**University of São Paulo
“Luiz de Queiroz” College of Agriculture**

**The role of colostrum for the newborn thermogenesis and feeding strategies
for calves raised in low temperatures**

Fernanda Lavínia Moura Silva

Thesis presented to obtain the degree of Doctor in Science.
Area: Animal Science and Pastures

**Piracicaba
2018**

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versão revisada de acordo com a resolução CoPGr 6018 de 2011

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1. Programa alimentar 2. Tolerância ao frio 3. Termogênese 4. Desempenho 5. Economia I. Título

DEDICATION

To God for the unconditional love.

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SUMMARY

	RESUMO	8
	ABSTRACT	9
1	INTRODUCTION	11
	REFERENCES	12
2	LITERATURE REVIEW	13
2.1	Factors affecting calf survival	13
2.2	Ambient temperature and thermoneutrality	14
2.3	Mechanisms of thermogenesis	15
2.4	Colostrum as an energy source for newborn calves' thermogenesis	17
2.5	Management practices for young calves' thermogenesis	20
2.6	Requirements for calves' maintenance under thermoneutral conditions	21
2.7	Cold weather feeding strategies	23
2.8	Costs of feeding systems	25
	REFERENCES	26
3	THERMOREGULATION AND PERFORMANCE OF DAIRY CALVES FED DIFFERENT AMOUNTS OF COLOSTRUM	31
	ABSTRACT	31
3.1	INTRODUCTION	31
3.2	MATERIAL AND METHODS	33
3.3	RESULTS AND DISCUSSION	36
3.4	CONCLUSION	48
	REFERENCES	49
4	PERFORMANCE AND PRODUCTION COST OF CALVES FED MILK REPLACER OR WHOLE MILK AND TRADITIONAL OR AN ALTERNATIVE STARTER FEED PROGRAM IN LOW ENVIRONMENTAL TEMPERATURE	53
	ABSTRACT	53
4.1	INTRODUCTION	54
4.2	MATERIAL AND METHODS	55
4.3	RESULTS AND DISCUSSION	58
4.4	CONCLUSION	72

	REFERENCES	72
5	FINAL CONSIDERATION	77

RESUMO

O papel do colostro na termogênese de bezerros neonatos e estratégias alimentares para bezerros criados em baixas temperaturas

Bezerros leiteiros necessitam de nutrientes para manutenção e crescimento, contudo, em baixas temperaturas, o corpo altera os processos fisiológicos para controlar a temperatura corporal por meio da termogênese, aumentando a exigência. Neste sentido, se torna importante uma melhor compreensão de como diferentes manejos alimentares atuam na termogênese, no desempenho e na saúde de bezerros criados em temperaturas abaixo das condições de termoneutralidade. Com base nisso, dois estudos foram realizados. O primeiro estudo avaliou a termorregulação, o desempenho e os metabólitos sanguíneos de trinta bezerros recém nascidos alimentados com 10%, 15% ou 20% de colostro em porcentagem de peso corporal. Vinte e quatro horas após o nascimento, os bezerros foram colocados em uma câmara de temperatura controlada a 10°C por 150 min. Após o desafio do frio, os bezerros foram individualmente alojados em instalação à temperatura ambiente ($26,8 \pm 5,9^{\circ}\text{C}$) até o desaleitamento. Bezerros que receberam 15% ou 20% de colostro exibiram aumento das respostas termorreguladoras durante o desafio pelo frio e aumento das respostas de imunidade durante o aleitamento. O segundo estudo comparou o desempenho e os custos de produção de 75 bezerras alimentadas com sucedâneo (S) ou leite (L) e uma ração tradicional (RT) ou uma ração inicial alternativa (RA) durante baixas temperaturas ambientais ($1,4 \pm 9,2^{\circ}\text{C}$). As bezerras foram designadas para um dos cinco tratamentos em fatorial $2 \times 2 + 1$. O primeiro tratamento foi o controle negativo (CN), 4 L/d de S do d1 ao 49; 2L/d de S do d 50 ao 56 do estudo e RT *ad libitum* (ração comercial texturizada). Os outros quatro tratamentos foram alta taxa (AT) de S ou L (6L/d do d 1 ao 7, 8 L/d do d 8 ao 35, 2L/d do d 36 ao 42, e 1 L/d do d 43 ao 49 do estudo) e RA *ad libitum* (milho quebrado do d 1 ao 21, ração de crescimento com baixa proteína do d 22 ao 28, ração de crescimento alta proteína do d 29 ao 49 do estudo) ou RT. Após o desaleitamento, os animais foram mantidos em baias coletivas até 12 semanas de vida. Dieta líquida restrita promoveu maior eficiência econômica. No entanto, o peso final foi maior para bezerras alimentadas com ATRT. Adicionalmente, alimentar bezerras com maior volume de L aumentou o crescimento e diminuiu morbidade. Além disso, substituir RT comercial por uma RA em altas taxas de nutrição apresentou mínimo impacto no desempenho.

Palavras-chave: Programa alimentar; Tolerância ao frio; Termogênese; Desempenho; Economia

ABSTRACT

The role of colostrum for the newborn thermogenesis and feeding strategies for calves raised in low temperatures

Dairy calves require nutrients for maintenance and growth, but in cold weather, the body alters physiologic processes to control body temperature through thermogenesis, which increases its requirements. In this regard, it is important a better understanding of how different feeding managements act in the calf thermogenesis, performance and health when raised in temperatures below thermoneutral conditions. Based on that, two studies were performed. The first study evaluated thermoregulation, performance and blood metabolites in thirty newborn calves fed 10%, 15% or 20% BW of colostrum. At 24h of life, calves were placed in a temperature-controlled chamber at 10°C for 150 min. After the cold challenge, calves were individually housed in ambient temperature facilities ($26.8 \pm 5.9^{\circ}\text{C}$) until weaning. Calves given 15% or 20% of BW as colostrum exhibited increased thermoregulatory responses during cold challenge and increased immunity responses during preweaning. The second study compared performance and production cost of 75 calves fed milk replacer (MR) or whole milk (WM) and a traditional starter (TS) or an alternative starter (AS) during low environmental temperatures ($1.4 \pm 9.2^{\circ}\text{C}$). Calves were assigned to one of five treatment groups in a $2 \times 2 + 1$ factorial. The first treatment was a negative control (NC), 4 L/d of MR from d1 to 49; 2L/d of MR from d 50 to 56 of the study and *ad libitum* TS (commercial texturized ration). The others four treatments were a high rate (HR) of MR or WM (6L/d from d 1 to 7, 8 L/d from d 8 to 35, 4L/d from d 36 to 42, and 2 L/d from d 43 to 49 of the study) and *ad libitum* AS (cracked corn from d 1 to 21, low protein grower from d 22 to 28, high protein grower from d 29 to 49 of the study) or TS. After weaning, animals were maintained in group hutches in the same environment until 12 weeks of life. Restricted liquid feeding provided higher economic efficiency. However, the final BW was higher for calves fed HRTS. In addition, feeding calves higher volumes of WM increased growth and decreased morbidity. Besides, replacing commercial TS with an AS in high rate of nutrition presented minimal impact on performance.

Keywords: Feeding-programs; Cold tolerance; Thermogenesis; Performance; Economics

1. INTRODUCTION

Efficient growth of young dairy calves are extremely important to decrease replacement heifer raising costs and increase the potential of future milk production. The achievement of the efficiency and success in dairy calf rearing is a function of an excellence feeding management that start soon after birth. In addition, to maximize profit and maintain young calves healthy is important to create an environment that keeps stress down (Hulbert and Moisé, 2016). Environmental temperature affects directly newborn and young calves' performance, health and survival (Bellows, 1997). One of main problems that affect newborn calves and cause morbidity and mortality is hypothermia due to excessive heat loss in low environmental temperature (Mellor and Stafford, 2004). Thus, some strategies allow to reduce cold stress induced by heat loss.

The first strategy starts immediately after birth, with colostrum supply. A major role of colostrum is the passive immunity transfer by provision of immunoglobulins. However, colostrum feeding is also important to neonatal thermogenesis (Hammon et al., 2012). It was evidenced that colostrum is of utmost importance in the provision of nutrients to the calf, constituting an excellent energy source to heat production (Vermorel et al., 1983). Some nutrients from colostrum are responsible for a metabolic heat production. This metabolic response, referred to as postprandial thermogenesis, represents the energy cost associated with nutrients metabolism and brown adipose tissue (BAT) metabolism (Herpin et al., 1994). Herpin (2005) reported that body temperature and heat production are positively related to the amount of colostrum intake in cold conditions.

Others strategies may also starts right after birth. Besides feeding colostrum, there are several ways to aid the young calf in low environmental temperature (Davis and Drackley, 1998). It is necessary to ensure that calf has access to dry, well-bedded shelter with a bedding sufficiently deep, and in some situations the use of calf jackets, blankets or coats. In addition, calves should be fed extra energy to meet the increase in energy maintenance requirements to produce heat. Drackely (2008) suggests that during low environmental temperature producers may increase the volume of milk or milk replacer fed at each feeding or increase solids content to each feeding, increasing energy intake. Another way to increase the energy intake is by feeding a higher energy content milk replacer or supplement the milk replacer with added fat or additional milk solids.

Despite the knowledge on the positive effect of the colostrum and feeding supply at the heat production and the performance of young calves, little is known about specific amount of colostrum and preweaning feeding system that result in greater heat production, health, performance and reduced rearing costs. Considering the above, a better understanding of the volume of colostrum and different feeding programs for calves submitted to cold environment is crucial to optimize calves rearing managements.

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2. LITERATURE REVIEW

Efficient performance of young dairy calves are extremely important to profitability of the dairy enterprise (Quigley et al., 2006). Calves should grow at an increasing rate throughout the preweaning period. Effective management of a calf rearing program ensures the best possible start for optimum growth, reduced veterinarian and medical costs, and increased future milk yield (Faber et al., 2005). Calves who had lower growth, often related to lower feed intake, presented lower milk production during the first lactation (Chester-Jones et al., 2017; Van De Stroet et al., 2016).

Achieving success in calf rearing is a function of excellent nutrition, good hygiene practices and prevention of disease that start as soon as the calf is born (Pineda et al., 2016). One of the keys to maximizing profit and keeping young calves healthy is creating an environment that keep stress down, since stress contributes to low performance and increased rates of morbidity and mortality in the first few weeks of life (Hulbert and Moisés, 2016). Morbidity and mortality of young calves are economic concerns for dairy cattle operations (Nagy, 2009). Poor calf health can lead to high death losses and have a serious impact on net income for the cattle producer (Bellows, 1997).

2.1. Factors affecting calf survival

The most challenging period for calves is from birth through weaning. During this time, calves experience remarkable physiological, metabolic, and environmental changes (Davis and Drackley, 1998). Survival of the calf can be compromised for many reasons including dystocia, nutrition and suboptimal environmental temperatures (Okamoto et al., 1986). At parturition, among other factors, the survival of the newborn depends on its ability to rapidly adapt to the new environmental conditions. At this moment, the calf moves from the controlled, warm uterine environment, to the hostile external environment. This transition demands many physiological actions to maintain normal body temperature especially during cold weather (Bellows, 1997). Climatic conditions affect newborn and young calves' survival and at low environmental temperature, morbidity and mortality may increase (Bellows, 1997). Patterson et al. (1987) found a neonatal mortality approximately 13% due to severe weather conditions during the calving season, resultant of chilling/exposure, pneumonia and scours.

2.2. Ambient temperature and thermoneutrality

One of main problems that affect newborn calves and cause morbidity and mortality is hypothermia due to excessive heat loss in low ambient temperature (Mellor and Stafford, 2004). After birth, the survival of the newborn depends on its ability to adapt to the environmental conditions (Slee, 1977). The ability of the neonate to maintain normal core body temperature is a function of its ability to produce enough heat to balance the loss of heat by evaporative and nonevaporative heat losses (Bellows, 1997). However, body temperature regulation in neonatal calves is metabolically immature (Davis and Drackley, 1998). Diesch et al. (2004) reported that calves born during windy and wet weather and when temperatures were $< 10^{\circ}\text{C}$, had lower rectal temperatures and also took longer to stand compared to calves born in dry weather and when air temperatures were $> 10^{\circ}\text{C}$.

There is a range of ambient temperatures for all mammals, within which the general metabolism of the organism generates sufficient heat as a byproduct of the metabolism, so that its predetermined body temperature can be maintained. This temperature range is known as the zone of thermoneutrality, and at this temperature, the organism demonstrates its basal metabolic rate (Cannon and Nedergaard, 2010). The thermoneutral zone of the young calf varies with age, weight, environmental conditions, and other stressors and ranges from 15 to 25°C (Davis and Drackley, 1998). The point in environmental temperature at the lower end of the thermoneutral zone is termed the lower critical temperature. With increased age, the lower critical temperature declines due an increase in hair thickness and length, skin thickness, and stores of subcutaneous fat (Table 1).

Table 1. Effect of age of Ayrshire bull calf on lower critical temperature

Age (d)	Critical temperature ($^{\circ}\text{C}$)
1	13.4
5	12.2
10	10.8
15	9.5
20	8.4
25	7.3
30	6.4

Source: Adapted from Gonzalez-Jimenez and Blaxter (1962)

Lower critical temperature are observed in temperate countries mainly during winter. However, some subtropical countries as Brazil present temperatures below lower critical temperature during winter in some regions (National Institute of Meteorology - INMET, 2017).

According to Coelho (2005) in part of south and southwest of Brazil, calves are exposed to cold weather, wind and humidity, requiring body temperature regulation of calves. At lower critical temperature, the body needs to produce heat (thermogenesis) to keep the animal alive (Davis and Drackley, 1998).

