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Effects of Adding Lipid Supplements to Milk Replacers in Young Bull Calves

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Topic description:	This Master thesis is undertaken as a part of the project SmartCalfFat funded by Kvægafgiftsfonden. This thesis evaluates the effect of increasing the fat content of milk replacer and compares the effects of two different lipid supplements on the performance of crossbred Holstein × Belgian Blue bull calves. Body weight gains, body conditions scores, records of dry matter intake, fecal scores, and biometrics were obtained in order to compare the effects of two different lipid supplements on the performance of calves.
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Preface & acknowledgments

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Abstract

Bull calves face serious stressors when they are sent to beef farms, which affects their health and can lead to reduced growth rates. Increasing the dietary fat content has been reported to improve weight gains and feed efficiencies in calves.

The objective of this study was to determine the effects of saturated and unsaturated dietary lipid supplements in milk replacer on body weight (BW) gains, body condition scores (BCS), fecal scores, body temperature, the biometrical measurements hip height (HH), withers height (WH), body length (BL) and heart girth (HG), along with dry matter intakes (DMI) and feed efficiency of preweaned calves.

A feeding trial was conducted with 18 Holstein × Belgian blue bull calves blocked by bodyweight (70.3 ± 11.9 kg, 69.0 ± 11.5 kg, 70.2 ± 11.8 kg) into three groups to receive one of three diets for 21 days. Three liters of milk replacer with 24% crude protein (CP), and 16% fat, were fed twice daily. Calves in the control group were fed 811 g DM of milk replacer. Calves in experimental groups were fed 753 g DM of milk replacer and 58.2 g unsaturated lipids supplement or 50.6 g DM saturated lipid supplement to reach 21% fat in the milk replacer. Bodyweights, BCS, fecal scores, rectal temperatures, and biometrics were recorded on days 0, 7, 14, and 21.

The BL and WH were reduced in calves fed saturated fat. In calves fed unsaturated lipids, HG and HH were reduced which may be ascribed to the inclusion of *n*-6 polyunsaturated fatty acids that can reduce bone formation. Fecal scores and rectal temperatures were recorded as parameters of health. Rectal temperatures were constant during the trial period, and fecal scores tended to decrease at day 21. Growth rates and feed efficiency were not significantly improved by lipid supplementation with may be ascribed to differences in energy requirements arising from differences in body sizes, gastrointestinal tract development, and inflammatory responses, caused by the supplemented lipids.

In conclusion, increasing the fat content of milk replacer reduced some biometric measures, but did not significantly affect BW gains, BCS, feed efficiency, or health parameters, and did not reduce DMI. Further investigations on the effects of increasing the fat content of diets for preweaned calves are needed to propose recommendations on fat supplementation of calves.

Abbreviations

AAT	Amino acids absorbed in the small intestine
ADG	Average daily gain
BCS	Body Condition Score
BL	Body length
BW	Bodyweight
CLA	Conjugated linoleic acid
CP	Crude protein
DM	Dry matter
DMI	Dry matter intake
FA	Fatty acids
HG	Heart girth
HH	Hip height
MCFA	Medium chain fatty acids
ME	Metabolizable energy
NE	Net energy
PUFA	Polyunsaturated fatty acids
SCFA	Short chain fatty acids
VFA	Volatile fatty acids
WH	Withers height

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1. Introduction

1.1. Background

In Denmark, around 600.000 calves of dairy breeds are born each year. In 2020, 44% of calves born were bull calves (Martin, 2021). More than 180.000 bull calves and young bulls were slaughtered in Denmark, while around 40.000 calves were exported in 2021 (Landbrug & Fødevarer, 2021). From 2022 a ban from the Danish Agriculture and Food Council on the culling of bull calves was set in (SEGES, 2020), and this was expected to lead to a production of up to 30.000 extra calves per year (Danish Crown, 2021).

Bull calves of dairy breeds are most often sent to beef farms, where they are firstly fed milk replacer until eight weeks of age and then fed only with concentrate and roughage (Fertner *et al*. 2016). The bulls are slaughtered at either 8-12 months of age as veal or as young bulls older than 12 months of age. The calves are usually housed in cubicles or straw-bedded pens, in groups of six to 50 calves of similar age or bodyweight (BW). While an all-in-all-out principle is commonly applied at pen level, this is not necessarily the case for the whole "compartment", which means that calves of varying ages and from different suppliers can be housed in the same building or pen (Fertner *et al*. 2016).

Housing calves from several farms in the same pen may lead to spreading pathogens between animals (Fertner *et al.* 2016; Masmeijer *et al*. 2020). Also, the stress related to transport, adaptation to a new environment, and new feed affect calves' health and immunity, leading to increased disease risk, especially for underweight calves (Fertner *et al.* 2016; Masmeijer *et al*. 2020). Lastly, these factors lead to a decrease in growth rates (Timmerman *et al.* 2005; Marcato *et al*. 2018). In this regard, an analysis on Danish dairy beef calves estimated that if calves had been sick at 56 to 91 days of age, the average daily gain (ADG) would be 20 g lower than that of healthy calves, and the risk of death before slaughtering could be almost doubled (Kjeldsen *et al*. 2017).

As the stressful events of transportation and relocation into a new environment at a beef farm is related to energy mobilization from adipose tissue and an increase in energy demand for initiation of immune responses (Marcato *et al.* 2018; Devant and Marti, 2020; Masmeijer *et al*. 2020), it could be considered that increasing the dietary energy density, could help calves to cope with the stress related to new housing and management systems. In this regard, increasing dietary fat content is related to reduced mortality rate in preweaned dairy calves (Urie *et* al. 2018) and dietary lipid supplements have been shown to improve

immunity, growth and reduce the need for medical treatments in calves (Hill *et al.* 2011a; Welboren *et al.* 2021). For example, Ballou and DePeters (2008) found that dietary supplements of oils rich in polyunsaturated fatty acids (PUFA) altered the immunological response in Jersey calves by increasing the phagocytic rate of monocytes, oxidative burst capacity of neutrophils, and humoral response (Ballou and DePeters, 2008). Similarly, Muturi *et al.* (2005) found that supplements rich in PUFA can reduce infection and aid in the development of immunity against parasites such as *Ostertagia ostertagi* and *Cooperia onchophora.* Also, short-chain fatty acids (SCFA) and medium-chain fatty acids (MCFA), and glycerol esters of these, are known to possess antimicrobial and antiviral properties, but also immunomodulatory properties. Immunomodulatory properties of SCFA and MCFA have been demonstrated by Masmeijer *et al.* (2020) when supplementing Holstein calves with mono- and tri-esters of butyrate (C4:0), caprylate (C8:0), caprinate (C10:0), and laurate (C12:0) for a 14-week period. Also Hill *et al.* (2011a) demonstrated immunomodulatory properties of fatty acids (FA), in terms of higher serum titers following vaccination of Holstein calves fed a supplement of linolenic acid (C18:3), butyrate, and MCFA, compared to calves without the FA supplement. They (Hill *et al.* 2011a) also reported increased ADG, feed efficiency, and reduced duration of scours when supplementing calves with linolenic acid, butyrate, and MCFA. Not only the FA profile but also increasing the fat content of the diet has been demonstrated to increase ADG and feed efficiency (Bascom *et al.* 2007; Hill *et al.* 2007; Litherland *et al*. 2014).

With the advantages of feeding lipid supplements to growing calves and the health-related challenges experienced by beef calves, the use of lipid supplements seems to be a viable option for improving beef calves' growth and health, however, this strategy has not been used in Danish beef farms.

1.2. Problem definition

With the potential benefits of increasing dietary lipid contents on the health and performance of beef calves, the aim of this project was to determine the effects of feeding two different dietary lipid supplements in milk replacers on productive traits of young calves. Specifically, this study aims at determining the effect of dietary lipid supplements, based on saturated and unsaturated fatty acids, on bodyweight gains, body condition scores, selected biometrical measurements, fecal scores, and body temperature, along with feed intake.

The hypothesis of this study was that increasing the dietary content of saturated fatty acids would improve productive traits. Whereas increasing the dietary content of unsaturated fatty acids would have positive effects on the calves' health, which might also result in improved growth rates.

2. Literature review

2.1. Nutritional requirements of calves

Despite the gastrointestinal tract (GIT) of calves not being fully developed at birth, calves are expected to be able to cover a part of their nutritional requirements from solid feed intake already at an early age (NRC, 2001). Due to delayed development of the ruminal function, the GIT of young calves is functionally comparable to that of monogastric animals (NRC, 2001), but only hours after birth, the rumen starts becoming colonized by microorganisms (Khan *et al.* 2016). When the calf starts consuming solid feed, the microorganisms ferment dietary carbohydrates and produce volatile fatty acids (VFA). Physical stimulation from feedstuffs and VFA are crucial for the development of ruminal epithelium and papillae (Baldwin *et al.* 2004; Drackley, 2008; Khan *et al.* 2016). As reflexive closure of the reticular groove during milk feeding promotes milk to be delivered directly to the abomasum, fermentation of milk carbohydrates is prevented, and thus feeding milk does not contribute to the development of ruminal function (Baldwin *et al.* 2004). Therefore, consumption of solid feed already from the first weeks of life is necessary for the development of a functional GIT (NRC, 2001).

The National Research Council (NRC) has estimated the nutritional requirements of calves. The requirements are dependent on several factors including age and development of the digestive tract, growth rate, and environmental temperature (NRC, 2001). According to the NRC (2001), a calf with 60 kg of bodyweight (BW), gaining 600 g/day, fed a diet of milk or milk replacer and starter feed, has a daily metabolizable energy (ME) requirement of 4.31 Mcal and 217 g of crude protein. The NRC (2001) estimates the net energy (NE) requirement for maintenance (NE_M) and growth (NE_G) using two equations (Eq.) shown in Eq. 1 and 2. The NE requirements are converted to ME using Eq. 3 and 4.

Eq. 1 $\mathrm{NE_M\ (Mcal)} = 0.086 * \mathrm{LW^{0.75}(kg)}$

Eq. 2 $\mathrm{NE_G\ (Mcal)} = (0.84 * \mathrm{LW\ kg^{0.355}} * \mathrm{LWG\ kg^{1.2}}) * 0.69$

Eq. 3 $\mathrm{ME_M\ (Mcal)} = \mathrm{NE_M}/0.75$

Eq. 4 $\mathrm{ME_G\ (Mcal)} = \mathrm{NE_G}/0.57$

Calculating the energy requirements for growing calves using the NorFor model is more complex, and no standard or table values are provided for calves. The NorFor model includes BW, ME, and gross energy (GE) of the diet, a factor dependent on breed and sex of the animal, coefficients for utilization of ME for maintenance and growth, and coefficients for retention of protein and fat in order to calculate the NE requirements for growth and maintenance (Volden, 2011).

