g propionic acid and 15 g benzoic acid per kg; Addcon Nordic AS, Porsgrunn, Norway). Early and late harvested forages were wrapped in 8 and 12 layers of 0.025 mm thick and 750 mm wide white plastic film (Trio Wrap, Trioplast AB, Sweden), respectively. Before feeding, silages were processed through Siloking Kverneland Duo 1840 TMR mixer (Kverneland Group, Klepp, Norway) to decrease particle length (30–50 mm), which reduce the spillage into the pen and enhance feed intake, and were fed within three to four days.

From one month prior to mating and until ultrasound measurements (about gestation day 50), the ewes were offered late-harvested silage *ad libitum*. Thereafter, and until gestation day 96 the ewes were offered the same silage, but restrictively fed. From gestation day 96, after grouping into the three dietary treatments, the diets consisted of a restricted portion of grass silage from the early harvest and an adjusted level of concentrate (CONC) (Norgesfôr, Norway) in all diets. In addition to CONC the ewes in all treatments were supplied with 60 g of protein concentrate (42% CP CONC) (Felleskjøpet Agri, Lillestrøm, Norway) due to low protein level in the grass silage. Ewes allocated to the 85 and 100% NEL treatment were offered chopped barley straw (300 and 100 g dry matter (DM) per day, respectively) treated with 2.5 kg urea per 100 kg straw. From day 96 through 131 of gestation, ewes allocated to 85% NEL treatment were restricted to receive 80% of their ration's DM from roughage (straw included) and 20% from concentrate, and ewes allocated to the 100% NEL and 120% NEL treatments received 85% from roughage and 15% from concentrate. From gestation day 132 through parturition, the same procedure remained for 100 and 120% NEL treatments, while ewes allocated to 85% treatment were restricted to receive 75% of their ration from roughage and 25% from concentrate. This was done to ensure that all ewes received adequate level of protein through late pregnancy, and resulted in ewes offered the 85% NEL treatment had about a 10% lower intake of CP and ewes offered the 120% NEL treatment having about a 20% higher intake of CP, when compared to those offered the 100% NEL treatment.

After lambing, ewes in all treatments were offered the same feed ration. Early harvested grass silage was fed ad libitum and supplemented with CONC according to the number of suckling lambs. All ewes were also supplied with 100 g of a protein concentrate (32% CP CONC) (Felleskjøpet Agri, Lillestrøm, Norway).

The ingredients of CONC were 49.5% barley, 25.0% oats, 6.0% rapeseed meal, 5.6% soybean meal, 5.0% wheat bran, 4.0% molasses, 3.0% minerals and vitamins, 1.0% maize gluten meal, 0.5% soybean oil and 0.4% limestone. The main ingredients of 42% CP CONC were 65% Soypass®, 14% extracted soybean meal and 7% rapeseed cake, and the main ingredients of 32% CP CONC were 40% extracted soybean meal, 18% wheat, 8% molasses and 5% Soypass®. Mineral and vitamin requirements were covered according to NRC (2007), both in gestation and lactation. The mineral and vitamin mixture (Vilomix, Hønefoss, Norway) consisted of 10% Ca, 9% Na, 6.5% Mg, and 5.5% P. The sheep had free access to salt lick (Felleskjøpet, Norway: 39% Na, 0.2% Ca, 0.2 Mg) and water. Grass silage, straw, and concentrates were offered twice daily. Feed intake was recorded during four consecutive days per week. For ewes offered straw, grass silage and straw were mixed by hand in the through. Although silage and straw were chopped to approximately 30–50 mm, ewes were able to select silage. Therefore, feed refusals consisted mainly of straw, and were assumed straw only, unless, as in a few cases, refusals weighed more, and the surplus was silage. If the ewes consumed the straw, the straw consumption was added to the intake of the ewe when calculating the total intake. After an indoor feeding period of 24 to 31 days postpartum, ewes and lambs were let out to summer pasture, a cultivated lowland pasture.

2.3. Ewe measurements

In the period prior to mating and until gestation day 96 ewe BW were recorded every third week, and BCS was assigned to the sheep approximately every 6th week. Later, ewes were weighed and assessed for BCS at gestation day 104 and 135, 4 and 10 days postpartum, and when turning out on pasture. All weights were recorded on two consecutive days at 12:15 h using an electronic weight (BioControl, Rakkestad, Norway). Body condition score assessments were made by two trained technicians on a scale of 1 to 5 according to Russel (1984). The concentration of non-esterified fatty acids (NEFA) in blood plasma was measured as an indicator of the energy status of the ewes (Bowden, 1971). Blood samples were collected before morning feeding at gestation day 135 and 144, 1, 3 and 18 days postpartum. About 1 hour after sampling the samples were centrifuged at 3000 × g, and serum was frozen at −80 °C until analysis.