2.3. Mechanisms of thermogenesis

Thermogenesis depends on exercise, diet, and climatic conditions, in particular environmental temperature (Harper et al., 2002; Vermorel et al., 1983). Heat is generated as a by-product of metabolic reactions in biological systems (Girardier and Michael, 1984). Heat production of animals is the result of several phenomena: the metabolic rate of body tissues, the metabolism of brown adipose tissue, shivering, physical activity and the feeding heat increment (Vermorel et al., 1983).

Mammalian thermogenesis can be classified as obligatory or facultative. Obligatory reactions include basal metabolic rate and essential reactions such as ingestion and digestion. The latter reactions account for a significant proportion of diet-induced thermogenesis, the energy costs of assimilating nutrients and retaining net energy (Harper et al., 2002). On the other hand, facultative reactions include everything needed by the animal beyond the basal metabolism. All cells and tissues of the body contribute to obligatory thermogenesis. However, facultative thermogenesis is predominantly the result of metabolic reactions in two types of tissue: skeletal muscle and brown adipose tissue (BAT). In muscle, these processes include exercise-induced thermogenesis and cold-induced shivering thermogenesis; both are mechanisms that require coupled oxidative phosphorylation. In BAT, facultative thermogenic processes include diet-induced thermogenesis and cold-induced non-shivering thermogenesis; both require uncoupled oxidative phosphorylation (Harper et al., 2002). Production of heat to maintain homeothermy in the neonate is dependent on shivering thermogenesis in the muscle and nonshivering thermogenesis in BAT (Bellows, 1997).

Cold-induced shivering thermogenesis

Shivering involves episodic or sustained vigorous contractions of antagonistic muscle fibers without efficient work output, which causes an increased turnover of the myofibrillar ATP pool and thus heat dissipation (Klingenspor and Fromme, 2012). Shivering appears soon after birth in calves housed at 10°C and stops when the hair coat is almost dry. It first affects skin

muscles and then skeletal muscles. According to Vermorel et al. (1983), in 15 h old calves lying in a 37 °C water bath, shivering starts when water temperature drops to 32 °C. Shivering is immediately followed by an increase in heat production ranging from 33 % to more than 100%. Shivering is a major factor in thermoregulation for newborn calves (Vermorel et al., 1983).

Bellows and Lammoglia (2000) classify shivering using a scale from 1 to 3: (1) no shivering; (2) moderate shivering of muscles in the back and legs; (3) intense shivering of muscles in back, legs and face of the calf. According to Lammoglia et al. (1999) shivering thermogenesis is associated with higher concentrations of plasma glucose.

Cold-induced non-shivering thermogenesis

Non-shivering thermogenesis contributes to a large proportion of total heat production of newborn calves in environments below the thermoneutral zone. In newborns, cold-induced non-shivering thermogenesis occurs almost exclusively in BAT (Himms-Hangen, 1985), which is present in newborn calves and other mammals (Vermorel et al., 1983). In smaller mammals at thermoneutrality nearly one half of their energy metabolism goes towards BAT metabolism (Cannon and Nedergaard, 2004). However, in environments below thermoneutrality, the predominant energy utilizer is BAT. The capacity of the BAT for the animal's metabolism alters based on environmental conditions: it atrophies when not needed and is recruited when a chronic, high demand is encountered (Cannon and Nedergaard, 2004).

Brown adipose tissue is located in the perirenal, inguinal and prescapular body regions and accounts to about 2 % of body weight (Figure 1) (Vermorel et al., 1983). The cells of BAT are usually multilocular, containing several drops of stored triacylglycerol, and are characteristically packed with many large mitochondria (Cannon and Nedergaard, 2004). This tissue represents the major portion of the depot fat reserve in a newborn calf, and is about 40% lipid on a wet weight basis (Okamoto et al., 1986). Although that, the BAT can also use exogenous triacylglycerol and glucose for thermogenesis (Himms-Hangen, 1985). The glucose can serve as a thermogenic substrate when it is abundantly available, i.e. after a meal.

The BAT grows when it is stimulated by prolonged and intense activation of its sympathetic nerve supply (Vermorel et al., 1983). Information on body temperature, feeding status, and body energy reserves is coordinated in an area in the brain to the BAT. With a cold stimulation, a signal is transmitted via the sympathetic nervous system to the individual brown adipocytes. The released transmitter, norepinephrine, initiates triglyceride breakdown in the

brown adipocytes. The intracellular signal is transmitted via cAMP and protein kinase A, leading to mitochondrial combustion of substrates and heat production (Cannon and Nedergaard, 2004). Mitochondria in BAT have a unique proton conductance mechanism that permits them to become reversibly uncoupled and thus to oxidize substrates at an extremely high rate. This mechanism is controlled by the intracellular concentration of fatty acids, mainly generated by the breakdown of endogenous triacylglycerol. The mechanism involves uncoupling protein (UCP) which has one binding site for purine nucleotides per dimer on the outer surface of the inner mitochondrial membrane (Himms-Hangen, 1985).

Figure 1. Brown adipose tissue distribution in the animal body (Cannon and Nedergaard, 2004)

The activity of BAT lipoprotein lipase increases very rapidly in response to acute sympathetic stimulation. When the cells are thermogenically inactive they become filled with lipid and may superficially resemble white adipose tissue cells (Himms-Hangen, 1985). This occurs during the first month of calves' life, when BAT is rapidly converted to white adipose tissue, which has decreased reactivity to norepinephrine (Vermorel et al., 1983).

2.4. Colostrum as an energy source for newborn calves' thermogenesis

Bovine colostrum consists of a mixture of lacteal secretions and constituents of blood serum, most notably Ig and other serum proteins, which accumulate in the mammary gland during the prepartum dry period (Godden, 2008). Colostrum is known to be important for passive immunity transfer (Uruakpa et al., 2002). Newborn calves are born hypogammaglobulinemic or agammaglobulinemic due to the absence of transplacental transfer

of antibodies during gestation. The calf is passively immunized by the ingestion of colostrum and absorption of antibodies early after birth (Olson et al., 1980). The efficiency of immunoglobulin transfer across the gut epithelium is optimal during the first 4 hours of life with a progressive decline 6 hours after birth (Godden, 2008). The most important management factor in determining health and survival of the neonatal calf is achieving early and adequate intake of high quality colostrum (Godden, 2008).

Colostrum intake also aims to stimulate maturation and function of the neonatal gastrointestinal tract (Hammon et al., 2014). Colostrum contain several peptide growth factors, which stimulate the growth and differentiation of mammalian cells. The important growth factors of colostrum include Insulin-like growth factors (IGF-1 and IGF-2), transforming growth factor beta (TGF- β 1 and TGF- β 2), growth hormone (GH), epidermal growth factor, and insulin (Pakkanen and Aalto, 1997). These hormones promote gastrointestinal tract development, production of digestive enzymes, and absorption capacity of nutrients (Bach, 2012). Yang et al. (2015) reported that calves fed high quality colostrum presented higher improvements in IgG absorption, antioxidant activities and serum growth factors, villus length and width, crypt depth, and mucosal thickness compared to transitional milk or bulk tank milk. Additionally, calves that received bulk tank milk presented villi severely atrophied, and some histological changes were detected.

In addition to passive immunity and grow factors, colostrum feeding is important to neonatal thermogenesis (Hammon et al., 2012). According to Herpin et al. (2005) in cold environment, body temperature and heat production are positively related to the amount of colostrum intake due to metabolic heat production represented by the energy cost associated with digestion and absorption of nutrients. Vermorel et al. (1983) reported that 24 Friesian calves held at 10 °C increased heat production on average by 18% and 9% respectively during the first and the second hour following colostrum consumption at 12 h of age. In a study with newborn pigs at the first day of life, colostrum provided as much as 75% of energy required for heat production at a low critical temperature (Herpin et al., 1994).

Additionally to heat production by the energy costs of assimilating nutrients and retaining net energy, colostrum may promote thermogenesis as source of substrate for the BAT. Colostrum supplies lactose, amino acids, and triglycerides, constituting an excellent energy source (6.7 MJ/kg) to heat production both by diet-induced thermogenesis and by nonshivering thermogenesis (Vermorel et al., 1983; Himms-Hagen, 1990; Godden, 2008; Kirovski, 2015) (Table 2).

Table 2. Characteristics and composition of Holstein colostrum and milk

Variable	Colostrum (milking postpartum)			
	1	2	3	Milk
Specific gravity	1.056	1.040	1.035	1.032
Total solids (%)	23.9	17.9	14.1	12.5
Fat (%)	6.7	5.4	3.9	3.6
Solids-not-fat (%)	16.7	12.2	9.8	8.6
Total protein (%)	14	8.4	5.1	3.2
Casein (%)	4.8	4.3	3.8	2.5
Albumin (%)	0.9	1.1	0.9	0.3
Immunoglobulins (%)	6.0	4.2	2.4	0.09
IgG (g/100 mL)	3.2	2.5	1.5	0.06
Nonprotein nitrogen (% of total N)	8.0	7.0	8.3	4.9
Lactose (%)	2.7	3.9	4.4	4.9
Calcium (%)	0.26	0.15	0.15	0.13
Magnesium (%)	0.04	0.01	0.01	0.01
Potassium (%)	0.14	0.13	0.14	0.15
Sodium (%)	0.14	0.13	0.14	0.15
Chloride (%)	0.12	0.10	0.10	0.07
Zinc (mg/100mL)	1.22	-	0.62	0.30
Manganese (mg/100mL)	0.02	-	0.01	0.004
Iron (mg/100 mL)	0.2	-	-	0.05
Copper (mg/100g)	0.06	-	-	0.01
Cobalt (µg/100g)	0.5	-	-	0.1
Vitamin A (µg/100 mL)	295	190	113	34
Vitamin E (µg/g fat)	84	76	56	15
Carotene (µg/g fat)	103.3	-	-	11.3
Riboflavin (µg/mL)	4.83	2.71	1.85	1.47
Pantothenic acid (µg/mL)	1.73	-	3.20	3.82
Vitamin B12 (µg/100mL)	4.9	-	2.5	0.6
Folic acid (µg/100mL)	0.8	-	0.2	0.2
Choline (mg/mL)	0.70	0.34	0.23	0.13
Ascorbic acid (mg/100mL)	2.5	-	2.3	2.2

Source: Adapted from Davis and Drackley (1998)

Therefore, efficient management practice feeding calves' high volume of colostrum during the first hours of life promote passive immunity and thermogenesis, ensuring the best possible start for optimum growth, reduced veterinarian and medical costs, and increased milk yield as a mature animal (Faber, 2005). The United States Department of Agriculture–National Animal Health Monitoring System (USDA-NAHMS, 2014) recommends feeding colostrum at 10% of body weight. However, Conneely et al. (2014) showed that calves fed 8.5% of BW in colostrum within 2 h of birth had greater passive immunity in the first 3 d of life compared with

calves fed either 7 or 10% of BW, probably due the saturation of a shared macromolecular transport mechanism across the calf intestinal epithelium. Additionally, Liang et al. (2015) reported that healthy neonatal calves that received 3 L of colostrum had the capability to digest and absorb additional nutrients from milk replacer with a higher plane of nutrition during the first week of postnatal life.

Faber et al. (2005) showed that heifer calves fed 4 L of high quality colostrum immediately after birth had lower veterinary costs, greater ADG, and produced more milk/d across their first two lactations compared with cohorts fed 2 L of colostrum. The improvements for calves fed a higher volume of colostrum are probably due to greater immunity but also energy supply during the first hours of life. Another study reported that calves fed unlimited amount of colostrum and milk were able to digest and metabolize high amounts of feed even during the first week of life compared with calves fed restrict amount (Hammon et al., 2002). Moreover, higher colostrum intake was reflected by a low plasma concentrations of NEFA and cortisol and higher insulin concentrations. Thus, when calves are fed an adequate volume of colostrum within the first hours of life, environment conditions and the energy obtained from liquid or solid diet become more important for thermogenesis (Davis and Drackley, 1998). Successful adaptation of the dairy calf to a cold environment is mainly dependent upon the availability of adequate housing, management and nutrition, resulting in decrease morbidity and mortality, and similar growth rates as calves in the zone of thermoneutrality (Pineda et al., 2016; Nonnecke et al., 2009; Davis and Drackley, 1998).

2.5. Management practices for young calves' thermogenesis

There are several ways to aid the young calf in reducing its heat loss from the body and increase thermogenesis. An important consideration that help to reduce heat losses is that the calf has access to a dry, well-bedded shelter that provides protection from wind and extreme environmental conditions. Calf pens should be bedded deeply to provide insulation in cold weather (Davis and Drackley, 1998). Bedding is potentially effective to reduce calves' heat loss. If the bedding is sufficiently deep, the calf can nest and trap a boundary layer of warm air around itself, which reduces the lower critical temperature of the calf (Nordlund, 2008).

Lago et al. (2006) assigned a nesting score based on how visible the calf's legs are when the calf is lying down. Nesting score 1 is assigned when calves lie on top of the bedding with legs exposed. Score 2 is assigned when calves nestle slightly into the bedding, but part of the legs are visible above the bedding. Score 3 is used when the calf appear to nestle deeply into

the bedding material and legs are not visible. The potential for the calf to nest deeply seems to reduce the risk for chilling and allows for colder and better-ventilated spaces (Nordlund, 2008). Hutches in cold climates are best bedded with long straw, to provide greater isolative effects. According to Sutherland et al. (2017), rearing calves on surfaces with lower insulation properties in cold environment conditions could result in lower skin surface temperature and consequently calves thermal discomfort. A minimum of 15 cm of bedding is recommended. A variety of bedding material can be used. Adequate bedding absorbs moisture, which will help keep the calf's haircoat dry to maintain its insulating function. (Davis and Drackley, 1998).

