UNIVERSIDADE FEDERAL DE MINAS GERAIS
ESCOLA DE VETERINÁRIA
Programa de Pós-graduação em Zootecnia

EFEITOS DA UTILIZAÇÃO DE LEITE INTEGRAL, LEITE DE DESCARTE E LEITE DE DESCARTE PASTEURIZADO SOBRE O CONSUMO, PARÂMETROS RUMINAIS, SAÚDE E DESEMPENHO DE BEZERROS LEITEIROS

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Belo Horizonte
Escola de Veterinária da UFMG
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Efeitos da utilização de leite integral, leite de descarte e leite de descarte pasteurizado sobre o consumo, parâmetros ruminais, saúde e desempenho de bezerros leiteiros

Dissertação apresentada ao Programa de Pós-Graduação em Zootecnia da Escola de Veterinária da Universidade Federal de Minas Gerais como requisito parcial para obtenção do grau de Mestre em Zootecnia.

Área de concentração: Produção Animal / Ruminantes
Orientador: Profª. Sandra Gesteira Coelho
Coorientadores: Drª. Mariana Magalhães Campos
Profª. Hemilly Cristina Menezes de Sá

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FOLHA DE APROVAÇÃO

"Efeitos da utilização de leite integral, leite de descarte e leite de descarte pasteurizado sobre o consumo, parâmetros ruminais, saúde e desempenho de bezerros leiteiros"

Sabrina de Freitas Vieira

Dissertação de Mestrado defendida e aprovada, no dia **vinte e cinco de fevereiro de dois mil e vinte e um**, pela Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em Zootecnia da Universidade Federal de Minas Gerais, constituída pelos seguintes professores:

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Belo Horizonte, 30 de novembro de 2021.

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“Para grandes conquistas é necessário grandes sacrifícios!”

Pr. Claudio Duarte

RESUMO

O objetivo deste ensaio foi descrever os efeitos dos tratamentos com leite integral, leite de descarte e leite de descarte pasteurizado sobre o consumo, parâmetros ruminais, saúde e desempenho de bezerros leiteiros. Foram utilizados 45 bezerros machos (Holandês x Gir), sendo eles: três animais puro sangue (P), um animal $^{1}/_{2}$ sangue, 25 animais $^{3}/_{4}$, nove animais $^{4}/_{5}$ e, sete animais $^{5}/_{8}$. Nos primeiros três dias de vida, os bezerros foram alojados em gaiolas individuais suspensas com cama de feno. No quarto dia de vida, foram agrupados de acordo com peso corporal, proteína sérica e composição genética e distribuídos em três tratamentos: leite integral (LI, n = 15); leite de descarte (LD, n = 15), oriundo de vacas até três dias após o parto, em tratamento com antimicrobianos (mastite clínica, retenção de placenta e afecção podal) e leite de descarte pasteurizado (LDP, n = 15), submetido ao processo de pasteurização rápida, 72 a 74^{o}C por 16 s. O delineamento experimental utilizado foi em blocos ao acaso, em parcelas sub divididas. A composição genética foi blocada, o tratamento foi a parcela e os tempos avaliados a sub parcela. Durante o período experimental (4 a 60 dias de idade), os animais receberam 6 L de leite/dia, divididos em duas refeições (LI = 09 e 15h; LD = 10 e 16 h e LDP = 11 e 17 h) em baldes contendo bicos. A dieta sólida foi fornecida à vontade a partir do quarto dia de vida (10% de sobra). O consumo de leite, concentrado e água foram mensurados diariamente pela diferença entre a quantidade oferecida e as sobras. Os animais foram pesados no dia do nascimento, no quarto dia de vida, ao entrarem para o tratamento e, semanalmente, até os 60 dias do experimento. As amostras de líquido ruminal e sangue para análise de AGV, pH e N-amoniacal, glicose e β-hidroxibutirato (BHBA) foram coletadas semanalmente, 3h após a alimentação da manhã, até os 60 dias do experimento. Os escores de saúde foram realizados diariamente, durante o período da manhã, antes da alimentação. O tratamento LI apresentou maior ingestão de matéria seca do leite, seguido pelo LD e LDP. Para o consumo de concentrado, houve efeito para semana e interação tratamento x semana. Em relação ao consumo de MS total,

houve efeito para semana de avaliação. O ganho de peso médio diário não diferiu em relação aos tratamentos, mas foram observadas diferenças entreas semanas avaliadas. Não houve diferença no peso final entre os tratamentos. Os tratamentos LI e LD apresentaram maior eficiência alimentar em relação ao tratamento LDP. Os bezerros do tratamento LDP apresentaram concentrações superiores de acetato ruminal em relação ao tratamento LI e LD. Não foram observadas diferenças entre os tratamentos para concentração de amônia ruminal, concentração de glicose e BHBA. As concentrações de BHBA foram diferentes entre as semanas avaliadas. Na avaliação de escore de fezes houve efeito apenas para semana. Não foi observada diferença entre os tratamentos para dias em diarreia, dias com febre e bezerros com escore ≥ 1 cm^2 de área de consolidação pulmonar. A utilização de LD e LDP não apresentou efeitos negativos nos parâmetros de consumo, parâmetros ruminais, saúde e desempenho de bezerros leiteiros. No entanto, os efeitos dos resíduos de antibióticos presentes no LD e LDP sobre a resistência bacteriana não foram mensurados no presente estudo.

Palavras chaves: aleitamento, dieta líquida, leite não comercializável, pasteurização.

Abstract: The aim of this study was to evaluate the effects of bulk tank milk (BTM), WM, and PWM on the intake, ruminal parameters, blood parameters, health, and performance of dairy calves. Forty-five male crossbred dairy calves (Gyr x Holstein) were used. On their fourth day of age, animals were grouped according to body weight, serum protein levels, and genetic composition. Three treatments were assessed: BTM (n = 15), WM from cows in antibiotic treatment (n = 15), and PWM via high-temperature short-time pasteurization (72–74 °C for 16 s) (n = 15). During the experimental period (from 4 to 60 d of age), animals were fed 6 L of milk/d, divided into two equal meals. Water and concentrate were provided *ad libitum*. Daily measurements were made for milk, concentrate, and water intakes, as well as for fecal and respiratory scores. Rumen fluid and blood were sampled weekly. The following parameters were evaluated: volatile fatty acids (VFAs), pH and ammonia-N in rumen fluid, and β-hydroxybutyrate (BHB) and glucose in blood. Animals were weighed at birth, 4 d of age, and weekly up to 60 d of age. At the end of the experimental period (60 ± 1 d), all animals were euthanized for pulmonary evaluation. The randomized complete design with an interaction between treatment and week was the experimental method of choice for testing the hypothesis of the treatment's effect on all evaluated outcomes. Animals in the BTM treatment had higher milk drymatter intake (DMI), followed by WM and PWM calves. Concentrate DMI was lower for BTM in comparison to WM and PWM calves. However, total DMI showed no significant differences between treatments. The rumen fluid from calves receiving PWM had higher concentrations of acetate and propionate than that of BTM and WM animals. No differences were observed between treatments for blood glucose and BHB concentrations. Health parameters (fecal and respiratory scores) and pneumonia occurrence showed no significant difference between treatments. No differences were observed for average daily gain (ADG) or body growth. Feeding WM and PWM did not show significant negative effects on the intake, ruminal parameters, blood parameters, health, or performance of dairy calves.

Keywords: growth; pasteurization; volatile fatty acids; weight gain

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LISTA DE ABREVIATURAS E SIGLAS

AC: acetato

BHBA: beta hidroxibutirato

C2:C3: relação acetato x propionato

CBT: contagem bacteriana total

CCS: contagem de células somáticas

cm: centímetros

CPP: contagem padrão em placas

CEUA: Comissão de Ética no Uso de Animais

d: dia

EDTA: Ácido etilenodiaminotetracético

EE: Extrato Etéreo

EMBRAPA: Empresa Brasileira de Pesquisa Agropecuária

EPM: erro padrão da média

FDA: fibra em detergente ácido

FDN: fibra em detergente neutro

g: grama

GMD: ganho médio diário

GPD: ganho de peso médio diário

h: horas

IMS: ingestão de matéria seca

kg: quilograma

L: litro

LA: leite acidificado

LD: leite de descarte

LDP: leite de descarte pasteurizado

LI: leite integral

LIP: leite integral pasteurizado

Mcal/d: megacaloria por dia

MG: Minas Gerais

mg/dL: miligrama por decilitro

mmol/dL: milimol por decilitro

mmol/L: milimol por litro

min: minutos

mL: mililitro

mm: milímetro

mmol: milimol

MS: matéria seca

n: número

PB: proteína bruta

PR: propionato

PV: peso vivo

S: semana

T: tratamento

T X S: interação semana e tratamento

UFC: unidade formadora de colônias

UI: unidade internacional

ºC: graus celsius

%: porcentagem

SUMÁRIO

3.1. INTRODUÇÃO

O leite de descarte (LD) é uma secreção láctea não comercializável, produto da ordenha de vacas no pós-parto imediato (colostro e leite de transição), vacas em tratamento com bases farmacológicas eliminadas no leite (antibióticos, anti-inflamatórios e antiparasitários) e vacas já tratadas, que ainda cumprem o prazo de carência do medicamento aplicado (Zou et al., 2017). Diante da impossibilidade legal de comercialização, o destino do LD se torna problema para propriedades leiteiras. Sua eliminação exige a presença de sistemas adequados de tratamento de dejetos, como biodigestores e lagoas de decantação. Entretanto, poucas propriedades brasileiras trabalham com esse tipo de sistema, fazendo com que o LD se torne entrave, inclusive financeiro, visto que apenas 0,05 % das propriedades (de suínos e bovinos) possuem, por exemplo, biodigestor (IBGE, 2009).