2.4. Lambing, behavioral data and lamb measurements

All ewes lambed in their individual pens, on plastic slats. The ewes were kept under continuous surveillance during the expected parturition dates. The ewes were video monitored using a Hikvision DS-2CD2722 camera, and in addition, at least one observer was always present once lambing had commenced. As far as possible ewes were allowed to give birth unaided. However, lambing assistance was provided in cases where no lamb parts were visible 30 min after the appearance of fluids, if no lamb parts were seen 1 h after fluid breaking, and/or 1 h after lamb parts had been seen with no other obvious progress. Certain specific presentations (breech, head back, two lambs together) needed immediate assistance. The time of birth was recorded for every lamb. The lambs were assessed a birth assistance score (0–4) and a lamb vigor score (0–4) 5 min after birth, as presented by Matheson et al. (2011). The lambs were marked for birth order using a colored nylon ribbon around their neck. Immediately after the lamb vigor score was set, the lamb was weighed, sex and lamb rectal temperature were recorded. The time that the lambs were standing on all four feet were recorded, and the time for successfully sucking. The lambs were allowed 2 h to stand and successfully suck unaided. Lambs that had not sucked after this time were assisted. A sucking assistance score (0–4) was assessed to the lambs as presented by Matheson et al. (2011). After all lambs had sucked and all measures had been taken, the navel of each lamb was dipped in a 10% iodine solution, to aid in the control of polyarthritis, and the lambs got ZooLac® Propaste® (ChemVet dk, Denmark) to stabilize the bacterial flora in the intestinal tract (routine treatment).

The weight and sex of stillborn lambs were recorded. The time of death was recorded for lambs dying in the neonatal period. Dead lambs were subjected to post-mortem autopsy. Ewes and lambs remained in their individual pens until turning out on pasture. The lambs had access to grass silage in a corner divided from their mother. Lamb's weights were recorded during two consecutive days, at 12:15 p.m., every week for 4 weeks before turning out on pasture. Body weights of the lambs were also recorded at weaning, 94–127 days postpartum.

2.5. Sampling and chemical analysis

Silage samples were collected twice a week during feed out and stored frozen at −20 °C until analysis. A portion of these samples was oven-dried at <60 °C to constant weight and weighed warm to obtain DM concentration and formed the basis for daily DM intakes of silage after correction for the volatile loss according to the Norfor DM determination method (Åkerlind et al., 2011). Another portion was kept undried and were further composited to six samples of the late harvest, and four samples of the early harvest. These undried samples were analyzed for NH_3−N, pH, organic acids, and ethanol as described by Randby et al. (2010). A portion of the composited samples was freeze-dried, equilibrated to room humidity overnight, and milled to pass a 1.0 mm screen (Retsch GmbH cutting mill, Haan, Germany) prior to analyses of DM, ash, neutral detergent fiber (NDF), acid detergent fiber (ADF), water-soluble carbohydrates (WSC), crude fat, lignin (ADL), total nitrogen (TN), selenium and rumen digestible organic matter (VOS). Analyses of DM, ash, crude fat, WSC, and ADF were done as described by Randby et al. (2010). Contents of NDF were determined as described by Licitra et al (1996), using amylase and not using sodium sulphite. ADL was analyzed with H_2SO_4 and modified according to AOAC (1984). Contents of ADF, NDF, and ADL were corrected for ash. Kjeldahl-N was analyzed at Kjeltec 2460 (Foss Electric, Hillerød, Denmark). Selenium content was determined using microwave utraCLAVE III (MLS Dresden) and measured by ICP-AES IRIS Intrepid II (Thermo Scientific). VOS was determined by in vitro incubation for 96 h in a buffer solution and rumen fluid (Åkerlind et al., 2011). One composite silage sample for each harvesting time was analyzed for α-tocopherol and indigestible NDF (iNDF). α-tocopherol was analyzed as described by Dønnem et al. (2015). The iNDF samples were milled through a 1.5 mm screen and 2 g was incubated *in sacco* for 288 h according to Åkerlind et al. (2011) using polyester bags from Sefar Petex (Sefar AG, Heiden, Switzerland) with a pore size of 11 µm and a pore area equal to 5% of the total surface area. The sample size to surface area ratio was 10 mg/cm^2.

Two composite samples of each concentrate were analyzed for DM, NDF, ADF, ADL, TN, starch, ash, crude fat, and WSC. Content of DM was determined at 103 °C and starch was determined by an enzymatic method (α-amylase and amyloglucosidase) (Megazyme, Wicklow, Ireland). The rest of the analyses were made with the same methods as for the grass silage.

Non-esterified fatty acids in ewe plasma were determined using RANDOX kits (RANDOX Laboratories, Ardmore, UK) in an ADVIA 1800 chemistry analyzer (Diamond Diagnostics, Holliston, MA, USA).

2.6. Calculations

The concentration of metabolizable energy (ME) in grass silage was calculated according to Lindgren (1983) by the equation: MJ ME/kg DM = = (0.160 × VOS-1.91) × OM. Net energy lactation in silage was calculated from ME according to Van Es (1978). Metabolizable protein content in silage, expressed as amino acids absorbed in the small intestine (AAT) and protein balance in the rumen (PBV), was calculated according to Madsen et al. (1995), based on a fixed value for ruminal efficient protein degradability in grass silages of 0.80 and a fixed value of 0.82 for intestinal digestibility of undegradable amino acids (Spörndly, 2003). Concentrate ME, NEL, AAT, and PBV were set as declared by the manufacturers (Norgesfôr and Felleskjøpet).