Another way to reduce heat losses is using calf jackets, blankets or coats. Rawson et al. (1989) observed that the insulated coat worn by calves housed at -30 to -18°C provided a 52% increase in animal insulation. Additionally, calves should be fed an extra energy to meet the increase in maintenance energy requirements to produce heat (Davis and Drackley, 1998). With prolonged exposure to even mildly cold conditions, physiological adaptation occurs in animals resulting in increases in thermal insulation, appetite and basal metabolic intensity, as well as alterations in digestive functions. Primary among these changes are an increased resting metabolic rate, and hence an increased energy requirement for maintenance (Young, 1983; Young, 1981). Therefore, temperature has an important effect on the energy requirement of the young calf (Scibilia et al., 1986).

2.6. Requirements for calves' maintenance under thermoneutral conditions

In cold weather, the body alters physiologic processes, and hence, requires more nutrients to control temperature through heat production (Drackley, 2008). When temperatures fall below the lower critical temperature, the energy needed to maintain core body temperature is supplied either by the increased energy intake or from the increased metabolism of tissue reserves (Nonnecke et al., 2009).

The National Research Council (NRC) established energy requirements for calves less than 100 kg body weight (BW) in units of metabolizable energy (ME), which in calves is determined by subtracting losses of energy in feces and urine from total feed (or intake) energy.

The ME requirements for maintenance under thermoneutral conditions are approximately 1.75 Mcal/d for a 45-kg calf. Whole milk contains about 5.37 Mcal /kg of solids, which means that a 45-kg calf requires about 325 g of milk solids just for maintenance at thermoneutrality. Because most milk replacers are lower in fat content than whole milk, they

have less ME per unit of solids (4.6–4.7 Mcal/kg). Consequently, a 45-kg calf requires about 380 g of milk replacer for maintenance at thermoneutrality. As environmental temperature decreases, maintenance requirements for ME increase. At -20°C a 45-kg calf requires about 725 g/d of milk replacer powder just to meet maintenance requirements and maintain body temperature. Energy consumed above maintenance can be used for growth. Table 2 shows the effects of BW and environmental temperature on maintenance ME requirements in calves less than 21 days of age (Drackley, 2008). In concentrate starters, the main portion of energy is derived from cereal grains such as corn.

Table 2. Maintenance requirements for metabolizable energy as affected by body weight and environmental temperature in calves less than 21 days old

BW (kg)	Environmental temperature (°C)				
	20	10	0	-10	-20
	(Maintenance ME, Mcal/day)				
30	1.28	1.63	1.97	2.38	2.67
40	1.59	2.02	2.45	2.96	3.31
50	1.88	2.39	2.9	2.5	3.91
60	2.16	2.74	3.32	4.01	4.48

Source: Adapted from Drackley (2008)

Like energy, protein is required for maintenance and growth as a source of amino acids. However, the protein requirements for maintenance are small (about 30 g/d for a 45-kg calf) and are not believed to be substantially altered by cold stress. Protein requirements are mostly determined by the rate of growth. On average 188 g of protein are deposited for every kilogram of BW gain in calves above or below thermoneutral conditions, which would require 250 to 280 g of crude protein intake from milk replacer (Drackley, 2008).

2.7. Cold weather feeding strategies

According to Drackley (2008), practical feeding systems can be made simple, although nutrients requirements for calves are more complex than the industry has recognized. Dairy replacement calves are usually fed limited amounts of whole milk or milk replacer (4 L) and have *ad libitum* access to a dry grain mixture (calf starter) prior to weaning. According to

USDA-NAHMS (2014), from the 1950s to the 1970s the approach was to minimize the cost reducing amount of milk/milk replacer feed. The restricted feeding system is known as a conventional milk-feeding program. This practice provides milk or milk replacer (MR) at approximately 10% of calf's BW at birth (Jasper and Weary, 2002). This volume of liquid feed is much lower than *ad libitum* intakes, which are in the range of 16% to 20% of BW. When MR is fed, it contains 20 to 22% CP and 15 to 20% fat and are traditionally reconstituted to approximately 12.5% solids (Cowles et al., 2006).

Conventional program is designed to meet or slightly exceed maintenance requirements of the young calf, about 200 to 300 g/d of growth under thermoneutral conditions (Drackley, 2008). Nutrients for growth are met by voluntary consumption of the starter concentrate rather than solids from milk. Typically, this practice encourages calf starter intake, and stimulates early rumen development, allowing a smoother weaning transition (Bush and Nicholson, 1986). A fully functional rumen allows the calf to utilize short chain fatty acids (SCFA) as its primary energy substrate. However, very young calves fed milk replacer at a conventional program rate during the winter do not consume enough energy to achieve maintenance under thermoneutral conditions (Drackley, 2008). Therefore, they do not grow at the targeted rates.

Alternatively to conventional milk feeding, some intensive milk-feeding systems allow calves to receive greater or *ad libitum* volume of milk. The first alternative is named step-down milk-feeding. In this procedure, animals receive more milk than in the conventional method during the early weeks of the milk feeding period (6 L/d milk from d 1 to 29), followed by a fixed volume of milk until weaning (4 L/d milk from d 30 to 60). Another procedure, called step-up/step-down, provides milk to calves with the aim of encouraging starter intake and promoting early rumen development and performance in calves. In this method, milk feeding is gradually increased to reach a peak in the middle of the milk-feeding period before it is gradually decreased to the original level toward the end of the period (Ommidi-Mizaei et al., 2015). A recent research showed that calves fed milk replacer *ad libitum* in step-down system presented greater average weight gain in the first phase of life and milk yield in the first lactation 612 kg above that animals fed approximately 50% less milk replacer (Korst et al., 2017). According to Soberon et al. (2012) for every 1 kg of preweaning average weight gain, milk yield increase about 1,113 kg in the first lactation.

Intensive programs achieve increased early and future life performance in general providing more dry matter per day from milk replacer generally containing greater than 25% CP with fat content similar to conventional (Davis Rincker et al., 2011). Solids content of

intensive MR at feeding ranges from 12.5 to 17.5% (Cowles et al., 2006). A recent study using medium CP milk replacer reported that calves fed intensive or step-up/step-down method presented lower concentrate intake (100.0 and 189.7 g of DM/d, respectively) during preweaning than calves in the conventional program (362.1 g of DM/d) (de Paula et al., 2017). On the other hand, concentrate intake showed no difference among treatments postweaning. Omid-Mirzaei et al. (2015) observed that calves fed a step-down, or step-up/step-down system, improved the total DMI, BW and some body measurements during pre and post-weaning compared to calves in the conventional diet. According to the authors, the better performance could be explained by the higher nutrient availability due to the greater milk intake, since improvements in growth and feed efficiency occur because of feeding greater amounts of liquid diet.

From birth to the first 2 to 3 weeks of age, the calf consumes negligible amounts of dry feed and relies almost entirely on milk or milk replacer to meet nutrient requirements. During this phase, the solids in milk and milk replacer are digested by enzymes in the abomasum and small intestine and the rumen is undeveloped (Xu, 1996). The digestive enzymes allow highly efficient digestion of milk proteins, lactose, and triacylglycerides, and smaller digestion of non-milk proteins or polysaccharides such as starch (Drackley, 2008). As the calf begins to consume starter, its fermentation leads to a fast increase in rumen volume and differentiation of the rumen epithelium so that the SCFA produced from microbial fermentation can be absorbed and metabolized. The animal will depend exclusively on fermentation of the solid diet only after weaning (Drackely, 2008).

Because of their lack of rumen functionality, young calves should be fed extra energy by increasing the amount of a good quality liquid diet to meet the increase in maintenance energy requirements when housed below the thermoneutral zone. According to the NRC (2001), dietary requirements during the preruminant phase are best met with high-quality liquid diets formulated with a rich source of carbohydrates, proteins and fats that are efficiently digested, with the addition of starter during the transition phase. Allowing calves greater intake of liquid feed during early life is closer to natural conditions in which calves would have *ad libitum* access to milk (Drackley, 2008).

Drackely (2008) suggests that during low environmental temperature some producers could use feeding strategies to improve calf growth and health. Producers may increase the volume of milk or milk replacer fed at each feeding or increase solids content to each feeding, increasing energy intake. For those that are already feeding larger amounts of milk or replacer, a third meal

may be added to the feeding routine. Another way to increase the energy intake is by feeding a higher energy content milk replacer or supplement the milk replacer with added fat or additional milk solids. Ghasemi et al. (2017) showed that supplementation with fat may be beneficial when feeding calves experiencing cold stress. Although these strategies increase energy/nutrient intake, it also increase feed costs.

2.8. Costs of feeding systems

Feeding systems have a strong influence on productions costs but also on the future milk production potential, turning the understanding of costs into a better approach of investments. According to USDA-NAHMS (2014), rumen development was studied extensively from the 1950s to the 1970s, and the primary approach at that time was to minimize the cost, reducing amount of milk/milk replacer fed. However, as showed in recent studies, preweaned calves are more efficient at converting milk to body mass. Quigley et al. (2006) reported that calves fed additional milk replacer presented greater body weight, body weight gain and feed efficiency, additionally, greater feed costs and cost per kilogram of body weight gain. On the other hand, a previously study reported that calves fed intensive diet and higher rearing costs during preweaning phase presented no difference of total costs measured through first lactation compared to calves fed conventional diet (Davis Rincker et al., 2011). Raeth-Knight et al. (2009) showed that calves fed intensive diet when cows calved 27.5 d earlier than calves fed conventional milk replacer, potentially decreasing costs associated with heifer replacer program (Tozer and Heinrichs, 2001). According to Korst et al. (2017), the economic returns from milk yield by animals previously fed whole milk *ad libitum* compared to animals fed in conventional system seems to be able to compensate the additional expenses during the preweaning phase, supporting the positive effect of higher investment during the nursery period without negative effect on economics over the first lactation. Additionally, Bittar et al. (2009) suggest that earlier weaning of calves may reduce the rearing costs by decreased calves' time on milk. The recommended weaning age for heifers is as early as 6 to 8 weeks and should occur when calves are consuming at least 900 g of starter daily (USDA-NAHMS, 2014). However, weaning calves as early as 4 weeks save time, money and reduce labor without apparent negative effects on calves through 8 weeks of age (Kehoe et al., 2007).

Costs of feeding programs may also increase substantially because of calf starter costs. Calf starter is usually made with good nutrients (balanced concentrate mixture, comprising ground cereal grains, protein supplements, mineral and vitamins), that are highly palatable, and are

provided *ad libitum* from the first week of life until weaning (Drackley, 2008). Good quality ingredients increase calf starter production cost. According to Drackley (2008), nutritional inputs are some of the major costs of calf production. Therefore, some alternatives in production and processing of starter are being developed to reduce costs. Bittar et al. (2009) showed that coarsely ground starter concentrate may be an alternative to reduce production costs since the physical form of the concentrate had no effect on performance or in the development of forestomach of dairy calves, but had a lower production cost.

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3. THERMOREGULATION AND PERFORMANCE OF DAIRY CALVES FED DIFFERENT AMOUNTS OF COLOSTRUM

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ABSTRACT

Colostrum is an important source of immune protection and an excellent energy source for thermogenesis in the newborn calf. However, the amount of colostrum required to promote optimal performance and produce heat is not well established. The objective of this study was to evaluate newborn thermoregulation, performance and blood metabolites in calves fed different amounts of colostrum. Thirty newborn Holstein bull calves were blocked by birth body weight (BW) in a randomized experimental design and fed one of 3 different volumes of high quality colostrum (60 mg of Ig/mL): 10%, 15% or 20% of BW. Colostrum was split into 2 feedings: within 2 hours after birth and 6h after the first feeding. At 24h of life, each calf was placed in a temperature-controlled chamber at 10°C for 150 min. Rectal and skin surfaces temperature, heart rate, respiratory rate and shivering were measured every 15 min, and blood samples were taken every 30 min. After the temperature challenge, calves were individually housed in ambient temperature facilities ($26.8 \pm 5.9^{\circ}\text{C}$), with free access to water and starter (20% crude protein), and received 6L/d of milk replacer (MR; 12.5% solids and 19.25% crude protein and 16.16% fat on a dry matter basis) until week 8 of life, when they were weaned. During the cold challenge, there was a quadratic effect on rectal, foot and tail temperature; prescapular temperature linearly increased and shivering behavior decreased linearly in response to increased colostrum supply. Additionally, feeding a higher volume of colostrum linearly decreased serum lactate and increased serum total protein. A linear increase in fecal score 24 h after birth was seen as volume of colostrum increased. Increased colostrum supply linearly increased heart girth and tended to increase quadratically hip width during the preweaning period. Starter intake tended to increase linearly at week 4 and quadratically at week 5 as volume of colostrum increased. Concentrations of beta-hydroxybutyrate tended to increase quadratically during the preweaning whereas non-esterified fatty acids decreased quadratically during preweaning and linearly at weaning. Furthermore, leukocytes increased linearly with increased volume of colostrum. In conclusion, newborn calves given 15% or 20% of BW as colostrum exhibited increased thermoregulatory responses during cold challenge, improved growth performance and increased immunity responses.