Por outro lado, o fornecimento de dieta líquida de alto valor nutricional e baixo custo para as bezerras, em fase de cria, constitui outro importante desafio para o sistema. Dessa forma, muitas propriedades optam por utilizar o LD no aleitamento, com intuito de contornar dois grandes problemas, a dificuldade do descarte desse material e a redução do custo da dieta (Brunton et al., 2012).

A utilização do leite de descarte parece ser alternativa economicamente viável para o aleitamento, no entanto, pode representar risco sanitário para humanos e animais, uma vez que sua eliminação, sem tratamento adequado, contamina o meio ambiente (Duse et al., 2013). Além disso, o LD apresenta composição nutricional variável, decorrente da contribuição do colostro e leite de transição, bem como das alterações oriundas de afecções, principalmente, as mastites. Assim, a utilização do produto apresenta alto risco, dada a elevada carga microbiana e a presença de resíduos de medicamentos (Zou et al., 2017).

É preciso, ainda, levar em conta o aumento exponencial da preocupação do

mercado consumidor de proteína de origem animal e de profissionais, das áreas de saúde e agrárias, em relação ao uso de antimicrobianos e ao desenvolvimento de linhagens microbianas multirresistentes (Junza et al., 2014). Diante disso, muitos estudos têm sido desenvolvidos para compreender melhor as características do LD e sua influência sobre o desempenho, saúde e microbiota dos animais.

Edrington et al. (2012) observaram que bezerros aleitados com leite integral (LI) e com LD obtiveram taxas semelhantes de crescimento e ganho de peso médio diário (GMD), e em relação à saúde, a incidência de diarreias entre eles também foi semelhante.

É de suma importância estudar o custo de produção de bezerras, dentro do sistema de produção de leite, pois auxilia o proprietário na tomada de decisões, o que permite obter melhores resultados econômicos (Reis, 1999). Diante da sua importância no sistema de produção, o custo da cria e recria de bezerras precisam ser analisados à parte, dado que essas categorias representam 20% da despesa no sistema, perdendo apenas para alimentação das vacas em lactação. O produtor precisa ter conhecimento dos custos de cada produto do seu sistema, seja do litro de leite, de uma novilha até a idade ao parto e principalmente da alimentação dos animais (dieta líquida e sólida) (Santos & Lopes, 2014). Porém, ainda há poucos estudos na literatura (Lopes et al., 2011).

3.2 OBJETIVOS

Objetivo Geral

O objetivo desse trabalho é descrever o impacto do aleitamento com leite integral, leite de descarte e leite de descarte pasteurizado sobre o consumo, parâmetros ruminais e sanguíneos, saúde e desempenho de bezerros leiteiros.

Objetivos específicos

Os objetivos específicos são:

- Avaliar o consumo da dieta líquida, sólida e água dos bezerros diante dos diferentes tipos de tratamentos.
- Avaliar o desempenho dos bezerros diante dos diferentes tipos de tratamentos.

- Avaliar impactos na saúde dos bezerros diante dos diferentes tipos de tratamentos.
- Avaliar o perfil metabólico, hormonal e ruminal dos bezerros diante dos diferentes tipos de tratamentos.

3.3 HIPÓTESES

- Bezerros alimentados com leite integral apresentam desempenho superior comparado aos bezerros alimentados com leite de descarte e aos alimentados com leite de descarte pasteurizado.
- O aleitamento com leite de descarte pasteurizado reduz a ocorrência de diarreia e pneumonia quando comparado com a utilização do leite de descarte e integral.
- A utilização de leite de descarte não altera os parâmetros sanguíneos e ruminais.

3.4 REVISÃO DE LITERATURA

3.4.1 Aleitamento na fase de cria

Nos primeiros 60 dias de vida, é fundamental o fornecimento de dieta líquida. Em fazendas leiteiras, os bezerros geralmente são alimentados com leite integral ou substituto do leite (leite de descarte ou sucedâneo) e recebem quantidade de aproximadamente 10% do peso ao nascer, podendo consumir até 240 litros de leite, desde o nascimento até o desaleitamento aos 60 dias de idade (Boito et al., 2015).

3.4.2 Composição nutricional do leite integral, descarte, descarte pasteurizado

O leite integral se destaca entre as opções para aleitamento de bezerras, por ser alimento materno natural. Sua composição nutricional, em geral, conta com 12,5 % de sólidos totais, dos quais aproximadamente 3,7 % de gordura; 3,2 % proteína; 4,6 % lactose e o restante minerais, vitaminas e água (Quigley, 2010).

Os teores podem variar influenciados por alguns fatores, como raça, ordem de parto, dias em lactação do rebanho, volume de produção e dieta dos animais (Quigley, 2010). Entretanto, em sistemas leiteiros, o LI obtido de acordo com as normas vigentes (IN 76,

2018) é a unidade de produto comercializável. Sua utilização para aleitamento das bezerras representa custo elevado para a criação, embora seja o produto mais indicado para tal.

O leite de descarte apresenta composição nutricional e qualidade microbiológica variáveis. A mastite é uma das três principais afecções que acometem rebanhos leiteiros (Demeu et al., 2011). Dessa forma, grande parte do LD provém de vacas com mastite e outros processos inflamatórios e infecciosos, que podem resultar em flutuação da secreção de constituintes do leite. Uma mudança frequentemente observada é a redução da concentração de gordura. Harmon (1994) relatou variação entre a composição do leite de 3,5 para 3,2 % de gordura no LI e LD, respectivamente. Para proteína e lactose do LD, houve redução de, respectivamente, 0,05 e 0,5 %, tendo os maiores teores sido observados no LI (3,61 % para proteína e 4,9 % para lactose). Durante o processo inflamatório da glândula mamária, apesar da redução na síntese de proteínas pelas células epiteliais, a concentração de proteína pode se manter normal ou aumentar devido ao aumento do influxo de proteínas plasmáticas para o alvéolo (Auldist & Hubble, 1998).

O LD pode apresentar ampla variação na sua carga microbiana. Vários patógenos, como: *Escherichia coli*, *Salmonella* spp, *Streptococcus*, *Staphylococcus e Mycoplasma* podem estar presentes. A pasteurização reduz estes microrganismos e a possibilidade de transmissão de doenças, como a leucose bovina e diarreia viral bovina, melhorando a qualidade do leite. Embora a pasteurização seja positiva por reduzir a carga microbiana, o processo pode eliminar bactérias benéficas, como *Lactobacillus*, que podem prevenir a colonização do trato gastrointestinal por *Salmonella* spp e, melhorar a função gastrointestinal (Kulkarni & Kaliwal, 2013).

Com o intuito de reduzir a carga bacteriana do LD e efeitos dos microrganismos patogênicos, algumas propriedades leiteiras passaram a utilizar a pasteurização do leite (Menezes et al., 2014). O aleitamento com LD pasteurizado pode levar a melhor desempenho dos animais, tendo ainda como vantagem alteração mínima na composição do leite (Butler, 2000).

Uma das principais causas de variação na composição do leite pelo efeito da mastite, pode ser, por exemplo, a redução de gordura, devido à ação das lípases leucocitárias e lipoproteicas (Bueno et al., 2005). Porém, ao longo do processo inflamatório, há aumento na concentração de proteína plasmática do sangue e redução na síntese de proteína pelas células epiteliais, o que pode explicar a manutenção das concentrações de proteína em valores normais (Auldist & Hubble, 1998).

A literatura ainda é escassa quanto a trabalhos em relação à eficiência da pasteurização sobre a contagem de células somáticas (CCS) e contagem padrão em placa (CPP) do LD pasteurizado. Os poucos trabalhos demonstram valores muito variáveis, devido ser leite oriundo de vacas não sadias, pois apesar da pasteurização reduzir esses valores, eles ainda são relativamente mais altos, quando comparados com o LI (Grant et al., 2005; Godden et al., 2005; Ruzante et al., 2008).