2.7. Statistical analysis

Data were analyzed using the REML method of SAS mixed model (version 9.4; SAS Inst., Cary, NC). For ewe parameters the fixed effects of treatment, time (as the repeated constant), age (gimmer or adult), rearing rank (for parameters measured postpartum) the 2-way interaction of treatment x time, and random effect of ewe within treatment were included in the model. The repeated measures were fit using variance-covariance structures; the most appropriate (lowest Bayesian information criterion values) was used for the analyses.

For lamb parameters, the fixed effects of treatment and sex, and random effect of ewe within treatment were included in the model.

Table 1
Chemical composition of silage and concentrates.

	Early HT[1]	Late HT[2]	Barley Straw	Concentrate	42% CP concentrate	32% CP concentrate
Dry matter, g/kg	358	262	829	848	924	890
g/kg DM						
Organic matter	911	929	931	935	931	893
Crude protein	138	103	88	140	522	361
Starch				497	62.9	191
NDF	493	587	744	210	275	204
iNDF	49.4					
ADF	289	348		98.8	94.7	104
ADL	19.3	29.9		22.6	9.39	23.2
Fat	25.9	21.0		31.1	32.6	44.2
Water soluble carbohydrates	127	77.9		48.7	84.3	76.7
Lactic acid	25.0	52.2				
Formic acid	8.5	0				
Acetic acid	3.2	8.3				
Propionic acid	4.6	1.7				
Ethanol	10.0	11.0				
Ammonia N (g/kg N)	94.7	112				
pH	4.72	4.21				
Selenium, mg/kg DM	0.0113	0.0431		0.402		
Vitamin E, IU/kg DM	18.9	15.4		64.0[7]		
ME, MJ/kg DM [3]	11.3	9.83	6.20	11.5	11.9	11.0
NEL, MJ/kg DM [4]	6.72	5.67	2.07	6.93	7.23	6.66
AAT, g/kg DM[5]	71.6	67.7		101	252	159
PBV, g/kg DM[5]	14.7	-19.3		-25.3	73.4	125
D-value[6]	717	635				

[1] Fed to ewes from gestation day 96 to lambing, and in lactation.
[2] Fed to ewes before mating and in early gestation until gestation day 96.
[3] Metabolizable energy in silage calculated according to Lindgren (1983). ME in concentrate based on the manufacturers analysis. ME in barley straw according to feed table (STIL, 1992).
[4] Net energy lactation in silage calculated according to Van Es (1978). NEL in concentrate as declared by the manufacturer. NEL in barley straw according to feed table (STIL, 1992).
[5] Amino acids absorbed in small intestine (AAT) and protein balance in rumen (PBV) in silage calculated according to Madsen et al. (1995) and Spörnly (2003). AAT and PBV in concentrate as declared by the manufacturer.
[6] Digestible organic matter in dry matter.
[7] Alfatocoferyl-acetate added to the concentrate mixture, and here calculated as Vitamin E.

Rearing rank (for postnatal parameters) was included in lamb weight models. Lamb age was included as a covariate in lamb growth rate models and lamb behavioral models. In addition, birth weight and birth order were included in lamb behavioral models. All live-born lambs were included in the lamb behavioral models. All data presented in the tables are expressed as least square means ± SEM. Mean separation was done by least significant differences, and treatment effects were declared significant at $P < 0.05$ and trends at $0.05 \leq P < 0.10$. One ewe (85% NEL) was reduced to rear a single lamb (one stillborn lamb and one lamb dying 2 days after birth). That ewe and her singleton lamb were excluded from the data set after lambing because rearing rank one, used as covariate for postnatal parameters for one single animal, was meaningless in the statistics. However, the singleton lamb had one sibling the first two days, and both were included in the behaviour scores in Tables 4 and 5.

3. Results

3.1. Feed nutritive characteristics

Table 1 presents the chemical composition of the silages and the concentrates. Silages were of good fermentation quality, with no butyric acid.

3.2. Ewe feed intake

The mean intake of MJ NEL (± SD, min to max) before mating and in early pregnancy until ultrasound measurement was 5.83 (± 0.06, 5.77 to 5.90) and 5.61 (± 0.16, 5.43 to 5.77) per ewe, respectively. The effect of treatment on intake from gestation day 96 through 146 is shown in Table 2.

After gestation day 96 the 85% NEL ewes consumed all the silage they were offered. The 100% and 120% NEL ewes also consumed all their offered silage from gestation day 96 and 5 weeks onwards, however, during the last two weeks before parturition ewes in these two treatments consumed 87 and 67% of the offered silage, respectively. Hence, they had an equal intake of grass silage and total NEL in this period (Figure 1). Ewes in the 85% NEL treatment had persistently lower silage and total NEL intake than the other two treatments. All ewes consumed all concentrate offered, except for one ewe in the 120% NEL treatment that refused 10% of her concentrate during the last two weeks before lambing.

