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## Effect of solid feed level and types of roughage on nitrogen and energy balance and circadian activity patterns in veal calves

Annemarie J.W. Mens, Sanne van Gastelen, Gisabeth P. Binnendijk<sup>1</sup>, and Walter J. J. Gerrits

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Annemarie J. W. Mens<sup>1</sup>, Sanne van Gastelen<sup>1</sup>, Gisabeth P. Binnendijk<sup>1</sup>, and Walter J. J. Gerrits<sup>2</sup>

<sup>1</sup> Wageningen Livestock Research, Wageningen University & Research, P.O. Box 338, 6700 AH, Wageningen, the Netherlands

<sup>2</sup> Animal Nutrition Group, Wageningen University & Research, P.O. Box 338, 6700 AH, Wageningen, the Netherlands

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P.O. Box 338, 6700 AH Wageningen, The Netherlands, T +31 (0)317 48 39 53,  
E [info.livestockresearch@wur.nl](mailto:info.livestockresearch@wur.nl), [www.wur.nl/livestock-research](http://www.wur.nl/livestock-research). Wageningen Livestock Research is part of Wageningen University & Research.



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# Abstract

The aims of this study were to (1) quantify the effect of increasing intake of solid feed (**SF**) containing 90% of concentrates and 10% of chopped straw, on protein and fat deposition rates in veal calves and (2) to measure the influence of the different SF levels on circadian patterns of activity heat, heat production and methane production. As a reference, a treatment was included with a high SF intake containing 70% of concentrates and 30% of long hay. In total, 48 Holstein-Friesian calves (6 weeks of age;  $68 \pm 7.7$  kg BW) were assigned to 1 of the 4 dietary treatments. Three treatments contained a combination of concentrate and chopped straw as roughage in the SF mixture in a concentrate to roughage ratio of 90:10 (as fed). The SF level varied, being 20 g/kg BW<sup>0.75</sup>/d (**LowSF**), 30 g/kg BW<sup>0.75</sup>/d (**MiddleSF**), or 40 g/kg BW<sup>0.75</sup>/d (**HighSF**). The fourth treatment (**Hay**) contained a combination of concentrate and long hay as roughage in the SF mixture, in a concentrate to roughage ratio of 70:30 (as fed). The SF level of the Hay treatment was similar to the HighSF treatment, viz. 40 g/kg BW<sup>0.75</sup>/d. The quantity of milk replacer (**MR**) was fixed for the HighSF treatment (i.e., 39 g/kg BW<sup>0.75</sup>/d) and during the adaptation period, the amount of MR for the other treatments was calculated based on a paired-gain strategy, to achieve comparable body weight gain (**BWG**) across treatments. During the adaptation period, calves within treatments were paired and assigned to one of three groups according to body weight. The grouped pairs of each treatment were transferred to the climate respiration chambers (CRC) from highest BW to lowest BW, resulting in 6 batches of measurements. In the CRC, the complete energy and nitrogen balance was measured and the physical activity was recorded continuously by a radar device. One CRC was the experimental unit, which housed two calves. The nitrogen intake decreased with the relative amount of MR ( $P < 0.001$ ) and increased with the relative amount of SF ( $P = 0.001$ ), which was consistent with the experimental set-up. Total nitrogen intake only differed between the LowSF and Hay and between the HighSF and Hay treatment. The nitrogen retention did not differ between treatments. Between treatments, the energy intake and energy excreted via faeces and urine did not differ. The treatments did not affect energy retention, protein or fat deposition, heat production, activity related heat production or the resting metabolic rate. Methane production increased with increasing SF intake, with the lowest methane production for the LowSF treatment and the highest for the Hay treatment (13.07 and 26.74 kJ/kg<sup>0.75</sup>/d respectively;  $P = 0.002$ ). These results were confirmed by the circadian rhythms, where methane production during the day was increased after a SF meal, but reciprocal difference between the level of SF remained. Overall, the Hay treatment had the highest methane production, followed by the HighSF, MiddleSF and the LowSF treatment respectively. The circadian rhythm of heat production did not differ between treatments but showed clear effects in the provision of a meal (both MR and SF). The activity related heat production rhythm showed the same results, the calves became active because of the provision of a meal. Disregarding the heat production by activity, the resting metabolic rate rhythm clearly visualizes the MR meals and the effects of exchanging MR by SF. During the dark period the LowSF and MiddleSF fed calves had a lower RMR than HighSF and Hay fed calves ( $P < 0.05$ ), indicating an increase in nutrient absorption from fermentation processes, but in part also reflecting an increase in metabolic processes related to an increase in gastro-intestinal tissues (i.e., rumen development). Furthermore, the provision of MR mirrors the response of RMR numerically, indicating differences in metabolic processes due to increasing amount of SF. Since the daily deposition rates of protein and fat are not affected, and BWG did not differ between treatments, the exchange of MR by SF has striking effects on metabolic processes. Overall, this study provides results of nitrogen and energy retention in veal calves fed different levels of SF, which were exchanged at the expense of MR in a paired-gained strategy. It is concluded that increasing levels of SF do not affect the fat and protein deposition. In addition, methane production increases according to the level of roughage and concentrates, with provision of hay resulting in the highest methane production. The circadian pattern of methane production supports these results and shows that these differences between treatments are stable throughout the day. The circadian rhythms of heat production, activity related heat production and RMR illustrate the effects of MR exchange with increasing roughage and concentrate levels and show strong shifts in metabolic processes. The chemical composition of body weight gain is not affected by these shifts.

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# Samenvatting NL

## **Nederlandse samenvatting - Het effect van de hoeveelheid ruw- en krachtvoer en van het type ruwvoer op de energie- en stikstof huishouding en circadiane activiteit patronen van vleeskalveren.**

Vleeskalveren worden traditioneel voornamelijk gevoerd met kalvermelk. De afgelopen 25 jaar is er een sterke stimulans om een deel van de kalvermelk te vervangen door ruw- en krachtvoer. De verstrekking van ruw- en krachtvoer heeft positieve gevolgen voor de ontwikkeling van de pens en het welzijn van de kalveren. Sinds de introductie van een verplicht deel ruw- en krachtvoer in het rantsoen voor kalveren in de EU, is in Nederland het aandeel van de metaboliseerbare energie-opname uit ruw- en krachtvoer met 30-50% toegenomen. Het bepalen van de nutritionele waarde van ruw- en krachtvoer in het rantsoen wordt belemmerd door interacties tussen kalvermelk en ruw- en krachtvoer in het maagdarmkanaal. Recent wetenschappelijk onderzoek heeft de hoeveelheid kalvermelk bepaald, welke nodig is om bij twee verschillende ruw- en krachtvoer niveaus een identieke toename in karkasgewicht te bereiken. Dit kan worden beschouwd als een uitwisseling van kalvermelk en ruw- en krachtvoer op basis van een netto voederwaarde.

Het is vrijwel onbekend in hoeverre een uitwisseling van metaboliseerbare energie uit kalvermelk door een verhoogde bijdrage van ruw- en krachtvoer in het rantsoen leidt tot veranderingen in de eiwit- en vetaanzet bij vleeskalveren. Het doel van dit onderzoek was dan ook om het effect van een toename in de opname van ruw- en krachtvoer op de vet- en eiwitaanzet ratio te kwantificeren. Het tweede doel was om de invloed van verschillende niveaus van ruw- en krachtvoer op de circadiane patronen van warmteproductie, activiteitswarmte en methaanproductie te meten. Het experiment is uitgevoerd met 48 vleeskalveren (verdeeld over 2 ronden met elk 24 kalveren) van 6 weken leeftijd bij de start van de proef. Na aankomst werden de kalveren direct random toegewezen aan één van de vier behandelingen en gehuisvest in groepen van 6 kalveren per behandeling. Drie behandelingen betroffen een combinatie van krachtvoer met kort stro als ruwvoer, waarbij de krachtvoer tot ruwvoer verhouding 90:10 was. De hoeveelheid ruw- plus krachtvoer was 20 g / kg metabool lichaamsgewicht / dag (**LowSF**), 30 g / kg metabool lichaamsgewicht / dag (**MiddleSF**), of 40 g / kg metabool lichaamsgewicht / dag (**HighSF**). De vierde behandeling (**Hay**) betrof een combinatie van krachtvoer met lang hooi als ruwvoer, waarbij de krachtvoer tot ruwvoer verhouding 70:30 was, met een vergelijkbare hoeveelheid ruw- en krachtvoer als de HighSF groep (40 g / kg metabool lichaamsgewicht). De Hay groep diende als referentie behandeling. De hoeveelheid kalvermelk was vastgezet op de HighSF behandeling, namelijk 39 g / kg metabool lichaamsgewicht / dag, waarbij de hoeveelheid kalvermelk voor de andere behandelingen werd bepaald op basis van een *paired-gain strategy* om een gelijke lichaamsgroei bij alle behandelingen te realiseren. Na 4 weken adaptatieperiode werden de kalveren op basis van lichaamsgewicht ingedeeld in paren, resulterend in 3 paren per hok met een oplopend lichaamsgewicht. De kalveren met het hoogste lichaamsgewicht hadden een korte adaptatieperiode (van 5 weken) en de kalveren met het laagste lichaamsgewicht een lange adaptatieperiode (van 7 weken) alvorens de meetperiode begon. Na de adaptatieperiode werden de kalveren in de toegewezen paren gehuisvest in klimaatrespiratiekamers. Er waren 4 klimaatrespiratiekamers beschikbaar, waardoor er 6 subgroepen binnen elke ronde zijn gevolgd. In elke ronde zijn de zwaarste groepen kalveren als eerste gevolgd, en de lichtste als laatste. De meetperiode duurde 7 dagen, experimentele eenheid was één klimaatrespiratiekamer, waarin 2 kalveren gehuisvest waren. Tijdens de meetperiode werd een complete energie- en stikstofbalans gemeten, waarbij de uitwisseling van O<sub>2</sub>, CO<sub>2</sub> en CH<sub>4</sub> elke 13 minuten werd gemeten. De lichaamsactiviteit per paar kalveren werd continu gemeten door een activiteitsmeter.