Keywords: Cold tolerance; Newborn thermogenesis; Calf performance

3.1. INTRODUCTION

Colostrum is known to be important for passive immunity. The calf acquires passive immunity by ingestion and absorption of colostral immunoglobulins soon after birth (Olson et al., 1980). In addition to passive immunity, colostrum feeding is important for neonatal thermogenesis (Hammon et al., 2012). According to Herpin et al. (2005) in cold environments, body temperature and heat production are positively related to the amount of colostrum intake

due to the metabolic heat production represented by the energy cost associated with digestion and absorption of nutrients. Vermorel et al. (1983) reported that 24 Friesian calves held at 10 °C increased heat production by 18% and 9% respectively during the first and the second hour following colostrum consumption at 12 h of age. In a study with newborn pigs on the first day of life, colostrum provided as much as 75% of the energy required for heat production at a low critical temperature (Herpin et al., 1994). Colostrum may also promote thermogenesis as a source of substrate for brown adipose tissue (**BAT**). Colostrum supplies lactose, amino acids, and triglycerides, which constitutes an excellent energy source (6.7 MJ/kg) for heat production both by diet-induced thermogenesis and by non-shivering thermogenesis (Vermorel et al., 1983; Himms-Hagen, 1990; Hammon et al., 2012).

Therefore, the management practice of feeding calves a high volume of colostrum during the first hours of life promotes not only efficient passive immunity transfer, but also thermogenesis, ensuring the best possible start for optimum growth, reduced veterinarian and medical costs, and increased milk yield as a mature animal (Faber et al., 2005). The United States Department of Agriculture–National Animal Health Monitoring System (USDA-NAHMS, 2014) recommends feeding colostrum at 10% of body weight. Faber et al. (2005) showed that heifer calves fed 4 L of high quality colostrum immediately after birth had lower veterinary costs, greater ADG, and produced more milk/d across their first two lactations compared with cohorts fed 2 L of colostrum. Another study reported that calves fed unlimited amounts of colostrum and milk were able to digest and metabolize high amounts of feed during the first week of life compared with calves fed restrict amount (Hammon et al., 2002). Moreover, higher colostrum intake was reflected by lower plasma concentrations of non-esterified fatty acids (NEFA) and cortisol and higher insulin concentrations in calves. As the amount of colostrum required to produce heat and to promote better performance is not well established, we hypothesized that greater colostrum supply to newborn dairy calves would increase heat production and improve physiologic characteristics and blood metabolites during early life, and improve performance and blood metabolites pre-weaning. The objective of this study was to evaluate the newborn heat production, physiologic characteristics and blood metabolites during cold challenge (10°C) at 24 hours of life for 150 minutes, and the performance and blood metabolites in the pre-weaning period in dairy calves fed different amounts of colostrum.

3.2. MATERIAL AND METHODS

The Animal Research Ethics Committee of Escola Superior de Agricultura “Luiz de Queiroz”/ University of São Paulo approved all procedures involving animals in this study (Protocol no. 2014-18).

Animals, Experimental Design and Treatments

This experiment was conducted on Experimental Calf Facility of the Luiz de Queiroz College of agriculture, University of São Paulo, Brazil. From November 2015 to February 2016 ($26.8 \pm 5.9^{\circ}\text{C}$), 30 male Holstein newborn calves (birth weight 39.4 ± 6.5 kg) were enrolled. Immediately after birth, the calves were separated from their mothers and weighed. The cows were milked and colostrum quality was measured using a colostrometer (Suprivet, Divinópolis, MG, Brazil). Colostrum was diluted with commercial whole milk to always achieve 60 mg of Ig/mL of quality, which is considered to be of high quality (Godden, 2008). If a cow had colostrum below this quality, then a frozen colostrum with 60 mg of Ig/mL of quality was thawed and fed to the newborn. Calves were blocked according to birth weight and were randomly distributed to one of the three different volumes of colostrum: low volume (**10% BW** of colostrum; n=10), medium volume (**15% BW** of colostrum; n=10), or high volume (**20% BW** of colostrum; n=10). Within 2 h after birth the calf was fed half the colostrum amount and 6 h after the first feeding the second half was given. An esophageal feeder was used for calves that did not voluntarily consume all colostrum.

Cold challenge

At 24 h of life, each calf was fed 7.5% BW of whole milk (≈ 3 L) and then placed in a temperature controlled chamber at 10°C , for 150 min. Rectal temperature, skin surface temperatures, heart rate, respiratory rate and shivering were measured every 15 min starting with placement into the chamber (time 0). The rectal temperature was taken using a digital thermometer and the skin surface temperatures were taken using an infrared thermometer (Instrutemp, São Paulo, Brazil). Skin surface temperatures were measured at the: prescapular, thorax wall, muscular part of the thigh, shin, foot, tail and ears (Figure 1). For shivering measurements, a score was assigned: (1) no shivering; (2) moderate shivering of muscles in the back and legs; (3) intense shivering of muscles in back, legs and face of the calf (Bellows and Lammoglia, 2000).

Figure 1. Skin surface measured throughout cold challenge. Ears (1), foot (2), shin (3), tail (4), muscular part of the thigh (5), prescapular (6) and thorax wall (7). Adapted from Gonzalez-Jimenez and Blaxter (1962).

Calf performance

After the challenge, calves were individually housed in wood shelters in a grassy field with free access to water and a pelleted commercial starter concentrate (21.71% CP; 4.46% Fat, 71.11% TDN; Agrocere Multimix, Rio Claro, SP, Brazil). Calves received 6L/d of milk replacer (19.25% CP, 16.16% Fat, 12.5% solids; Sucelac, Agrocere, Rio Claro, SP, Brazil) split between 2 feedings at 700h and 1700h until the eighth week of life, when they were weaned. Pelleted commercial starter was fed *ad libitum* every morning, and orts were weighed to monitor daily intake. Intake of milk replacer was recorded daily until weaning. Animals were weighed weekly using a mechanical scale (ICS-300, Coimma Ltda., Dracena, SP, Brazil), and withers height, heart girth and hip width were measured before the morning milk feeding. The wither height and hip width were measured using a ruler, and the heart girth with a flexible tape. Every morning, fecal scores was recorded by a single observer using a scale from 0 to 3 (http://www.vetmed.wisc.edu/dms/fapm/fapmtools/8calf/calf_health_scoring_chart.pdf) according to fluidity as (0) normal; (1) semi-formed, pasty; (2) loose, but stays on top of bedding; (3) watery, sifts through bedding. Weekly averages of all scores were generated per calf for statistical analysis. Calves given a fecal score ≥ 2 were considered to have diarrhea. When diarrhea was diagnosed, an oral electrolyte solution was offered. Calves rectal temperature was measured daily from day 1 to week 8 of life.

Blood Sampling and Analysis of Metabolites and Hormones

Blood samples were collected via jugular venipuncture into two vacuum tubes, one tube without anticoagulant and the other containing sodium fluoride and potassium EDTA (Vacuette of Brazil, Campinas, SP, Brazil). Sampling was done at birth and every 30 min during the cold challenge. Blood samples were also collected weekly, 2 h after morning feeding until 8 weeks of age. Samples were centrifuged at 2,000 x g, for 20 min at 4°C to obtain plasma or serum, and were stored at -20°C for subsequent analysis. Specific commercial enzymatic kits from LABTEST Diagnóstica S.A. (Lagoa Santa, MG, Brazil) were used to analyze total protein (Ref.: 99), albumin (Ref.: 19), glucose (Ref.: 85), lactate (Ref.: 116), alkaline phosphatase (Ref.: 40); and from Randox Laboratories (Life Sciences Ltd., Crumlin, UK) to analyze β -hydroxybutyrate (Ref.: RB1007) and NEFA (Ref.: FA115) in an automatic biochemistry system (SBA – 200, CELM, Barueri, SP, Brazil). Insulin from blood samples taken during the cold challenge was determined by a chemiluminescence immunoassay using the Immulite 1000 (Siemens Healthcare Diagnostics, Deerfield, IL), using components of commercial kit (Diagnostic Products Corp., Los Angeles, CA). The assay sensitivity was 2.0 μ IU. Concentrations of T3, T4 (h 0 and 120) and cortisol (h 0, 60 and 120) were measured with commercial ELISA kits from Monobind Inc. (Lake Forest, CA, USA) and a microplate reader. An aliquot of blood from the tube containing anticoagulant was used for hematocrit determination, after centrifugation (SPIN 1000 – MICROSPIN) at 12,000 x g for 10 min. Blood samples (0.02 mL) were diluted with 4 mL of Gower solution (12.5g sodium sulfate and 33.3 mL glacial acetic acid in 100 mL on distilled water) for the cells preservation. The dilution was pipetted into the Neubauer chamber and observed under a microscope (400X, Bioval, PR, Brazil) for the total count of erythrocytes in μ L. For the leukocytes count, blood samples (0.02 mL) were diluted with 0.4 mL of Turk solution (2 mL of acetic acid, 1 mL of gentian violet, 100 mL distilled water), pipetted into the Neubauer chamber and observed under a microscope (400X, Bioval, PR, Brazil).

Statistical Analysis

Continuous data with or without repeated measures over time were analyzed using the PROC MIXED procedure of SAS version 9.3 (SAS/STAT, SAS Institute Inc., Cary, NC) with models fitting a Gaussian distribution. Data were tested for normality of residuals. Daily feed intake, fecal score and rectal temperature during preweaning data were averaged for each week before statistical analysis. For data without repeated measures, the model used was: $Y_{ij} = \mu + T_i + B_j + E_{ij}$ where μ = Overall average; T_i was the treatment effect (colostrum volume); B_j was the random block effect; E_{ij} = random experimental error. The repeated measures were analyzed

according to the model: $Y_{ijk} = \mu + T_i + B_j + I_k + TI_{ik} + E_{ijk}$, where Y_{ijk} was the response variable; μ was the overall mean; T_i was the treatment effect (colostrum volume); B_j was the random block effect; I_k was the time or age effect; TI_{ik} was the effect of the interaction of treatment and time or age; and E_{ijk} was the residual effect. Orthogonal contrasts performed were as follows: (1) linear effect of treatment, (2) quadratic effect of treatment, (3) time effect, (4) interaction of time and treatment. In all analyses, differences were considered significant when $P \leq 0.05$, whereas tendencies were considered when $0.10 \geq P > 0.05$.

3.3. RESULTS AND DISCUSSION

Cold challenge

The results of rectal and skin surface temperature during the cold challenge are presented in Figure 2. Rectal temperature tended to increase quadratically as colostrum intake increased ($P = 0.07$). Le Dividich et al. (2005) suggests a direct linear relationship between rectal temperature and the amount of colostrum consumed by the newborn pig during the first day postpartum, when kept under thermoneutral conditions. Rectal temperature decreased ($P < 0.001$), as time of cold challenge increased. Contrary to the current finding, some authors reported an increase in rectal temperature with time of cold exposure (Lammoglia et al., 1999a,b; Bellows and Lammoglia, 2000). However, the authors observed those results at 0 °C. According to Klingenspor et al. (2017), the mechanism of increased temperature involves skin thermoreceptors activated by cold sensation, resulting in heat production, which 10°C may not have been cold enough to elicit a response from.

Ear temperature was not affected by treatment. However, prescapular temperature increased linearly with increasing intake of colostrum ($P = 0.03$). This result suggests that the brown adipose tissue present in prescapular region, using substrates from colostrum for thermogenesis, produced more heat as the volume of colostrum increased. Skin temperature of the thorax, thigh and shin did not differ due to treatments, but there was a time effect for all of them, with decreased temperature as cold challenge time passed ($P < 0.001$). There was a quadratic effect on foot temperature ($P < 0.05$) and a tendency for a quadratic effect on tail temperature. Thus, foot and tail temperatures were positively correlated with rectal temperature, since constriction of peripheral blood supply reduces rectal temperature (Gonzalez-Jimenez and Blaxter, 1962).

Figure 2. Rectal and the skin surface temperature of newborn dairy calves fed different volume of colostrum, across time during a cold challenge. 10% BW as colostrum (n = 10); 15% BW as colostrum (n = 10); 20% BW as colostrum (n = 10). (A) Rectal temperature; linear effect ($P = 0.01$), quadratic effect ($P = 0.07$), time ($P < 0.001$) and treatment by time interaction ($P = 0.32$). (B) Ear; linear effect ($P = 0.38$), quadratic effect ($P = 0.86$), time ($P < 0.001$) and treatment by

time interaction ($P = 0.72$). (C) Prescapular; linear effect ($P = 0.03$), quadratic effect ($P = 0.69$), time ($P = 0.11$) and treatment by time interaction ($P = 0.54$). (D) Thorax wall; linear effect ($P = 0.16$), quadratic effect ($P = 0.92$), time ($P < 0.001$) and treatment by time interaction ($P = 0.83$). (E) Thigh; linear effect ($P = 0.91$), quadratic effect ($P = 0.61$), time ($P < 0.001$) and treatment by time interaction ($P = 0.97$). (F) Shin; linear effect ($P = 0.18$), quadratic effect ($P = 0.45$), time ($P < 0.001$) and treatment by time interaction ($P = 0.62$). (G) Foot; linear effect ($P = 0.41$), quadratic effect ($P = 0.05$), time ($P < 0.001$) and treatment by time interaction ($P = 0.11$). (H) Tail; linear effect ($P = 0.39$), quadratic effect ($P = 0.10$), time ($P < 0.001$) and treatment by time interaction ($P = 0.34$).

There was no difference among treatments for heart rate, but respiratory rate tended to increase linearly at 150 min of the cold challenge (treatment by time interaction; $P = 0.09$, Figure 3) for the lowest colostrum amount (10%), probably due a reduced respiratory stimulation caused by increasing rectal temperature (Conlon et al., 2011).