3.3. Ewe BW, BCS and energy status

The effect of treatment on ewe BW, BCS, and BCS change is shown in Table 3. The BW gain from gestation day 104 to 135 increased with increasing NEL allowance ($=P = 0.0004$). At gestation day 135 the 120% NEL ewes had a 5 and 9 kg higher mean BW than 100 and 85% NEL ewes, respectively.

The 120% NEL ewes gained ($=P = 0.002$) BCS between gestation days 104 and 135, while the 85 and 100% NEL ewes lost ($=P = 0.04$) BCS in this period. Hence, the 120% NEL ewes had a higher BCS than the two other treatments at gestation day 135 ($=P = 0.01$). However, at turnout to pasture, there was no difference between the treatments ($=P = 0.27$). The 120% NEL ewes lost more body condition in lactation compared with the 100% NEL ewes ($=P = 0.02$).

At gestation day 144 the serum NEFA concentration was higher for the 85% NEL ewes than the two other treatments ($=P = 0.04$; Table 3). However, the 120% NEL ewes had higher serum NEFA concentration

Table 2
Daily feed intake of ewes on restricted feeding from gestation day 96 to parturition (gestation day 148) and on grass silage libitum for all groups during lactation day 1–30.

	Treatment 85% NEL	100% NEL	120% NEL	SEM	*P*-value
Gestation day 96–148					
Grass silage, kg DM	0.98	1.25	1.53		
Barley straw, kg DM	0.16	0.05	0		
Concentrate, kg DM	0.28	0.26	0.30		
42% CP[1] concentrate, kg DM	0.055	0.055	0.055		
Total ration, kg DM	1.48	1.61	1.89		
ME[2], MJ	15.9	18.0	21.4		
NEL[3], MJ	9.25	10.7	12.8		
CP, g	218	242	283		
CP, g/NEL	23.6	22.8	22.2		
AAT[4], g	112	129	154		
PBV[5], g	11.3	15.6	19.1		
Lactation day 1–30					
Silage, kg DM	2.57	2.52	2.55	0.158	0.96
Concentrate, kg DM	0.41	0.40	0.46		
32% CP concentrate, kg DM	0.093	0.093	0.093		
Total ration, kg DM	3.08	3.00	3.09	0.139	0.79
ME, MJ	34.9	34.0	35.0	1.52	0.78
NEL, MJ	20.8	20.3	20.9	0.946	0.78
CP, g	453	443	454	19.00	0.80
CP, g/NEL	21.8	21.8	21.7	0.107	0.59
AAT, g	245	238	246	10.11	0.72
PBV, g	35.1	35.0	33.8	3.36	0.92

[1] CP = crude protein.
[2] Metabolizable energy.
[3] Net energy lactation.
[4] Amino acids absorbed in the small intestine.
[5] Protein balance in the rumen.

Table 3
Body weight (BW), BW gain, body condition score (BCS) and non-esterified fatty acids (NEFA) (as indicator of nutritional status) of ewes.

	Treatment 85% NEL	100% NEL	120% NEL	SEM	*P*-value
BW					
Gestation day 104	89.2	90.1	92.3	2.770	0.72
Gestation day 135	96.2$^{(a)}$	100$^{(ab)}$	105$^{(b)}$	2.770	0.07
Lactation day 3	79.3	84.6	85.1	4.176	0.33
Lactation day 10	78.2	82.6	83.7	4.179	0.41
Lactation day 29[1]	79.3	80.3	80.1	4.179	0.96
BW gain gestation day 104–135, g/day	227^{a}	325^{b}	416^{c}	28.55	0.0004
BW gain lactation day 3–29, g/day	−6.1	−111	−114	61.95	0.40
BCS					
Gestation day 104	2.99	3.24	3.17	0.128	0.36
Gestation day 135	2.89^{a}	3.07^{a}	3.43^{b}	0.128	0.01
Lactation day 3	2.75$^{(a)}$	2.94$^{(ab)}$	3.12$^{(b)}$	0.123	0.06
Lactation day 29	2.32	2.61	2.42	0.173	0.27
BC change gestation day 104–135	−0.09^{a}	−0.15^{a}	0.28^{b}	0.086	0.003
BC change lactation day 3–29	−0.39^{ab}	−0.18^{a}	−0.51^{b}	0.093	0.04
NEFA mmol/l					
Gestation day 135	0.52	0.38	0.35	0.119	0.530
Gestation day 144	0.77^{a}	0.50^{b}	0.44^{b}	0.101	0.040
Lactation day 1	0.69	0.43	0.53	0.101	0.190
Lactation day 3	0.44^{a}	0.51^{a}	0.90^{b}	0.101	0.004
Lactation day 18	0.45^{a}	0.69^{ab}	0.92^{b}	0.104	0.009

a,b,c Means with different subscripts within the same row differ ($P < 0.05$).
[1] Lactation day 29 = turnout to spring pasture.

than the two other treatments at day 3 after lambing ($=P = 0.004$), and higher than the 85% NEL ewes at day 18 after lambing ($=P = 0.002$).