Requirements for dietary energy, protein, and fat of calves related to the Danish standards will be further elaborated in the following paragraphs.

2.1.1. Energy requirements

Requirements for metabolizable energy for maintenance and growth are dependent on bodyweight and growth rate. For calves fed milk or milk replacer and starter feed, 60% of DMI from milk or milk replacer with an energy content of 4.75 Mcal ME/kg DM, and 40% of DMI from starter feed with 3.28 Mcal ME/kg DM is estimated to cover the energy requirements (NRC, 2001).

The lower critical temperature for calves has been reported to range between 8°C and 15°C (NRC, 2001; Litherland *et al.* 2014). When the temperature falls below this limit, the amount of energy available for growth decreases as more energy is needed to maintain a normal body temperature (NRC, 2001; Litherland *et al.* 2014). With an average temperature of 8.7°C in Denmark (DMI, 2021), Danish calves are subjected to temperatures under their lower critical temperature for most parts of the year. According to the NRC (2001) additional 2.15 Kcal ME/kg $BW^{0.75}$ is needed for each degree the environmental temperature is below the lower critical temperature.

While the NorFor model does not directly include effects of environmental temperature when estimating energy requirements, this model considers the DMI when calculating the ME and GE of the diet (Volden, 2011). As the DMI during periods with temperatures below the thermoneutral zone tends to be increased due to increased energy requirements (Litherland *et al.* 2014) the Norfor model indirectly accounts for environmental temperature.

2.1.2. Protein requirement

As with energy requirements, protein requirements depend on growth rate and BW (NRC, 2001). In the NorFor model, protein requirements for growing cattle are calculated from the

amount of retained protein and efficiency of deposition of amino acids absorbed in the small intestine (AAT) (Volden, 2011).

Not only dietary protein supply but also CP-to-energy ratio is important for optimal protein retention in lean tissue. If CP-to-energy ratios are not optimal, ADG can be reduced, and deposition of adipose tissue will be favored over lean tissue (Hill *et al*. 2009). The optimal ratio in milk replacer has been estimated by Hill *et al*. (2009) to range from 51.5 g CP/Mcal ME to 55.0 g CP/Mcal ME for calves fed whey-based milk replacer.

Hill *et al.* (2009) fed two rates of milk replacer (545 and 654 g DM/day) with four different protein concentrations (23%, 25%, 27%, and 29% CP), to eight treatment groups of twelve calves (Table 1.) When feeding 545 g DM the highest growth rate (0.47 kg/day) was achieved by the 25% crude protein milk replacer corresponding to 51.5 g CP/Mcal ME. When feeding 654 g DM the 27% crude protein milk replacer, corresponding to 55.0 g CP/Mcal ME, led to the highest growth rate (0.53 kg/day). The 23% crude protein milk replacer resulted in the lowest growth rate and least for both rates of milk replacer (0.44 kg/day and 0.45 kg/day, respectively) (Hill *et al.* 2009).

Table 1. Experimental groups and results from Hill et al. (2009).

Dry matter from milk replacer	**545 g/day**				**654 g/day**				**P-value**		
									Rate	**CP**	**Rate × CP**
g CP/Mcal ME	48.1	51.5	55.0	59.3	48.1	51.5	55.0	59.3			
Crude protein	23%	25%	27%	29%	23%	25%	27%	29%			
Growth rate, kg/day	0.44	0.47	0.47	0.47	0.45	0.50	0.53	0.52	0.03	0.02	0.05

The trial included eight treatments groups of twelve Holstein bull calves fed one of two rates of milk replacer, 545 g and 654 g DM/day with either 23%, 25%, 27%, or 29% crude protein.

2.1.3. Fat requirement

Neither NRC (2001) nor NorFor report any specific minimum requirement for dietary fat content or for essential FA for calves, but Danish recommendations are a maximum of 50-60 g. crude fat per kg DM (Volden, 2011). And while Suarez-Mena *et al*. (2021) consider 16% fat in milk replacer inadequate because of low body fat deposition, Tiksofky *et al.* (2001) found no difference in growth performance of calves fed isocaloric and isonitrogenous diets with increasing fat concentration.

Garcia *et al.* (2014) evaluated the effects on growth and health parameters of varying milk replacer concentrations of the essential FA linoleic (18:2) and α-linolenic acid (18:3) in Holstein calves. While no specific requirements for essential FA are proposed elsewhere, Garcia *et al*. (2014) estimated dietary requirements for linoleic acids of at least 0.187 to 0.321 g linoleic acid per kg $BW^{0.75}$ and requirements for α-linolenic acid of at least 0.017 to 0.036 g per kg $BW^{0.75}$. This means that a calf of 70 kg BW would have a requirement of 4.5 to 7.7 g linoleic acid and 0.4 to 0.9 g α-linolenic.

2.2. Lipid digestion in calves

Despite the NRC (2001) and NorFor not mentioning specific dietary fat requirements for calves, fat from milk is a natural part of their diet (Litherland *et al.* 2019). During the pre-ruminant stage, the feed, which at this stage consists mainly of milk or milk replacer, is digested in the abomasum and small intestine. When the milk coagulates in the abomasum of calves, the fat is incorporated in the curd where it starts enzymatic digestion by pregastric lipase. The pregastric lipase acts only on the third position of the triglycerides to hydrolyze the ester bonds and produce diacylglycerols and free FA. Because the rumen epithelium is not functional to absorb FA in young calves, the free FA are absorbed in the small intestine. The diacylglycerides are further hydrolyzed in the small intestine by pancreatic lipase, while bile salts allow monoacylglycerols to cross the epithelial layer of the small intestine (Drackley, 2008).

The development of ruminal function allows for another route of lipid digestion as lipids originating from solid feed are exposed to the microorganisms in the forestomach. Lipids are hydrolyzed by microbial lipase, producing free FA. Following hydrolysis, biohydrogenation of unsaturated FA takes place, firstly by isomerization to *trans* formation, and secondly by hydrogenation of the double bonds to create either monounsaturated or saturated FA. For example, linoleic acid (*cis*-9, *cis*-12-18:2) is first

converted to CLA (*cis*-9, *trans*-11-18:2), then hydrogenated to *trans*-11-18:1 which is then hydrogenated to stearic acid (18:0) (Jenkins *et al*. 2008). The majority of SCFA and MCFA are absorbed over the epithelium in the rumen, while long-chain FA are usually passed on to the abomasum to be absorbed in the small intestine (Schmidely *et al.* 2008; Sjaastad *et al.* 2016). However, if the concentration of lipids in the rumen becomes high enough, absorption of long-chain FA in the rumen is possible (Schmidely *et al.* 2008).

2.3. Effects of dietary lipid supplements on calf performance

The effects of adding lipids into the milk replacer and starter feed have been investigated by numerous authors with contrasting results (Kuehn *et al.* 1994; Bascom *et al.* 2007; Hill, *et al*. 2007; Suarez-Mena *et al*. 2021).

Litherland *et al.* (2014) found that during a three week period with an average temperature below the thermoneutral zone of calves (5.3 ± 1.1° C), feeding Holstein and Holstein-cross calves milk replacer (28% CP, 15% fat) with 113 to 227 g fat supplement until 21 days of age (Table 2), increased ADG but did not affect body measures (hip height (HH), withers height (WH), body length (BL), heart girth (HG), and hip width) significantly, compared to calves without fat supplement. By day 42, the growth rate was similar amongst the calves (0.78, 0.76, and 0.82 kg/day for calves fed low-fat, 113 g, and 227 g fat supplement, respectively) and after weaning, the calves fed fat supplements had decreased HH, and a tendency for lowered WH and BL compared to calves fed low-fat diet (Litherland, *et al*. (2014). Also, the intake of starter feed recorded by Litherland *et al.* (2014) was affected by fat supplementation. Before weaning, intake of starter feed decreased with increasing fat supplement which led to equal intakes of ME across groups, while after weaning, starter intake was similar between groups. The reason for the similar growth rates at day 42 may be found in the starter feed intake. Calves fed low-fat milk replacer had a higher intake of starter feed, which is necessary for rumen development (Litherland *et al.* 2014). Therefore, their rumen development allowed them to digest sufficient nutrients from starter feed to sustain a growth rate similar to that of fat supplemented calves by day 42. Litherland *et al.* (2014) mentioned that the reduced HH may be a sign of reduced bone or muscle growth or both, but the growth rate in terms of BW gain after weaning was not reduced. According to Fonseca *et al.* (2017), HH is not only an indicator for skeletal growth but can also be an indicator for fat deposition. Accordingly, reduced HH could be perceived as a sign of decreased fat deposition after weaning due to delayed rumen development following fat supplementation and decreased starter intake, compared to calves fed low-fat diet.

Similarly, Hill *et al.* (2007) found that feeding Holstein calves a high-fat milk replacer (26% CP, 27% fat) at a high rate (817 g/day) from day 1 to 21 reduced growth compared to feeding a low-fat milk replacer (26% CP, 17% fat) at a high rate (908 g/day) from day 5 to 21, also during a period with temperatures below the thermoneutral zone (average 7.2° C). Feeding the high-fat milk replacer increased hip width change from day 0 to 14 (0.2 cm vs. 0.5 cm for calves fed low-fat milk replacer at 681 g/day compared to calves fed high-fat milk replacer at 817 g/day). Feeding the high-fat milk replacer reduced starter intake even after weaning and also growth rate after weaning. It was considered by Hill *et al.* (2007) that the poor response to the high-fat milk replacer might be due to a metabolic response to the high-fat concentrate or due to the CP:energy ratio being reduced from 54.5 g CP/Mcal DE in the low-fat milk replacer to 41.8 g CP/Mcal DE in the high-fat milk replacer.