Consistent aan de experimentele opzet nam de stikstofopname uit de kalvermelk af met de relatieve melkgift (1,47 g/ kg<sup>0,75</sup> LowSF, 1,29 g/ kg<sup>0,75</sup> MiddleSF, 1,13 g/ kg<sup>0,75</sup> HighSF en 1,21 g/ kg<sup>0,75</sup> Hay; P < 0,001). De stikstofopname uit het ruw- en krachtvoer steeg met de relatieve ruw- en krachtvoergift (0,3 g/ kg<sup>0,75</sup> LowSF, 0,77 g/ kg<sup>0,75</sup> MiddleSF, 0,89 g/ kg<sup>0,75</sup> HighSF en 1,02 g/ kg<sup>0,75</sup> Hay; P = 0,001). De stikstofbalans verschilde niet tussen de behandelingen (gemiddeld 1,21; P = 0,47). De energie-opname



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en de verliezen door feces en urine waren vergelijkbaar tussen behandelingen. De methaanproductie verschilde tussen de vier behandelingen. Deze was het hoogste in de Hay groep en steeg met het toenemende niveau van ruw- en krachtvoer in de andere drie behandelingsgroepen. Tussen LowSF en MiddleSF was een verschil van 4,92 KJ/kg<sup>0,75</sup>, tussen MiddleSF en HighSF een verschil van 2,87 KJ/kg<sup>0,75</sup> en tussen LowSF en HighSF was het verschil 7,79 KJ/kg<sup>0,75</sup>. De Hay kalveren verschilden van de LowSF kalveren (+13,67 KJ/kg<sup>0,75</sup>), de MiddleSF kalveren (+8,75 KJ/kg<sup>0,75</sup>) en de HighSF kalveren (+5,88 KJ/kg<sup>0,75</sup>). De behandelingen hadden geen invloed op de warmteproductie, warmteproductie gerelateerd aan activiteit, resting metabolic rate (RMR), energiebalans of de vet- en eiwitaanzet. Deze resultaten impliceren dat kalvermelk vervangen kan worden door verschillende ruw- en krachtvoer mengsels zonder de weefselcompositie te beïnvloeden.

De circadiane patronen van de methaanproductie laten een piek in methaanproductie zien na elke ruw- en krachtvoermaaltijd. Gedurende de gehele periode is de methaanproductie in de Hay behandeling het hoogste, gevolgd door respectievelijk HighSF, MiddleSF en LowSF. Aangezien de methaanproductie evenredig toenemen met het niveau van de SF-inname, deze studie bijdragen aan de illustratie van de wisselwerking tussen voerkosten, kalverwelzijn en methaanemissies. De totale warmteproductie gedurende de dag was niet beïnvloed door de behandelingen. Tijdens zowel de ruw- en krachtvoer maaltijden, als de melkmaaltijden is een toename in warmteproductie te zien bij alle behandelingen. Deze toename is te verwachten, aangezien de kalveren actief worden door fysiek te gaan eten en door de metabole processen die in werking treden na de maaltijd. De toename in activiteit wordt bevestigd door de patronen van activiteit-gerelateerde warmteproductie, waarin de pieken rondom de maaltijden ook gevonden zijn. De patronen van de RMR laten duidelijk de pieken zien van de melkmaaltijd. Tijdens de donkerperiode (uren 1 t/m 6), wanneer er geen invloed is van de melkmaaltijden, hadden de LowSF en MiddleSF kalveren een lagere RMR dan de HighSF en Hay behandelingen ( $P < 0,05$ ). Deze verschillen wijzen op een toename van de opname van voedingsstoffen door fermentatieprocessen, maar deels ook op een toename van metabole processen gerelateerd aan een toename van gastro-intestinale weefsels wanneer de kalveren een hogere hoeveelheid ruw- en krachtvoer ontvangen. De effecten tijdens de donkerperiode werden numeriek gespiegeld door de melkmaaltijd, waarbij de LowSF de hoogste respons in RMR had. Deze resultaten wijzen op een proportioneel verschil in metabole processen door de verschillende ruw- en krachtvoer giften. Dit is terug te zien in de dagelijkse vet- en eiwitaanzet ratio. Aangezien deze niet beïnvloed worden door de behandelingen en de kalveren ook een gelijke groei hadden, moet de uitwisseling van kalvermelk met ruw- en krachtvoer invloed hebben op de voorafgaande metabole processen. Ruw- en krachtvoer zorgen voor een meer geleidelijke toevoer van nutriënten (microbiële eiwitten, korte-keten vetzuren) gedurende de dag, terwijl het thermische effect van het verstrekken van kalvermelk de piekabsorptie van glucose, lange-keten vetzuren en eiwitten volgt. De toename van de minimale RMR bij de hoogste dosering ruw- en krachtvoer die binnen een dag wordt waargenomen, kan ook een weerspiegeling zijn van een toename van de energiebehoefte voor onderhoud, gerelateerd aan een toename van de ontwikkeling van gastro-intestinale weefsels, met name de pens.

Op basis van deze studie kunnen we concluderen dat ruw- en krachtvoer, verstrekt in toenemende hoeveelheden en daarbij uitgewisseld met een hoeveelheid kalvermelk, bij een paired-gain strategy, de vet- en eiwitaanzet niet beïnvloedt. De methaanproductie neemt toe naarmate het niveau van ruw- en krachtvoer toeneemt, waarbij de verstrekking van hooi resulteert in de hoogste methaanproductie. Het circadiane patroon van methaan bevestigt deze toenames en illustreert dat deze verschillen in methaan gedurende de dag stabiel zijn. Op basis van de circadiane ritmes toonde de studie aan dat het niveau van ruw- en krachtvoer de warmteproductie beïnvloedt. Hoewel er geen significante effecten werden gevonden tussen behandelingen, vertoonden de melkmaaltijden duidelijke pieken in het RMR-ritme, waarbij de LowSF behandeling numeriek het hoogste piekte, gevolgd door de MiddleSF, HighSF en Hay behandelingen. Deze resultaten illustreren de effecten van het uitwisselen van kalvermelk met toenemende ruw- en krachtvoer niveaus en laten sterke verschuivingen in metabole processen zien wanneer de kalvermelkmaaltijd wordt verstrekt. Opvallend is dat de chemische samenstelling (d.w.z. vet en eiwit) van de lichaamsgewichtstoename niet wordt beïnvloed door deze verschuivingen.



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# 1. Introduction

In veal calf nutrition, there is a strong incentive to replace a considerable portion of the milk replacer (MR) by solid feeds (SF) in the diet. Since the introduction of a compulsory portion of solid feed provision to calves in the EU (European Union 97/2/EC Directive by the EU Council), the proportion of metabolizable energy intake from solid feed has increased in veal calves to 30-50% in the Netherlands.

Interactions between MR and SF occurring in the gastro-intestinal tract, complicate the prediction of the nutritional value of these ration components. Recent research by Berends (2014) has illustrated the complexity of evaluating the feeding value of SF and MR when combining them in a nutritional strategy. Addition of SF to different groups of calves fed a similar quantity of MR has the disadvantage of feed restriction of the low SF groups or overfeeding the high SF groups, while designing an exchange factor between SF and MR almost inevitably leads to confounding of the study objective (quantify the exchange factor) with the assumptions that are inherent to the study design (always related to some exchange factor). Berends et al. (2014) therefore proposed a paired-gain approach to solve this problem. While feeding calves different quantities of each of two SF mixtures (20:80 and 50:50 roughage:concentrate), they determined the quantity of MR required to achieve identical rates of carcass gain (estimated from body weight gain and assumed quantities of gastro-intestinal contents). This can be considered an exchange based on a net feeding value. They found that for replacing 1 kg of a SF mixture of 20:80 concentrate:roughage, 0.52 kg of MR was required. This was 0.41 kg of MR when the concentrate:roughage ratio was 50:50. The roughage in the study of Berends et al (2014) consisted of a 50:50 mixture straw and corn silage on a DM basis. Hence, for optimizing nutrient economy and profitability, higher concentrate proportions can be advantageous. However, there are clear indications that for animal welfare, the optimum SF composition contains higher roughage proportions (Webb et al., 2015). Increased roughage provision, but not concentrate, increased rumination and decreased tongue playing. Calves have been demonstrated to be highly motivated to consume hay, particularly long hay compared to chopped hay and chopped or long straw (Webb et al., 2014). Long hay stimulates rumination behaviour more strongly than straw, and can trigger recycling of nutrients into the rumen, for example through saliva. Recycling of urea back into the rumen has been demonstrated to exist, but its quantitative contribution to nitrogen balance (i.e., protein gain) has been questioned (Berends et al., 2015). Recycling of phosphorus into the rumen can be important and has been demonstrated to be stimulated by rumination. It is unknown to what extent an increased contribution of SF to metabolizable energy intake leads to changes in protein and fat gain in calves. The only studies available in published literature are at relatively low levels of SF feeding (e.g. Berends et al., 2012a; Labussiere et al., 2009). For the measurement of protein and fat gain, energy balance studies are often conducted using indirect calorimetry. Such studies are almost exclusively performed in individually housed calves, quite often in social isolation. It has been demonstrated that prolonged social isolation in combination with housing on metabolism cages increases activity heat production, associated abnormal oral behaviours, particularly during standing (Gerrits et al., 2015). Hence, whenever possible, such measurements would be more relevant for veal calf production when performed under conditions avoiding social isolation.