Figure 3. Heart and respiratory rate of newborn dairy calves fed different volume of colostrum, across time during a cold challenge. 10% BW as colostrum ($n = 10$); 15% BW as colostrum (n

= 10); 20% BW as colostrum (n = 10). (A) Heart rate; linear effect ($P = 0.17$), quadratic effect ($P = 0.91$), time ($P = 0.25$) and treatment by time interaction ($P = 0.17$). (B) Respiratory rate; linear effect ($P = 0.26$), quadratic effect ($P = 0.59$), time ($P < 0.001$) and treatment by time interaction ($P = 0.09$).

Volume of colostrum affected shivering during the cold challenge, showing a linear decrease ($P = 0.03$, Figure 4). Respiratory rate could have had an effect on shivering amplitude because according to Pozos and Danzl (2001) inspiration of cold air causes an increase in rhythmic and tonic muscle activity, increasing shivering behavior, as observed in treatment 10%. Moreover, increased volume of colostrum probably provided more triglycerides for heat production by non-shivering thermogenesis, decreasing cold stress and then shivering, since increased triglycerides are positively related to the amount of colostrum intake (Rauprich et al., 2000).

Figure 4. Shivering of newborn dairy calves fed different volume of colostrum, across time during a cold challenge. 10% BW as colostrum (n = 10); 15% BW as colostrum (n = 10); 20% BW as colostrum (n = 10). Linear effect ($P = 0.03$), quadratic effect ($P = 0.98$), time ($P < 0.001$), treatment by time interaction ($P = 0.77$).

Mean blood concentrations of metabolites during the cold challenge are presented in Figure 5. A linear increase in total protein was observed 24 h after birth as colostrum intake increased (6.78, 7.03, and 7.28 g/d for 10%, 15% and 20% BW of colostrum, respectively; SEM 0.21, $P = 0.03$). Additionally, during challenge concentrations of total protein increased linearly with increasing volume of colostrum ($P = 0.03$). As expected, mean concentrations of serum total protein exceeded 5.5 g/dL, since the current study provided a sufficient volume of

colostrum to meet or exceed adequate passive transfer immunity (McGuirk and Collins, 2004; Godden, 2008). Serum albumin of calves fed more colostrum tended to decrease linearly ($P = 0.06$). Morin et al. (2010) reported that expansion in the plasma volume may decrease serum albumin due dilution. In a previous study, albumin concentrations decreased after milk feeding (Muri et al., 2005). Plasma glucose concentrations were not affected by colostrum volume, but a time effect was observed ($P < 0.001$) with increased glucose achieving high levels as time of cold challenge increased, possibly due to cold stress. Knowles and Warriss (2007) suggest that an initial response to stress is release of adrenaline and noradrenalin stimulating hepatic glycogenolysis, leading to increased plasma glucose levels. On the other hand, according to the authors, those hormones have a rather short half-life in blood stream. Lammoglia et al. (1999b) observed an increased in glucose concentrations when the calves were placed in the 0 °C room with a peak concentration reached at 50 min of cold exposure, then decreased to approximately the level noted at the start of cold exposure. Thus, 150 min on 10 °C may not have been time enough to observe the peak.

Colostrum levels linearly decreased concentrations of lactate ($P = 0.04$). Additionally, it was observed that lactate increased right after cold exposure for all groups ($P = 0.03$). Gruber et al. (2014) reported that more stressed behavior post-transportation was not correlated with concentrations of glucose, but was associated with increased lactate, which agrees with results observed in the present study. According to the authors, exposure of animals to adverse stimuli resulted in a cascade of effects, including increased metabolic rate leading to greater concentrations of lactate.

Concentrations of NEFA tended increase quadratically with increasing volume of colostrum ($P = 0.09$). Increased concentrations of NEFA generally indicates mobilization of fat due to low nutrient intake. However, feeding more colostrum may have provided higher amount of triglycerides to the body, thus allowed more NEFA mobilization as substrate for non-shivering thermogenesis (Vermorel et al., 1983; Himms-Hagen, 1990).

Figure 5. Blood metabolites of newborn dairy calves fed different volume of colostrum, across time during a cold challenge. 10% BW as colostrum (n = 10); 15% BW as colostrum (n = 10); 20% BW as colostrum (n = 10). (A) Total protein; linear effect ($P = 0.03$), quadratic effect ($P = 1.00$), time ($P < 0.001$), treatment by time interaction ($P = 0.86$). (B) Albumin; linear effect ($P = 0.06$), quadratic effect ($P = 0.21$), time ($P = 0.01$), treatment by time interaction ($P = 0.48$). (C) Glucose; linear effect ($P = 0.20$), quadratic effect ($P = 0.55$), time ($P < 0.001$), treatment by time interaction ($P = 0.90$). (D) Lactate; linear effect ($P = 0.04$), quadratic effect ($P = 0.37$), time ($P = 0.03$), treatment by time interaction ($P = 0.17$). (E) NEFA; linear effect ($P = 0.64$), quadratic effect ($P = 0.09$), time ($P < 0.001$), treatment by time interaction ($P = 0.73$). (F) Alkaline phosphatase; linear effect ($P = 0.37$), quadratic effect ($P = 0.24$), time ($P < 0.001$), treatment by time interaction ($P = 1.00$).

Mean blood hormones during the cold challenge are presented in Table 1. Insulin concentrations were not affected by treatments, but was observed increased levels as time of cold exposure increased ($P = 0.01$). On the contrary, Bassett and Alexander (1971) observed that during cold exposure insulin declined. According to the authors, suppression of insulin secretion will minimize glucose uptake by peripheral tissues and so conserve glucose for use by central nervous tissue and mainly, this suppression of insulin secretion will minimize its antilipolytic effects in BAT allowing the triglyceride breakdown proceeding at a maximum rate. Despite that, the increased glucose levels in the present study may stimulated insulin secretions. There was no difference among treatments for cortisol. In contrast to our data, other authors reported that cortisol concentrations were affected by time during cold stress, with cortisol reaching peak concentration after approximately 10 to 20 min of cold exposure, and returning to initial concentrations after 80 min of exposure (Lammoglia et al., 1999a,b; Bellows and Lammoglia, 2000). No difference among treatments was observed for T3 and T4. Contrary to the current finding, Stojić et al. (2002) reported an increase of T3 and T4 concentrations in calves fed a full ration of colostrum compared with calves feed half that amount.

Table 1. Blood hormones during a cold challenge of newborn calves fed different volumes of colostrum

Item ¹	Treatments ²			SEM	P ³ <			
	10%	15%	20%		L	Q	Time	T*Time
Insulin, μ UI	3.8	4.7	4.0	0.68	0.88	0.33	0.01	0.71
Cortisol, μ g/dL	6.8	7.1	8.1	0.85	0.16	0.60	0.12	0.75
T3, ng/dL	4.2	3.5	4.3	0.55	0.95	0.29	0.81	0.35
T4, ng/dL	1.3	1.2	1.1	0.19	0.27	0.91	0.30	0.36

¹T3 = Triiodothyronine; T4 = thyroxine.

²10%, 15% e 20% = BW as colostrum.

³P value for orthogonal contrasts of treatments, time and treatment by time interaction: L = linear effect of treatment, Q = quadratic effect of treatment; T*Time = treatment by time interaction.

Cortisol and thyroid hormones are mainly involved in thermogenesis of the neonate. Cortisol stimulated by cold stress enhances the maturation of the thyroid axis leading to increased thyroid hormones levels and conversion of T4 to T3 in the BAT (Klingenspor, 2003; Kirovski, 2015). Triiodothyronine enhances the expression of uncoupling protein-1 (UCP1), which produces an increase in proton leakage in the mitochondrial inner membrane surface area thus, leading to increased heat production and regulated cold thermogenesis (Harper et al., 2002; Cannon and Nedergaard, 2004).

Calf performance

The results of feed intake, BW, average weight gain, feed efficiency and body measurements are showed in Table 2. Was observed an increased tendency for starter intake (treatment by age interaction; $P = 0.08$) during preweaning, increasing linearly at week 4 and quadratically at week 5 (Figure 3). In a previous study, calves fed unlimited amounts of colostrum presented greater dry matter intake compared with animals fed a restricted amount (Hammon et al., 2002). No differences were observed among treatments for total DMI, ADG or feed efficiency during preweaning. Additionally, treatment did not affect preweaning or weaning BW. A previous study with dairy calves reported that animals fed 4 L had greater BW compared with calves fed 2 L (Faber et al., 2005). However, those authors provided only 2 L of colostrum, which is below recommendations (USDA-NAHMS, 2014). According to the authors, although a direct assessment of Ig effect cannot be determined, since blood Ig was not measured, a high quality colostrum was fed to each treatment group, assuming that the heifers fed the greater volume likely had greater concentrations of Ig, which could have contributed to increased growth. Preweaning heart girth increased linearly with increasing volume of colostrum ($P = 0.05$) and at weaning ($P = 0.04$), and hip width at weaning tended to increase quadratically ($P = 0.09$). A study using different liquid-feeding systems also reported an effect of treatment on heart girth, but no differences in BW or ADG (de Paula et al., 2017). Moallem et al. (2010) suggests that there may be a different mechanism in the altered pattern of skeletal growth as compared with BW. According to Stamey et al. (2012), it is assumed that increased crude protein content of starter supports the greater rates of lean tissue growth. Thus, these improvements in growth may have occurred as a result of tendency to increased starter intake.

Figure 5. Preweaning starter intake of dairy calves previously fed different volumes of colostrum. 10% BW as colostrum (n = 15); 15% BW as colostrum (n = 15); 20% BW as

colostrum ($n = 15$). Age effect of treatment ($P < 0.001$) and treatment by age interaction ($P = 0.08$).

Table 2. Growth and intake during preweaning and at weaning (d 56) of dairy calves previously fed different volumes of colostrum

Item	Treatments ¹			SEM	P ² <			
	10%	15%	20%		L	Q	Age	T*Age
Prewaning								
Concentrate intake, g of DM/d	407.1	488.2	456.5	50.05	0.50	0.35	<0.001	0.08
Total intake, g of DM/d	1098.0	1178.4	1156.2	77.66	0.52	0.50	<0.001	0.14
Body weight, kg	47.1	50.9	50.2	4.88	0.18	0.25	<0.001	0.73
Average weight gain, g/d	481.4	544.4	552.8	55.24	0.27	0.61	<0.001	0.42
Feed efficiency	0.4	0.4	0.5	0.03	0.14	0.31	<0.001	0.42
Heart girth, cm	84.1	86.9	86.6	2.65	0.05	0.13	<0.001	0.10
Hip width, cm	22.9	23.6	23.3	0.69	0.29	0.12	<0.001	0.34
Wither height, cm	82.7	84.2	82.6	1.88	0.94	0.13	<0.001	0.94
d 56								
Concentrate intake, g of DM/d	1051.8	1212.6	1108.6	183.85	0.78	0.41	-	-
Total intake, g of DM/d	1803.5	1945.6	1859.6	183.49	0.78	0.49	-	-
Body weight, kg	65.4	71.9	70.6	4.89	0.25	0.29	-	-
Heart girth, cm	92.3	96.5	96.6	2.30	0.04	0.21	-	-
Hip width, cm	24.9	26.1	25.4	0.17	0.40	0.09	-	-
Wither height, cm	87.7	89.3	87.3	1.94	0.84	0.27	-	-

¹10%, 15% e 20% = BW as colostrum.

²P value for orthogonal contrasts of treatments, time and treatment by time interaction: L = linear effect of treatment, Q = quadratic effect of treatment; T*Age = treatment by Age interaction.

Mean results of blood metabolites during preweaning and weaning are presented in Table 3. No differences were observed in blood total protein, albumin, glucose and lactate concentrations among treatments. Hammon et al. (2002) also observed that concentrations of those metabolites were not different among treatments when calves were provided unlimited or restricted amounts of colostrum. As expected, glucose concentrations in young calves exceed adult values (45–55 mg/dL) during the first weeks of life (Nonnecke et al., 2009) due to milk feeding and a non-functional rumen. Blood concentrations of BHB during the preweaning period tended to increase linearly as intake of colostrum increased ($P = 0.06$). As expected, an age effect was seen on BHB concentrations ($P < 0.001$) with increased BHB concentrations closer to weaning as concentrate intake increases, contributing to rumen development (Naeem et al., 2012) and ketogenesis (Quigley, 1996). Increasing consumption of colostrum after birth resulted in quadratically decreasing ($P = 0.04$) concentrations of NEFA in blood of calves during preweaning, whereas at weaning concentrations of NEFA decreased linearly as colostrum supply increased ($P = 0.03$).

Table 3. Blood metabolite concentrations during preweaning and at weaning (d 56) of dairy calves previously fed different volumes of colostrum.

Item ¹	Treatments ²			SEM	P ³ <			
	10%	15%	20%		L	Q	Age	T*Age
Preweaning								
Total protein, g/dL	5.4	5.5	5.6	0.09	0.13	0.89	<0.001	0.17
Albumin, g/dL	2.8	2.8	2.9	2.83	0.57	0.15	<0.001	0.52
Glucose, mg/dL	98.6	107.9	102.7	6.00	0.63	0.32	<0.001	0.95
Lactate, mg/dL	13.5	14.2	14.3	0.89	0.56	0.75	<0.001	0.30
BHB, mmol/L	0.07	0.09	0.09	0.005	0.06	0.29	<0.001	0.50
NEFA, mmol/L	0.21	0.16	0.19	0.19	0.39	0.04	<0.001	0.49
d 56								
Total protein, g/dL	5.7	5.3	5.5	0.16	0.46	0.22	-	-
Albumin, g/dL	3.0	2.9	3.1	0.08	0.27	0.12	-	-
Glucose, mg/dL	111.0	126.7	124.0	10.04	0.32	0.38	-	-
Lactate, mg/dL	8.5	8.5	8.4	0.57	0.91	0.95	-	-
BHB, mmol/L	0.13	0.17	0.14	0.03	0.74	0.18	-	-
NEFA, mmol/L	0.31	0.18	0.17	0.04	0.03	0.27	-	-

¹BHB = beta-hydroxybutyrate; NEFA = Non esterified fatty acids.