3.4. Lamb behavioral data and lamb survival

There was no effect of ewe nutritional treatment on the lambs behavioral scores (birth assistance, lamb vigour and sucking assistance) ($P \geq 0.56$; Table 4). As a mean for all the treatments, 47% of the lambs were born unassisted or uncomplicated (scores 0 and 1; Table 5). Further, 85% of the lambs born were active and vigorous lambs (scores 0, 1 and 2), but almost half of the lambs needed assistance to suck (scores 2, 3, 4; Table 5).

Increasing birth weight increased the birth assistance score ($=P = 0.02$). The nutritional treatments had no effect on the length of time from birth until each lamb stood successfully ($=P = 0.46$). The first lamb born had a lower sucking assistance score than the third lamb, with the second lamb intermediate ($=P = 0.03$; Table 4). The first and second lamb born in each litter tended to successfully stand quicker than their third littermate ($=P = 0.07$). The rectal temperature of the lambs was not affected by the nutritional treatment of the ewes ($=P = 0.39$).

There were 4 stillborn lambs (3 due to dystocia and 1 was malformed) (Table 6). One lamb died 2 days old due to *E. coli*-infection. Four lambs needed life support and were bottle-fed the indoor period. At summer pasture, another 6 lambs either died or needed life support (taken from their mother and bottle-fed), ending in 20, 21 and 25 weaned lambs for ewes in the 85, 100 and 120% NEL treatment,

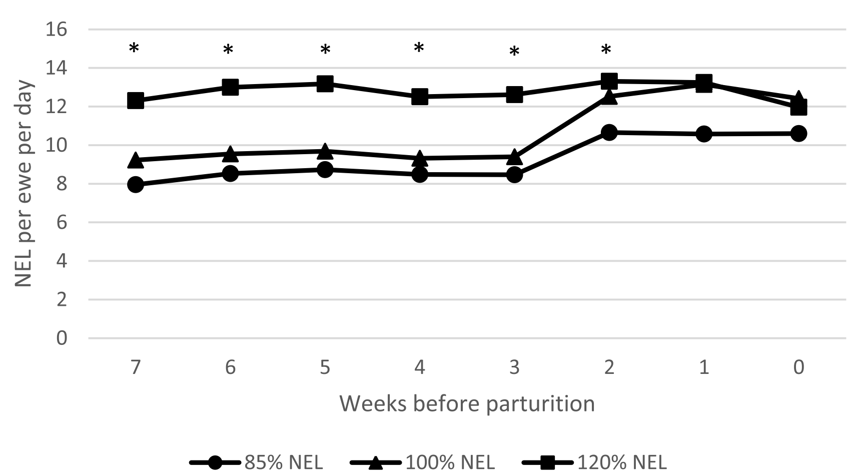

Figure 1. Intake of net energy lactation (NEL) from gestation day 96 to parturition (week 7 to 0). An asterisk (*) indicates that means of treatments 85, 100 and 120% NEL differ ($P < 0.05$).

Table 4
Behavior scores and behavior of lambs and rectal temperature of lambs.

	Treatment 85% NEL	100% NEL	120% NEL	SEM	Birth rank 1	2	3	SEM	P-values Trt	BR
Birth assistance score[1]	1.53	1.34	1.30	0.248	1.45	1.28	1.45	0.230	0.78	0.83
Lamb vigour score[2]	1.94	1.66	1.73	0.192	1.68	1.60	2.05	0.184	0.56	0.16
Sucking assistance score[3]	1.51	1.12	1.34	0.285	0.85[a]	1.44[ab]	1.67[b]	0.241	0.63	0.03
Time to successful stand, s	1142	1214	886	196.1	965[(a)]	902[(a)]	1375[(b)]	171.2	0.46	0.07
Rectal temperature, °C	39.6	39.3	38.9	0.328	39.1	39.7	39.1	0.332	0.39	0.37

a,b,c Means with different subscripts within the same row differ ($P < 0.05$).

[1] Score 0 = unassisted or easy uncomplicated delivery of short duration (<30 min), 1 = unassisted or easy uncomplicated delivery of long duration (>30 min), 2 = minor assisted required. Presentation corrected, little effort to deliver lamb, 3 = major assisted required. Difficult delivery needing effort to deliver lamb, 4 = veterinary assistance required.

[2] Score 0 = extremely active and vigorous lamb has been standing on all four feet, 1 = very active and vigour lamb, standing, on back legs and on knees, 2 = active and vigorous lamb, on chest and holding head up, 3 = weak lamb, lying flat, able to hold head up, 4 = very weak lamb, unable to lift head, little movement.

[3] Score 0 = lamb sucking well without assistance within 1 h, 1 = lamb sucking well without assistance within 2 hours, 2 = lamb sucking given assistance, fed by stomach tube once or twice in first 24 hours after birth, 3 = lamb given sucking assistance, fed using a stomach tube more than twice, needing help after 1 day of age, but able to suck by 3 days of age, 4 = lamb still needing help to suck when more than 3 days of age.

respectively. Of these, 9, 15 and 21 lambs, respectively, were still in complete triplet litters.