Zou et al. (2017) avaliaram a composição nutricional de quatro tipos de leite: LI, LD, LDP e leite acidificado (LA). Não houve diferença na proporção de proteína entre os produtos utilizados. Entretanto, houve diferença no percentual de gordura do leite ($P< 0,05$) no LD, sendo superior aos outros (LD 6,3± 0,44 %, LI 4,22± 0,24 %, LDP 4,50± 0,07%, LA 4,33± 0,10%). O valor de 6,3 % relatado pode ter sido oriundo do leite de quartos mamários com mastite, de leite de transição e do colostro. Entretanto, o valor de lactose observado foi baixo, ou seja, apesar da gordura estar alta, o LD não estaria atendendo a todas as necessidades dos animais.

3.4.3 Saúde e desempenho

Em relação ao desenvolvimento e saúde dos animais, Walz et al. (1997) observaram que bezerros aleitados com LD provenientes de vacas com mastite apresentaram aumento dos casos de otite média. Diversos microrganismos presentes no LD podem conduzir à maior ocorrência de afecções e queda no desempenho, como por exemplo, *Mycoplasma bovis*, frequentemente associado à ocorrência de mastite em vacas e otite nos animais jovens (Maunsell et al., 2011). Apesar disso, Butler (2000) havia relatado que o

fornecimento do LD pasteurizado teve benefícios que levaram ao melhor desenvolvimento dos animais, como o aumento do ganho de peso, tendo sido observadas poucas alterações na composição do leite.

Zou et al. (2017) avaliaram o efeito de diferentes tipos de leite sobre o desempenho dos bezerros, crescimento, metabolismo, imunidade e desenvolvimento intestinal. Foram utilizados 84 bezerros Holandês, distribuídos em quatro grupos. No grupo 1, os animais receberam LI; grupo 2: LD; grupo 3: LDP e no grupo 4: LA. O leite foi fornecido duas vezes ao dia, durante 21 dias. Em relação ao consumo de concentrado, os animais do grupo LI apresentaram consumo semelhante ao grupo LDP (34,4 e 29,4 g/d, respectivamente), entretanto superiores aos observados no grupo LD (21,3 g/d, $P < 0,05$). Os animais do grupo LD e LDP apresentaram maior ganho de peso médio diário (525 e 454 g/d), comparado ao grupo LI (258 g/d, $P < 0,05$). De acordo com os pesquisadores, o maior ganho de peso observado no grupo LD e LDP se deve aos elevados teores de gordura e à redução da carga microbiana do leite, respectivamente. A ocorrência de diarreia foi maior no grupo 2 (14 animais) em relação aos grupos: 1 (12 animais), grupo 3 (12 animais) e grupo 4 (11 animais) ($P< 0,05$), o que pode ter sido causado pela elevada carga microbiana no LD não processado. Do ponto de vista nutricional e de saúde, o LI seria a melhor escolha para a alimentação de bezerros em comparação ao LD (Zou et al., 2017).

Dennis et al. (2019) utilizaram 32 bezerros Holandês, distribuídos em dois grupos, para avaliar os efeitos do fornecimento de leite com resíduo de drogas antimicrobianas sobre o desempenho e a saúde. No grupo um os animais receberam sucedâneo (25% de proteína e 18% de gordura), enquanto o grupo dois recebeu o mesmo sucedâneo, porém com adição de antimicrobiano à base de neomicina-oxitetraciclina. Todos os bezerros foram alimentados com 0,66 kg de matéria seca de sucedâneo em duas refeições diárias, com 14% de sólidos totais, durante os primeiros 39 dias. Em seguida, foi fornecido 0,33 kg na mesma concentração, uma vez ao dia, durante três dias, totalizando 42 dias de aleitamento. Não foram observadas alterações no consumo de matéria seca. Porém, os animais do grupo

dois apresentaram menor período de tratamento de diarreia e pneumonia em relação ao grupo 1 (1,1 dias para o grupo 1 e 0,3 dias para o grupo 2) (P = 0,03).

Maynou et al. (2019), em estudo com 114 bezerros alimentados com LD, LD pasteurizado, LI e LIP, relataram que não foram observadas diferenças para o consumo de leite, ganho de peso médio diário e ocorrência de diarreia.

3.4.4 Pasteurizadores

Com o intuito de reduzir a carga bacteriana do LD e efeitos dos microrganismos patogênicos, algumas propriedades leiteiras passaram a utilizar a pasteurização do leite, eliminando por volta de 98-99% dos microrganismos. O LDP pode ser submetido ao processo de pasteurização tanto lento (realizado entre 62 a 65^{o}C, por 30 min), quanto ao rápido (realizado entre 72 a 75^{o}C, de 15 a 20 s), em equipamento de pasteurização pelo sistema de placas. Para avaliar a eficiência da pasteurização, é ideal que sejam realizados testes rápidos, após o processo, para garantir que foi adequado, avaliando a presença ou não de enzimas do leite: fosfatase alcalina e peroxidase (Menezes et al., 2014).

O aleitamento com LDP pode levar ao melhor desempenho dos animais, tendo ainda, como vantagem, mínima alteração na composição do leite (Butler, 2000). De acordo com Oh & Marshall (1995) o método de pasteurização também possui dificuldades, tais como: mão de obra qualificada, controle da temperatura e tempo ideal de pasteurização, limpeza correta do equipamento, recontaminação do leite pelo próprio manipulador do equipamento.

3.4.5 Custos com a criação de bezerras

Diversos itens compõem o custo total para a produção de uma bezerra na atividade leiteira, tais como, alimentação, mão-de-obra, sanidade, ordenha, reprodução, entre outras despesas. Portanto, uma análise de custo permite ao produtor verificar o que gera mais impacto, seja positivo ou negativo, tanto no desempenho quanto na saúde dos animais,

e principalmente, no quesito financeiro (Oaigen et al., 2008).

Reis et al. (2001) relataram que o rebanho estabilizado é o argumento para o cálculo do custo específico do leite, de tal modo que, com as mudanças no decorrer no tempo conservaria o tamanho e a capacidade produtiva do rebanho, do começo ao fim do tempo de análise. Os pesquisadores observaram, ainda, que as bezerras e novilhas, além de despesa, são a receita da atividade leiteira, pois, no rebanho, são a reposição das matrizes e animais de descarte. E a partir da utilização desse valor no custo total da atividade, tem-se o custo de produção do leite.

3.4.6 Custo da nutrição

De acordo com Santos & Lopes (2014), na fase da cria do sistema de produção de leite, a alimentação de bezerras com dieta sólida, que teve como itens incluídos concentrado e forragem, estes representaram 58,49% e 55,69% do custo, para bezerras mestiças (Holandês-Gir) e Holandês, respectivamente.

Em se tratando da dieta líquida, o aleitamento das bezerras, com duas refeições diárias, totalizando seis litros de leite integral/dia, representou 11,76% (R$235,91) e 6,12% (R$178,69) do custo, de bezerras mestiças e puras, respectivamente. A diferença de custo no aleitamento pode ser explicada pelo menor período de aleitamento das bezerras puras (80 versus 95 dias) e a utilização de sucedâneo. O uso dos sucedâneos pode ser uma opção interessante em sistemas de produção, no momento em que o preço do leite esteja alto. No sistema de bezerras puras, o que resultou em maior ganho de peso diário (0,481 versus 0,454 kg/dia, em comparação ao LI), foi, possivelmente, o fato por gastarem menor quantidade de energia para se locomoverem, devido a menor área. Tal fato contribuiu para reduzir a idade ao primeiro parto dos animais puros em 89 dias, porém com maior custo. Outros fatores, como a alimentação e genética, também contribuíram para o aumento no ganho de peso dos animais. No entanto, a comparação de desempenho na primeira lactação e saúde não foram avaliados (Santos & Lopes, 2014).

Santos et al. (2016) em estudo com bezerras da raça Holandês, que receberam seis

litros de leite integral por dia em período de aleitamento de 60 dias, relataram que a dieta líquida (leite) representou 52,3% do custo total dessa fase. Para o suposto custo de produção, utilizou-se a média das despesas entre os meses, em função do valor total,

dividido pelo número de animais. Observou-se custo total de uma bezerra na fase de aleitamento em torno de R$ 857,43, a média da despesa diária de cada uma foi de R$ 14,29. Já a dieta sólida (concentrado), representou 12,2% do custo. Essa diferença da dieta líquida para sólida ocorre devido ao custo do leite ser mais elevado, além do consumo do concentrado ser reduzido na fase de aleitamento. Em relação às demais despesas como mão-de-obra, medicamento e outras, representaram apenas 13,4% do custo total.

O fornecimento do concentrado na alimentação de bezerras é de extrema importância para o desenvolvimento ruminal, acelerando a fase do desaleitamento e, consequentemente, os tornando ruminantes verdadeiros mais rápido. Porém, reduzir a quantidade do leite oferecido, a fim de reduzir custo diário, não é indicado, pois, além de ser a principal fonte de alimentação para desenvolvimento dos animais, nessa fase, irá retardar o período de desaleitamento, o que se torna uma estratégia inviável (Faber et al., 2005).