²10%, 15% e 20% = BW as colostrum.

³P value for orthogonal contrasts of treatments, age and treatment by age interaction: L = linear effect of treatment, Q = quadratic effect of treatment; T*Age = treatment by Age interaction.

Circulating concentrations of BHB are highly correlated with concentrate intake while concentrations of NEFA are negatively correlated with intake of solid feed by the preruminant calf (Quigley, 1996). Therefore, in the current study the tendency to increase starter intake at week 4 and 5 probably was sufficient to promote an effect on mean of metabolites concentrations among treatments, leading to increased BHB and decreased NEFA, as colostrum intake was higher.

Health parameters, mean values of hematocrit and blood cells observed preweaning are presented in Table 4. A linear increase in fecal score was observed 24 h after birth ($P = 0.05$), potentially due decreased curd formation. Miyazaki et al. (2017) suggest that calves exhibiting incomplete and no curd formation may be unable to absorb colostrum IgG efficiently due rapid emptying. No difference was observed among treatments for fecal score preweaning, similar to Conneely et al. (2014). However, there was an age effect on fecal score ($P < 0.001$) with an increase during the second week of age for all treatments (Figure 6), in agreement with other studies (Garcia et al., 2015; Miqueo et al., 2017). There was no difference among treatments for rectal temperature and diarrhea days, suggesting adequate immune passive transfer for the lowest volume fed (10%).

Table 4. Fecal score, diarrhea occurrence, rectal temperature, hematocrit, erythrocytes and leukocytes during preweaning of dairy calves previously fed different volumes of colostrum.

Item	Treatments ¹			SEM	P ² <			
	10%	15%	20%		L	Q	Age	T*Age
Fecal score								
24h after birth	1.0	1.5	1.5	0.18	0.05	0.21	-	-
Prewaning	1.8	1.8	1.7	0.07	0.26	0.50	<0.001	0.46
Diarrhea days	10.3	12.4	9.3	2.08	0.61	0.13	-	-
Rectal temperature, °C	38.3	38.3	38.3	0.06	0.61	0.97	<0.001	0.27
Hematocrit, %	20.1	20.0	20.5	0.95	0.58	0.60	0.03	0.96
Erythrocytes, 10 ⁶ /μL	7.1	7.2	6.9	0.27	0.47	0.57	<0.001	0.23
Leukocytes, 10 ³ / μL	6.3	6.3	7.4	0.37	0.02	0.14	0.02	0.85

¹10%, 15% e 20% = BW as colostrum.

²P value for orthogonal contrasts of treatments, age and treatment by age interaction: L = linear effect of treatment, Q = quadratic effect of treatment; T*Age = treatment by Age interaction.

Figure 6. Average weekly fecal score of preweaning dairy calves previously fed different volumes of colostrum. 10% BW as colostrum (n = 15); 15% BW as colostrum (n = 15); 20% BW as colostrum (n = 15). Possible scores were 0 for firm feces, no diarrhea; 1 for soft feces, no diarrhea; 2 for mild diarrhea; and 3 for watery, severe diarrhea. Time effect of treatment ($P < 0.001$).

Mean values for hematocrit were not affected by treatment, but there was an age effect ($P = 0.03$) with an increase at the second week of life for all treatments coinciding with increased fecal score, usually related to increased dehydration (Leal et al., 2012; Garcia et al., 2015). Erythrocytes were also not affected by treatments. However, mean concentration of leukocytes increased linearly with increasing volume of colostrum ($P = 0.02$). Increased concentrations of leukocytes are usually related to innate responses to disease (Hulbert and Moisé, 2016). However, mean values of leukocytes cells were within the reference intervals for preweaning health dairy calves (Jezek et al., 2011). Thereby, the increasing concentration of leukocytes detected in calves fed increased volume of colostrum could be due to increased intake of colostral leukocytes that have a significant effect on the leukocyte response in calves, stimulating the development of neonatal immune responses (Reber et al., 2005)

3.4. CONCLUSION

Feeding higher volumes of colostrum had a positive effect on newborn calves' thermoregulatory responses during cold challenge. However, even though there was a benefit for the calf submitted to cold stress at the first day of life, feeding higher volumes of colostrum resulted in smaller differences on performance pre and at weaning. Nevertheless, calves fed higher volume of colostrum presented increased immunity responses during preweaning.

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4. PERFORMANCE AND PRODUCTION COST OF CALVES FED MILK REPLACER OR WHOLE MILK AND TRADITIONAL OR AN ALTERNATIVE STARTER FEED PROGRAM IN LOW ENVIRONMENTAL TEMPERATURE

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ABSTRACT

Traditional calf starters are formulated to balance nutrient needs of calves fed restricted amounts (4L/d) of milk replacer or whole milk. The objective of this study was to compare performance and production cost of calves fed milk replacer (MR) or whole milk (WM) and a commercial textured starter (TS) or an alternative starter (AS) feeding program during low environmental temperatures ($1.4 \pm 9.2^{\circ}\text{C}$). Holstein heifers ($n=75$) received 4 L of high quality colostrum within 6 hours of birth and were assigned randomly to one of five treatment groups in a $2 \times 2 + 1$ factorial. The first treatment was a negative control (NC): 4 L/d of MR from d1 to 49, 2L/d of MR from d 50 to 56 of the study and *ad libitum* TS (commercial texturized ration). The other 4 treatments were a high rate (HR) of MR or WM (6L/d from d 1 to 7, 8 L/d from d 8 to 35, 4L/d from d 36 to 42, and 2 L/d from d 43 to 49 of the study) and *ad libitum* AS (cracked corn 8% CP from d 1 to 21, low protein grower 11% CP from d 22 to 28, high protein grower 20% CP from d 29 to 49 of the study) or TS (19% CP). After weaning, animals were maintained in group hutches in the same environment with free access to water and grower (18% CP) until 12 weeks of life. Daily intake and health data were recorded until wk 7. Growth rates, blood metabolites and hemogram were recorded until wk 12. Calves on NC presented lower BW at wk 12 than calves on HRAS. Calves fed WM had increased BW at wk 7 and 12, compared to those fed MR. Calves fed AS had lower BW at wk 12 than calves fed TS. Calves on the NC consumed more concentrate and less total DMI during the preweaning period than calves in HR with TS or AS and thus had lower feed costs. Calves fed TS showed greater starter and total intake compared to AS. Mean for ADG during all trial was greater for calves fed WM than calves that received MR. Calves fed MR had higher health scores (higher morbidity) and lower plasma glucose preweaning than those fed WM. Concentrations of BHB were greater for NC compared to HRAS, and were greater for TS compared to AS. The AS program had lower total costs compared to the TS program. In conclusion, feed restricted liquid diet provide economic body weight gains. However, the final BW was higher for calves fed HRAS suggesting an increased future productivity. In addition, feeding calves WM showed superior performance and decreased morbidity. Besides, replacing commercial TS with an AS in high rate of nutrition present minimal impact on performance with reduced rearing costs during the first months of life.

Keywords: Feeding system; Early weaning; Health; Economics

4.1. INTRODUCTION

For many years dairy replacement calves were fed limited amounts (typically 8% to 10% of birth BW) of whole milk or milk replacer (MR) and had *ad libitum* access to a dry grain mixture (calf starter) prior to weaning (Lesmeister and Heinrichs, 2004; Lesmeister and Heinrichs, 2005; Zhang et al., 2010). The restricted milk feeding program have been used to encourage starter feed intake and reduce feeding costs of rearing young calves. This practice provides liquid diet to meet or slightly exceed maintenance requirements of the young calf, about 200 to 300 g/d of growth (Drackley, 2008). In this type of feeding management, nutrients for growth are met by voluntary consumption of the starter concentrate rather than solids from milk (Rosenberger et al., 2017). Typically, the restricted milk feeding encourages calf starter intake, and stimulates early rumen development, allowing a smoother weaning transition (Khan et al., 2011). However, as showed in recent studies, preweaned calves are more efficient at converting milk to body mass (Stamey et al., 2012; Omid-Mirzaei et al., 2015; Hill et al., 2016). Preweaned calves do not yet have a functional rumen (MacPherson et al., 2016). Because of the initial low intake, during first 3 weeks of life, the calf relies almost entirely on liquid diet to meet nutrient requirements (NRC, 2001). A recent research showed that calves fed milk replacer *ad libitum* presented greater average weight gain in the first phase of life and first lactation milk yield 612 kg above that of animals fed approximately 50% less milk replacer (Korst et al., 2017). According to Soberon et al. (2012) for every 1 kg of preweaning average weight gain, milk yield increase about 1,113 kg in the first lactation.

Feeding systems have not only a strong influence on productions costs, but also on the potential of future milk production, turning the understanding of costs into a better approach of investments. Quigley et al. (2006) reported that calves fed additional MR presented greater body weight, body weight gain and feed efficiency. Yet, even with the higher feed efficiency, great cost per kilogram of body weight gain. However, Raeth-Knight et al. (2009) showed that calves fed intensive diet calved 27.5 d earlier than calves fed conventional MR, potentially decreasing costs associated with heifer replacer program (Tozer and Heinrichs, 2001). According to Korst et al. (2017), the economic returns from increased milk yield over the first lactation by animals previously fed milk *ad libitum* compared to conventional system seems to be able to compensate the additional expenses during the preweaning phase.

Costs of starter feeding programs may also increase substantially because of ingredient costs. According to Drackley (2008), nutritional inputs are some of the major costs of calf production. Therefore, the present study hypothesized that calves fed higher rates of milk or

MR and an alternative starter, being weaned a week early than the conventional system would improve performance and reduce rearing costs. The objective of this study was to compare performance and production cost of calves fed milk replacer or whole milk and a commercial textured starter or an alternative starter feeding program of calves raised at low environmental temperatures.

4.2. MATERIALS AND METHODS

The animal experiment was performed in strict accordance with the Animal Care and Use Committee of the College of Agriculture and Life Science at the University of Wisconsin-Madison.

Animals, Experimental Design and Treatments

This study was conducted from October 2016 to March 2017 ($1.4 \pm 9.2^{\circ}\text{C}$) at the UW – Emmons Blaine Dairy Cattle Research facility in Arlington, Wisconsin. Seventy –five Holstein heifer calves (birth weight 38.3 ± 3.7) were separated from their dams immediately after birth, weighed and fed 4L of high quality colostrum within 4 to 6 h of birth. Calves were housed in individual hutches and assigned randomly to one of five treatment groups. The first treatment was a negative control (NC): calves were offered 4 L/d of a commercial MR (12.5% Solids, 23% CP, 23% fat, DM basis,) as a restrict amount of liquid diet from d1 to 49, and 2L/d from d 50 to 56 and commercial TS. The other 4 treatments were a 2x2 factorial arrangement of a high rate (HR) of MR or pasteurized WM (6L/d from d 1 to 7, 8 L/d from d 8 to 35, 4L/d from d 36 to 42, and 2 L/d from d 43 to 49 of the study, Figure 1); and AS (cracked corn 8% CP from d 1 to 21, low protein grower 11% CP from d 22 to 28, high protein grower 20% CP from d 29 to 49 of the study) or pelletized TS (19% CP). After weaning at 56 (NC) or at 49 days of age (for all other treatments), calves were moved to collective hutches with free access to calf grower (18% CP) remained in the study until 12 weeks old. Water was available at all time for all treatments. The chemical composition of the experimental diets are present in Table 1.

Figure 1. The schematic represents the amounts of whole milk and milk replacer provided (L/d) by calves fed different diets and procedures. NC: negative control (n = 15) and HR: MRTS as milk replacer and traditional starter (n = 15); MRAS as milk replacer and alternative starter (n = 15); WMTS as whole milk and traditional starter (n = 15); WMAS as whole milk and alternative starter (n = 15).

Table 1. Chemical composition of experimental diets, milk replacer and whole milk on DM basis.

Item	Calf Starter	Calf Grower	Cracked Corn	Low protein grower	High protein grower	Milk Replacer	Whole Milk
Dry Matter %	89.5	86.9	89.5	88.6	87.2	96.2	12.2
CP	18.8	17.9	7.7	11.4	19.8	23.0	27.4
Fat	5.5	4.8	3.8	4.2	4.6	23.2	26.8
Lactose	-	-	-	-	-	46.8	38.1
NDF	21.4	13.0	8.0	9.8	12.8	-	-
Starch	29.6	38.9	68.0	57.5	36.7	-	-
Ash	5.6	6.2	1.4	3.1	6.3	7.0	7.8
NFC	49.19	58.1	80.42	72.4	57.1	-	-
TDN 1x	76.19	77.73	88.34	84.52	78.0	-	-
ME ¹ (Mcal/kg)	2.9	3.0	3.5	3.3	3	4.8	5.0

¹ME = Metabolized energy calculated as NRC2001 recommendation, pg 220.

Measurements and Sample Collections

Calves received the liquid diet split between 2 feedings at 500h and 1600h until the weaning. Traditional and alternative starter were fed *ad libitum* every morning, and orts were weighed to monitor daily intake. Intake of liquid diet also was recorded daily until weaning.