Therefore, the objectives of this study were (1) to quantify the effect of increasing the intake of SF containing 90% of concentrates and 10% of straw, on protein and fat deposition rates, and (2) to measure the influence of the different SF levels on circadian patterns of activity heat, heat production and methane production. As a reference treatment, a group of calves was included with a high SF intake containing 70% of concentrates and 30% of long hay. All calves received a supplemental quantity of MR to enable equal rates of BW gain, and measurements were done on pair-housed calves.

## 2. Materials and Methods

This study was conducted from September 2017 until March 2019 at the animal research facilities of Wageningen University & Research (Wageningen, the Netherlands)], in accordance with Dutch law and approved by the Central Committee of Animal Experiments (The Hague, the Netherlands).

### 2.1. Animals and Housing

In total, 48 Holstein-Friesian calves (6 weeks of age;  $68 \pm 7.7$  kg of BW) were purchased from commercial veal calf farms and selected based on uniformity and clinical health. The 48 calves could not be housed simultaneously, hence calves arrived in 2 batches of 24 calves each, following the exact same procedure and timeline.

### 2.2. Diets

The 4 dietary treatments consisted of different levels of SF as well as different types of roughage in the SF mixture (Table 1). Three treatments contained chopped straw as roughage in the SF mixture in a concentrate to roughage ratio of 90:10 (as fed). The SF level was 20 g/kg  $BW^{0.75/d}$  (**LowSF**), 30 g/kg  $BW^{0.75/d}$  (**MiddleSF**), or 40 g/kg  $BW^{0.75/d}$  (**HighSF**). The fourth treatment (**Hay**) contained long hay as roughage in the SF mixture in a concentrate to roughage ratio of 70:30 (as fed). The SF level of the Hay treatment was similar to the HighSF treatment, viz. 40 g/kg  $BW^{0.75/d}$ . Based on our expected maximum voluntary intake, the quantity of MR was fixed for the HighSF treatment at 39 g/kg  $BW^{0.75/d}$  and during the adaptation period, the amount of MR for the other treatments was calculated based on a paired-gain strategy, to achieve similar rates of body weight gain (**BWG**) across treatments. After the adaptation period (i.e., shortest duration was 5 weeks), the quantity of MR for the other treatments was fixed to the levels reported in Table 1.

The same MR (produced by Denkavit, Voorthuizen, the Netherlands) was used for all 4 treatments, whereas the concentrates were adjusted to the type of roughage fed, based on equal protein content of the SF mixture. Both concentrates were produced as a mash by Denkavit (Voorthuizen, the Netherlands) with mineral premix produced by Twilmij B.V. (Stroe, the Netherlands). Table 2 shows the nutrient and chemical composition of the different feed components (i.e., MR, concentrates, and roughages).

**Table 1** Paired-gain feeding schedule of the different diets.

Diet	MR <sup>1</sup> (g/kg $BW^{0.75/d}$ )	SF <sup>2</sup> (g/kg $BW^{0.75/d}$ )	Concentrates (g/kg $BW^{0.75/d}$ )	Roughage (g/kg $BW^{0.75/d}$ )	Roughage type
LowSF	50.6	20	18	2	Straw
MiddleSF	44.8	30	27	3	Straw
HighSF	39.0	40	36	4	Straw
Hay	41.4	40	28	12	Hay

<sup>1</sup> Milk replacer mixed with water in a concentration of 130 g/L. The amount of MR for the HighSF treatment was fixed. The amount of MR for the other treatments was based on paired-gain strategy for the first 6 weeks (adaptation period), after which it was fixed as well.

<sup>2</sup> Solid feed.

**Table 2** Nutrient and chemical composition of milk replacer (MR), concentrates and roughages in g/kg DM, unless stated otherwise.

Nutrient <sup>1</sup>	MR <sup>2</sup>	Concentrates		Roughages	
		C_Straw <sup>3</sup>	C_Hay <sup>4</sup>	Straw	Hay
Dry matter (g/kg product)	968	885	900	936	903
Organic matter	940	953	953	n.d. <sup>5</sup>	n.d.
Crude protein	184	168	181	41	101
Crude fat	212	52	48	n.d.	n.d.
Gross energy (MJ/kg DM)	21.1	18.7	18.6	18.9	18.7
Calcium	8.00	6.40	6.40	n.d.	n.d.
Phosphorus	6.56	4.62	4.93	0.39	2.55

<sup>1</sup> Presented as g/kg dry matter, unless stated otherwise.

<sup>2</sup> Ingredient composition of MR: 38% lactose, 21% fatfilled wheypowder (i.e., 80% palm and 20% coconut), 20% whey protein concentrate, 10% vegetable oil (i.e., 70% palm, 20% coconut, and 10% rapeseed), 7% wheypowder, and 4% mineral premix (i.e., Vitamin A, 25,000 IU; Vitamin D3, 4,000 IU; Vitamin E, 100 mg; Vitamin K3, 2.0 mg; Vitamin B1, 5.0 mg; Vitamin B2, 7.5 mg; Vitamin B3, 40 mg; Vitamin B5, 20 mg; Vitamin B6, 5.0 mg; Vitamine B12, 40 mcg; Biotin, 125 mcg; Choline, 350 mg).

<sup>3</sup> Ingredient composition of concentrates fed with straw-based diets lowSF, middleSF, and highSF: 55% corn, 37% lupins, 5% barley, and 3% mineral premix (i.e., Magnesium, 1 g; Vitamin A, 4,000 IU; Vitamin D3, 5,000 IU; Vitamin E, 50 mg; Vitamin B1, 2.0 mg; Vitamin B2, 2.5 mg; Vitamin B6, 2.0 mg; Manganese, 20 mg; Copper, 10 mg; Zinc, 25 mg; Potassium iodide, 0.80 mg; Cobalt, 0.10 mg; Selenium, 0.15 mg)

<sup>4</sup> Ingredient composition of concentrates fed with the hay-based diet Hay: 44% lupins, 22% maize feed flour, 26% corn, 5% barley, and 3% mineral premix (i.e., Magnesium, 1 g; Vitamin A, 4,000 IU; Vitamin D3, 5,000 IU; Vitamin E, 50 mg; Vitamin B1, 2.0 mg; Vitamin B2, 2.5 mg; Vitamin B6, 2.0 mg; Manganese, 20 mg; Copper, 10 mg; Zinc, 25 mg; Potassium iodide, 0.80 mg; Cobalt, 0.10 mg; Selenium, 0.15 mg).

<sup>5</sup> Not determined.

## 2.3. Adaptation

Within each batch, calves were allocated to pens (6 calves per pen; 4 pens) based on BW to ensure similar mean BW across pens. The pens were randomly assigned to 1 of the 4 dietary treatments. Upon arrival, calves were adapted to the designated dietary treatment to allow rumen development and to become adapted to the levels and composition of SF. During the adaptation period, calves were housed in groups of 6 calves per pen (3 by 3 m) equipped with wooded-slatted floors and fences.

The experiment followed a staggered time-line due to the limited capacity of the climate respiration chambers (**CRC**). Hence, the adaptation period lasted 5 to 7 weeks. After 4 weeks of adaptation, the calves within a pen were paired according to BW, resulting in 3 pairs per pen (#1 to #3) with increased mean BW. Striving for equal BW upon measurements, the pair of calves with the highest BW had the shortest adaptation period (i.e., 5 weeks) and the pair of calves with the lowest BW had the longest adaptation period (i.e., 7 weeks). The mean BW of the calves at the end of the adaptation period was  $117 \pm 3.4$  kg,  $115 \pm 3.9$  kg,  $126 \pm 7.7$  kg,  $127 \pm 8.9$  kg,  $125 \pm 3.5$  kg, and  $130 \pm 8.9$  kg for pairs 1, 2, and 3 of batch 1 and pairs 1, 2, and 3 of batch 2, respectively.

### 2.3.1. Feeding

During the adaptation period, calves were allowed ad libitum access to water provided via drinking nipples. The concentration of MR was 130 g/L and was supplied individually in buckets at 40 to 42°C, provided twice daily in equally sized meals at 0730 h and at 1530 h throughout the experiment. The calves were allowed to drink for 15 minutes. Residual MR was subsequently collected and weighed. Except during the last week of adaptation, the SF was provided as a mixture (roughage and concentrates) directly after the MR meal, in a long feed trough in front of the pen. Refusals of the SF were removed and weighed twice daily (i.e., just before the next MR meal). During the last 5 days of

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the adaptation period, the pair of calves was separated from the other calves of their pen, by placing a fence within the original pens. The pair of calves was subsequently accustomed to steady state feeding of the SF. During these 5 days, the SF was provided as a mixture in 4 equal portions per day (i.e., 0530, 0930, 1330, and 1730 h). Refusals of the SF were removed and weighed 4 times daily (i.e., just before the next SF meal).