3.5. Lamb BW and BW gain

There was no significant effect of ewe nutritional treatment on lamb birth weight or weight at 1 week of age ($P \geq 0.17$; Table 7). At turnout to pasture 24–31 days after birth, the lambs born to the 120% NEL ewes were heavier than lambs born to 85% NEL ewes ($=P = 0.03$). At weaning, average litter size per ewe was 2.4, 2.6 and 2.8 for ewes in the 85, 100 and 120% NEL treatment, respectively. However, when only the complete triplet litters were regarded at weaning, the lambs born to the 120% NEL ewes were heavier than lambs born to the 100% NEL ewes ($=P = 0.002$), but did not differ from lambs born to the 85% NEL ewes ($=P = 0.14$). Further, across treatments, there was an effect of litter size ($=P = 0.01$) at turnout to pasture, where twins were 1.9 kg heavier than triplets. Male lambs were 0.4 kg heavier than ewe lambs at birth ($=P = 0.03$) and 3.2 kg heavier at weaning ($=P = 0.01$).

There was no significant effect of ewe nutritional treatment on the BW gain of the lambs (Table 7). When regarding only the complete triplet litters, the BW gain from birth to weaning was greater for the lambs born to the 120% NEL ewes than lambs born to the 100% NEL ewes ($=P = 0.002$). Male lambs had a greater BW gain (26 g/d) ($=P = 0.007$) from birth to weaning than ewe lambs, and from turnout to pasture to weaning (31 g/d) ($=P = 0.005$).

Table 6
Mortality of lambs and number of lambs bottle-fed and weaned.

	Treatment 85% NEL	100% NEL	120% NEL
Lambs born	27	27	27
Stillborn lambs	3	1	0
Dead indoors	1	0	0
Bottle-fed lambs indoors	2	1	1
Dead/bottle-fed during summer	1	4	1
Weaned lambs	20	21	25
Weaned lambs in complete litters	9	15	21

4. Discussion

Previous studies have investigated the effects of gestational nutrition on both ewe- and lamb performance (Kenyon et al., 2011b; McGovern et al., 2015a; McGovern et al., 2015b; Campion et al., 2016; Nadeau et al., 2016). However, this study focused on assessing the performance of triplet-bearing ewes, which has been studied to a much lesser extent than singlet- and twin-bearing ewes. Another aspect is the different sheep production systems. Whereas Morris and Kenyon (2004) and Kenyon et al. (2011a) have examined gestational nutrition for multiple litter size in year-around grazing systems, the present study assessed systems where animals are housed during the whole gestation, and pastured in the postpartum period. Previous studies have found that nutrition during late gestation may influence lamb birth weight, colostrum production and subsequent lamb growth rate and weaning weight (Gardner et al., 2007; Castro et al., 2011; McGovern et al., 2015a; McGovern et al., 2015b). This shows that controlling the plane of nutrition in late gestation may influence the production.

The high energy allowance to the ewes in the 120% NEL treatment caused a high intake from day 96 and 5 weeks onwards, demonstrating that they are able to ingest feed above the requirements, as opposed to the consideration of Jarrige et al. (1989). However, during the last two

Table 5
Behaviour scores with the proportion (number) of lambs attaining the scores.

	Treatment	Score 0	1	2	3	4
Birth assistance	85% NEL	0.33 (8)	0.13 (3)	0.30 (7)	0.25 (6)	0 (0)
	100% NEL	0.46 (12)	0.04 (1)	0.27 (7)	0.23 (6)	0 (0)
	120% NEL	0.30 (8)	0.19 (5)	0.33 (9)	0.19 (5)	0 (0)
	total	**0.36 (28)**	**0.11 (9)**	**0.30 (23)**	**0.22 (17)**	0 (0)
Lamb vigour	85% NEL	0.08 (2)	0.21 (5)	0.50 (12)	0.13 (3)	0.08 (2)
	100% NEL	0.12 (3)	0.15 (4)	0.69 (18)	0.03 (1)	0 (0)
	120% NEL	0.07 (2)	0.33 (9)	0.41 (11)	0.15 (4)	0.04 (1)
	total	**0.09 (7)**	**0.23 (18)**	**0.53 (41)**	**0.10 (8)**	**0.04 (3)**
Sucking assistance	85% NEL	0.25 (6)	0.29 (7)	0.25 (6)	0.08 (2)	0.13 (3)
	100% NEL	0.44 (11)	0.12 (3)	0.32 (8)	0.08 (2)	0.04 (1)
	120% NEL	0.37 (10)	0.74 (2)	0.52 (14)	0 (0)	0.04 (1)
	total	**0.36 (27)**	**0.16 (12)**	**0.37 (28)**	**0.05 (4)**	**0.07 (5)**

Table 7
Body weight and weight gain of lambs.