Milk replacer, traditional starter and the alternative starter ingredients were sampled weekly and grounded through a 1 mm for analysis in a commercial laboratory (DairyLand Laboratories, Arcadia, WI). Whole milk samples were collected from the bulk tank daily and analyzed also by a commercial laboratory (AgSource Milk Analysis Laboratory, Menominee, WI).

Animals were weighed at birth and week 2, 4, 6, 7, and 12 after birth using a digital scale. Heart girth and hip height were measured using a flexible tape and a ruler, respectively. Growth parameters were taken 2 h after morning milk feeding. Health was monitored daily using a scale from 0 to 3 through a digital scoring program (Calf Health Scorer App) developed by McGuirk and Peek (2014). Fecal scores was recorded according to fluidity as (0) normal; (1) semi-formed, pasty; (2) loose, but stays on top of bedding; (3) watery, sifts through bedding. Calves given a fecal score ≥ 2 were considered to have diarrhea. When diarrhea was diagnosed an oral electrolyte solution was offered until fecal score decreased and behavior returned to normal. Calves given a score ≥ 2 for other criteria were considered as having the disorder and were treated according to UW-Madison Dairy Herd standard operating procedures. Weekly averages of all scores were generated per calf for statistical analysis.

Blood samples were collected via jugular venipuncture into two vacuum tubes at 2, 4, 6, 7 and 12 weeks of age, 2 h after morning feeding. Tubes containing sodium fluoride and potassium EDTA were centrifuged at 2,000 x g, for 10 min at 4 °C to obtain plasma, and were stored at -20°C for subsequent glucose, nonesterified fatty acids (NEFA) and β -hydroxybutyrate (BHB) analysis. Specific commercial enzymatic kits (Wako Chemicals USA Inc., Richmond, VA) were used to analyze glucose and NEFA, and from Stanbio (Diagnostics-Stanbio, Boerne, TX; certified for serum and plasma) to analyzed BHB using a colorimetric assay. The other tubes contain K₃EDTA were sent to a commercial laboratory (Marshfield Labs, Marshfield, WI) to determinate the erythrocytes, hematocrit, hemoglobin, leukocytes, segmented neutrophils, lymphocytes, monocytes and eosinophils.

Costs

Feed costs were calculated based on intake from first treatment day to weaning assuming that WM price was \$3.31/kg (price for saleable milk), and MR price was \$3.09/kg. Costs of TS were \$0.57/kg, whereas costs for AS based on costs of \$0.15/kg, \$0.42/kg and \$0.35/kg for corn, calf grower and soybean meal, respectively. Electrolyte was calculated assuming that dose used in calf with diarrhea cost \$0.90 (Quigley et al. 2006).

Statistical Analysis

The data were analyzed as a completely randomized design with repeated measures when applicable using the PROC MIXED procedure of SAS version 9.4 (SAS/STAT, SAS Institute Inc., Cary, NC) with models fitting a Gaussian distribution. Data were tested for normality of residuals, and data with residuals not normally distributed were transformed before analysis. Data from starter intake and total intake during the preweaning period were not normally distributed, so they were transformed on a square root and base-10 logarithm, respectively. However, respective tables present nontransformed data. Data from BHB and NEFA pre and postweaning were not normally distributed, so they were transformed on a square root. However, respective figures present nontransformed data. Daily feed intake and health score data were averaged for each week before statistical analysis. The models included the fixed effects of treatment, age, interactions between treatment and age, and the random effects of animals nested within treatment. Preplanned contrasts performed were as follows: (1) NC versus HRTS, (2) NC versus HRAS, (3) MR versus WM, (4) TS versus AS. In all analyses, differences were considered significant when $P \leq 0.05$, whereas tendencies were considered when $0.10 \geq P > 0.05$.

4.3. RESULTS AND DISCUSSION

The results of body growth, feed intake, feed efficiency and average daily gain are presented in Table 2. During the preweaning period calves in NC consumed more concentrate than those fed the HR, regardless of the solid diet fed ($P = 0.01$). Several others studies have also reported that allowance increases of the liquid diet decreases the starter feed intake (Hill et al., 2016; MacPherson et al., 2016; de Paula et al., 2017). Additionally, Nonnecke et al. (2009) observed that calves fed restricted amount of milk replacer in cold environmental consumed more starter to maintain a growth rate comparable to calves in warm environment. Calves fed less milk likely attempted to compensate for the lack of nutrients by consuming more starter (Rosenberger et al., 2017). Starter intake from birth trough week 7 tended to be greater for calves that received WM than calves fed MR ($P = 0.06$). Contrary to the current finding, Moallem et al. (2010) observed that calves offered WM or MR *ad libitum* presented no difference in starter intake. Calves fed TS presented greater concentrate intake ($P < 0.01$) than calves fed AS, maybe due to the high palatability of traditional starter ingredients. According to Drackley (2008), calves need to eat easily fermentable ingredients with highly palatability to drive growth and differentiation of the ruminal absorptive epithelium. Bush (1989) offered dairy calves 4 different starter feeds containing barley, corn, oats, or hull-less oats preweaning

and observed that calves preferred the barley starter feeds, ingredient present in most traditional starter. Additionally, our calf starter ration contained molasses, commonly used to increase palatability (Morales et al., 1989). Even though calves in NC presented higher starter intake, their total intake was lower than calves in HR associated with TS or AS ($P < 0.01$), because of the reduced volume of liquid diet fed (4 L/d, 12.5% solids). Calves fed TS presented greater total feed intake as compared to calves fed AS ($P < 0.01$). As expected, starter and total feed intake increased with age ($P < 0.01$) for all treatments (Figure 2 and 3).

Prewaning gain and feed efficiency was greater for calves in WM treatments than calves fed MR ($P < 0.01$). Overall ADG (d0 to 12 wk) also was greater for calves fed WM than calves that received MR ($P < 0.01$). According to the model presented in the NRC (2001), feeding the same volume of whole milk would support a gain of 97g/d greater than milk replacer gain. Average daily gain tended to be greater when calves were fed TS as compared to AS ($P = 0.07$) from birth until week 7. Although those differences, calves presented expected average growth rates for calves fed accelerated milk or milk and *ad libitum* starter in thermoneutral conditions (Drackley, 2008). Additionally, no difference was observed considering the overall trial (d0 to 12 wk). A previous study providing high plane of WM and traditional starter *ad libitum* observed a BW gain of 700g/d from d1 until d49, whereas in the current study calves fed WM and AS presented a BW gain of 764.8g/d from d1 until d49 (Khan et al., 2007a). Contrary to the current finding, Scibilia et al. (1986) reported that calves housed in low environmental temperature had lower ADG and required higher fat diets to maintain similar rates of gain as calves housed in warmer environments. However, those authors housed calves in environments below 4°C.

Table 2. Growth, feed intake and feed efficiency of dairy calves fed conventional or high rate of liquid diet and traditional or alternative starter.

Item	Treatments ¹					SEM	P ² <			
	NC	MRTS	MRAS	WMTS	WMAS		NC vs HRTS	NC vs HRAS	MR vs WM	TS vs AS
d 0 to wk 7										
starter intake, g of DM/d	856.3	693.8	598.4	770.2	633.9	45.41	0.01	<0.01	0.06	<0.01
total intake, g of DM/d	1330.0	1436.9	1340.3	1490.2	1351.4	44.19	<0.01	<0.01	0.48	<0.01
feed efficiency	0.47	0.46	0.43	0.52	0.53	0.02	0.33	0.64	<0.01	0.54
average weight gain, g/d	701.3	713.4	634.6	803.3	764.8	33.3	0.15	0.97	<0.01	0.07
wk 7 to 12 average weight gain, g/d	821.77	876.3	839.6	928.0	901.1	40.9	0.11	0.33	0.16	0.43
Overall average weight gain, g/d	755.9	762.0	706.7	836.8	805.7	86.8	0.21	0.99	<0.01	0.12
d 0										
Body weight, kg	38.3	38.3	38.8	38.0	38.0	0.97	0.90	0.98	0.56	0.85
Hip height, cm	80.2	79.4	80.9	80.4	79.7	0.79	0.74	0.93	0.86	0.60
Heart girth, cm	79.6	79.6	79.7	79.1	79.3	0.68	0.81	0.93	0.55	0.84
wk 7										
Body weight, kg	73.7	74.0	70.5	78.3	76.5	1.75	0.24	0.94	<0.01	0.13
Hip height, cm	92.97	93.0	93.3	94.2	93.4	0.79	0.49	0.70	0.40	0.71
Heart girth, cm	99.0	99.8	98.3	100.4	99.3	1.03	0.40	0.84	0.40	0.20
wk 12										
Body weight, kg	101.9	102.8	97.5	107.7	105.6	0.89	<0.01	0.78	<0.01	<0.01
Hip height, cm	101.0	100.9	101.4	102.0	100.9	0.89	0.68	0.86	0.70	0.76
Heart girth, cm	112.1	111.5	112.2	111.7	112.7	0.91	0.64	0.77	0.66	0.35

¹NC = Negative control; MRTS= high rate of milk replacer and traditional starter; MRAS = high rate of milk replacer and alternative starter; WMTS = high rate of whole milk and traditional starter; WMAS = high rate of whole milk and alternative starter.

²P value for contrasts. NC vs HRTS = negative control versus high rate of liquid diet and traditional starter; NC vs HRAS = negative control versus high rate of liquid diet and alternative starter; MR vs WM = high rate of milk replacer versus high rate of whole milk; TS v s AS = traditional starter versus alternative starter.

Figure 2. Preweaning starter intake of dairy calves fed conventional or high rate of liquid diet and traditional or alternative starter. NC: negative control (n = 15); MRTS: milk replacer and traditional starter (n = 15); MRAS: milk replacer and alternative starter (n = 15); WMTS: whole milk and traditional starter (n = 15); WMAS: whole milk and alternative starter (n = 15). NC versus HRTS ($P = 0.01$), NC versus HRAS ($P < 0.01$), MR versus WM ($P = 0.06$), TS versus AS ($P < 0.01$).

Figure 3. Preweaning total intake of dairy calves fed conventional or high rate of liquid diet and traditional or alternative starter. NC: negative control (n = 15); MRTS: milk replacer and traditional starter (n = 15); MRAS: milk replacer and alternative starter (n = 15); WMTS: whole milk and traditional starter (n = 15); WMAS: whole milk and alternative starter (n = 15). NC versus HRTS ($P < 0.01$), NC versus HRAS ($P < 0.01$), MR versus WM ($P = 0.48$), TS versus AS ($P < 0.01$).

Initial calves' measurements (d 0) did not differ among treatments. Body weight was greater ($P < 0.01$) for calves fed WM compared to calves fed MR replacer at the 7th and 12th weeks of age. In a previous study, calves offered WM presented greater BW at weaning compared to calves fed same amount of MR with gain on fat and no differences in other skeletal measurements (Moallem et al., 2010). The authors suggest that the increased BW in the WM calves at weaning was due to greater fat deposition. Calves fed HRTS presented greater BW ($P < 0.01$) at week 12, than calves fed the conventional program (NC). However, no difference was observed between NC and HRAS for BW during the trial. Omid-Mirzaei et al. (2015) observed that calves fed according to intensive MR system and starter formulated according to the Cornell Net Carbohydrate and Protein System, version 5.1 (CNCPS; Fox et al., 2000), presented higher total DMI, BW and some body measurements pre and post-weaning, compared to calves in the conventional diet. According to the authors, the better performance could be explained by the higher nutrient availability due to the greater milk intake. However, liquid diet and starter both contribute to meet the nutrient requirements of the calf (NRC, 2001). Thus, lower starter intake associated with low nutrient source from the alternative starter (HRAS)

presented no benefit for BW. Calves fed TS presented higher BW ($P < 0.01$) compared to calves fed AS at week 12. Despite this difference, BW for calves fed WM and the AS was 2.8 kg higher than BW for calves fed MR and TS. Feeding systems did not affect hip height and heart girth in the present study.

The health parameters, hematocrit and concentrations of select blood cells, observed preweaning are presented in Table 3. Only fecal and respiratory score are presented since a score ≥ 2 was observed only in those variables. No difference was observed between NC and high rate of nutrition with TS or AS. Contrary to the current finding, Hill et al. (2016) reported increased fecal score of calves fed additional milk replacer. However, they worked reconstituting high rate to 15% solids, whereas moderate feeding program was reconstituted to 13% solid, thus calves fed high rate presented increased fecal score possibly due a higher osmolality of the liquid feed (Glosson et al., 2015). Calves fed MR presented greater fecal score as compared to calves in WM treatments ($P < 0.01$), suggesting a positive effect of WM feeding on abomasal emptying. However, there was no difference between TS and AS for fecal score.

Table 3. Health score, diarrhea days, hematocrit and concentrations of blood cells during preweaning period of dairy calves fed conventional or high rate of liquid diet and traditional or alternative starter.

Item	Treatments ¹					SEM	P ² <			
	NC	MRTS	MRAS	WMTS	WMAS		NC vs HRTS	NC vs HRAS	MR vs WM	TS vs AS
Fecal score	0.3	0.3	0.3	0.2	0.2	0.03	0.30	0.35	<0.01	0.91
Respiratory score	0.1	0.2	0.2	0.1	0.2	0.04	0.81	0.56	0.55	0.69
Health score	0.5	0.5	0.5	0.4	0.4	0.06	0.71	0.85	0.03	0.83
Diarrhea days	1.3	1.8	1.7	0.7	0.7	0.40	0.94	0.80	0.01	0.82
Hematocrit, %	37.8	40.9	39.5	36.2	34.9	0.74	0.36	0.54	<0.01	0.07
Erythrocytes, 10 ⁶ /μL	10.1	10.0	9.8	9.8	9.4	0.19	0.30	0.03	0.08	0.17
Hemoglobin, g/dL	11.8	12.4	12.1	11.3	10.7	0.23	0.84	0.18	<0.01	0.06
Leukocytes, 10 ³ /μL	9.7	9.4	10.0	10.2	9.5	0.39	0.93	0.95	0.68	0.97

¹NC = Negative control; MRTS= high rate of milk replacer and traditional starter; MRAS = high rate of milk replacer and alternative starter; WMTS = high rate of whole milk and traditional starter; WMAS = high rate of whole milk and alternative starter.