## 2.4. Energy and nitrogen balance

Within each batch, after the adaptation period of minimal 5 weeks, the pairs were assigned to one of 3 subgroups according to BW (high to low BW). The heaviest pairs of each treatment group were transferred to the climate respiration chambers (CRC) for a balance period of 7d (mean BW:  $116.5 \pm 3.4$  kg). Right after this period, the calves from the second subgroup were transferred to the CRC for a balance period of 7d (mean BW:  $115.3 \pm 3.9$  kg). Due to practical reasons, after the second balance week the CRC were empty for one week before the third subgroup was transferred for a balance period of 7d (mean BW:  $126 \pm 7.7$  kg). The same procedure was executed for the second batch of calves (mean BW subgroup 1:  $127.1 \pm 8.9$  kg; BW: subgroup 2:  $125.1 \pm 3.5$  kg; BW subgroup 3:  $129.8 \pm 8.9$  kg). The experimental unit was one chamber, in which two calves were housed. During the measurement periods, all calves were housed in pairs in 1 of 4 identical 40 m<sup>3</sup> indirect calorimetry chambers (Heetkamp et al., 2015). Within each chamber the two calves were housed in one pen (225 by 240 cm) on slatted, plastic flooring without bedding material. Lights were on from 0600h to 1800h. After the light period a small light was provided to illuminate the feeding trough. The rooms were kept on 18°C, with relative humidity of 65% and air velocity of <0.2m/s.

### 2.4.1. Feeding

During the 7d measurement period in the CRC, calves were allowed ad libitum access to water provided via drinking nipples. The concentration of MR was 130 g/L and was supplied individually in buckets at 40 to 42°C, provided twice daily in equally sized meals at 0730 h and at 1530 h throughout the experiment. The calves were allowed to drink for 15 minutes. Residual MR was subsequently collected and weighed. The SF was provided as a mixture in 6 equal portions per day (i.e., 0000, 0400, 0800, 1200, 1600, and 2000 h), approaching steady state feeding.

## 2.5. Measurements

All calves were weighed before and after the balance period. During the balance period, the complete energy and nitrogen balance was measured. The exchange of O<sub>2</sub>, CO<sub>2</sub> and CH<sub>4</sub> was measured every 13 minutes. A CO<sub>2</sub> recovery test was performed as a full system check, prior to the start of the experiment (Heetkamp et al., 2015). In the four CRC, 100.1%, 100.4%, 99.6% and 100.5% of the released CO<sub>2</sub> was recovered. At the end of the balance period, a pooled sample of the manure (i.e., faeces + urine) was taken. Feed intake (MR and SF) was measured and refusals were weighed and sampled for analysis. The faeces of the calves continued to stick to the plastic flooring. Since all the faeces are necessary for the complete balance, faeces were manually scraped from the flooring as much as possible. The scrapings were added to the collection of liquid manure of the balance period and mixed properly. Liquid manure samples were taken out of that collection. The faeces could not all be removed manually, therefore the last remainders were gathered by using water. Samples were taken from the cleaning water and the remainders of faeces as well. Furthermore, aerial NH<sub>3</sub> was collected from a quantified sample of outgoing air in sulphuric acid. Water that condensed on the heat exchanger was collected quantitatively and sampled for NH<sub>4</sub><sup>+</sup> measurements. Per pair of calves, physical activity was recorded continuously by a radar device.

Milk replacer, concentrates, chopped wheat straw and hay were sampled weekly in the second batch. Samples were pooled for a period of two weeks, resulting in two samples per feed source.

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## 2.6. Chemical Analyses

Samples of MR, concentrates, straw, and hay were collected weekly, pooled over the experimental period, and stored at  $-20^{\circ}\text{C}$  pending analysis. Samples of the feed components and faeces were thawed at room temperature, oven-dried at  $60^{\circ}\text{C}$ , ground to pass a 1-mm screen using a cross beater mill (Peppink 100AN, Olst, the Netherlands), and subsequently analyzed. The feed components and mixed faeces were analyzed for DM, N, gross energy (GE), nitrogen and phosphorus.

Dry matter content was determined by drying to a constant weight according to ISO 6496 (ISO, 1998). Bomb calorimetry (ISO 9831; International Organization for Standardization, 1998) was used to determine GE. Crude protein was calculated as  $\text{N} \times 6.25$ , where N was determined using the Kjeldahl principle (ISO 5983; International Organization for Standardization, 2008). The phosphorus content was determined by incinerating a test portion at  $550^{\circ}\text{C}$  and digesting with concentrated hydrochloric acid. Molybdovanadate reagent was added, resulting in a characteristic yellow color after reacting with phosphorus, which was subsequently measured spectrophotometrically at 430 nm (ISO 6491; International Organization for Standardization, 1998).

The DM, ash, and calcium content of the MR and concentrates was determined by Denkvit Nederland B.V. (Voorthuizen, the Netherlands). Dry matter content was determined by drying to a constant weight according to ISO 6496 (ISO, 1998). Ash content was determined according to ISO 5984 (ISO, 2003) and calcium content was determined according to ISO 15510 (ISO, 2017).

## 2.7. Calculations

Gross energy (GE) intake was calculated by multiplying the actual feed intake (feed provision minus refusals) by the GE of each feed component. The metabolizable energy (ME) was calculated by subtracting energy losses via both manure and methane production from the GE intake. Heat production was calculated with Brouwer equation without protein coefficients (Brouwer, 1965). Energy retention was determined by the difference between ME intake and heat production. Nitrogen (N) retention was calculated by subtracting N losses via manure, aerial  $\text{NH}_3$  and  $\text{NH}_4^+$  in water that condensed on the heat exchanger from the N intake from feed. Energy retention as protein was calculated as  $\text{N retention} \times 6.25 \times 23.7 \text{ kJ/g}$ . Energy retention as fat was calculated as the difference between energy retention and energy retention as protein. Resting metabolic rate and heat production related to activity were estimated from total heat production and activity data using penalized b-spline regression procedures (Van Klinken et al., 2012).

## 2.8. Statistical Analyses

Each pair of calves housed within a CRC was the experimental unit, and both the dependent variables and covariables were expressed as average of the pair. Dependent variables of the energy and N balance and the activity patterns were analyzed with the mixed model procedure (PROC MIXED in SAS 9.4; SAS Institute, 2000), including the fixed effects of treatment, batch, and the period of testing in the CRC within the batch.

When model residuals were not normally distributed, data was quadratically transformed to obtain homogeneity of variance. Data are presented as non-transformed means with the associated SEM.

Differences between treatment means were compared using the least square means procedure and the Tukey-Kramer method for multiple comparisons when an treatment effect was detected at  $P \leq 0.05$ . All results are presented as least square means and their SEM with significance of effects declared at  $P \leq 0.05$  and trends at  $0.05 < P \leq 0.10$ .

Because of health issues or reduced milk intake, 4 pairs of calves were excluded from this study. One calf was identified as a ruminal drinker, one calf was suffering from pneumonia, one calf had four times milk refusals higher than 20% of the provided meal and one calf suffered from various infections and had a fever.

## 3. Results

### 3.1. Animal Performance

The DMI of the various ration components (i.e., MR, roughage and concentrates) of the different treatments is consistent with the experimental design, and have not been statistically evaluated (Table 3). With the paired-gain feeding strategy, we aimed for similar body weight gain among the treatments. Average body weight gain during the experimental period was 9.6 kg for LowSF, 10.6 kg for MiddleSF, 10.1 kg for HighSF and 9.5 kg for the Hay treatment, and was not affected by treatment ( $P = 0.43$ ). Due to similar body weight gain and a higher total DMI for the calves receiving the Hay treatment, feed conversion ratio tended to be higher for the Hay treatment compared to the straw containing treatments ( $P = 0.087$ ).

**Table 3** Effect of increasing solid feed intake (LowSF, MiddleSF, HighSF) at a concentrate:straw ratio of 90:30, and of Hay at a concentrate:hay ratio of 70:30, with complementary milk replacer to achieve paired-gain, on animal performance during the experiment.

Item	Treatment				SEM	P-value
	LowSF	MiddleSF	HighSF	Hay		
DMI <sup>1</sup> milk replacer (kg/d)	1.90	1.74	1.49	1.62	0.023	n.d. <sup>2</sup>
DMI roughages (kg/d)	0.08	0.11	0.13	0.39	0.008	n.d. <sup>2</sup>
DMI concentrates (kg/d)	0.59	0.87	1.08	0.88	0.021	n.d. <sup>2</sup>
DMI total (kg/d)	2.53 <sup>a</sup>	2.70 <sup>b</sup>	2.67 <sup>b</sup>	2.85 <sup>c</sup>	0.035	< 0.001
Average body weight (kg)	127	130	128	130	2.6	0.75
Metabolic body weight (kg <sup>0.75</sup> ) / calf	37.8	38.4	38.0	38.5	0.57	0.75
Body weight gain (kg)	9.6	10.6	10.1	9.5	0.56	0.43
Average daily gain (kg/d)	1.38	1.51	1.44	1.35	0.079	0.43
Feed conversion ratio <sup>3</sup>	1.91	1.84	1.93	2.26	0.131	0.087

<sup>1</sup> Dry matter intake.

<sup>2</sup> Not determined; differences in DMI milk replacer, DMI roughages, and DMI concentrates between treatments were due to the design of the treatments.

<sup>3</sup> Ratio between DMI total (kg/d) and average daily gain (kg/d).

### 3.2. Nitrogen balance

Consistent with the experimental design, N intake from MR decreased with the amount of MR provided in the dietary treatments – decreasing from LowSF to Hay ( $P < 0.001$ ; Table 4). The N intake from SF was lower for the LowSF treatment compared with all other treatments, and lower for the MiddleSF treatment compared with the Hay treatment ( $P = 0.001$ ; Table 4). Total N intake was higher for the Hay treatment compared to both the LowSF and HighSF treatment ( $P = 0.052$ ). The N retention did not differ between treatments.