Recently Suarez-Mena *et al*. (2021) investigated the effects of increasing the fat concentration of milk replacer. Milk replacers with 17% and 24% fat were fed to Jersey calves, at a low rate (465 g DM/day) and a high rate (656 g DM/day) (Table 2). The calves fed milk replacer with 24% fat had a lower starter feed intake and higher feed efficiency than calves fed the 17% fat milk replacer. Feeding a 24% fat milk replacer reduced the starter intake by 12% when feeding 465 g milk replacer daily, and by 13% when feeding 656 g milk replacer daily. However, feeding the 24% fat milk replacer did not affect the final growth rate, bodyweight, or body measures compared to the 17% fat milk replacer. For calves fed 656 g DM of milk replacer, the starter intake was lower, and the growth rate and final bodyweight were higher than for calves fed 465 g DM milk replacer, while body measures were similar. Suarez-Mena *et al.* (2021) mentioned that replacing carbohydrates with fat in milk replacer, might reduce protein deposition, and consequently, frame growth may also be reduced. Accordingly, no differences in body measures across groups were observed (Suarez-Mena *et al*. 2021).

The effect of varying both fat and protein levels in milk replacers has been investigated by Bascom *et al.* (2007). They demonstrated that increasing the fat content in milk replacer did not affect the percentage of carcass yield but feeding a milk replacer of 27% CP and 33% fat increased fat deposition compared to feeding a milk replacer with 29% CP and only 16% fat (Table 2). The same tendency has been observed by other authors and described by Tikofsky *et al*. (2001) as the more fat the calves consume, the more fat they deposit. Even though the calves fed the 27% CP, and 33% fat milk replacer had a higher energy intake than the calves fed 29% CP and 16% fat milk replacer (6.2 Mcal/kg vs. 5.3

Mcal/kg) the two milk replacers led to similar growth rates and feed efficiencies. Due to the higher energy intake in calves fed 27% CP and 33% fat milk replacer they were expected to have a higher growth rate.

Garcia *et al.* (2014) measured the growth performance of calves fed increasing concentrations of linoleic and α-linolenic acid. Four ratios of coconut oil and soybean oil were added to milk replacer to obtain four milk replacers with increasing amounts of linoleic and α-linolenic acid, but equal fat content (Table 3). Growth rate and feed efficiency were increased during the first 30 days of life for calves fed a moderate amount of the FA (0.187 g linoleic and 0.017 g α-linolenic per kg $BW^{0.75}$) but were not different across treatments at 60 days of age. Skeletal growth, measured by HH and WH change, was increased by feeding the two moderate concentrations of the FA (0.187 g linoleic and 0.017 g α-linolenic acid, and 0.320 g linoleic and 0.036 g α-linolenic acid). According to Garcia *et al.* (2014), the reason why growth rate and feed efficiency was decreased by feeding the highest amount of linoleic and α-linolenic is most likely due to the amount of linoleic acid exceeding the optimum range, which has been shown in growing rats to lead to impaired performance (Garcia *et al.* 2014).

Table 2. Treatments and results from day 1 to 21 from Litherland *et al.* (2014), Hill *et al.* (2007), Suarez-Mena *et al.* (2021), and Bascom *et al.* (2007).

Treatment	ADG, kg/day	Feed efficiency kg BW gain/kg DMI	Hip height change cm	Withers height change cm	Body length change cm	Heart girth change cm	Hip width change cm[a]	Group size	
	0.78	0.52	8.0	7.8	4.4	6.2	3.7	27 Holstein & Holstein -cross bulls and heifers	Litherland *et al.* 2014
+ 113 g fat supplement	0.76	0.58	7.4	6.7	3.5	6.5	3.6		
+ 227 g fat supplement	0.82	0.57	7.3	-	3.7	5.7	3.8		
26% CP, 17% fat	0.371	0.459	-	-	-	-	3.0	16 Holstein bull calves	Hill *et al.* 2007
	0.449	0.456	-	-	-	-	3.0		
22% CP, 27%fat	0.404	0.455	-	-	-	-	2.8		
Low rate 17% fat	0.53	0.45	15.7	-	-	-	4.8	25 Jersey heifer calves	Suarez-Mena *et al.* 2021
Low rate 24% fat	0.51	0.47	15.5	-	-	-	4.4		
High rate 17% fat	0.57	0.46	16.8	-	-	-	4.7		
High rate 24% fat	0.55	0.49	15.6	-	-	-	4.4		
20% protein, 20% fat	0.11	0.28	-	-	-	-	-	8 Holstein bull calves	Bascom *et al.* 2007
27% protein, 33% fat	0.36	0.55	-	-	-	-	-		
29% protein, 16% fat	0.37	0.57	-	-	-	-	-		
Whole milk	0.50	0.72	-	-	-	-	-		

[a] Hip width change in Hill *et al.* (2007) was measured on day 42.

[b] Results from Bascom *et al.* (2007) were measured on day 26.

Table 3. Concentrations of linoleic and linolenic acid added and results on growth and metabolic performance by Garcia *et al.* (2014). Results from day 0 to weaning at 60 days of age. Bodyweight gain and feed efficiency are shown for male calves only.

G per kg $BW^{0,75}$	0.119 g linoleic 0.007 g α-linolenic acid	0.187 g linoleic 0.017 g α-linolenic acid	0.321 g linoleic 0.036 g α-linolenic acid	0.593 g linoleic 0.076 g α-linolenic acid	P-value
Bodyweight gain, kg day 0 to 30	2.8	5.3	2.8	2.7	0.02
Bodyweight gain, kg day 0 to 60	26.2	26.6	26.5	26.8	0.90
Feed efficiency kg BW gain/kg DMI	0.49	0.52	0.50	0.52	0.38
Hip height change, cm	8.0	9.1	9.4	8.5	0.04
Wither height change, cm	7.6	8.6	9.1	8.3	0.04

It seems that most authors investigate the effects of unsaturated dietary fat. Garcia *et al.* (2014), evaluated the effect of the essential FA linoleic and α-linolenic acid, and Litherland *et al*. (2014) used a commercial fat supplement containing mainly unsaturated FA (>56% unsaturated FA) while Bascom *et al*. (2007) fed milk replacers with lard as fat source which contains 42 to 61% unsaturated FA (Spanski *et al.* 1996; Huuskonen *et al*. 2005).

In contrast, Karimi *et al.* (2021) compared the effects of unsaturated and saturated FA supplements and alfalfa hay in calf starter feed. Soybean oil (81% unsaturated FA) was used to supply unsaturated FA and rumen protected palm fat (96.6% saturated FA) was used to supply saturated FA (Karimi *et al*. 2021). Four isocaloric, isonitrogenous, and isolipidic diets containing either soybean oil or palm fat (3% of DM), and alfalfa hay (15% of DM) or no alfalfa hay were produced and fed *ad libitum* to four groups of 10 Holstein calves as shown in Table 4. Regardless of the alfalfa hay, calves fed palm fat diets had the highest growth rates and a tendency for higher feed efficiency (Table 4). The fat source did not affect HG, BL, WH, or HH before weaning, while calves fed soybean oil had a tendency for lower hip width and lower starter feed intake. After weaning, calves fed soybean oil had significantly lower starter feed intake (P = 0.03) (Karimi *et al*. 2021). Several possible reasons for the reduced starter feed intake of the calves fed soybean oil are proposed by

Karimi *et al.* (2021). Lower digestibility of organic matter in the soybean supplemented diets was measured (P = 0.01), and lower palatability has been suggested. It was also mentioned that fiber fermentation may be negatively affected by unsaturated FA, especially by linoleic and linolenic acid. This is due to these FA being toxic to rumen microorganisms, but also partially because fat can physically coat the fibers. Saturated FA are believed to be less toxic to rumen microorganisms because they rapidly form salts with metal ions (Karimi *et al.* 2021).

It was suggested that the reduced starter feed intake in combination with reduced digestibility, leads to reduced VFA production, and this might be the reason for reduced growth of calves fed soybean oil compared to calves fed palm fat. In addition, it was also claimed that the reduced growth could be due to the inflammatory properties of the FA found in soybean oil (Karimi *et al.* 2021). Karimi *et al.* (2021) concluded that calves' growth performance was improved by supplementing saturated FA.

Table 4 Results from Karimi *et al*. (2021). 40 Holstein calves supplemented with soybean oil or palm and either 0 or 15% of DM alfalfa hay for 63 days

	Soybean oil no alfalfa	Soybean oil + alfalfa	Palm fat no alfalfa	Palm fat + alfalfa	P-value		
					Fat	Alfalfa	Fat × alfalfa
ADG, kg/day	0.58[ab]	0.54[b]	0.60[ab]	0.67[a]	0.02	0.59	0.05
Starter intake, kg/day	0.55	0.44	0.54	0.64	0.09	0.85	0.04
Total DMI, kg/day	1.2	1.1	1.2	1.3	0.15	0.85	0.07
Feed efficiency, kg BW gain/kg DMI	0.49	0.50	0.50	0.54	0.06	0.74	0.51
Heart girth, cm	102.2	99.7	100.2	100.5	0.61	0.32	0.26
Body length, cm	62.8	60.1	61.1	63.6	0.57	0.99	0.10
Withers height, cm	98.6[a]	93.5[b]	95.4[ab]	97.7[a]	0.61	0.19	<0.01
Hip height, cm	95.6[a]	92.4[b]	94.2[a]	95.5[a]	0.30	0.22	0.01
Final hip width, cm	20.8	19.9	20.7	20.3	0.73	0.26	0.71

[a, b] Values in the same row with different superscripts are different (P < 0.05).

3. Experimental Study

With the hypothesis that the growth of calves may be improved by increasing the dietary content of FA, the objective of this study was to determine the effects of saturated and unsaturated dietary lipid supplements in milk replacer, on productive traits in calves.

3.1. Methods

3.1.1. Experimental design, animals, and diets

The experiment was performed at a beef farm located in Eskilstrup, Denmark (54°52'24.5"N, 11°56'23.8"E). Eighteen Holstein-Belgian Blue crossbred bull calves at 34 ± 8.0 days of age (± SD) were blocked by bodyweight (± SD) (70.3 ± 11.9 kg, 69.0 ± 11.5 kg, 70.2 ± 11.8 kg) and randomly assigned to receive one of three treatments.

Calves arrived at the farm on day 0, from five different dairy herds. Calves received a milk replacer (16% fat) and then, lipid supplements (BoviLM and Bovi85) were added at 5% of DM to the experimental groups. Calculations on the amount of lipid supplements added to the milk replacer can be found in Appendix I. Both BoviLM and Bovi85 are commercial feedstuffs for dairy cattle to be mixed in the feed or as topdressing on the feed. Bovi85 contained 45% palmitic acid (C16:0) and 40% oleic acid (C18:1) while BoviLM contained saturated FA 40-55% palmitic acid and 40-55% stearic acid (C18:0) (Table 5) (Lipitec, 2021).