Portanto, de acordo Santos et al. (2016), duas estratégias são viáveis na produção de bezerras, visando diminuir os custos durante a fase de aleitamento: aprimorar o processo produtivo, reduzindo despesas operacionais e desperdícios, sem que isso gere impacto negativo no desempenho dos animais, e reduzir, ao máximo, a taxa de mortalidade.

4.1 REFERÊNCIAS BIBLIOGRÁFICAS

AULDIST, M. J.; HUBLLE, I. B. Effects of mastitis on raw milk and dairy products. Austr. *J. Dairy Technol.*, v.53, p.28-36, 1998.

BOITO, B.; MENEZES, L. F. G.; ZIECH, M. F. et al. Uso de sucedâneo em substituição ao leite no desempenho de bezerros da raça holandesa durante a cria e recria. Cienc. Anim. Bras., v.16, p. 498-507, 2015.

BUENO, V. F. F.; MESQUITA, A. J. de; NICOLAU, E. S. et al. Contagem celular somática: relação com a composição centesimal do leite e período do ano no Estado de Goiás. Ciência Rural, v.35, 2005.

BUTLER, J. A.; SICKLES, S. A.; JOHANNS, C. J. et al. Pasteurization of Discard Mycoplasma Mastitic Milk Used to Feed Calves: Thermal Effects on Various Mycoplasma. *J Dairy Sci*., v.83, p.2285–2288, 2000.

BRUNTON, L. A.; DUNCAN, D.; COLDHAM, N. G. et al. A survey of antimicrobial usage on dairy farms and waste milk feeding practices in England and Wales. *Vet. Rec*.,v.171, p.296–302, 2012.

DEMEU, F. A.; LOPES, M. A.; COSTA, G. M. et al. Influência do descarte involuntário de matrizes no impacto econômico da mastite em rebanhos leiteiros. *Ciênc. Agrotec*., v. 35, p. 195-202, 2011.

DENNIS, T. S.; SUAREZ-MENA, F. X.; HU, W. et al. Effects of milk replacer feeding rate and long-term antibiotic inclusion in milk replacer on performance and nutrient digestibility of Holstein dairy calves up to 4 months of age. *J. Dairy Sci*., v.102, p.1–9, 2019.

DUSE, A.; WALLER K, P.; EMANUELSON, U. et al. Farming practices in Sweden relatedto

feeding milk and colostrum from cows treated with antimicrobials to dairy calves. *Acta Vet. Scand*., v.55, p.49–57, 2013.

EDRINGTON, T. S.; S. E. DOWD, S. E.; †FARROW, R. F. et al. Development of colonic microflora as assessed by pyrosequencing in dairy calves fed waste Milk. *J. Dairy Sci*., v.95, p.4519–4525, 2012.

FABER, S. N.; FABER, N. E.; MCCAULEY, T. C. Effects of colostrum ingestion on lactational performance. The Professional Animal Scientist, v. 21, p. 420-425, 2005.

GODDEN, S.M.; FETROW, J. P.; FEIRTAG, J. M.; GREEN, L R. AND WELLS, S. J. Economic analysis of feeding pasteurized nonsaleable milk versus conventional milk replacer to dairy calves. *J Am Vet Med Assoc*., v.226, p.1547-54, 2005.

GRANT, I.R.; BALL, H.J.; ROWE, M.T. Thermal inactivation of several *Mycobacterium* spp in milk by pasteurization. *Lett Appl Microbiol*., v.22. v.253-256, 1996.

HARMON, R. J. Physiology of mastitis and factors affecting somatic cell counts. *J. Dairy Sci*., v.77, p.2103-2112, 1994.

IBGE- Instituto Brasileiro de Geoestatísticas. Censo Agropecuário 2006. Agricultura Familiar: grandes regiões e unidades da federação. Rio de Janeiro: IBGE, 2009.

IN 76- Instrução Normativa nº 76, 2018. Disponível em: <http://www.in.gov.br>. Acessado em: 05 maio. 2020.

JUNZA, A.; MONTANÉ, A.; BARBOSA, J. et al. High resolution mass spectrometry in the identification of transformation products and metabolites from β-lactam antibiotics in thermally treated milk. *J Chromatogr. A.*, v.1368, p.89-99, 2014.

KULKARNI, A. G. & KALIWAL, B. Bovine mastitis: a review. Int. *J. Recent Sci*. Res., v.4, p.543-548, 2013.

LOPES, M. A.; RESENDE, M. C.; CARVALHO, F. M. et al. Estudo da rentabilidade de sistemas de produção de leite na região de Nazareno (MG). Ciência Animal Brasileira, v. 12, p. 58-69, 2011.

MAUNSELL, F. P.; WOOLUMS, A. R.; FRANCOZ, D. et al. *Mycoplasma bovis* infections in cattle. *J. Animal Sci. Biotec*h., v.8, p.53, 2011.

MAYNOU, G.; BACH, A.; TERRÉ, M. Feeding pasteurized waste milk to preweaned dairy calves changes fecal and upper respiratory tract microbiota. *Frontiers Vet. Sci*., v.6, p. 1-10, 2019.

MENEZES, M. F. C.; SIMEONI, C. P.; ETCHEPARE, M. A. et al. Microbiota e conservação do leite. *Rev. do Centro do Ciências*, 2014.

OAIGEN R. P.; BARCELLOS J. O. J.; CHRISTOFARI LF, NETO J. B. et al. Melhoria organizacional na produção de bezerros de corte a partir dos centros de custos. Rev. Bras. Zootec. v. 37, p. 580-87, 2008.

OH, D. H. & MARSHALL, D. L. Infuencie of packaging method, lactic and monolaurin on Listeria monocytogenes in crawfish tail meat homogenate. Food Microbiology, v. 12, p. 159-163, 1995.

QUIGLEY, J. Supplementing waste milk. 2010. Disponível em: http://www.calfnotes.com/pdffiles/CN148.pdf. Acessado em: 02 maio. 2020.

REIS, R. P. Como calcular o custo de produção. Lavras: FAEPE, p. 15, 1999.

REIS, R. P.; MEDEIROS, A. L. E.; MONTEIRO, L. A. Custo de produção da atividade leiteira na região sul de Minas Gerais. Organizações Rurais e Agroindustriais, v. 3, p. 45-54, 2001.

RUZANTE, J.M.; GARDNER, I. A.; CULLOR, J. S.; SMITH, W. L.; KIRK, J. H. AND ADASKA, J. M. Isolation of *Mycobacterium avium* ssp. *paratuberculosis* from waste milk delivered to California calf ranches. *Foodborne Pathog. Dis*., v.5, p.681- 686, 2008.

SANTOS, G.; BELONI, T. Custo de produção de bezerras e novilhas leiteiras. Revista iPecege, v. 2(1), p. 29-39, 2016.

SANTOS, G & LOPES, M. A. Custos de produção de fêmeas bovinas leiteiras do nascimento ao primeiro parto. *Cienc. Anim. Bras*., v.15, p. 11-19, 2014.

WALZ, P. H.; MULLANEY, T. P.; RENDER J. A. et al. Otitis media in preweaned Holstein dairy calves in Michigan due to Mycoplasma bovis. *J. Vet. Diagn. Invest*., v.9, p.250–254, 1997.

ZOU, Y.; WANG, Y.; DENG, Y. et al. Effects of feeding untreated, pasteurized and acidified waste milk and bunk tank milk on the performance, serum metabolic profiles, immunity, and intestinal development in Holstein calves. *J. Animal Sci. Biotech*., v. 8, p. 53, 2017.

Embrapa Gado de Leite

Comissão de Ética no Uso de Animais

CERTIFICADO

Certificamos que a proposta intitulada "Efeito do fornecimento de leite cru e de leite de descarte cru ou pasteurizado sobre o desempenho e a saúde de bezerras leiteiras e a resistência a antimicrobianos", protocolada sob o CEUA nº 9849040419, sob a responsabilidade de **Mariana Magalhães Campos** *e equipe; Hilton do Carmo Diniz Neto; Sabrina de Freitas Vieira* - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Embrapa Gado de Leite (CEUA/EGL) na reunião de 25/04/2019.

We certify that the proposal "Effect of the supply of raw milk and raw or pasteurized performance and health of dairy heifers and antimicrobial resistance", utilizing 60 Bovines (60 males), protocol number CEUA 9849040419, under the responsibility of **Mariana Magalhães Campos** *and team; Hilton do Carmo Diniz Neto; Sabrina de Freitas Vieira* - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the Embrapa Gado de Leite Corporate (CEUA/EGL) in the meeting of 04/25/2019.