### 3.3. Energy balance

The energy intake and losses via manure were similar between treatments (Table 4). Energy losses via methane production increased with increasing SF level in the diet and were highest for the Hay treatment ( $P < 0.001$ ). The provision of different combinations of MR and SF had no effect on the heat production,



RMR or activity related heat production. Energy retention and deposition as protein or fat were not affected by the treatments as well.

**Table 4** Effect of increasing solid feed intake (LowSF, MiddleSF, HighSF) at a concentrate:straw ratio of 90:30, and of Hay at a concentrate:hay ratio of 70:30, with complementary milk replacer to achieve paired-gain, on energy and nitrogen balance traits of calves.

item	Treatment <sup>1</sup>				SEM	P-value
	LowSF	MiddleSF	HighSF	Hay		
No. of groups, no. of animals	4, 8	5, 10	6, 12	5, 10		
<i>N balance (g/kg<sup>0.75</sup>/d)</i>						
Total N intake	2.00 <sup>a</sup>	2.06 <sup>abc</sup>	2.02 <sup>ab</sup>	2.23 <sup>c</sup>	0.053	0.052
N from MR	1.47 <sup>a</sup>	1.29 <sup>b</sup>	1.13 <sup>c</sup>	1.21 <sup>d</sup>	0.005	<0.001
N from SF	0.53 <sup>a</sup>	0.77 <sup>b</sup>	0.89 <sup>bc</sup>	1.02 <sup>c</sup>	0.052	0.001
N retention <sup>2</sup>	1.26	1.13	1.12	1.31	0.091	0.47
<i>Energy balance (kJ/kg<sup>0.75</sup>/d)</i>						
Total GE <sup>3</sup> intake	1412	1437	1396	1540	37.0	n.d.
ME <sup>3</sup> intake <sup>4</sup>	1294	1275	1224	1305	292.2	0.33
CH <sub>4</sub> production	13.1 <sup>a</sup>	18.0 <sup>b</sup>	20.9 <sup>c</sup>	26.7 <sup>d</sup>	0.21	<0.001
Heat production	727 <sup>a</sup>	725 <sup>a</sup>	725 <sup>a</sup>	748 <sup>b</sup>	15.4	0.10
Activity heat	89	85	77	85	19.4	0.78
Resting metabolic rate	640	639	651	660	87.9	0.37
Energy retention <sup>4</sup>	565	550	506	557	187.0	0.55
As protein <sup>2</sup>	185	166	165	192	14.4	0.46
As fat <sup>4</sup>	381	383	338	365	367.5	0.48

<sup>1</sup> Treatments correspond to SF levels.

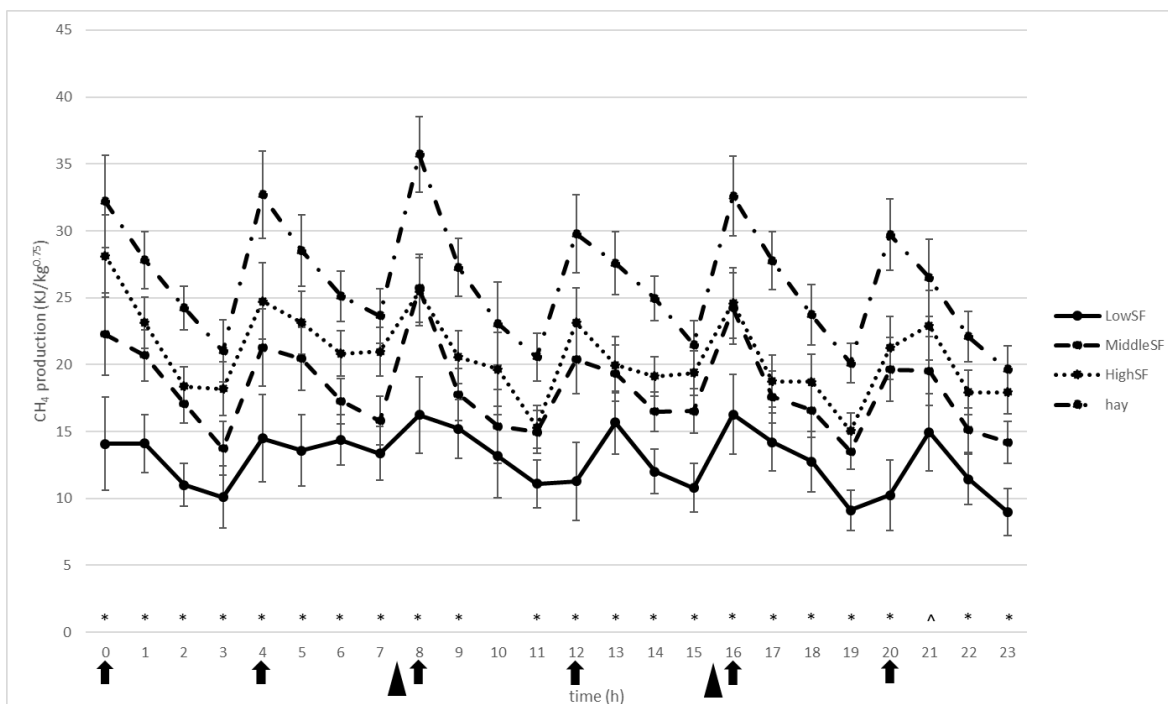
<sup>2</sup> Residuals non-normally distributed; unable to transform data.

<sup>3</sup> GE = gross energy; ME = metabolizable energy.

<sup>4</sup> Transformed values by using  $x^2$ ; means are backtransformed values.

### 3.4. Circadian rhythms

Differences in methane production among the dietary treatments followed patterns of SF meals (Figure 1; Appendix 1: Table 5). Overall, the Hay treatment had the highest methane production, followed by the HighSF, MiddleSF and the LowSF treatment respectively. Increasing the amount of SF results in more methane production during the day. In all treatments, methane production increased during the provision of the SF meal. The methane production of the Hay treatment was always the highest and the methane production of LowSF was always the lowest, which indicates consistency during the day.

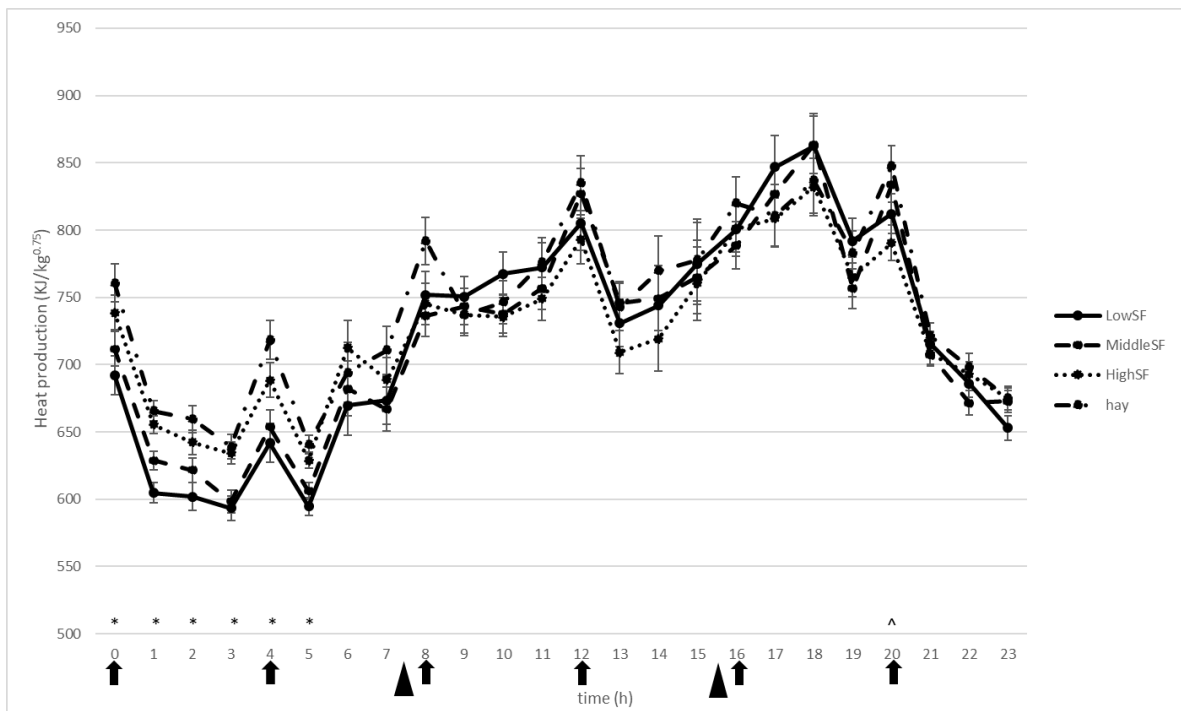


**Figure 1** Circadian pattern of  $\text{CH}_4$  production in veal calves fed different levels of solid feed (SF). Solid feed was provided as low (straight line), middle (striped line) or high (dotted) amounts of chopped straw or as hay (dotted & striped line). Arrows represent feeding times of SF and triangles represent feeding times of milk replacer. \* represents  $P < 0.05$  and ^ represents  $0.05 < P < 0.10$ .