	Treatment			SEM	*P*-value
	85% NEL	100% NEL	120% NEL		
Live weights, kg					
Birth weight	4.41	4.37	4.69	0.166	0.34
1 week[1]	6.99	7.68	8.17	0.421	0.17
Turnout[2]	11.7$^{(a)}$	12.3$^{(ab)}$	13.3$^{(b)}$	0.511	0.08
Weaning[3]	35.3	35.6	38.0	1.28	0.26
Weaning triplets[4]	35.6^{ab}	32.5^{a}	38.3^{b}	1.16	0.007
Live weight gain, g/d					
Birth-1 week[1]	293	318	356	22.2	0.14
Birth- turnout[2]	266	284	305	15.4	0.20
Turnout-weaning[3]	262	263	277	12.1	0.60
Birth-weaning	264	269	283	11.3	0.47
Birth-weaning triplets[4]	266^{ab}	240^{a}	284^{b}	9.04	0.008

a,b,c Means with different subscripts within the same row differ ($P < 0.05$).

[1] Age at "one-week weight":6 to 12 days. Number of lambs in each group: 85% NEL = 20, 100% NEL = 25, 120% NEL = 26. All data onwards is excluded the singleton lamb in 85% NEL group.

[2] Age at turnout: 24-31 days. Number of lambs in each group: 85% NEL = 20, 100% NEL = 25, 120% NEL = 26.

[3] Age at weaning: 94 to 127 days. Number of lambs in each group: 85% NEL = 19, 100% NEL = 21, 120% NEL = 25.

[4] Including lambs in complete triplet litters, only, i.e. lambs with one or two dead or bottle-fed siblings are excluded. Number of lambs in each group: 85% NEL = 9, 100% NEL = 15, 120% NEL = 21.

weeks before lambing they refused to eat all the grass silage, and had probably reached the limit of their intake potential (Kenyon et al. 2007). Even if the grass silage was of early cut and highly digestible, the physical fill effect probably regulated the intake (Weston, 2002), together with the large uterine volume containing the three fetuses (Forbes, 1986). These observations suggest that it would be advantageous to offer a higher concentrate ratio to the ewes these last weeks, as also suggested by Kenyon et al. (2007).

Despite differences in DM intake and NEL intake between the treatments during the late gestational period, there was no observed difference in lamb birth weight. However, it seems to be only cases of severe undernutrition during late gestation that significantly reduce birth weight. In a study by Gardner et al. (2007) the ewes fed restrictively were provided 50–60 % of AFRC (1993) ME requirement in late gestation, and birth weight was reduced by 0.2 kg per MJ decrease in energy allowance. Meyer et al. (2010) fed the restrictively treated ewes 60% of NRC (1985) requirement, and birthweight of lambs born to the restrictively fed ewes was 0.4 kg less than lambs born to the control ewes. In both studies, the ewes were more undernourished than in the present experiment. For this reason, lamb birth weight is not suitable as a sole index of dietary energy in late gestation nutrition. This is also emphasized by McGovern et al. (2015b), where altering the nutrition (80, 100 or 120% of AFRC (1993) ME requirements) to twin ewes in late gestation did not affect birth weight or organ weight of newborn lambs.

Ewes fed the highest energy level in late gestation had a higher BW gain, and also an increased body condition (BC) in the prepartum period, but an increased level of BC loss in the postpartum period compared to the other treatments. This coincides with the study of Campion et al. (2016), where twin-bearing ewes were fed different energy levels in late gestation and responded in the same pattern. This suggests that different levels of energy intake during late gestation can alter the body reserve mobilization pattern. The mobilization of body fat reserves is indicated through an elevation of plasma NEFA concentration (Bowden, 1971). The shift of NEFA concentration in plasma between few days before lambing and L3 supports the altered tissue mobilization pattern; ewes fed the highest energy level shifted from the lowest to the highest NEFA concentration (Table 3). It is apparent that the 120% NEL ewes had greater body fat stores and could mobilize fat in the early postpartum period to increase the milk yield, even though the mechanism behind is not fully clear. Campion et al. (2016) found that the ewes fed the highest energy level in late gestation maintained the highest milk yield through the first 6 weeks of lactation. Thompson et al. (2011) found that increased ewe live weight gain during pregnancy increased lamb growth rate to weaning. In the present study, the early live weight gain and weaning weights of lambs increased nominally with increasing prepartum energy allowance of their mother, but only for lambs in complete triplet litters the increase in live weight gain until weaning was significant. This suggests that the body reserves acquired by the 120% NEL ewes pre-lambing to a greater extent were utilized to feed three lambs compared to fewer to weaning. This is in line with Mathias-Davis et al. (2013), where triplets had the highest growth rate when their mother had a high BCS at lambing and a negative BCS change to weaning. The growth from birth to weaning of the triplet-reared lambs in the 85% NEL treatment was between the 120% NEL and 100% NEL treatments, which may be due to the low number of triplet litters still complete until weaning: only three of the initial nine litters. Also, the body reserve mobilization of the 85% NEL ewes both in late gestation and early lactation appears to have compensated for the energy deficiency in late gestation. The energy reserves they had left at lambing were adequately utilized to feed their lambs. However, it has earlier been demonstrated (Mathias-Davis et al., 2013; Kenyon et al., 2014) that there is not a simple association between ewe BCS at lambing and lamb growth rate. In goats, high body mass index attained through high prepartum energy feeding increased body mass mobilization postpartum and increased milk production, whereas this was not the case in goats with similar high body mass at parturition that was not attained by high prepartum feeding (Randby et al., 2015). Lamb growth will also be affected by the nutrition of the ewes during lactation. There was no difference in intake between the treatments from lambing and until the grazing period, despite the potential reduced gut capacity of the 120% NEL ewes due to higher BC and hence internal fat stores (Forbes, 1986). On summer pasture there was no control of the feed intake, with the exception that all ewes were at the same pasture.