Finalidade da Proposta: Pesquisa

Vigência da Proposta: de 05/2019 a 10/2019 Área: Núcleo Produção E Bem Estar Animal

Origem:	Campo Experimental José Henrique Bruschi						
Espécie:	Bovinos	sexo:	Machos	idade:	0 a 60 dias	N:	60
Linhagem:	Holandês x Gir			Peso:	25 a 120 kg		

Local do experimento: Biotério Campo Experimental José Henrique Bruschi, Coronel Pacheco - MG - Retiro da Genizinha

Juiz de Fora, 25 de abril de 2019

Dra. Letícia Sayuri Suzuki
Coordenadora da Comissão de Ética no Uso de Animais
Embrapa Gado de Leite

Virginia de Souza Columbiano Barbosa
Vice-Coordenadora da Comissão de Ética no Uso de Animais
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4.3 Artigo Científico

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EFFECTS OF BULK TANK MILK, WASTE MILK, AND PASTEURIZED WASTE MILK ON THE INTAKE, RUMINAL PARAMETERS, BLOOD PARAMETERS, HEALTH, AND PERFORMANCE OF DAIRY CALVES

Simple Summary: Waste milk (WM) is commonly used in the feeding of calves. Due to its legal prohibition in commercialization, the destination of WM has become an environmental issue for dairy farms. Many dairy farms pasteurize WM, focusing on reducing the microbial load and related sanitary challenges. However, pasteurized milk may still contain toxins of bacterial origin, spores, and antibiotic residues. Few studies have evaluated the effects of whole milk, WM, and pasteurized WM (PWM) on the intake, ruminal parameters, blood parameters, health, and performance of dairy calves. In our study, feeding WM or PWM did not show significant negative effects on the intake, ruminal parameters, blood parameters, health, or performance of dairy calves. Understanding the effects of using WM and PWM on the health and performance of dairy calves requires further investigation.

Abstract: The aim of this study was to evaluate the effects of bulk tank milk (BTM), WM, and PWM on the intake, ruminal parameters, blood parameters, health, and performance of dairy calves. Forty-five male crossbred dairy calves (Gyr x Holstein) were used. On their fourth day of age, animals were grouped according to body weight, serum protein levels, and genetic composition. Three treatments were assessed: BTM (n = 15), WM from cows in antibiotic treatment (n = 15), and PWM via high-temperature short-time pasteurization (72–74 °C for 16 s) (n = 15). During the experimental period (from 4 to 60 d of age), animals were fed 6 L of milk/d, divided into two equal meals. Water and concentrate were provided *ad libitum*. Daily measurements were made for milk, concentrate, and water intakes, as well as for fecal and respiratory scores. Rumen fluid and blood were sampled weekly. The following parameters were evaluated: volatile fatty acids (VFAs), pH and ammonia-N in rumen fluid, and β-hydroxybutyrate (BHB) and glucose in blood. Animals were weighed at birth, 4 d

of age, and weekly up to 60 d of age. At the end of the experimental period (60 ± 1 d), all animals were euthanized for pulmonary evaluation. The randomized complete design with an interaction between treatment and week was the experimental method of choice for testing the hypothesis of the treatment's effect on all evaluated outcomes. Animals in the BTM treatment had higher milk dry matter intake (DMI), followed by WM and PWM calves. Concentrate DMI was lower for BTM in comparison to WM and PWM calves. However, total DMI showed no significant differences between treatments. The rumen fluid from calves receiving PWM had higher concentrations of acetate and propionate than that of BTM and WM animals. No differences were observed between treatments for blood glucose and BHB concentrations. Health parameters (fecal and respiratory scores) and pneumonia occurrence showed no significant difference between treatments. No differences were observed for average daily gain (ADG) or body growth. Feeding WM and PWM did not show significant negative effects on the intake, ruminal parameters, blood parameters, health, or performance of dairy calves.

Keywords: growth; pasteurization; volatile fatty acids; weight gain

1. Introduction

Waste milk (WM) is a milk secretion not suitable for commercialization and originates from cows milked immediately after calving (colostrum and transition milk), undergoing treatment with pharmacological products (antibiotics, anti-inflammatory or antiparasitic drugs), or cows fully treated but still within the withdrawal period [1]. Because it is legally prohibited for commercialization, WM's destination has become an environmental issue for dairy farms. The appropriate discharge of such substances requires waste treatment systems such as biodigesters and settling ponds.

On the other hand, providing a liquid diet of high nutritional value and low cost for young calves is another challenge in dairy production. Attempting to avoid the obstacles and high costs of correct discharge and reduce feeding expenses, many dairy farms commonly use WM in the feeding of calves.

Although feeding WM is an economically viable option, this practice may pose health risks for calves, given the high microbial load and presence of antibiotic residues in WM [2]. Using WM of cows affected by infections, such as mastitis, in the feeding of calves constitutes another controversial subject [1]. In addition, WM presents variable nutritional composition, mainly due to the presence of transition milk and low-quality colostrum [3].

In this sense, many dairy farms pasteurize WM, focusing on reducing the microbial load and overcoming the sanitary challenges related to such milk. Besides decreasing 99% of pathogenic microorganisms [4], pasteurization causes minimal alterations in the composition of milk, and pasteurized waste milk (PWM) can stimulate weight gain and improve the development of calves [5]. The use of PWM in the feeding of animals may reduce the development of diseases such as diarrhea and pneumonia [6].

However, many different and controversial results have been reported in the scientific literature regarding WM and PWM use. Studies have been done to evaluate the differences in health parameters and performance in calves fed WM and bulk tank milk (BTM), but no differences were observed between the evaluated groups [7,8]. In contrast, in another study, the animals receiving WM had higher concentrate intake and more weight gain than animals fed BTM [1]. When evaluating milk pasteurization, calves that consumed PWM presented better health parameters than those that consumed milk replacer [9,10]. However, a recent study did not find any development or health improvements in calves fed PWM in comparison to WM or BTM [1].

During the milk-feeding of calves, small amounts of milk pass through the rumen. In this sense, the antibiotic residue and the microbial properties of WM and PWM may alter the rumen microbiota, directly affecting the ruminal parameters of animals in these treatments [11, 12]. However, this subject is little discussed in the scientific literature.

The aim of this study was to evaluate the effects of BTM, WM, and PWM on the intake, ruminal parameters, blood parameters, health, and performance of dairy calves. Our hypothesis is that although feeding WM does not hinder the growth or development of calves, it may negatively affect their

health. Therefore, pasteurization may be a valid strategy in avoiding these negative consequences.

2. Materials and Methods

The experiment was conducted at the Embrapa Gado de Leite Experimental Farm, located in Coronel Pacheco, Minas Gerais, Brazil. During the trial period, the average temperature was 27 °C (maximum 37.4 °C, minimum 8.2 °C). The mean relative humidity during the study period was 72% (maximum 85 °C, minimum 40 °C). Procedures were approved by the Ethics Committee on Animal Use of Embrapa Dairy Cattle (CEUA number: 9849040419).

2.1. Calves, housing, management, and treatments

Forty-five male Holstein × Gyr crossbred dairy heifers, with genetic composition of 5/8 or more Holstein and 3/8 or less Gyr were used. After birth, newborn animals were immediately separated from their dams and had their umbilical cord immersed in iodine solution (10%) for three consecutive days. Before completing 6 h of age, animals were weighed and received 10% of their body weight in colostrum with Brix = 25% (79.8 g/L of IgG) [13]. Colostrum with a result Brix < 25 was densified using a colostrum replacer (Saskatoon Colostrum Company, Saskatoon, Canada; DM = 67.1) until reaching a Brix value of 25%.

In the first three days, calves were housed in individual suspended cages (1.50 m x 0.80 m; Intergado Ltda, Contagem, Brazil) with hay beds. Blood samples (5 mL) for the assessment of passive immunity transfer were obtained 48 h after the initial colostrum intake. Blood samples were collected via jugular venipuncture with a clot activator tube (Labor Import, Osasco, Brazil), centrifuged at 1,800 × g for 10 min at room temperature (22–25 °C), to measure total serum protein using a refractometer (Serum protein REF-301, Biocotek, Ningbo, China). Male calves with low serum protein (< 5.5) were not enrolled in the present study [14]. There were no differences (P = 0.38) between groups for passive immune transfer, with average and standard deviations for total serum protein of 6.6 ± 0.81, 6.95 ± 0.88 e 6.63 ± 0.65 g/dL for calves in BTM, WM, and PWM groups, respectively.

At 2 to 3 d of age, heifers were fed 6 L/d of transition milk divided into two equal meals (0800 and 1600 h) offered in buckets provided with rubber teats (Milkbar®, New Zealand). Water was provided ad libitum from day one.

On their 4 d of age, calves were randomly distributed according to body weight, serum protein levels, and genetic composition into three treatments: BTM (n = 15), WM from cows in antimicrobial treatments (n = 15) (cows with clinical mastitis, placental retention, metritis, or foot infections), and PWM (n = 15), pasteurized by high-temperature short-time pasteurization (72–74 °C for 16 s) with a plate pasteurizer (West, Juiz de Fora, Brazil).