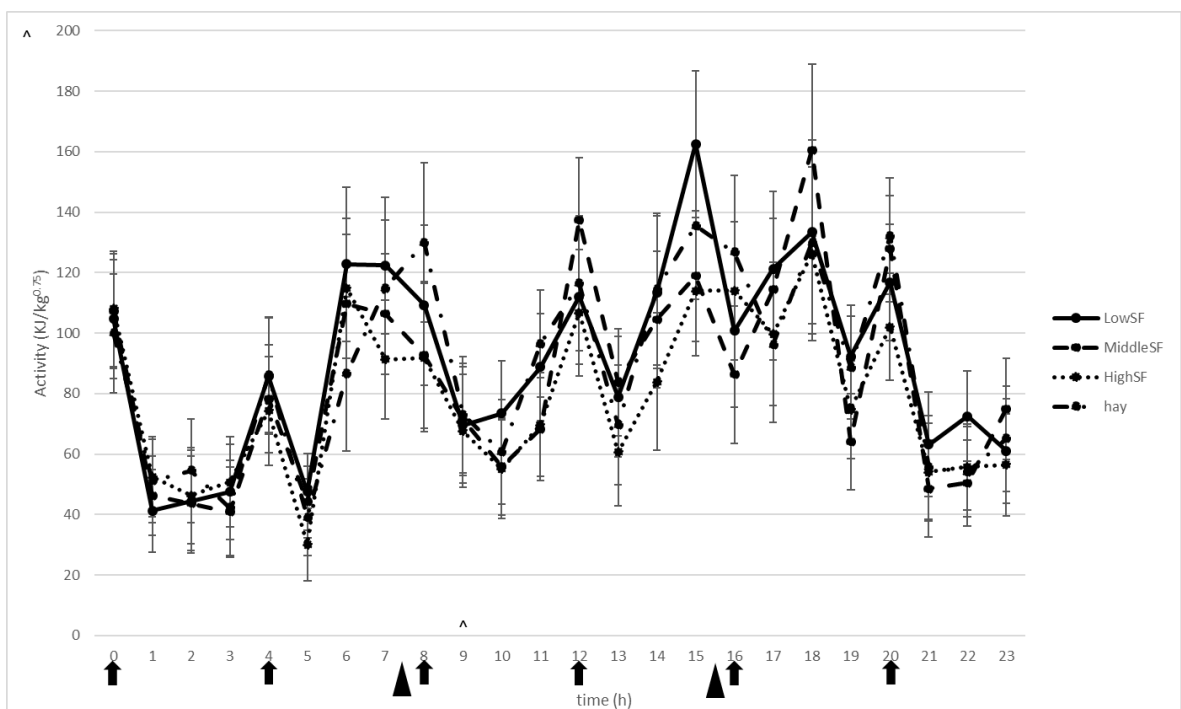
Total heat production did not differ between the treatments with straw as SF source, however the Hay treatment tended to increase heat production (HP) compared with the straw treatments ( $P=0.10$ ; Table 4). The influences of the SF treatments on the circadian rhythms of HP are shown in Figure 2 (means in table can be found in Appendix 1: Table 6). All treatments showed an increase in HP during a SF (6 x daily) or MR meal (2x daily). The response in heat production to the 1530h MR meal coincided with the response to the SF meal fed at 1600h. During the first part of the night (hours 0 to 5) the LowSF calves had a lower HP than the HighSF and Hay calves. Furthermore, numerically, in the same period the HP increased with increasing SF and was the highest in Hay calves.

Activity related HP responded to the meals provided, but averaged over the day, did not differ between treatments (Table 4, Figure 3; Appendix 1: Table 7). All calves were more active at the moments when solely a SF meal was available (0000h, 0400h, 1200h and 2000h). The patterns furthermore show increased activity for 30 min to 1.5h before the MR meals were provided.

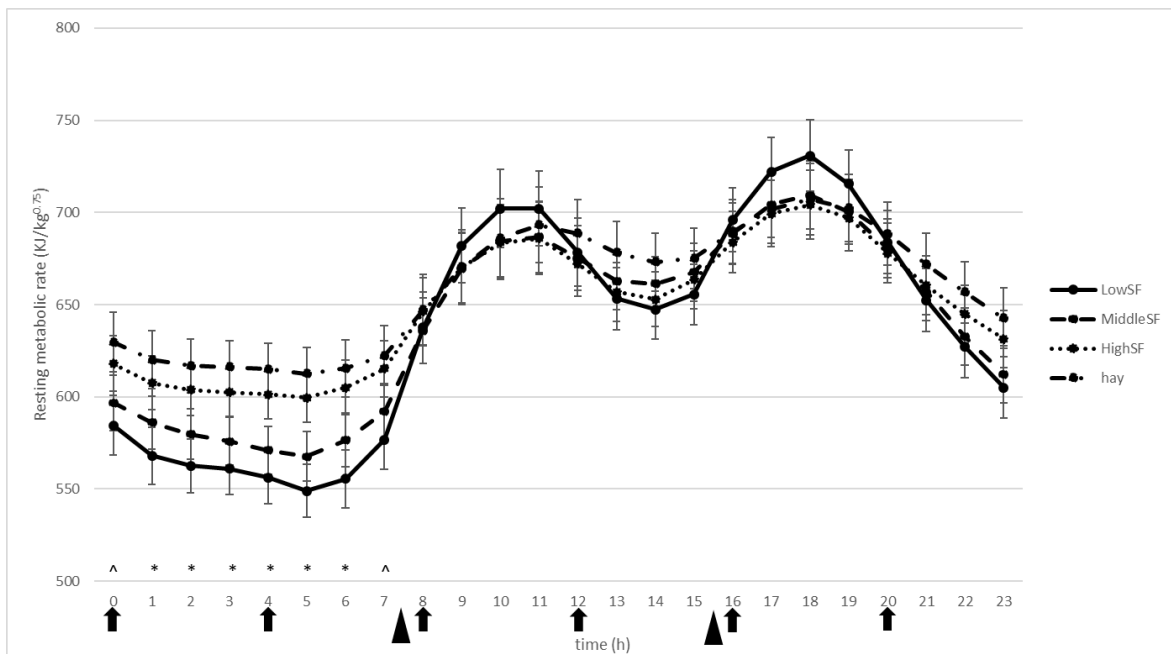
Within the circadian rhythms of the RMR, the MR meals are clearly visible (Figure 4; Appendix 1: Table 8). For all treatment, RMR increases after both MR meals. Before the first meal at 0730h (hours 1 to 6) calves fed the LowSF and MiddleSF treatments had a lower RMR than the calves fed the HighSF and Hay treatments ( $P<0.05$ ). The response in RMR to the MR meals mirrored the RMR numerically, where the LowSF calves had the highest RMR.



**Figure 2** Circadian pattern of heat production in veal calves fed different levels of solid feed (SF). Solid feed intake was provided as low (straight line), middle (striped line) or high (dotted) amounts of straw or as hay (dotted & striped line). Arrows represent feeding times of SF and triangles represent feeding times of milk replacer. \* represents  $P < 0.05$  and ^ represents  $0.05 < P < 0.10$ .



**Figure 3** Circadian pattern of activity related heat production in veal calves fed different levels of solid feed (SF). Solid feed intake was provided as low (straight line), middle (striped line) or high (dotted) amounts of straw or as hay (dotted & striped line). Arrows represent feeding times of SF and triangles represent feeding times of milk replacer. ^ represents  $0.05 < P < 0.10$ .



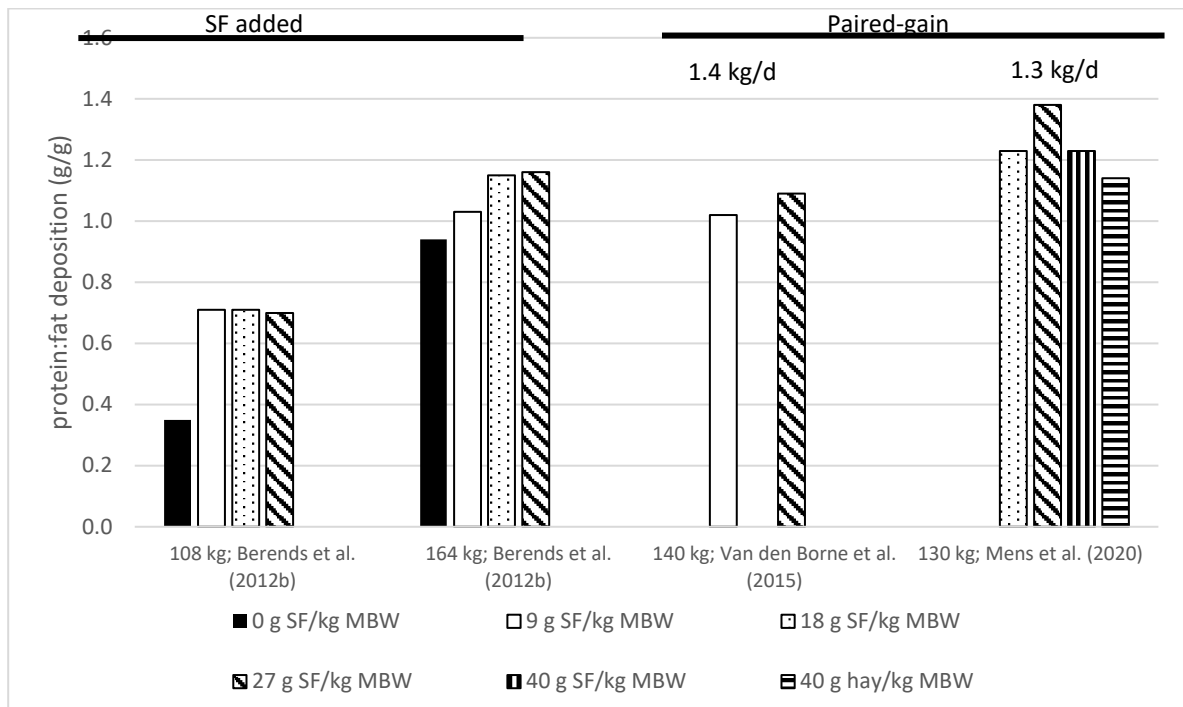
**Figure 4** Circadian pattern of resting metabolic rate in veal calves fed different levels of solid feed (SF). Solid feed intake was provided as low (straight line), middle (striped line) or high (dotted) amounts of straw or as hay (dotted & striped line). Arrows represent feeding times of SF and triangles represent feeding times of milk replacer. \* represents treatment effects at each point in time,  $P < 0.05$  and ^ represents treatment effects at  $0.05 < P < 0.10$ .