Early milk production is sensitive to periods of undernutrition, with 70% of udder development taking place during the final 4 weeks of gestation (Mellor and Murray, 1985). Also, energy supply in the final week of gestation is one of the primary drivers of colostrum yield (Banchero et al., 2015). As the 100% NEL and 120% NEL ewes had similar energy intake the last two weeks of gestation, this could partly affect the lack of significant early live weight gain of the lambs between the treatments. During early lactation, ewe milk production significantly affects lamb growth rate (Snowder and Glimp, 1991; Morgan et al., 2007), while in later lactation the correlation between milk production and lamb growth is weakened. Snowder and Glimp (1991) found milk yield to be insignificant after day 42 of lactation, indicating the lamb's decreasing dependence on milk as the main nutrient source. In the second half of the summer pasture period in the present study the lambs could have compensated for low ewe milk production by increased pasture intake. However, the heaviest lambs at turnout apparently had a growth advance until weaning, although not significantly between the treatments. Greater milk production in early lactation has been previously shown to give lambs a weight advance that remains present up until weaning (Morgan et al., 2007).

In the present study, there is a low conformity between BC change and live weight change in ewes in the postpartum period. However, energy mobilization from body tissue in early lactation may greatly exceed apparent weight loss, suggesting that changes in the energy reserves of lactating animals may be more accurately assessed from their condition score rather than from measurements of live weight (Freer et al, 2007). Differences in rumen fill will also affect measured live weight, and possibly give an unreliable live weight change.

Birth difficulties are associated with increased lamb mortality (Dwyer, 2003). Three of the four stillborn lambs in the present experiment died because of dystocia during birth. Also, half of the lambs as a mean of the treatments were assisted in the delivery to a minor or major extent. Prolonged and difficult labours will slow the lambs' behavioral landmarks and viability, and cause impaired sucking ability, which again is linked to increased lamb mortality (Dwyer, 2003; Matheson et al., 2011). As in McGovern et al. (2015a; 2015b) the present study found no effects of gestational nutrition on maternal and lamb neonatal behavior, which contrasts with the study of Dwyer et al. (2003) where a reduction (35%) in nutritional intake in pregnant Scottish Blackface ewes adversely affected lamb neonatal behavior and the mothering ability of the ewe.

The present dataset contained triplet-bearing ewes and intended to study the performance of lambs raised as triplets. However, throughout the study, there was a considerable reduction of lambs. The stillborn rate in the experiment was close to the stillborn rates of sheep enrolled in the Norwegian Sheep Recording System in 2017, 4.9% and 4.8%, respectively. In addition, some of the lambs died due to weakness, or were taken from their mother as life support. The majority of the lamb loss was in the 85% and 100% NEL treatments, and we cannot rule out that some of the causes of lamb loss were related to poor milk yield of the ewe, which in turn may be due to low energy allowance in late gestation and low fat mobilization after lambing.

The most common lamb management protocol in Norway is to reduce litters of 3 or more lambs to 2 lambs when let out on summer pasture. Surplus lambs are either fostered to other ewes or "orphaned" for artificial rearing. This is an experience that farmers have gained over the years. The present study confirms that it may be challenging for an ewe to rear 3 lambs, concerning the high loss of lambs. This is supported by the study of Notter et al. (2018), where ewe productivity and lamb survival were compared in triplet and twin litters. Ewes that reared triplets weaned 0.20 more lambs per litter than ewes that had twins but also had 0.75 additional dead lambs per litter, and thus a lamb mortality overhead of 3.75 additional dead lambs for each additional weaned lamb.

5. Conclusion

This study shows that it is possible for triple-bearing ewes to meet the increased nutritional demand via intake, and even gain BC, in late gestation when feed quality is high. Energy intake during late gestation influenced the pattern of body reserve deposition or mobilization in ewes, simultaneously, as well as after parturition. For lambs reared in triplet litters the highest energy allocation seemed to give a growth advance from birth up until weaning. This study suggests that if a farmer wants to minimize the number of surplus lambs and let the ewes rear three lambs, the energy allowance should be increased above the current requirements.

CRediT authorship contribution statement

I. Dønnem: Conceptualization, Formal analysis, Investigation, Data curation, Writing - original draft. **E.G. Granquist:** Data curation, Investigation, Writing - review & editing. **E. Nadeau:** Formal analysis, Writing - review & editing. **Å.T. Randby:** Conceptualization, Project administration, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors confirm that there are no known conflicts of interest associated with this publication.

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Supplementary materials

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