Table 5 Chemical composition and fatty acids of Bovi85 and BoviLM fat supplements.

Chemical composition	**Lipid supplements**	
% in DM	**Bovi85**	**BoviLM**
g DM/day	58.2	50.6
DM	96.6	99.3
Ash	20.5	0
Fat	89.9	100
Fatty acid profile, %		
C14:0	1	<1.5
C16:0	45	40-55
C18:0	5	40-55
C18:1	40	<8
C18:2	9	<3

Lipitec, 2021

A commercial milk replacer (e-Lac aps, Broby, Denmark) (Table 6) was supplied to all groups. The control group was fed 811 g DM of milk replacer containing 16% fat, group Bovi85 were fed 753 g DM of milk replacer + 57.9 g DM Bovi85 fat supplement (Lipitec, NLM Vantinge, Ringe, Denmark) (Table 5), and group BoviLM were fed 753 g DM of milk replacer + 51.6 g DM BoviLM fat supplement (Lipitec, NLM Vantinge A/S, Ringe, Denmark) (Table 5). Group Bovi85 and BoviLM were fed the corresponding diets to achieve 21% fat in the diets. The fat supplements were fed over a 21-day period during November 2021.

Table 6 Composition of milk replacer and daily DM from milk replacer supplied to all calves.

Milk replacer	
Composition, in DM	
DM, %	96.6
Crude protein, %	24.0
Fat, %	15.7
Ash, %	6.0
Calcium, %	0.8
Phosphorus, %	0.78
Lignin, %	0.52
Cellulose, %	0.1
Iron, mg/kg	100
Zink, mg/kg	64
Selenium, mg/kg	0.3
Cupper, mg/kg	4
Manganese, mg/kg	64
Iodine, g/kg	0.16

e-Lac, 2022.

Calves in the control group were fed 811 g DM and calves from Bovi85 and BoviLM were fed 753 g DM from milk replacer per day.

3.1.2.Housing and feeding

All groups were housed in pens measuring 3.7 m × 3.6 m (13.32 m^2) and bedded with fresh straw supplied daily. The front of the pens was equipped with headlock panels where the calves were fixed during milk replacer feeding time. All groups had *ad libitum* access to water, meadow grass haylage, and starter feed (Table 7). Haylage was fed in hayracks placed in the wall of the pen. In each pen, the starter feed was supplied from a wood-made hopper feeder, with a capacity of 31 kg. Intakes of haylage and starter feed were monitored daily to ensure feed was always available. All calves were fed a fixed rate of three liters of milk replacer. Milk replacer was fed at around 40 ° C in buckets twice daily at 0800 h and 1700 h. After feeding, all calves were offered three liters of water with sodium chloride (1 g/L), sodium bicarbonate (0.5 g/L), dextrose (10.6 g/L), and E vitamin supplement (1.36 g/L) (Vitral, R2Agro A/S, Hedensted, Denmark).

Table 7 Composition and chemical composition of starter feed and haylage offered to all groups.

Ingredients, % of inclusion	Starter feed	Haylage
Pelleted concentrate[1]	40	
Wheat	30	
Barley	24	
Pea	6	
Grass		100
Chemical composition, % of DM		
Dry matter	84.2	69.9
Crude protein	21.0	11.9
Crude fat	3.4	-
Ash	8.0	5.9
NDF	20.7	70.4

[1] Pelleted concentrate consisting of 50% soybean meal, 25% dried sugar beet pulp, calcium carbonate, dried Brewers spent grain, sodium chloride, sugar beet molasses, pre-mix minerals, and vitamins. Per kg. containing 40000 i. e. A-vitamin, 8000 i. e. D3-vitamin, 300 mg. E-vitamin, 15 mg. cupper, 100 mg. manganese, 167 mg. zinc, 4.7 mg iodine, 2.0 mg selenium.

3.1.3. Measuring and monitoring

Calves' body condition scores, fecal scores, rectal temperature, and biometrics (BL, HG, WH, and HH) were measured at the beginning of the study (day 0) and at 7, 14, and 21 days.

Body condition scores were determined by palpation of the vertical and transverse processes of the spine and visual assessment of the calves on a scale from 1 (thin) to 5 (fat) with 0.5-unit increments (Rasby *et al*. 2014; Suarez-Mena, 2021). The fecal scoring was a visual evaluation of feces collected from the rectum of each calf, ranging from 1 = watery, 2 = thin, not watery 3 = thick, batter-like, 4 = less firm, 5 = firm, normal.

The calves were weighed on a cattle scale (Bjerringbro Vægte, Bjerringbro, Denmark). Measures of the BL, WH, HH, and (Figure 1) were measured using a measuring tape for cattle. Body length was measured as the distance between the cranial point of scapulae and the distal point of the pinbone (*tuber ischii*). Withers height was measured as the vertical distance between the ground and the highest point over the scapulae. Hip height

was measured as the vertical distance between the ground and the highest point over the hook bone (*tuber coxae*). Heart girth was measured as the circumference of the body just posterior to the front legs.

The average daily gain was calculated using the weekly gains. Intakes of starter feed and grass haylage were measured on pen level and were used to calculate feed efficiency (kg BW gain per kg DMI). Intakes were calculated based on the amount of starter feed and haylage offered and the amount of starter feed left in the feeder and haylage left in the hayrack.

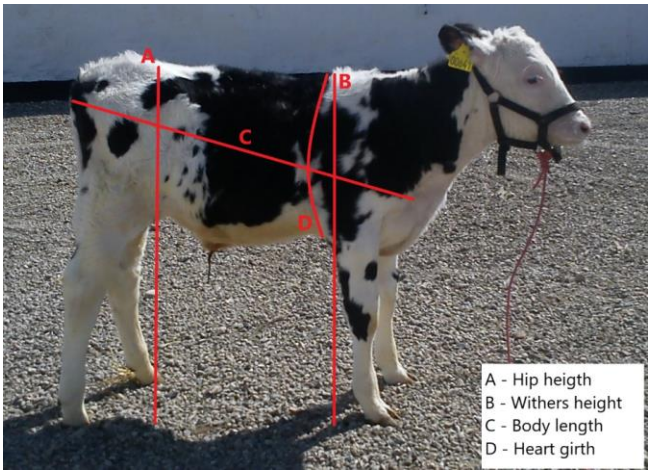

Figure 1 Illustration of recorded biometrics (own photo). A – hip height measured vertically from ground level to highest point over the hook bone (*tuber coxae*). B – withers height measured vertically from ground level to highest point over the *scapulae*. C – body length measured as the distance between the cranial point of *scapulae* and the distal point of the pinbone (*tuber ischii*). D – heart girth measured as the circumference of the body posterior to the front legs.

3.1.4. Statistical analysis

The software R (version 1.2.5042) was used to perform analysis of variance to determine differences in bodyweight gains, ADG, BCS, biometrics, fecal scores, rectal temperatures, feed intake. Descriptive statistics were done in Microsoft Excel. Significance was declared when the P-value < 0.05 and tendencies were declared where $0.05 \geq$ P-value ≤ 0.1. Shapiro-Wilk's normality test was performed to test for normal distribution of data. Normal distribution was declared at P-level > 0.05. For normal distributed data, a one-way analysis of variance was performed and in cases of significant variance, a Tukey's Honest Difference test was done for pairwise comparison of groups. For non-normal distributed data, Kruskal-Wallis test for variance was done, and when significant differences were detected, a Wilcoxon Rank Sum test was used for pairwise comparison of groups.

4. Results

4.1. Biometrics

Table 8 shows the changes in biometrics (an overview of the actual measures of biometrics are found in Appendix II). During day 0 to day 7 the change in HH was greatest in the control group (4.7 cm) and lowest in group BoviLM (0.7 cm) (P = 0.04) while through the entire treatment period, group Bovi85 had a tendency for lowered HH change (P = 0.1). No effect of time (P = 0.436) or interaction of time and treatment were detected (P = 0.35).

Group BoviLM had a lower total change in WH (P = 0.01) for the whole treatment period, and a lower change through day 0 to 7 (P = 0.03) compared to the control group. An effect of time on WH change was detected (P = 0.05), which was due to a reduced change in WH for all groups during day 7 to 14 compared to day 0 to 7. No interaction of time and treatment was detected (P = 0.4).

Change in BL through the entire treatment period was lowest in group BoviLM (P = 0.05) (Table 8). No differences between groups in the individual periods were detected. Body length changes across groups were lower (P = 0.01) through day 7 to 14 and day 14 to 21 compared to day 0 to 7. An interaction between period and treatment (P = 0.04) was detected and is visualized in the interaction plot in Figure 2. The plot shows that the largest change in BL for all groups was measured during day 0 to 7, with the change in this period being largest in group BoviLM.

Changes in HG did not differ significantly between groups (Table 8) but tended to be reduced in the control group (P = 0.10) during day 14 to 21. Changes in HG were not affected by time (P = 0.27) or interactions of time and treatment (P = 0.21).

Table 8 Changes in biometrics.

	Control	Bovi85	BoviLM	P-value		
				Treatment	Time	Time × Treatment
Hip height change, cm						
Day 0 to 21	9.3 ± 1.9	4.5 ± 1.5	6.1 ± 0.3	0.096	0.468	0.117
Day 0 to 7	4.7 ± 1.4[a]	1.0 ± 1.1[a,b]	0.7 ± 0.5[b]	0.038		
Day 7 to 14	2.3 ± 1.2	2.0 ± 0.7	3.8 ± 0.9	0.376		
Day 14 to 21	2.3 ± 0.99	1.5 ± 0.9	1.6 ± 0.4	0.714		
Withers height change, cm						
Day 0 to 21	7.7 ± 1.3[a]	3.8 ± 0.8[b]	3.0 ± 0.3[b]	0.008	0.048	0.421
Day 0 to 7	4.0 ± 0.9[a]	2.5 ± 0.9[a,b]	0.7 ± 0.3[b]	0.027		
Day 7 to 14	1.3 ± 1.0	0.2 ± 0.8	0.7 ± 0.7	0.464		
Day 14 to 21	2.33 ± 1.0	1.2 ± 0.5	1.7 ± 0.6	0.567		
Body length change, cm						
Day 0 to 21	8.7 ± 1.8[a]	4.5 ± 1.1[a,b]	3.7 ± 1.2[b]	0.048	0.007	0.044
Day 0 to 7	5.5 ± 1.9	3.8 ± 1.2	1.5 ± 0.6	0.244		
Day 7 to 14	0.7 ± 0.4[a]	0.2 ± 0.2[b]	1.5 ± 0.9[b]	0.391		
Day 14 to 21	2.5 ± 1.3[a]	0.5 ± 0.3[b]	0.7 ± 0.3[b]	0.319		
Heart girth change, cm						
Day 0 to 21	7.8 ± 1.0	6.7 ± 1.5	9.0 ± 0.9	0.396	0.272	0.206
Day 0 to 7	3.0 ± 0.9	1.3 ± 1.6	3.0 ± 1.1	0.544		
Day 7 to 14	2.5 ± 0.6	2.5 ± 0.6	1.3 ± 0.6	0.311		
Day 14 to 21	2.3 ± 0.7	2.8 ± 0.7	4.7 ± 0.8	0.097		

[a, b] Values in the same row with different superscripts differ between groups (P < 0.05).
All values are shown with SEM.