²P value for contrasts. NC vs HRTS = negative control versus high rate of liquid diet and traditional starter; NC vs HRAS = negative control versus high rate of liquid diet and alternative starter; MR vs WM = high rate of milk replacer versus high rate of whole milk; TS v s AS = traditional starter versus alternative starter.

During weeks 2 and 3 an increased fecal score was observed for all treatments (Figure 4), in agreement with McGuirk (2008). No difference was observed for respiratory score. Total health score was higher for calves fed MR than calves fed WM preweaning ($P = 0.03$), probably due difference in fecal score. Similar to the current research, Godden et al. (2005) observed higher morbidity risk for calves fed MR than calves fed pasteurized nonsaleable milk. Calves fed MR had diarrhea for a higher number of days than calves fed WM ($P = 0.01$). A previously study observed that calves fed WM tended to have more days with diarrhea requiring more electrolyte therapies as compared with calves fed MR (Górka et al. 2011).

Figure 4. Preweaning average weekly fecal score of dairy calves fed conventional or high rate of liquid diet and traditional or alternative starter. NC: negative control ($n = 15$); MRTS: milk replacer and traditional starter ($n = 15$); MRAS: milk replacer and alternative starter ($n = 15$); WMTS: whole milk and traditional starter ($n = 15$); WMAS: whole milk and alternative starter ($n = 15$). Possible scores were 0 for firm feces, no diarrhea; 1 for soft feces, no diarrhea; 2 for mild diarrhea; and 3 for watery, severe diarrhea. NC versus HRTS ($P = 0.30$), NC versus HRAS ($P = 0.35$), MR versus WM ($P < 0.01$), TS versus AS ($P = 0.91$).

Calves fed MR presented greater values of hematocrit and hemoglobin compared to calves fed WM ($P < 0.01$), probably due to the greater fecal score preweaning. Decreased concentrations of hematocrit and hemoglobin are in part result of hemodilution, whereas increased blood cells are usually related to dehydration caused by diarrhea (Muri et al., 2005; Garcia et al., 2015). Calves fed TS tended to present higher hematocrit ($P = 0.07$) and hemoglobin ($P = 0.06$) than calves fed AS. Calves fed solely milk, which is low in iron, a metal

required to hemoglobin synthesis, may present iron deficiency anemia (Mohri et al., 2004). Thus, calves fed AS probably tended to presented lower hemoglobin production due lower concentrate intake. In addition, the ingredients in AS perhaps presented lower iron concentration or availability. According to the NRC (2001) some of traditional starters and grower are supplemented with minerals including iron, whereas the cracked corn is not. It was observed that NC presented greater erythrocytes than HRAS ($P = 0.03$) and MR tended to present greater erythrocytes compared to WM ($P = 0.08$). As hemoglobin, low level of erythrocyte establish the occurrence of anemia in calves (Mandal et al. 2015). However, values of hemoglobin and erythrocytes were within the reference intervals for health dairy calves (Jezek et al., 2011). Mean values for leukocytes were not affected by treatments.

There was no difference between NC and HRTS preweaning for glucose concentrations, supporting previous studies (Omidi-Mirzaei et al., 2015; Chapman et al., 2017). However, plasma glucose was greater in calves HRAS from birth trough week 7 compared to calves fed NC ($P = 0.05$), possibly as a result of greater liquid feeding and the greater starch intake by cracked corn provided as the main ingredient in AS group until week 4 (Figure 5). Postweaning concentrations of glucose were lower in AS as compared to TS treatment ($P = 0.02$), possibly due to greater rumen development postweaning by increased feed intake (uncollected data). According to Khan et al. (2007b) lower blood glucose concentration may be ascribed to more solid feed consumption, likely better ruminal activity and fermentation. Decreases in blood glucose levels were observed in all feeding regimens as calves grew older, suggesting development of the ruminal function (Quigley et al., 1991). Mean concentrations of NEFA tented to be greater in NC versus HRAS ($P = 0.06$) from birth to week 7 (Figure 6), probably due to lower glucose levels. Concentrations of NEFA are sensitive to plasma glucose, and indicates greater mobilization of fat due to low nutrient intake (Abdelgadir et al., 1996; Zhang et al., 2010; Gilbert et al., 2017).

Figure 5. Glucose concentration of dairy calves fed conventional or high rate of liquid diet and traditional or alternative starter. NC: negative control (n = 15); MRTS: milk replacer and traditional starter (n = 15); MRAS: milk replacer and alternative starter (n = 15); WMTS: whole milk and traditional starter (n = 15); WMAS: whole milk and alternative starter (n = 15). Prewaning contrasts: NC versus HRTS (P = 0.12), NC versus HRAS (P = 0.05), MR versus WM (P = 0.14), TS versus AS (P = 0.62). Postweaning contrasts: NC versus HRTS (P = 0.40), NC versus HRAS (P = 0.28), MR versus WM (P = 0.48), TS versus AS (P = 0.02).

Figure 6. Nonesterified fatty acids concentration of dairy calves fed conventional or high rate of liquid diet and traditional or alternative starter. NC: negative control (n = 15); MRTS: milk replacer and traditional starter (n = 15); MRAS: milk replacer and alternative starter (n = 15); WMTS: whole milk and traditional starter (n = 15); WMAS: whole milk and alternative starter (n = 15). Prewaning contrasts: NC versus HRTS ($P = 0.17$), NC versus HRAS ($P = 0.06$), MR versus WM ($P = 0.32$), TS versus AS ($P = 0.56$). Postweaning contrasts: NC versus HRTS ($P = 0.69$), NC versus HRAS ($P = 0.92$), MR versus WM ($P = 0.24$), TS versus AS ($P = 0.55$).

From birth to week 7, blood concentrations of BHB were greater ($P = 0.01$) for calves fed NC as compared to calves that received HRAS (Figure 7), however no difference was observed between NC and HRTS. Despite lower starter intake compared to NC, calves fed HRTS consumed 116 g of DM/d more than HRAS. Additionally, according to Górká et al. (2011) rumen development may be affected by liquid feed type and composition, possibly affecting BHB production. This grain source effect on BHB concentrations in the current study was inconsistent with Khan et al. (2007c) that found greater BHB in calves fed corn diet as compared to calves fed wheat, oat and barley diets. However, in the present study the lower feed intake observed for AS treatments may have reduced BHB concentrations, since it is highly correlated with intake of solid feed and rumen development in pre-ruminant calves. (Nemati et al., 2015; Deelen et al., 2016). In addition, Meale et al. (2017) suggest that chemical composition of the diet seems to contribute to shifts in regulatory mechanisms in the rumen. According to the authors, CP present on diet has a regulatory role in ruminal epithelial cell development. In the current study the AS group provided cracked corn until the 3rd week of age providing less than half amount of CP compared to TS (Table 1). Mean of BHB tended to

be greater for calves fed WM that also tended to consume more starter than calves fed MR ($P = 0.06$). Previous study observed that calves fed MR indirectly slows down rumen development (Górka et al., 2011). No difference was observed among treatments for BHB from week 7 to week 12, whereas the same diet was provided postweaning for all of them. As expected, it was observed an increased concentration of BHB as a result of increased starter intake and ruminal development (Nemati et al., 2015; Deelen et al., 2016).

Figure 7. β -hydroxybutyrate concentration of dairy calves fed conventional or high rate of liquid diet and traditional or alternative starter. NC: negative control ($n = 15$); MRTS: milk replacer and traditional starter ($n = 15$); MRAS: milk replacer and alternative starter ($n = 15$); WMTS: whole milk and traditional starter ($n = 15$); WMAS: whole milk and alternative starter ($n = 15$). Prewaning contrasts: NC versus HRTS ($P = 0.76$), NC versus HRAS ($P = 0.01$), MR versus WM ($P = 0.06$), TS versus AS ($P = 0.01$). Postweaning contrasts: NC versus HRTS ($P = 0.61$), NC versus HRAS ($P = 0.50$), MR versus WM ($P = 0.97$), TS versus AS ($P = 0.84$).

Feed intake costs and electrolyte treatment costs of different feeding systems are summarized in Table 4. It was observed that NC presented greater starter cost ($P < 0.01$) and lower cost of liquid diet ($P < 0.01$); however lower total feed costs ($P < 0.01$). This occurred because of the higher solid intake and approximately 50% less amount of liquid feeding, compared to high rate of nutrition associated with TS or AS. These results are in agreement with those reported by Davis Rincker et al. (2011). Whole milk treatment tended to present higher starter cost than MR ($P = 0.10$), since there was a tendency for higher starter intake

(Table 1). As expected, AS presented lower starter cost as compared to TS group ($P < 0.01$), since costs of ingredients were lower. Total feed cost was higher for high rate of liquid diet independent of solid diet when compared to NC ($P < 0.01$). Whole milk presented greater total feed cost than MR ($P < 0.01$) did. Total feed cost was lower for AS feeding versus TS ($P < 0.01$). Feed cost per kilogram of BW gain was higher for calves fed additional liquid diet ($P < 0.01$) as compared to calves fed conventional system, in agreement with Quigley et al. (2006). Restricted liquid feeding encourages earlier starter intake and ruminal development, which in turn allows for earlier weaning and more economic body weight gains (NRC, 2001). Additionally, WM presented lower feed cost per kilogram of BW than MR ($P < 0.01$). However, no difference was observed among TS and AS. The cost of electrolytes was greater for calves fed MR compared to calves fed WM ($P < 0.01$), since diarrhea days was higher in MR (Table 3). Total cost was lower for calves fed NC as compared to calves fed HR of liquid diet and TS or AS ($P < 0.01$). Calves fed WM presented greater total cost than calves fed MR ($P < 0.01$). Contrary to our study, Godden et al. (2005) observed that feeding WM, rather than commercial MR, was associated with an economic advantage of \$0.69/calf per day or \$34/calf from birth to weaning. However, the authors used the real costs of pasteurized nonsaleable milk, whereas our study used price of saleable milk. Calves fed TS presented higher total cost compared to calves fed AS ($P < 0.01$).

Table 4. Economic analysis per animal fed different feeding programs during preweaning.

Item ¹	Treatments ²					SEM	P ³ <			
	NC	MRTS	MRAS	WMTS	WMAS		NC vs HRTS	NC vs HRAS	MR vs WM	TS vs AS
Starter cost, \$	31.86	19.31	11.15	21.51	12.05	0.96	<0.01	<0.01	0.10	<0.01
Liquid feed cost, \$	76.69	112.35	112.32	116.40	116.38	0.11	<0.01	<0.01	<0.01	0.80
Total feed cost, \$	108.57	131.64	123.47	137.91	128.43	0.98	<0.01	<0.01	<0.01	<0.01
Total feed/kg gain cost, \$	2.70	3.81	3.75	3.48	3.39	0.13	<0.01	<0.01	<0.01	0.54
Electrolytes cost, \$	1.16	1.59	1.50	0.66	0.60	0.36	0.94	0.80	0.01	0.82
Total cost, \$	109.72	133.51	124.97	138.57	129.03	0.94	<0.01	<0.01	<0.01	<0.01

¹Total cost = Total feed costs plus electrolytes costs.

²NC = Negative control; MRTS= high rate of milk replacer and traditional starter; MRAS = high rate of milk replacer and alternative starter; WMTS = high rate of whole milk and traditional starter; WMAS = high rate of whole milk and alternative starter.

³P value for contrasts. NC vs HRTS = negative control versus high rate of liquid diet and traditional starter; NC vs HRAS = negative control versus high rate of liquid diet and alternative starter; MR vs WM = high rate of milk replacer versus high rate of whole milk; TS v s AS = traditional starter versus alternative starter.

4.4. CONCLUSION

Feeding calves restricted liquid diet presented satisfactory results for the conventional system and provided economic body weight gains. However, the final BW suggest that feeding dairy calves an elevated plane of nutrition, mainly whole milk and traditional starter may be a better feeding strategy to increase future productivity, achieving economic returns. Besides, replacing commercial TS with an AS in high rate of nutrition present minimal impact on performance suggesting that the nutrient requirements from solid diet for young calves are not entirely understood. Additional studies are necessary to achieve better costs and benefits associated with liquid and solid diet provided for young calves.

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5. FINAL CONSIDERATION

This study showed that increased amount of colostrum within the first hours of life was an important tool to increase newborn calves' thermoregulatory responses during cold environment. However, even though a benefit for the calf submitted to cold stress at the first day of life was observed, feeding higher volumes of colostrum resulted in small differences on performance preweaning. Nevertheless, calves fed higher volume of colostrum presented increased immunity responses during preweaning.

For calves submitted to low environmental temperature within first month of life, restricted liquid diet presented satisfactory results for the conventional system, but the final BW suggest that feeding dairy calves an elevated plane of nutrition, mainly whole milk and traditional starter may increase future productivity, achieving economic returns. In addition, replacing commercial texturized starter with an alternative starter in high rate of nutrition present minimal impact on performance suggesting that the nutrient requirements from solid diet for young calves are not entirely understanding.