During the experimental period (4 to 60 d of age), animals were housed in individual sand-bedded pens (1.25 × 1.75 m, tethered with 1.2 m long chains). All experimental groups received 6 L/d of milk, divided into two meals (BTM = 0900 and 1500; WM = 1000 and 1600; and PWM = 1100 and 1700). The milk from each treatment was homogenized with the aid of a ladle always before the meal. Experimental treatments were fed in buckets provided with rubber teats (Milkbar®, New Zealand). A solid diet was offered ad libitum, starting at the fourth day of age (10% orts of solid feed). The diet comprised ground corn, soybean meal, and mineral and vitamin supplements (Prima/DSM, São Paulo, Brazil) (Table 1).

Table 1. Composition, somatic cell count (SCC) and total bacterial count (TBC) of bulk tank milk (BTM), waste milk (WM), and pasteurized waste milk (PWM) samples and composition of concentrate used during the period from 4 to 60 d

Item	Treatment[1]			SEM	Starter[2]
	BTM	WM	PWM		
Composition (%)					
DM	13.04 (0.58) a	12.82 (0.64) b	12.48 (0.53) c	0.51	94.53
Fat	4.24 (0.54) a	4.10 (0.58) a	3.76 (0.43) b	0.46	3.14
CP	3.30 (0.27) b	3.46 (0.44) a	3.49 (0.29) a	0.24	19.06
Casein	2.71 (0.19)	2.64 (0.36)	2.53 (0.44)	0.04	-
Non-protein nitrogen	0.10 (0.01) b	0.13 (0.02) a	0.12 (0.02) a	< 0.01	-
Lactose	4.46 (0.12) a	4.33 (0.20) b	4.33 (0.17) b	0.15	-
Ash	0.69 (0.03) b	0.72 (0.04) a	0.73 (0.03) a	< 0.01	8.81
NDF	-	-	-	-	12.70
ADF	-	-	-	-	5.60
GE (Kcal/kg)	-	-	-	-	4,168.63
Milk Quality					
SCC (x 10^3 cells / mL)	366.81 (175.13) c	1,740.15 (1,638.03) a	1,424.67 (784.44) b	901.00	-
TBC (x 10^3 UFC / mL)	19.79 (15.14) c	548.37 (695.11) a	295.41 (353.25) b	369.00	-

[*] Values in parentheses indicate standard deviation of the values of each treatment;
[1]Means followed by a lowercase letter represent statistical difference between treatments ($P < 0.05$; Tukey test);

1 Basic composition: soybean meal, ground corn, and mineral (Prima / DSM, São Paulo, Brazil).

Milk pasteurization was performed daily (1000 h) with a plate pasteurizer (West, Juiz de Fora, Brazil). After homogenization of WM, the daily volume required for feeding PWM calves was pasteurized. The presence of alkaline phosphatase and peroxidase was assessed daily with reagent strips (Cab-Lab, São Paulo, Brazil) to verify the efficiency of the pasteurization process. The PWM was only used after certification of the absence of alkaline phosphatase and presence of peroxidase. Calves in the PWM treatment had their first meal immediately after pasteurization (milk with temperature 38 °C), and the remaining milk was refrigerated for 6 h (4 °C) until the time of the second meal. At that time, PWM was heated to 38 °C and fed to calves.

2.2. Intake

Feed intake (milk, starter, and water) was measured daily by the difference between the supplied and orts. Starter and water were weighed on 5 g precision scales (Prix 3 Plus, Toledo®, São Bernardo do Campo, São Paulo, Brazil).

Dry matter intake (DMI) was calculated for milk and starter, considering their respective dry

matter (DM) content. Total DMI was calculated from the sum of the consumed quantities of each supplied feed (MS of the milk + starter).

Gross energy intake (GEI) was calculated as the difference between the gross energy (GE) of the supplied, consumed, and leftover feeds (milk + starter). Milk GE was calculated based on the calculation: GE (Mcal/kg milk) = (0.0911 x % fat) + (0.0586 x % protein) + (0.0395 x % lactose) [15].

2.3. Nutrient composition analysis

The supplied starter was sampled weekly. Samples were stored at -20 °C until analysis. Analysis began by oven-drying samples for 72 h at 55 °C. Then, samples were ground in a Wiley mill (Model 3, Arthur H. Thomas Co., Philadelphia, PA, USA) to pass a 1 mm sieve. Crude protein (CP) (988.05 AOAC method), ether extract (EE) (920.9 AOAC method), ashes (942.05 AOAC method), neutral detergent fiber (NDF), and acid detergent fiber (ADF) were quantified [16].

Milk samples for each treatment were obtained prior to feeding at both meal times (morning and afternoon). For the milk composition analysis (crude protein, fat content, lactose, and total dry extract) and somatic cell count (SCC), samples were stored in flasks containing Bronopol® (Prolab, São Paulo, Brazil). For the total bacteria count (TBC) analysis, milk samples were stored in flasks containing Azidiol (Prolab, São Paulo, Brazil).

Flow cytometry analyses were performed to assess the concentration of SCC and TBC in milk samples (Bentley 2300 Combi & Bactocount IBC, Laboratory of Milk Quality of Embrapa Dairy Cattle, Juiz de Fora, Brazil). To evaluate the efficiency of the pasteurization process, pre-pasteurization and immediately after pasteurization, milk samples were collected in flasks containing Azidiol (Prolab, São Paulo, Brazil).

Organic matter was incinerated at 550 °C to determine the ash content (ISO 936: 1998). Nitrogen content was determined by the Micro Kjeldahl method (FIL 20B: 1993), with a 6.35 factor for the conversion of crude protein and casein and a 3.6 factor for non-protein nitrogen.

2.4. Rumen Variables and Analyses

Rumen fluid was obtained weekly, 4 h after the morning meal of each treatment. Sampling was performed with an oroesophageal tube. Samples were filtered twice and had their pH measured (T-1000 pH Meter, Tekna, Araucária, Brazil).

For the determination of ammonia-N and volatile fatty acid (VFA) concentrations, 5 and 10 mL of rumen fluid were filtered and acidified with 1 mL sulfuric acid (500 mL/L) and 1 mL of metaphosphoric acid (20%), respectively. Samples were stored at -20 °C until analysis.

Ammonia-N was quantified by the colorimetric method [17]. After the Kjeldahl test, absorbance was measured at 630 nm (Thermo Fisher Scientific, Madison, USA) with magnesium oxide and calcium chloride according to the 920.03 method (AOAC, 1990). Samples for the VFA analysis were evaluated by high-performance liquid chromatography (Waters Alliance e2695 Chromatograph, Waters Technologies do Brasil LTDA, Barueri, São Paulo, Brazil).

2.5. Blood Variables and Analyses

Blood samples were obtained by jugular venipuncture after local asepsis with 70% alcohol and were used for β-hydroxybutyrate (BHB) and glucose analyses. Sampling was performed weekly, 3 h after the first meal of each treatment.

For the BHB analysis, tubes without anticoagulant were used, whereas tubes for the glucose analysis contained sodium fluoride (Vacutainer; Becton, Dickinson and Company, São Paulo, Brazil). Samples were immediately transported on ice to the laboratory and were centrifuged at 3000 x g for 10 min at room temperature (22–25 °C). Duplicates were made for serum and plasma aliquots and stored at -20 °C for later analysis.

BHB and glucose serum concentrations were determined with an auto-analyzer (Cobas Mira Plus, Roche Diagnostic Systems, Risch-Rotkreuz, Switzerland) using commercial kits for BHB (Ranbut-D-3-Hidroxibutyrate, Randox Laboratories Ltd., Antrim, UK) and glucose (Glucose, Doles, Goiás, Brazil).

2.6. Health parameters

Rectal temperature was measured daily at 0600 h with the aid of a digital thermometer (Ombo Electronics, iColor®, G-Tech model, Shenzhen, China) with a measurement range of 32.0 to 43.9 °C. Animals with temperatures higher or equal to 39.4 °C were classified with hyperthermia.

Fecal score was determined daily, according to the following scores: 0 – normal (firm but not hard); 1 – soft (does not hold form, piles but spreads slightly); 2 – runny (spreads readily to about 6 mm depth); and 3 – watery (liquid consistency, splatters) [18]. Fecal score ≥ 2 was classified as diarrhea and severe diarrhea if the score was equal to 3. All animals with diarrhea, during the days they presented symptoms, received oral fluid therapy twice a day (10 g NaCl, 12 g sodium acetate, 2 g KCl, and 40 g glucose for 2 L water). If hyperthermic, animals received anti-inflammatory drugs for three days (flunixin meglumine, JA Saúde Animal, São Paulo, Brazil - 1 mL/45 kg). If hyperthermia persisted for two consecutive days, animals received parenteral antibiotic therapy for five days (enrofloxacin, Bayer, São Paulo, Brazil - 1 mL/40 kg).

Respiratory disease assessment was performed daily based on rectal temperature and nasal discharge scores, where: 0 – normal, serous discharge; 1 – small amount of unilateral, cloudy discharge; 2 – bilateral, cloudy, or excessive mucus discharge; 3 – copious, bilateral mucopurulent nasal discharge [14]. Animals with pneumonia received anti-inflammatory drugs for three days (flunixin meglumine, JA Animal Health, São Paulo, Brazil - 1 mL/45 kg) and parenteral antibiotic therapy for five days (enrofloxacin, Bayer, São Paulo, Brazil - 1 mL/40 kg).