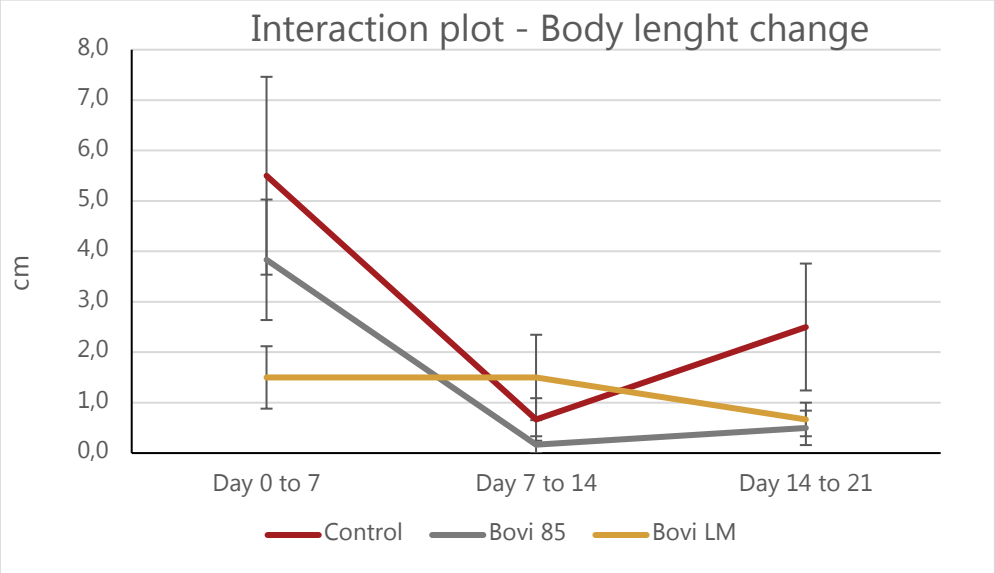

Figure 2 Interaction plot for the time × treatment interaction on change in body length. Changes in body length are affected by an interaction between group and period. Lines that are not parallel indicate an interaction between group and period.

4.2. Health

Rectal temperature was not affected by treatment (Table 9), or time (P = 0.24), and no interaction of time and treatment was detected (P = 0.45). No calves were found to have fever (rectal temperature > 39.5 °C (Garcia *et al.* 2014)) at any time during the treatment period.

While there were no significant differences in fecal scores between groups for individual periods (Table 9). There was a tendency (P = 0.09) for decreased fecal score on day 21 compared to day 7, and a tendency for decreased fecal score in group BoviLM (P = 0.07) compared to the control group overall during the 21 days treatment period was detected. No interaction of time and treatment was detected (P = 0.20).

4.3. Bodyweight gain and body condition score

Bodyweight gains are seen in Table 9 (averages for measured bodyweights are found in Appendix II). Through the entire treatment period, there was a tendency for lower BW gain and ADG in group Bovi85 (P = 0.08). Through day 14 to 21 group Bovi85 had significantly lower weight gain (P = 0.01) compared to the other groups. For both BW gain and ADG, there was an effect of time (P = 0.03). Pairwise comparison test shows that this is due to decreased weight gain across all groups through day 7 to 14 compared to day 0 to 7 and day 14 to 21. No interaction between time and treatment was detected for bodyweight gain or ADG (P = 0.32).

Body condition scores (Table 9) were not significantly different between groups or periods (P = 0.30), and no interaction of time and treatment was detected (P = 0.58), but group BoviLM had numerically higher scores on day 21.

4.4. Dry matter intake and feed efficiency

As seen in Table 9 the DMI for the entire 21 days period was not significantly different between the groups (P = 0.17) but was numerically lower in group Bovi85. Through day 0 to 7, the DMI was highest in group Bovi85 and lowest in group BoviLM (P <0.001) while during day 7 to 14 it was highest in the control group and lowest in group Bovi85 (P <0.001). Group Bovi85 tended to have decreased DMI through day 14 to 21 compared to the control group and group BoviLM (P = 0.08). DMI was affected by an effect of period (P = 0.03) as DMI across groups was significantly lower through day 7 to 14 compared to day 14 to 21 (P = 0.02). DMI was also affected by an interaction between time and treatment (P <0.001)

which is visualized in the interaction plot in Figure 3. The plot shows that the DMI for all groups was lowest during day 7 to 14, with group Bovi85 having the overall lowest DMI, while the overall highest DMI was measured for group BoviLM during day 14 to 21.

The feed efficiency (Table 9) over the entire period was numerically highest in group BoviLM, and lowest in group Bovi85 but this was not significant (P = 0.08). The feed efficiency was not significantly different between groups in any period, but during day 0 to 7 it was numerically highest in the control group (P = 0.69) and while group BoviLM had the highest feed efficiency during day 7 to 14 (P = 0.11) it was highest during day 14 to 21 in group Bovi85 (P = 0.97). Feed efficiency was not affected by time (P = 0.31) or by an interaction between time and treatment (P = 0.45).

Table 9 Results on BW gain, ADG, feed efficiency, and health parameters.

	Control	Bovi85	BoviLM	P-value		
				Treatment	Time	Time × Treatment
BW gain, kg						
Day 0 to 21	15.33 ± 1.99	10.17 ± 1.85	15.67 ± 1.48	0.082	0.028	0.322
Day 0 to 7	6.50 ± 2.32	4.50 ± 1.63	4.50 ± 1.57	0.689		
Day 7 to 14	2.17 ± 0.60	2.50 ± 0.85	4.17 ± 0.60	0.125		
Day 14 to 21	6.67 ± 0.92[a]	3.17 ± 0.95[b]	7.00 ±0.77[a]	0.014		
ADG, kg						
Day 0 to 21	0.73 ± 0.09	0.48 ± 0.09	0.75 ± 0.07	0.082	0.028	0.322
Day 0 to 7	0.93 ± 0.33	0.64 ± 0.23	0.64 ± 0.22	0.689		
Day 7 to 14	0.31 ± 0.09	0.36 ± 0.12	0.60 ± 0.09	0.125		
Day 14 to 21	0.95 ± 0.13[a]	0.45 ± 0.14[b]	1.0 ± 0.11[a]	0.014		
DMI[1], kg/day						
Day 0 to 21	1.27	1.11	1.24	0.171	0.031	<0.001
Day 0 to 7	1.19[a]	1.27[b]	1.15[c]	<0.001		
Day 7 to 14	1.20[a]	1.01[b]	1.09[c]	<0.001		
Day 14 to 21	1.42	1.07	1.44	0.08		
Feed efficiency (kg BW gain/kg DM)						
Day 0 to 21	0.58 ± 0.07	0.44 ± 0.08	0.60 ± 0.06	0.822	0.308	0.445
Day 0 to 7	0.78 ± 0.28	0.51 ± 0.18	0.56 ± 0.20	0.659		
Day 7 to 14	0.30 ± 0.08	0.41 ± 0.14	0.64 ± 0.09	0.114		
Day 14 to 21	0.44 ± 0.09	0.47 ± 0.15	0.43 ± 0.08	0.967		
Rectal temp. ° C						
Day 0	37.8 ± 0.40	38.6 ± 0.23	38.1 ± 0.17	0.177	0.244	0.451
Day 7	38.2 ± 0.21	38.5 ± 0.10	38.1 ± 0.31	0.562		
Day 14	37.8 ± 0.39	37.9 ± 0.31	38.2 ± 0.29	0.628		
Day 21	38.5 ± 0.12	38.4 ± 0.11	38.5 ± 0.13	0.842		
Fecal score,						
Day 0	3.7 ± 0.61	3.0 ± 0.73	3.5 ± 0.67	0.974	0.093	0.200
Day 7	4.2 ± 0.48	3.7 ± 0.21	2.8 ± 0.65	0.174		
Day 14	4.3 ± 0.49	2.8 ± 0.65	3.5 ± 0.22	0.112		
Day 21	3.2 ± 0.40	2.7 ± 0.67	2.2 ± 0.31	0.296		
Body condition score						
Day 0	3.80 ± 0.3	3.92 ± 0.4	4.2 ± 0.3	0.763	0.296	0.576
Day 7	3.46 ± 0.2	3.58 ± 0.3	3.7 ± 0.2	0.455		
Day 14	3.88 ± 0.2	3.54 ± 0.4	3.4 ± 0.2	0.441		
Day 21	3.54 ± 0.2	3.79 ± 0.3	4.0 ± 0.2	0.408		

[1]DMI includes daily DMI from milk replacer, lipid supplements, starter feed, and haylage.

[a, b, c] Values in the same row with different superscripts differ between groups (P < 0.05).

All values are shown with SEM.