## 4. Discussion

Animal performance was good throughout the experiment, and, in line with the paired-gain strategy, unaffected by the dietary treatments.

### 4.1. Nitrogen and Energy balance

In this study, energy retention, both as protein and as fat, was unaffected by the dietary treatments. This suggests that MR can be exchanged for different mixtures of solid feed without affecting the composition of the tissues deposited. Both the N and energy retention found in the current study were approximately twice as high as has been reported by Berends et al. (2012b). In their study N retention of the calves fed SF varied between 0.73 and 0.91 g N/ kg<sup>0.75</sup>/d (BW calves: 108 kg) and the energy retention varied between 236 and 294 kJ/kg<sup>0.75</sup>/d (BW calves 108 kg). However, the study of Berends et al. (2012b) provided SF in addition to MR, whereas this study exchanged part of the MR for SF, according to the paired-gain strategy.



**Figure 5** Comparison of studies (Berends et al., 2012b; Van den Borne et al., 2015; this study) reporting the ratio of protein to fat deposition (g/g) in veal calves fed different levels of solid feed (SF). SF intake was provided as 0 g/kg BW<sup>0.75</sup> straw (black bars), 9 g/kg BW<sup>0.75</sup> straw (white bars), 18 g/kg BW<sup>0.75</sup> straw (dotted bars), 27 g/kg BW<sup>0.75</sup> straw (diagonal striped bars), 40 g/kg BW<sup>0.75</sup> straw (vertical striped bar) or 40 g/kg BW<sup>0.75</sup> hay (horizontal striped bar). Studies on the left provided SF in addition to milk replacer (MR), studies on the right exchanged part of the MR for SF, according to the paired-gain strategy. The ADG of the paired-gain studies were 1.4 kg/d (Van den Borne et al., 2015, unpublished results) and 1.3 kg/d (present study)

The increasing allowance of SF did not affect the ratio of fat to protein deposition (g/g) (Figure 5). This is in contrast with the results by Berends et al. 2012b where an increasing intake of SF (i.e., a 50:25:25 mixture of concentrate, straw and corn silage, respectively) seemed to result in higher fat deposition relative to protein deposition. In the study of Berends et al. (2012b), the calves were fed SF on top of the MR. Hence, the total feed intake level increased with the increasing SF level, which could have resulted in the higher fat deposition. Van den Borne et al. (2015, unpublished results) also used the

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paired-gain strategy, and did not find an increase in fat deposition when SF allowance increased. In their study, feeding level and growth rates were comparable to this present study. Nevertheless, the ratio of fat to protein deposition was lower than in the current study. This difference corresponds to a higher heat production in the study by Van den Borne et al. (2015, unpublished data). Calves in the study by Van den Borne et al. (2015, unpublished results) were housed individually in metabolic cages within the CRC, whereas the calves in the present study were housed in pairs. The group housing might have been beneficial for the calves, reducing stress and the occurrence of abnormal oral behaviours. The latter is responsible for a disproportionate increase in heat production, particularly during standing (Gerrits et al., 2015).

## 4.2. Circadian rhythms

Methane production clearly followed patterns of SF supply, and increased with increasing SF levels, where the provision of Hay as a solid feed resulted in the highest methane production. These results are well in line with results in calves (Berends et al., 2012b) and adult dairy cows (Mills et al., 2001). Methane production in calves fed solely MR is negligible (Van den Borne et al., 2006a; Labussi re et al., 2009a; Berends et al., 2012b; Gerrits et al., 2014). As methane emissions increase proportionally to the level of SF intake, more so with roughage than with concentrate, this study illustrates the trade-off between feeding costs, calf welfare and methane emissions.

Even though the treatments did not affect total heat production, the circadian rhythm clearly shows an increase during every meal. These results were expected, as calves become active to eat and metabolic processes take place after the meal. The increase in activity is confirmed by the circadian rhythm of the activity related heat production, which follows the provision of a meal as well. The circadian rhythm of RMR only shows clear effects of the MR meals. This indicated the strategy of steady state feeding of the SF in small portions had succeeded, and that only MR meals provided differences. During the dark period, before the first MR meal, the RMR of the LowSF was the lowest, and the RMR increased with increasing SF allowance. Although the peaks in RMR after a MR meal showed no significant differences between treatments, the peak of the LowSF treatment was numerically the highest. These results indicate differences in metabolic processes due to the provision of SF. Effects of the dietary treatments on the RMR were clear and proportional to the SF level provided. This illustrates the effects of exchanging the MR fed twice daily for increasing quantities of SF, fed in 6 equal portions. Without affecting daily deposition rates of protein and fat, this exchange has marked effects on many metabolic processes occurring throughout the day. Solid feed provides a more gradual supply of nutrients (microbial proteins, short-chain fatty acids) throughout the day, whereas the thermic effect of feeding MR follows peak absorption of glucose, long chain fatty acids and dietary proteins. The increase in the minimum RMR observed within the day may also reflect an increase in maintenance energy requirement, related to an increase in development of gastro-intestinal tissues, particularly the rumen. It is remarkable that such strong shifts in metabolism observed within the day, even designed at equal rates of body weight gain, do not lead to changes in the chemical composition of body weight gain.

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# Conclusions

The results of this study indicate that increasing the allowance of SF at the expense of MR in a paired-gain feeding strategy, does not influence the protein and fat deposition. Moreover, methane production increases as the allowance of SF increases, where the provision of hay results in the highest methane production. The circadian pattern of methane production show that these differences in methane production are present throughout the day. Based on the circadian rhythms, the results showed that the level of SF affects heat production. The resting metabolic rate during the dark period was lowest for the calves fed the LowSF diets, and amplified with increasing SF allowance, indicating an increase in nutrient absorption from fermentation processes, but in part also reflecting an increase in metabolic processes related to an increase in gastro-intestinal tissues. Although no significant effects were found between treatments, the MR meals showed clear peaks in the RMR rhythm, with the LowSF treatment peaking numerically the highest, followed by MiddleSF, HighSF and Hay treatments. These results illustrate the effects of exchanging MR by increasing levels of SF and strong shifts in metabolic processes when fed the MR meal. Remarkably, the chemical composition of body weight gain is not affected by these shifts.

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# Appendix 1 Means of circadian rhythms

**Table 5** Effects of increasing the intake of a solid feed (SF) mixture of 90:10 concentrate:straw and a reference Hay treatment containing 70:30 concentrate:hay on circadian pattern of methane production (KJ/kg<sup>0.75</sup>), fed an additional quantity of milk replacer to achieve equal rates of BW gain.

Hour	Treatment <sup>1</sup>				SEM	P-value
	LowSF	MiddleSF	HighSF	Hay		
0	14.1 <sup>a</sup>	22.3 <sup>ab</sup>	28.1 <sup>b</sup>	32.2 <sup>c</sup>	3.27	0.014
1	14.1 <sup>a</sup>	20.7 <sup>b</sup>	23.2 <sup>bc</sup>	27.8 <sup>c</sup>	2.03	0.004
2	11.0 <sup>a</sup>	17.1 <sup>b</sup>	18.4 <sup>b</sup>	24.2 <sup>c</sup>	1.53	0.001
3	10.1 <sup>a</sup>	13.8 <sup>ab</sup>	18.2 <sup>bc</sup>	21.0 <sup>c</sup>	2.16	0.024
4	14.5 <sup>a</sup>	21.3 <sup>ab</sup>	24.8 <sup>bc</sup>	32.7 <sup>c</sup>	3.06	0.014
5	13.6 <sup>a</sup>	20.4 <sup>ab</sup>	23.1 <sup>bc</sup>	28.5 <sup>c</sup>	2.51	0.014
6	14.4 <sup>a</sup>	17.3 <sup>ab</sup>	20.8 <sup>bc</sup>	25.1 <sup>c</sup>	1.79	0.007
7	13.4 <sup>a</sup>	15.8 <sup>ab</sup>	21.0 <sup>bc</sup>	23.6 <sup>c</sup>	1.92	0.010
8	16.2 <sup>a</sup>	25.7 <sup>bc</sup>	25.5 <sup>c</sup>	35.7 <sup>d</sup>	2.69	0.002
9	15.2 <sup>a</sup>	17.8 <sup>bc</sup>	20.6 <sup>c</sup>	27.3 <sup>d</sup>	2.06	0.006
10	13.2	15.4	19.7	23.1	2.93	0.14
11	11.1 <sup>a</sup>	15.0 <sup>bc</sup>	15.3 <sup>c</sup>	20.6 <sup>d</sup>	1.70	0.018
12	11.3 <sup>a</sup>	20.4 <sup>b</sup>	23.2 <sup>bc</sup>	29.8 <sup>c</sup>	2.74	0.004
13	15.7 <sup>a</sup>	19.4 <sup>a</sup>	20.0 <sup>a</sup>	27.6 <sup>b</sup>	2.24	0.021
14	12.0 <sup>a</sup>	16.5 <sup>ab</sup>	19.1 <sup>b</sup>	25.0 <sup>c</sup>	1.56	0.001
15	10.8 <sup>a</sup>	16.5 <sup>bc</sup>	19.4 <sup>c</sup>	21.5 <sup>c</sup>	1.74	0.006
16	16.3 <sup>a</sup>	24.2 <sup>ab</sup>	24.6 <sup>b</sup>	32.6 <sup>b</sup>	2.82	0.015
17	14.2 <sup>a</sup>	17.6 <sup>a</sup>	18.8 <sup>a</sup>	27.8 <sup>b</sup>	2.05	0.004
18	12.8 <sup>a</sup>	16.6 <sup>a</sup>	18.7 <sup>ab</sup>	23.7 <sup>b</sup>	2.14	0.027
19	9.1 <sup>a</sup>	13.5 <sup>b</sup>	15.0 <sup>bc</sup>	20.1 <sup>d</sup>	1.41	0.001
20	10.3 <sup>a</sup>	19.6 <sup>b</sup>	21.3 <sup>bc</sup>	29.7 <sup>d</sup>	2.51	0.001
21	15.0	19.5	22.9	26.5	2.73	0.068
22	11.4 <sup>a</sup>	15.1 <sup>ab</sup>	17.9 <sup>bc</sup>	22.1 <sup>c</sup>	1.76	0.010
23	9.0 <sup>a</sup>	14.2 <sup>b</sup>	17.9 <sup>bc</sup>	19.7 <sup>c</sup>	1.67	0.003