2.7. Pulmonary consolidation

At the end of the experimental period (60 ± 1 d), all animals were euthanized following the procedures recommended by the Brazilian National Council of Veterinary Medicine. After euthanasia, the chest cavity was opened, and lungs were removed to assess the occurrence of respiratory disease. The area of lung consolidation was measured based on the score, where: 0 = normal or area of consolidation < 1 cm^2 and 1 = area of consolidation ≥ 1 cm^2 [19].

2.8. Performance, feed efficiency, and body measurements

Weight and body measurements were performed at the fourth and seventh day and weekly thereafter. All measurements were made prior to the first meal. Feeding efficiency (FE) was calculated by dividing the mean of average daily gain (ADG) by the total DMI (milk + starter). Animals were weighed in mechanical scales (COIMMA S16.742, Dracena, São Paulo, Brazil). Wither height (WH, distance from the base of the front feet to the withers) and rump height (RH, distance from the base of the rear feet to the rump) were measured using Teletape (Ketchum Deluxe Livestock Measure). The heart girth (HG, circumference of the chest) and rump width (RW, circumference of the chest) were measured with a measuring tape (38.68.150.000, Vonder, Curitiba, Brazil). Measurements were performed on plain ground, allowing animals to stand with limbs positioned symmetrically in relation to the floor.

2.9. Statistical Analysis

Data were analyzed using R software (R Core Team, 2019–version 4.1.2). Sample size calculations using a power of 80% and a significance level of 0.05 indicate that a difference in most of the parameters would be evident with 15 calves per group. The continuous outcomes such intake (milk, starter, total DMI, total GE, CP intake, and water intake), performance, feed efficiency, body measurements, blood and ruminal parameters were analyzed using a linear mixed-effect model (nlme package). Treatment, genetic composition, day/week, and interaction were included as factors for fixed effects and animals as random effects. Birth weight and total serum protein were tested as covariates and included in the model only if significant ($P < 0.05$). All models were verified graphically for normality and homoscedasticity of residuals and tested with the Shapiro–Wilk and Bartlett tests. A 95% confidence interval was adopted to verify the null hypothesis, and *P*-values were produced with a Tukey test.

Variables such as initial and final BW, total weight gain, and passive immunity transfer were analyzed using a linear mixed-effect model (nlme package). Treatment and genetic composition were included as fixed effects and animals as random effects. Variables of milk composition (DM, fat

content, crude protein, casein, non-protein nitrogen, lactose, and minerals) and quality (SCC and TBC) were analyzed using a linear mixed-effect model (nlme package). Treatment was included as fixed effects and samples as random effects. The SCC and TBC data were log-transformed (log10) prior to the analysis.

The categorical outcomes fecal and respiratory scores were analyzed using a non-parametric aligned rank transformation test, implemented in the R package ARTool. A 95% Confidence Interval was also adopted for the non-parametric tests. The occurrence of diarrhea and respiratory diseases (area of consolidation) were analyzed with a chi-squared test (stats package). For all analyses, a *P*-value of < 0.05 was considered statistically significant.

3. Results

3.1. Milk composition and efficiency of pasteurization

Milk fat content was higher for the BTM (4.24%) and WM (4.10%) treatments in comparison to the PWM treatment (3.76%) ($P < 0.05$) (Table 1). WM and PWM had higher crude protein content (3.46 and 3.49%, respectively) than BTM (3.30%; $P < 0.05$). No significant difference was observed for the percentage of casein in the BTM, WM, and PWM treatments (2.71, 2.64, 2.53%, respectively; $P = 0.22$). However, WM and PWM had a higher content of non-protein nitrogen (0.13 and 0.12%, respectively) than BTM (0.10%; $P < 0.05$). The lactose concentration was significantly higher in the BTM treatment (4.46%) than in the WM and PWM treatments (4.33%; $P < 0.05$)

BTM had a significantly lower concentration of minerals (0.69%; $P < 0.05$) than WM and PWM (0.72 and 0.73%, respectively). BTM had the highest DM content (13.04%), followed by WM (12.82%) and PWM (12.48%; $P < 0.05$). WM had a higher SCC (1,740.15 x 10^3 cells/mL) than BTM and PWM (366.81 and 1,424.67 x 10^3 cells/mL, respectively; $P < 0.05$). WM had a higher TBC (548.37 x 10^3 CFU/mL) than BTM and PWM (19.79 and 295.41 x 10^3 CFU/mL, respectively; $P < 0.05$).

Milk samples presented a TBC of 544 x 10^3 CFU/mL prior to the pasteurization and 195 x 10^3 CFU/mL immediately after the pasteurization process, resulting in a 64.15% reduction in the microbial load of the WM.

3.2. Intake

Milk intake was higher in the BTM treatment (772.37 g DM/d; Table 2), followed by WM (764.29 g DM/d) and PWM (740.87; $P < 0.05$).

Table 2. Intake, performance, feed efficiency, and body measurements of dairy calves fed bulk tank milk (BTM, n = 15), waste milk (WM, n = 15), and pasteurized waste milk (PWM, n = 15) during the period from 4 to 60 d of age

Item	Treatment[6]			SEM	*P*-value[1]		
	BTM	WM	PWM		T	W	T x W
Intake							
Milk (g of DM/d)	772.37 a	764.29 b	740.87 c	1.20	< 0.01	0.06	0.69
Starter (g of DM/d)	129.95	159.72	162.44	0.73	0.13	< 0.01	< 0.01
Total DMI[2] (g of DM/d)	895.34	911.17	899.98	11.81	0.93	< 0.01	0.64
Total gross energy[3] (Mcal/d)	3.48	3.91	4.23	1.21	0.21	< 0.01	< 0.01
Total CP[4] (kg/d)	50.75	57.35	59.22	5.19	0.27	< 0.01	< 0.01
Water (L/d)	1.87	1.84	1.86	0.08	0.95	< 0.01	0.09
Performance							
Initial weight (kg)	38.49	38.19	39.51	0.43	0.62	-	-
Final weight (kg)	76.03	77.43	74.09	0.82	0.77	-	-
Average of total period (kg)	37.55	39.25	34.58	0.36	0.13	-	-
ADG (kg/d)	0.67	0.71	0.62	0.03	0.25	< 0.01	0.26
Feed efficiency[5]	0.76 a	0.79 a	0.71 b	0.03	< 0.01	< 0.01	< 0.01
Body measures (cm)							
Heart girth	86.94	86.16	86.23	1.33	0.21	< 0.01	< 0.01
Withers height	84.41	84.15	84.28	0.79	0.74	< 0.01	0.73
Hip width	24.95	25.01	24.82	0.56	0.35	< 0.01	0.75
Hip height	87.19	87.35	87.36	0.92	0.88	< 0.01	0.06

[1]T = treatment effect; W = week effect; T × W = treatment × week interaction;
[2] Total DMI = starter DM + milk DM intakes;
[3] Total gross energy = starter GE + milk GE intakes;
[4] Total CP = starter CP + milk CP intakes;
[5] Feed efficiency was calculated by dividing ADG (g) by average daily DMI;
[6]Means followed by a lowercase letter represent statistical difference between treatments ($P < 0.05$; Tukey test).

Starter intake was significantly affected by week and week x treatment interaction ($P < 0.05$; Table 2). No difference regarding starter intake was found between treatments from weeks 1 to 5 (Figure 1b; $P > 0.05$). However, during weeks 6 and 7, calves fed BTM had a lower starter intake in comparison to PWM ($P < 0.05$) and WM animals (Figure 1). During week 8, a similar pattern was

found—calves receiving BTM had a lower starter intake (280.03 g DM/d) than WM, whereas WM (364.73 g DM/d) had similar values to PWM (339.73 g DM/d) ($P > 0.05$) (Figure 1). Regarding total DMI, only the week effect was significant ($P < 0.01$; Figure 1), with a gradual increase each week in total DMI.

Gross energy was significantly affected by week and week x treatment interaction ($P < 0.01$; Table 2). During weeks 6 and 7, the PWM treatment showed a higher GE intake (5.41 and 7.91 Mcal/d for weeks 6 and 7, respectively) in comparison to the BTM treatment (4.0 and 6.25 Mcal/d; $P < 0.05$) but was similar to WM (4.84 and 6.86 Mcal/d; $P > 0.05$). During week 8, both WM and PWM treatments had a higher GE intake (8.76 and 8.83 Mcal/d, respectively) than the BTM treatment (7.03 Mcal/d; $P < 0.05$). A significant effect for the week factor and a significant interaction between week and treatment were observed for the CP values ($P < 0.01$; Table 2). The PWM treatment (69.01 kg/d), from weeks 5 to 7, had a CP intake similar to the WM treatment (65.71 kg/d; $P > 0.05$) and a significantly higher CP intake than the BTM treatment (57.33 kg/d; $P < 0.05$). At week 8, both WM and PWM treatments had a higher CP intake (96.12 and 96.70 kg/d, respectively) than the BTM treatment (79.61 kg/d; $P < 0.05$).