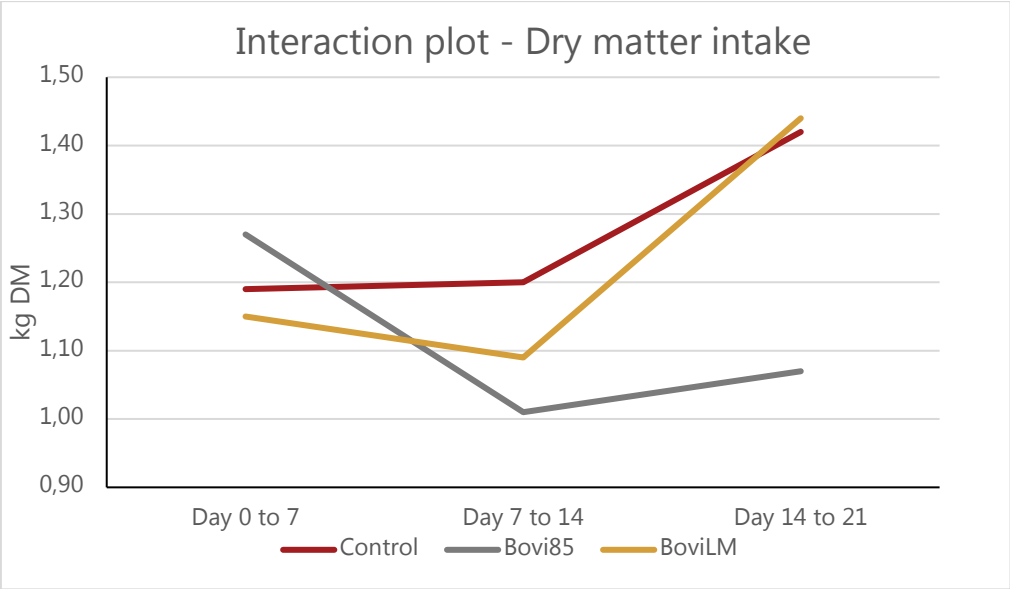

Figure 3 Interaction plot for the time × treatment interaction on DMI. Daily DMI is affected by an interaction between group and period. Lines that are not parallel indicate an interaction between group and period. p0 = day 0 to 7, p1 = day 7 to 14, p2 = day 14 to 21.

5. Discussion

5.1. Biometrics

The calves in group Bovi85 and BoviLM tended to have reduced structural body growth compared to the control group, only HG was increased in group BoviLM. Group BoviLM had the lowest change in BL (P = 0.05) and the overall lowest change in WH. Group Bovi85 had the numerically lowest change in HG and HH. Similarly, Suarez-Mena *et al.* (2020) reported reduced growth in HH from increasing milk replacer fat content. Also, Litherland *et al*. (2014) reported tendencies towards reduced HH, WH, and BL, but also reduced HG in calves. Litherland *et al.* (2014) suggested that increasing milk replacer fat content might lead to reduced muscle or bone growth or both, but this might be due to reduced starter feed intake. (Litherland, *et al.* 2014). Conversely, Mohtashami *et al*. (2021) reported increased BL in Holstein calves from feeding soybean oil compared to fish oil and no oil supplement, but no differences in heights. The increase in BL following soybean oil supplementation was attributed to the effect of PUFA on bone metabolism, while the lack of increased structural growth in calves fed fish oil, was ascribed to reduced nutrients available for growth due to decreased starter feed intake (Mohtashami *et al*. 2021).

Hip height, withers height, and body length are indicators of skeletal development and can also be used for estimating BW (Heinrichs *et al.* 1992; Enevoldsen and Kristensen, 1997). Hip height is mentioned by Fonseca *et al*. (2017) to be a good indicator

of empty body physically separable fat, while HG can be an indicator for carcass physical fat, and BL can be used to estimate subcutaneous fat (Fonseca *et al*. 2017). As is stated by Heinrichs *et al.* (1992), different anatomical points are used for measuring BL, but the exact points from which the BL is measured are not always mentioned by authors, making it difficult to compare BL across studies (Heinrich *et al*. 1992). While BL in the present study was measured from the cranial point of scapulae to the distal point of the pin bone (*tuber ischii*), Fonseca *et al*. (2017) measured BL from the cranial point of scapulae to the ventral point of the hook bone (*tuber coxae*). Heinrichs *et al.* (1992) also mention that WH and BL are rarely affected by fat deposition and body condition. Since the BCS in the present study were not different between groups but WH and BL growth were different, this could seem to be true. On the other hand, Belgian blue crossbreds, like the animals used in this experiment, inherit the myostatin gene leading to double muscling (Praharani *et al.* 2019). For double muscled Belgian blue bulls, the use of WH has been shown to be more accurate for estimating BW, than the use of HG, and in these animals, WH can also be indicative for muscle development (Coopman *et al.* 2009).

Karimi *et al*. (2021) did not mention how BL was measured but found no effect of lipid type on BL. However, similar to the reduced HH change, and reduced WH changes in the last two measuring days in group Bovi85, Karimi *et al.* (2021) also reported reduced HH and WH growth in calves fed unsaturated lipids. Similarly, Garcia *et al*. (2014) reported increased growth in HH and WH when increasing the concentration of unsaturated dietary FA from 0.12 g linoleic and 0.01 g linolenic acid per kg $BW^{0.75}$ to 0.32 g linoleic acid and 0.04 g α-linolenic acid per kg $BW^{0.75}$per day, while HH and WH were reduced when feeding 0.6 g linoleic and 0.08 g α-linolenic acid per kg $BW^{0.75}$. Karimi *et al.* (2021) fed soybean oil corresponding to 0.4 - 0.5 g linoleic acid per kg $BW^{0.75}$ and 0.06 - 0.07 g linolenic acid per kg $BW^{0.75}$ which led to reduced HH and WH, compared to feeding palm fat corresponding to 0.25 – 0.35 g linoleic acid and 0.03 – 0.05 g linolenic acid per kg $BW^{0.75}$ (Karimi *et al.* 2021). In this regard, the Bovi85 supplement, containing unsaturated FA, had 40% oleic acid and 9% linoleic acid, but no linolenic acid (Table 5). With 9% linoleic acid supplied, and an average BW across the period of 74.42 ± 13.25 kg the calves in group Bovi85 received on average 0.19 g linoleic acid per kg $BW^{0.75}$ per day from the lipid supplement. A FA profile of the starter feed and haylage supplied to the calves in the present study, would have revealed the total daily intake of both linoleic and linolenic acid. However, Garcia *et al*. (2014) suggest that calves can cover their requirements for essential FA through a daily intake of 500 grams of grain. In that case, it is possible that the intake of linoleic acid by

calves in group Bovi85 was exceeding their requirement. Since the Bovi85 supplement did not contain linolenic acid, this group did not receive more linolenic acid than the control group or group BoviLM, and it seems unlikely that the calves in the present study received amounts of linolenic acid similar to that supplied by Karimi *et al.* (2021) and Garcia *et al.* (2014).

The reason behind reduced structural growth reported in this and other studies in response to lipid supplementation may be found in the bioactivity of the supplemented FA. Watkins *et al*. (2001) reported that bone formation is increased by stimulatory effects on osteoblasts by dietary *n*-3 FA. A low ratio of *n*-6:*n*-3 PUFA has a stimulatory effect on osteoblasts, while a high ratio reduces osteoblast activity (Watkins *et al*. 2001). However, this does not explain the reduced growth in group BoviLM compared to the control group, as this group was not supplemented with *n*-6 PUFA (Table 5), while the supplement administered to group Bovi85 contain linoleic acid, and when only *n*-6 PUFA are supplied, it is unavoidable that the *n*-6:*n*-3 ratio is increased. Mohtashami *et al*. (2021) also fed a high ratio of *n*-6:*n*-3 PUFA by feeding soybean oil, which led to increased BL. However, as starter feed intake was increased when feeding soybean oil, it cannot be excluded that this might have affected the growth (Mohtashami *et al.* 2020).

5.2. Health

Rectal temperature, which increases in response to inflammation (Hill *et al.* 2011a), and fecal scores have been used as measures of health indices by Ballou and DePeters (2008) and Suarez-Mena *et al*. (2021).

In this study, fecal scores tended to be decreased on day 21, and while group BoviLM had the overall lowest fecal scores, group Bovi85 also had numerically lower scores than the control group. In previous studies, increasing dietary fat for unweaned calves does not usually lead to reduced fecal scores (Kuehn *et al.* 1994; Tikofsky *et al.* 2001; Litherland *et al.* 2014; Suarez-Mena *et al*. 2021). It is possible, that this is due to the calves not being able to fully digest the FA supplemented in the present study. According to Okada *et al*. (2009), the absorption rate of palmitic and stearic acid in newborn calves is only 42% and 26% respectively, and adding these FA to the milk replacer of 10 to 14 days old calves led to reduced fecal scores. The BoviLM supplement contained more or less only palmitic acid (40-55%) and stearic acid (40-55%) while the Bovi85 supplement contained 45% palmitic acid (Table 5), thus, the calves may not have been fully able to digest the supplements. When

the FA are not digested, their presence in the intestine causes water and sodium to leak into the intestine, leading to watery feces (Okada *et al*. 2009).

Why this issue has not been reported in other studies, may be due to differences in the fat sources supplied to calves. Animal fats are commonly used in milk replacers (NRC, 2001; Huuskonen *et al.* 2005; Bascom *et al*. 2007) but due to an industry agreement, the only animal fats allowed to use in milk replacers in Denmark are fats from milk and fish (SEGES, 2020b). Animal fats, like tallow and choice white grease as was used by Kuehn *et al.* (1994), Tikofsky *et al*. (2001), and Suarez-Mena *et al*. (2021), contain 23-25% palmitic acid and 13-19% stearic acid (NRC, 2001). Accordingly, animal fats contain less palmitic acid than the Bovi85 and BoviLM supplements, and less stearic acid than the BoviLM supplement, and may therefore have higher digestibility. In addition, the Bovi85 and BoviLM supplements are calcium soaps of palm fat (Lipitec, 2021). Calcium soaps are digested as free FA (Vandoni *et al.* 2010), and while it is stated by Okada *et al.* (2009) that the digestibility of free FA is lower than that of triglycerides, digestibility coefficients of calcium soaps between 60% and 100% have been reported (Vandoni *et al.* 2010). While the true digestibility of the lipid supplements used in the current study is not known, it is possible that the digestibility of the supplied FA was lower than the 42% and 26% reported for palmitic and stearic acid by Okada *et al*. (2009), and thus resulting in more watery feces than reported when using higher digestible lipids.

5.3. Bodyweight gain and body condition score

The bodyweight gain in the Bovi85 calves was lower without lowered BCS and overall lowered body size, only HH, and HG changes were smaller in this group.

By increasing the fat content of the milk replacer, the CP:energy ratio is decreased, which Hill *et al*. (2009) found could lead to reduced growth rates. The CP:energy ratio of the milk replacer fed to the control group was 52.8 g CP/Mcal ME, which is within the optimal range of 51.5 to 55.0 g CP/Mcal ME estimated by Hill *et al.* (2009). The CP:energy ratio of the Bovi85 and BoviLM milk replacers are both below this range, with 47.1 and 46.9 g CP/Mcal ME respectively. This may have affected the growth rates and caused the reduced growth rate in the Bovi85 calves. Calculations of CP:energy ratios are found in Appendix III.