<sup>1</sup>treatments correspond to SF levels.

<sup>abcd</sup> means within a row with no common superscript differ ( $P < 0.05$ ).

**Table 6** Effects of increasing the intake of a solid feed (SF) mixture of 90:10 concentrate:straw and a reference Hay treatment containing 70:30 concentrate:hay on circadian pattern of heat production ( $KJ/kg^{0.75}$ ), fed an additional quantity of milk replacer to achieve equal rates of BW gain.

Hour	Treatment <sup>1</sup>				SEM	P-value
	LowSF	MiddleSF	HighSF	Hay		
0	692.2 <sup>a</sup>	711.7 <sup>ab</sup>	738.6 <sup>bc</sup>	760.7 <sup>c</sup>	13.50	0.013
1	604.7 <sup>a</sup>	628.8 <sup>b</sup>	655.5 <sup>cd</sup>	665.5 <sup>d</sup>	7.10	0.002
2	602.0 <sup>a</sup>	621.8 <sup>ab</sup>	642.2 <sup>bc</sup>	659.5 <sup>c</sup>	9.65	0.007
3	593.3 <sup>a</sup>	598.3 <sup>a</sup>	634.2 <sup>b</sup>	638.8 <sup>b</sup>	8.69	0.004
4	641.7 <sup>a</sup>	653.7 <sup>ab</sup>	688.6 <sup>bc</sup>	718.4 <sup>c</sup>	13.55	0.009
5	594.5 <sup>a</sup>	606.1 <sup>a</sup>	629.0 <sup>b</sup>	640.7 <sup>b</sup>	6.50	0.001
6	669.6	682.3	712.6	694.1	21.33	0.35
7	673.5	667.0	688.7	710.7	17.10	0.22
8	751.7	736.5	745.2	791.9	16.40	0.12
9	750.5	743.5	737.3	737.0	14.38	0.86
10	767.6	737.9	735.5	746.4	15.18	0.47
11	772.2	756.8	748.9	776.1	17.10	0.59
12	805.3	827.1	793.1	834.9	19.53	0.42
13	730.9	745.9	709.3	742.8	16.73	0.39
14	744.1	749.4	719.2	769.8	24.84	0.18
15	775.1	765.2	760.2	777.8	28.86	0.95
16	800.5	788.5	801.0	819.9	18.63	0.70
17	847.0	827.3	808.7	810.9	21.79	0.56
18	862.7	863.6	832.0	836.7	22.74	0.60
19	791.9	756.6	765.2	782.9	15.73	0.39
20	812.2	833.8	790.5	847.7	14.14	0.055
21	715.3	707.4	708.4	721.6	8.95	0.58
22	685.9	671.6	693.0	698.1	9.74	0.27
23	652.9	672.7	674.6	675.1	8.43	0.26

<sup>1</sup>treatments correspond to SF levels

<sup>abc</sup> means within a row with no common superscript differ ( $P < 0.05$ )

**Table 7** Effects of increasing the intake of a solid feed (SF) mixture of 90:10 concentrate:straw and a reference Hay treatment containing 70:30 concentrate:hay on circadian pattern of activity ( $\text{KJ}/\text{kg}^{0.75}$ ), fed an additional quantity of milk replacer to achieve equal rates of BW gain.

hour	Treatment <sup>1</sup>				SEM	P-value
	LowSF	MiddleSF	HighSF	Hay		
0	104.7	107.4	108.1	100.0	19.35	0.91
1	41.3	46.3	52.4	51.1	13.45	0.69
2	44.3	43.7	46.3	54.4	16.42	0.92
3	47.5	40.8	50.8	42.3	15.31	0.83
4	85.7	78.3	74.3	86.2	18.57	0.86
5	47.5	44.2	30.0	39.0	12.30	0.45
6	122.8	109.8	114.9	86.5	24.21	0.65
7	122.4	106.4	91.3	114.9	21.18	0.73
8	109.3	92.7	91.9	130.0	25.42	0.46
9	69.6	71.3	67.6	73.0	18.98	0.98
10	73.4	55.9	55.0	60.7	16.77	0.65
11	88.8	68.3	69.7	96.5	17.41	0.096
12	112.1	137.4	106.8	116.4	21.54	0.50
13	78.9	83.7	60.7	69.6	18.82	0.75
14	113.4	104.6	83.9	114.6	23.92	0.65
15	162.5	118.9	114.1	135.5	22.92	0.47
16	100.7	86.3	114.0	126.7	24.10	0.58
17	121.3	114.4	99.7	96.2	24.60	0.78
18	133.5	160.5	126.3	130.0	29.43	0.55
19	92.3	64.1	74.7	88.7	16.47	0.26
20	116.8	127.8	102.1	132.1	18.40	0.41
21	63.2	48.5	54.1	55.6	16.70	0.82
22	72.5	50.5	55.8	54.0	14.57	0.29
23	61.0	75.0	56.6	65.1	17.05	0.44

<sup>1</sup>treatments correspond to SF levels.

**Table 8** Effects of increasing the intake of a solid feed (SF) mixture of 90:10 concentrate:straw and a reference Hay treatment containing 70:30 concentrate:hay on circadian pattern of resting metabolic rate(KJ/kg<sup>0.75</sup>), fed an additional quantity of milk replacer to achieve equal rates of BW gain.

Hour	Treatment <sup>1</sup>				SEM	P-value
	LowSF	MiddleSF	HighSF	Hay		
0	584.5	596.8	618.1	629.6	15.58	0.066
1	568.1 <sup>a</sup>	586.1 <sup>ab</sup>	607.6 <sup>b</sup>	620.0 <sup>b</sup>	15.07	0.033
2	562.6 <sup>a</sup>	579.8 <sup>ab</sup>	603.8 <sup>bc</sup>	616.8 <sup>c</sup>	14.22	0.015
3	561.0 <sup>a</sup>	575.8 <sup>a</sup>	602.5 <sup>b</sup>	616.3 <sup>b</sup>	13.57	0.007
4	556.2 <sup>a</sup>	571.0 <sup>a</sup>	601.4 <sup>b</sup>	615.1 <sup>b</sup>	13.54	0.005
5	549.0 <sup>a</sup>	567.7 <sup>a</sup>	599.5 <sup>b</sup>	612.6 <sup>b</sup>	13.94	0.004
6	555.6 <sup>a</sup>	576.6 <sup>ab</sup>	605.1 <sup>bc</sup>	615.4 <sup>c</sup>	15.13	0.012
7	576.7	592.0	615.2	622.3	15.63	0.056
8	637.5	635.9	646.3	647.1	18.63	0.90
9	682.1	669.8	670.1	670.3	19.79	0.88
10	702.1	684.4	683.7	686.1	20.56	0.71
11	702.2	686.8	686.0	693.3	19.90	0.73
12	678.3	675.3	672.3	688.6	18.00	0.73
13	653.4	662.9	657.0	678.1	16.41	0.43
14	647.4	661.3	653.0	673.1	15.38	0.35
15	655.5	667.6	663.5	675.2	16.06	0.57
16	696.0	688.4	684.0	689.6	17.08	0.78
17	722.1	704.3	699.4	702.0	18.38	0.35
18	730.8	709.3	704.1	707.2	18.93	0.27
19	715.5	700.4	697.0	702.3	17.93	0.53
20	684.0	680.4	678.0	688.5	16.54	0.87
21	652.4	657.1	660.4	671.8	16.47	0.68
22	627.2	632.6	644.9	656.7	16.23	0.32
23	605.1	612.2	631.4	642.6	15.94	0.13

<sup>1</sup>treatments correspond to SF levels.

<sup>abc</sup> means within a row with no common superscript differ ( $P < 0.05$ ).

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Wageningen Livestock Research  
P.O. Box 338  
6700 AH Wageningen  
The Netherlands  
T +31 (0)317 48 39 53  
E [info.livestockresearch@wur.nl](mailto:info.livestockresearch@wur.nl)  
[www.wur.nl/livestock-research](http://www.wur.nl/livestock-research)

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