Welboren *et al.* (2021) reported increased weight of the GIT following increased dietary content of palm and coconut oil and reduced lactose content of milk replacer. Increased GIT weight indicates improved development and increased capacity for

digestion (Welboren *et al.* 2021). The DMI in group Bovi85 was numerically lower than in the other groups which could be due to reduced digestibility, and together with the lowered weight gain without lowered body size in group Bovi85, this could indicate that the development and weight of the GIT were reduced in these calves compared to the other groups.

Welboren *et al.* (2021) also observed increased intestinal permeability following dietary lipid supplementation, probably due to the high content of PUFA in the supplemented lipids. PUFA, as the Bovi85 supplement also contained, are prone to oxidation which can cause oxidative stress in the intestine, consequently causing damage to the plasma membranes of the epithelial cells, leading to apoptosis (Welboren *et al.* 2021). Increased intestinal permeability is related to increased inflammatory response, which reduces the energy available for growth (Devant and Marti, 2020). This could probably also result in impaired development of the GIT. Therefore, the decreased weight gain in group Bovi85 could also be due to less energy available for growth because of inflammation in response to increased intestinal permeability caused by oxidative stress.

Moreover, Karimi *et al.* (2021) considered that the inflammatory properties of the unsaturated FA in soybean oil could be the cause of reduced weight gain in calves fed soybean oil compared to calves fed palm fat (Karimi *et al*. 2021). Similarly, Hill *et al*. (2011b) observed decreased growth rates in calves fed lipid supplements containing soybean oil, presumable due to the inflammatory properties of linoleic acid (Hill *et al*. 2011b). The Bovi85 supplement contained only 9% linoleic acid (Table 5), but as it did not contain any linolenic acid, this could have increased the dietary ratio of linoleic and linolenic acid to exceed the ratio of 10 which is mentioned by Hill *et al.* (2011b) to be detrimental. This is due to the inflammatory properties and interference of linoleic acid with the conversion of linolenic acid to eicosapentaenoic acid (C20:5 *n*-3) and docosahexaenoic acid (C22:6 *n*-3) which, in contrast, are anti-inflammatory (Hill *et al.* 2011b; Urrutia *et al.* 2020).

Body condition scores are based on visual assessment and palpation of the vertical and transverse processes of the spine of the calves. As the dietary fat content, and thereby the energy content of the diets was increased in groups Bovi85 and BoviLM, an increase in BCS was initially expected. However, this was not the case as BCS were not significantly different between groups. When assessing the BCS, the deposition of subcutaneous fat is evaluated (Fiems *et al*. 2006). Adipose tissue in cattle is firstly deposited on the kidneys and the pelvic cavity, secondly intermuscular and subcutaneous deposition is prioritized, followed by intramuscular deposition (Urrutia *et al*. 2020). Even though the bone

processes palpated when assessing the BCS are covered by muscle tissue and not adipose tissue in double muscled animals, Fiems *et al.* (2006) found that regular BCS scales were still applicable in double muscled animals.

As previously mentioned, it is possible that unsaturated FA in the Bovi85 supplement were causing inflammation in the intestine and thereby limiting the energy available for growth, hence these calves might not have deposited considerably more muscle and adipose tissue than the control group. The BoviLM group may have improved GIT characterized by increased weight gain and DMI, which can have led to a further increase in dietary energy intake. However, Welboren *et al.* (2021) also mentioned that increased GIT development can be related to increased energy expenses, which could have decreased the energy available for deposition of muscles and adipose tissue. Furthermore, the HG was increased in group BoviLM compared to the other groups (Table 8) which have contributed to a larger body surface of these calves, under the assumption that the body represents the shape of a cylinder (Fonseca *et al.* 2017). Calculations of body surface area can be seen in Appendix IV. The surface area of the body affects the maintenance energy requirements because a larger surface is related to larger heat loss (Fonseca, *et al.* 2017). The estimated body surface of the BoviLM calves of 4.37 m^2 was larger than the estimated body surface of the control and Bovi85 calves with 3.35 m^2 and 4.27 m^2 respectively. This trial was conducted during a period with the environmental temperature ranging from – 2.7° C to 13.8 ° C and an average temperature of 7.5 ° C (DMI, 2022), and thus the calves were exposed to temperatures below the lower critical temperatures of 8 ° C to 15 ° C reported by the NRC (2001) and Litherland *et al.* (2014). Due to the larger body surface of the BoviLM calves, they may have had a larger energy requirement due to increased heat loss, and less energy available for deposition, hence the BCS of these calves were not considerably different from the control calves despite higher energy intake.

5.4. Dry matter intake and feed efficiency

Despite the numerically reduced DMI in group Bovi85, the DMI was not significantly reduced following the lipid supplementation of group Bovi85 and BoviLM. Other studies reported decreased DMI following lipid supplementation (Hill *et al*. 2007; Litherland *et al*. 2014; Suarez-Mena *et al*. 2021). Increased energy intake from supplemental lipids, leading to increased satiety can be expected to reduce the DMI from starter feed (Litherland *et al.* 2014). As mentioned earlier, intake of solid feed is necessary for development of the GIT

(NRC, 2001; Baldwin *et al.* 2004; Drackley, 2008; Khan *et al.* 2016), and thus decreased starter feed intake may delay the development of the GIT (Suarez-Mena *et al.* 2021).

Instead of decreasing the DMI in response to increased satiety signaling from increased energy intake and oxidation of FA (Karimi *et al*. 2021), the cold stress experienced by the calves may have counteracted the appetite reducing effects of lipid supplementation, as it is stated by Litherland *et al*. (2014) that the increased energy requirements during cold stress, increases the appetite. However, increased feed intake in response to cold stress leads to increased passage rate and thereby reduced digestibility (Litherland *et al.* 2014). Both Bascom *et al.* (2007), Litherland *et al*. (2014) and Suarez-Mena *et al.* (2021) report increased feed efficiency from increasing the fat content of milk replacer. In the current study, the feed efficiency was not significantly different between groups (Table 9). As the DMI was also similar across groups, but the energy intake from milk replacer was expected to be higher in the Bovi85 and BoviLM groups, the similar feed efficiencies could indicate a low digestibility of the lipid supplements as the energy was not utilized for growth. But it could also reflect a lower stage of GIT development in the Bovi85 calves, as addressed earlier, as the weight of the GIT also affects the BW. Also, the energy expenses related to a potential inflammation caused by oxidative stress in the Bovi85 calves, and maintenance of larger amounts of GIT tissue and heat loss in the BoviLM calves, may account for the lack of improved feed efficiency that was initially expected from increasing fat content of the milk replacer.

6. Conclusions

Increasing the fat content of milk replacer reduced some biometric measures, but did not significantly affect bodyweight gains, body condition scores, feed efficiency, or health parameters, and did not reduce dry matter intake from solid feed. Further investigations on the effects of increasing the fat content of diets for preweaned calves are needed in order to propose recommendations on fat supplementation of calves.

In the present study, the body length and withers height were reduced in calves fed a saturated FA supplement. In calves fed a supplement rich in PUFA, heart girth, and hip height was reduced which may be ascribed to the dietary inclusion of *n*-6 polyunsaturated FA that can reduce bone formation.

Fecal scores and rectal temperatures were recorded as parameters of health. The rectal temperatures were constant during the trial period, while the fecal scores tended to decrease at day 21 and were slightly lower in calves fed lipid supplements based on saturated FA which may have been caused by low absorption rates of palmitic and stearic acid.

The lack of improvement in growth and feed efficiency may be ascribed to differences in energy requirements arising from differences in body sizes, gastrointestinal tract development, and inflammatory responses, caused by the supplemented lipids.

For future investigations in lipid supplementation of young calves, blood samples to analysis of markers of immune response and inflammation, along with markers of metabolism could provide further insight into the effects of lipid supplementation. Determination of digestibility of lipid supplements in young calves could also be beneficial. Also, for further investigations especially with regards to the use of PUFA, performing tests for measuring gut permeability could be of interest.

7. Perspectives

Many of the discussed topics are based on considerations, and additional registrations and tests could have strengthened the basis for discussion.

As calves originate from different farms, most likely with varying accessibility to solid feed, and varying types of solid feed offered, some animals may have been more accustomed to solid feed than others and may have had a higher intake. Differences in individual solid feed intakes have not been accounted for in this study, as equipment for registration of individual intakes during group housing is needed to do so.

This study was conducted during a 21-day period in November, which is a relatively short time. Feeding lipid supplements over a longer period could be beneficial to evaluate long-term effects and the response during weaning. Also, lipid supplementation during other environmental conditions should be evaluated, such as during the thermoneutral zone of calves.

Due to increased energy requirements during the cold season, it is recommended to increase the amount of milk replacer fed to calves in the winter (Martin, 2015). Fat supplementation could probably be used as a strategy to prepare the calves for the cold season, as fat deposition is important for heating and thermal insulation (Urie *et al.* 2018). By increasing the dietary energy intake before the environmental temperature falls below the thermoneutral zone and the energy requirements increase, the calves could deposit more adipose tissue and be better prepared for the cold environment, which might improve growth.

Even though there were no noteworthy differences in growth of the calves fed saturated fatty acids and the calves without, there might still be an economic advantage of the saturated fat supplement because the fat supplemented calves were fed less milk replacer. Calculation of daily economy is done below, without accounting for numerically lower DMI and higher BW gain by BoviLM calves.

- The price of BoviLM supplement is 182.25 DKK/25 kg = 7.57 DKK/kg (Linds.dk, n.d.).
- 51.6 g of BoviLM were fed per day. 51.6 g/day × 7.57 DKK/kg = 0.39 DKK/day.
- The price of the milk replacer used was 21 DKK/kg (Christensen, 2022).

- Control calves were fed 0.84 kg milk replacer. 0.84 kg/day × 21 DKK = 17.64 DKK/day.
- Calves fed BoviLM were fed 0.78 g milk replacer. 0.78 kg/day × 21 DKK = 16.38 DKK/day.
- The price of feeding the BoviLM calves was 16.38 + 0.39 = 16.77 DKK/day, compared to the 17.64 DKK/day for feeding the control calves. The daily saving per animal is 0.87 DKK.

Anticipating that calves grow according to our findings, and there is an economic advantage, it seems feasible to believe that farmers would be keen to implement the use of fat supplements into their calf feeding strategies as this strategy is also simple to use in practice and the fat supplements are available at conventional retailers of feed and agricultural articles. However, it seems unsure whether fat supplementation of young calves is a strategy that will be put into practice within the near future. Currently, increasing prices on energy, raw materials, and shipping costs, are causing feed prices to increase (Iversen, 2021). Therefore, it is uncertain that an economic advantage of adding fat supplements will persist. Also, the supplements used in this study, are both based on palm fat. And while fat supplementation is proposed as a strategy to reduce the climate impact from cattle production as a step in the direction of a climate-neutral food industry by 2050 (Landbrug & Fødevarer, n.d.), the use of palm fat is criticized due to the environmental impact of production (Vijay *et al*, 2016; Landbrugsavisen, 2021; Ankersen, 2022). If climate-friendly alternatives to palm fat could yield the same, or better, results as this study, that would still be economically responsible, it seems realistic that this strategy would be implemented by farmers.

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Appendix I - Calculation of fat supplements

The following information was used to calculate the amount of lipids, supplemented to match both the DM and fat of the milk replacer and the amount of liquid feed given, in order to reach 21% fat in the milk fed to the two experimental groups (group Bovi85 and BoviLM). It was desired that all groups received the same amount of DM and liquid feed.

- The Bovi85 had 96.60% DM and 89.70% fat of DM.
- The BoviLM had 99.28% DM and 99.28% fat of DM.
- 140 g of milk replacer was per liter of milk for the control group.
- The dry matter content of the milk replacer was 96.60% and contained 15.7% of DM.

Bovi 85 supplement:

Milk Replacer: 140 g L^{-1},

$x + y = 140$,

$y = 140 - x$

$$0.966 * 0.157 * x + 0.97 * 0.897 * y = 0.21(0.966x + 0.97y)$$

$$\rightarrow\ 0.152x + 0.870y = 0.203x + 0.204y$$

Replacing $140 - x$ in place of y:

$$0.152x + 0.870(140 - x) = 0.203x + 0.204(140 - x)$$

$$\rightarrow 0.152x + 121.8 - 0.870x = 0.203x + 28.56 - 0.204x$$

$$\rightarrow 93.24 = 0.717x \rightarrow x = 130.0g$$

$$\rightarrow y = 10.0g$$

Checking for fat content:

$$0.966 * 0.157 * 130.0 + 0.97 * 0.0897 * 10 = 19.7 + 8.7$$

$$= 28.4$$

$$DM = 0.966 * 130 + 0.97 * 10 = 135.28 \cong 135.3\ g\ DM$$

$$\rightarrow [fat] = \frac{28.4}{135.3} * 1000 \cong 210 g/kg^{-1}\ DM$$

$$Milk\ Replacer\ (x) = 130 g/L^{-1} \rightarrow 3 * 130 = 390g$$

$$Bovi\ 85\ (y) = 10 g/L^{-1} * 3 = 30g$$

Therefore, the calves in group Bovi85 were given 30 g of Bovi85 each, twice daily, meaning that each calf received 60 g/day.

Bovi LM supplement:

Milk Replacer: 140 g L^{-1}

$x + y = 140$

$y = 140 - x$

$$0.966 * 0.157 * x + 0.99 * y = 0.23(0.966x + 0.99y)$$

$$\rightarrow 0.152x + 0.99y = 0.203x + 0.208y$$

Replacing $140 - x$ in place of y:

$$0.152x + 0.99(140 - x) = 0.203x + 0.208(140 - x)$$

$$138.6 - 29.12 = 0.833x$$

$$x = 131.4g \rightarrow y = 140 - 131.4 = 8.6g$$

Checking for fat content:

$$0.966 * 0.157 * 131.4 + 0.99 * 1 * 8.6 = 28.44g\ fat$$

$$DM = 0.966 * 131.4 + 0.99 * 8.6 = 135.45\ g\ DM$$

$$[fat] = \frac{28.44}{135.45} * 1000 = 210g/kg^{-1}\ DM$$

$$Milk\ Replacer\ (x) = 3 * 131.4g/L^{-1} = 394.2g$$

$$Bovi\ LM\ (y) = 3 * 8.6g/L^{-1} = 25.8g$$

Therefore, the calves in group BoviLM were given 25.8 g of BoviLM each, twice daily, meaning that each calf received 51.6 g/day.

Appendix II – Averages of Measured Bodyweights and Biometrics

Average bodyweights and biometrics (± SEM) measured on day 0, 7, 14, and 21.

Group	**Control**	**Bovi85**	**BoviLM**
Bodyweight, kg,			
Day 0	70.3 ± 4.9	69.0 ± 4.7	70.2 ± 4.8
Day 7	76.8 ± 6.5	73.5 ± 5.4	74.7 ± 3.7
Day 14	79.0 ± 6.1	76.0 ± 5.9	78.8 ± 3.9
Day 21	85.7 ± 5.8	79.2 ± 5.9	85.8 ±4.4
Hip height, cm,			
Day 0	82.8 ± 3.4	87.5 ± 1.3	87.2 ± 1.28
Day 7	87.5 ± 2.2	88.5 ± 1.4	87.9 ± 1.3
Day 14	89.8 ± 1.6	90.5 ± 1.1	91.7 ± 1.2
Day 21	92.2 ± 1.7	92.0 ± 1.8	93.3 ± 0.9
Withers height, cm,			
Day 0	79.0 ± 2.3	81.2 ± 2.3	82.7 ± 1.1
Day 7	83.0 ± 2.2	83.7 ± 1.7	83.3 ± 1.1
Day 14	84.3 ± 2.0	83.8 ± 1.9	84.0 ± 1.0
Day 21	86.7 ± 1.7	85.0 ± 17	85.7 ± 1.0
Heart girth, cm,			
Day 0	93.3 ± 3.0	93.8 ± 2.8	94.3 ± 2.6
Day 7	96.3 ± 2.9	95.2 ± 2.0	97.3 ± 1.7
Day 14	98.8 ± 2.8	97.7 ± 2.2	98.7 ± 2.0
Day 21	101.2 ± 2.5	100.5 ± 2.6	103.3 ± 2.0
Body length, cm,			
Day 0	77.7 ± 2.0	80.5 ± 1.8	79.3 ± 1.2
Day 7	83.2 ± 1.9	84.3 ± 1.7	80.8 ± 1.3
Day 14	84.6 ± 2.2	84.7 ± 2.4	82.3 ± 1.8
Day 21	86.2 ± 1.6	85.0 ± 1.7	83.0 ± 1.7

Appendix III – Calculations of CP:energy ratios

NorFor and the NRC (2001) both propose equations for estimating the ME of feedstuffs, based on the content of fat, protein, and carbohydrates. Estimation of ME using the equations by NorFor may be more accurate due to the inclusion of total tract digestibility of the nutrients, but as these are unknown factors, the NRC's simple equation for calculating ME in milk replacer is used here:

$$\text{ME, Mcal} = 0.93 * \text{GE, Mcal}$$

The energy content of the milk replacer is stated to be 148 FE (feed units) per kg. As feed units are NE, this must be converted to ME, which is easiest done from the gross energy (GE) with the NRC equation for milk replacers:

$$\text{GE, Mcal} = 0.057 * \text{CP\%} + 0{,}092 * \text{Fat\%} + 0.0395 * \text{Lactose\%}$$

The CP and fat percentage, along with the other nutrient in the table below, are stated by the manufacturers of the milk replacer and fat supplements, and the lactose content is then estimated. In the table below, GE and ME are calculated using the equations mentioned, and the CP:energy ratio is then calculated.

Milk replacer contents			
	Control	**Bovi85**	**BoviLM**
Composition, % in DM			
Crude protein, %	24.0	22.3	22.5
Ether extract, %	15.7	21.2	21.4
Ash, %	6.0	7.0	6.0
Calcium, %	0.8	0.74	0.75
Phosphorus, %	0.78	0.72	0.73
Sodium, %	0.52	0.48	0.49
Lignin, %	0.1	0.09	0.09
Lactose, % estimated	51.80	47.47	48.04
GE, Mcal/kg	4.89	5.10	5.15
ME, Mcal/kg	4.54	4.74	4.79
CP, g/kg	240	223	225
CP:energy ratio, g CP/Mcal ME	52.82	47.05	46.99

Appendix IV – Calculation of Body Surface

Under the assumption that the body of the calves has the shape of a cylinder, the body surface is calculated as: $A_{surface} = 2 * \pi * r * h + 2 * \pi * r^2$

$r = \text{radius} = \frac{1}{2} * \text{heart girth}$

$h = \text{height} = \text{body length}$

Body surface of control calves:

Heart girth, day 21: 101.20 cm, $r = \frac{1}{2} * 101.20\ cm = 50.60\ cm,$

$r = 50.60\ cm$

Body length, day 21: 86.20 cm

$h = 86.20\ cm$

$A_{surface} = 2 * \pi * r * h + 2 * \pi * r^2$

$\Leftrightarrow 2 * \pi * 50.60\ \text{cm} * 86.20\ \text{cm} + 2 * \pi * 50.60^2 = 43{,}492.71\ cm^2$

$\frac{43{,}492.71\ cm^2}{10{,}000} = 4.35\ m^2$

The body surface of control calves is 4.35 m^2.

Body surface of Bovi85 calves:

Heart girth, day 21: 100.50 cm, $r = \frac{1}{2} * 100.50\ cm = 50.25\ cm,$

$r = 50.25$ cm

Body length, day 21: 85.00 cm

$h = 85.00$ cm

$A_{surface} = 2 * \pi * r * h + 2 * \pi * r^2$

$\Leftrightarrow 2 * \pi * 50.25\ \text{cm} * 85.00\ \text{cm} + 2 * \pi * 50.25^2 = 42{,}702.49\ cm^2$

$\frac{42{,}702.49\ cm^2}{10{,}000} = 4.27\ m^2$

The body surface of control calves is 4.27 m^2.

Body Surface of BoviLM calves:

Heart girth, day 21: 103.33 cm, $r = \frac{1}{2} * 103.33\ cm = 51.67\ cm$,

$r = 51.67$ cm

Body length, day 21: 83.00 cm

$h = 83.00$ cm

$A_{surface} = 2 * \pi * r * h + 2 * \pi * r^2$

$\Leftrightarrow 2 * \pi * 51.67\ \text{cm} * 83.00\ \text{cm} + 2 * \pi * 51.67^2 = 43{,}720.91\ cm^2$

$\frac{43{,}720.91\ cm^2}{10{,}000} = 4.37\ m^2$

The body surface of control calves is $4.37\ m^2$.