Water intake was significantly affected by week ($P < 0.01$; Table 2). Water intake increased gradually from weeks 1 to 5 ($P < 0.05$) and stabilized from weeks 6 to 8 ($P > 0.05$).

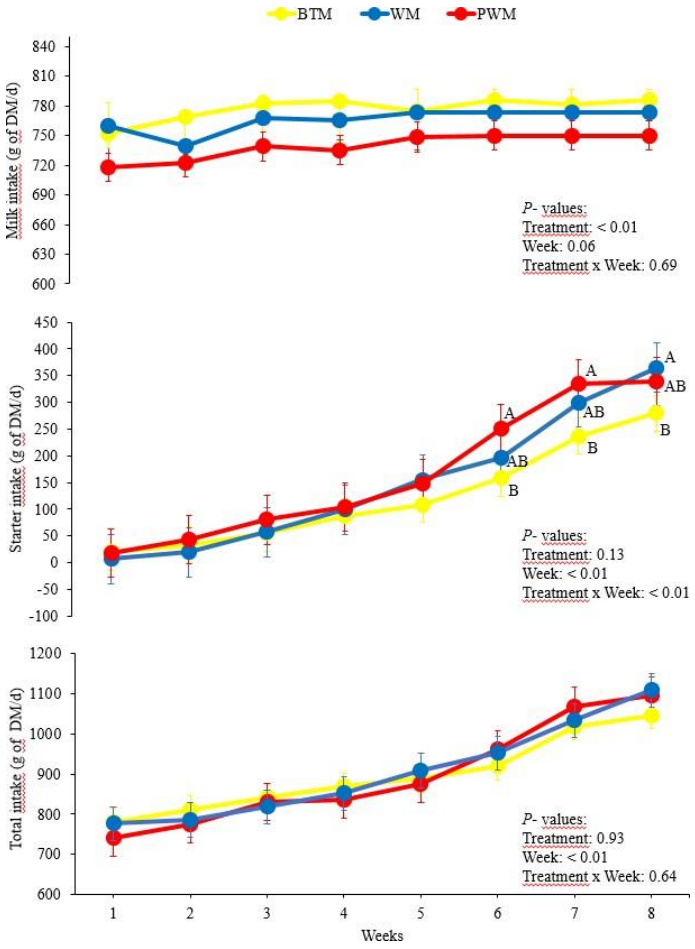

Figure 1. Dry matter intake (milk, starter, and total) of dairy calves fed bulk tank milk (BTM, n = 15), waste milk (WM, n = 15), and pasteurized waste milk (PWM, n = 15) during the period from 4 to 60 d. Bars represent SEM. Letters represent statistical difference between treatments ($P < 0.05$).

3.3. Rumen and blood parameters

Only the concentrations of acetate ($P = 0.01$) and propionate ($P = 0.03$) showed significant effects from the treatment. The rumen fluid of calves receiving PWM had higher concentrations of acetate and propionate than that of calves in the BTM and WM treatments (38.48, 33.02, and 32.89 mmol/L acetate and 26.21, 21.22, and 21.68 mmol/L propionate for PWM, BTM, and WM, respectively) (Table 3). The week factor affected acetate, propionate, butyrate, the acetate:propionate ratio, and pH ($P < 0.05$; Table 3). No significant differences were found between treatment for the ammonia-N ($P > 0.05$).

Glucose and BHB concentrations were neither affected by the treatment nor by the interaction between treatment and week ($P > 0.05$; Table 3). However, a significant effect of week was observed for BHB, where lower concentrations were found from weeks 1 to 3 (0.036 mmol/dL) in comparison to weeks 4 to 8 (0.13 mmol/dL; $P < 0.01$; Table 3).

Table 3. Ruminal and blood parameters of dairy calves fed bulk tank milk (BTM, n = 15), waste milk (WM, n = 15), and pasteurized waste milk (PWM, n = 15) during 4–60 d of age

Item	Treatment[1]			SEM	*P*–value[2]		
	BTM	WM	PWM		T	W	T x W
Rumen parameters							
pH	5.81	5.64	5.58	0.19	0.10	< 0.01	0.84
Ammonia-N (mg/dL)	17.71	19.02	21.18	0.08	0.36	0.48	0.35
Volatile Fatty Acids (mmol/L)							
Acetic (C2)	33.02 b	32.89 b	38.48 a	8.33	0.02	< 0.01	0.50
Propionic (C3)	21.68 b	21.22 b	26.21 a	0.71	0.03	< 0.01	0.41
Butyric (C4)	4.64	5.49	6.13	0.02	0.08	0.07	0.26
C2:C3	1.57	1.52	1.50	0.01	0.49	< 0.01	0.86
Blood parameters							
Glucose (mg/dL)	97.36	102.14	94.11	24.51	0.33	0.19	0.75
BHB (mmol/dL)	0.13	0.12	0.11	0.01	0.68	< 0.01	0.31

[1]Means followed by a lowercase letter represent statistical difference between treatments ($P < 0.05$; Tukey test);
[2]T = treatment effect; W = week effect; T × W = treatment × week interaction.

3.4. Health parameters

Fecal score analysis showed a significant effect of week ($P < 0.0001$), with higher values observed in week 2 (0.81; $P < 0.05$). No significant differences were observed between the BTM, WM, and PWM treatments for the number of animals with diarrhea (9, 6, and 7 animals, respectively; $P = 0.72$), days with diarrhea (8.21, 5.93, and 5.21 d, respectively; $P = 0.29$), days with fever (0.67, 1.13, and 1.81, respectively; $P = 0.79$), or calves with area of lung consolidation ≥ 1 (3, 7, and 5 calves, respectively; $P = 0.30$).

Table 4. Health parameters of dairy calves fed bulk tank milk (BTM, n = 15), waste milk (WM, n = 15), and pasteurized waste milk (PWM, n = 15) during the period from 4 to 60 d of age

Item	Treatment			SEM	*P*–value[1]		
	BTM	WM	PWM		T	W	T x W
Diarrhea							
Fecal score[2]	0.65	0.57	0.51	0.08	0.58	< 0.01	0.08
Days with diarrhea (d)	8.21	5.93	5.21	1.44	0.29	-	-
Days with fever (d)	0.67	1.13	1.81	0.94	0.79	-	-
Pneumonia							
Pulmonary consolidation ≥ 1 cm (n)[2]	3	7	5	-	0.3	-	-

[1]T = treatment effect; W = week effect; T × W = treatment × week interaction;
[2] Fecal score: 0 – normal (firm but not hard); 1 – soft (does not hold form, piles but spreads slightly); 2 – runny (spreads readily to about 6 mm depth); and 3 – watery (liquid consistency, splatters) [18];
[3]Number of animals with lung consolidation ≥ 1 cm.

3.5. Performance, feed efficiency, and body development

Average daily gain was not affected by any of the three treatments ($P > 0.05$). However, ADG was significantly different between weeks, with the second week showing smaller values of ADG (0.44 kg/d, $P < 0.01$; Table 2) No significant differences between BTM, WM, and PWM calves were found for final BW (76.03, 77.43, and 74.09 kg, respectively; $P > 0.05$). Feed efficiency was significantly affected by treatment, week, and treatment x week. BTM and WM calves showed higher FE (0.76 and 0.79, respectively) than PWM animals (0.71; $P < 0.05$). Calves receiving BTM, during weeks 2 and 3, had FE values (0.75) similar to WM animals (0.73; $P > 0.05$) and a significantly higher FE than PWM-fed calves (0.65; $P < 0.05$). For weeks 4 and 7, BTM and WM animals had higher GE values (0.75 and 0.81, respectively) than PWM calves (0.62; $P < 0.05$). In week 6, calves fed PWM had FE values (0.83) similar to animals receiving WM (0.76; $P > 0.05$) and a significantly higher FE than BTM fed calves (0.72; $P < 0.05$).

The week factor significantly affected the body measurement parameters of WH, RH, and RW ($P < 0.05$; Table 2). Values of WH (84.41, 84.15, and 84.28 cm for BTM, WM, and PWM, respectively), RH (87.19, 87.35, and 87.36 cm for BTM, WM, and PWM, respectively) and RW (24.95, 25.01, and 24.82 cm for BTM, WM, and PWM, respectively) gradually increased in each of the weeks of the experiment. The HG parameter was significantly affected both by week and by the interaction between

treatment and week. In week 5, calves fed BTM had CG values (89.42 cm) similar to WM animals (87.66 cm; $P > 0.05$), whereas these values were significantly higher than the PWM treatment (87.05; $P < 0.05$).

4. Discussion

To our knowledge, this study was the first to simultaneously assess the effects of feeding bulk tank milk (BTM), waste milk (WM), and pasteurized waste milk (PWM) on the intake, ruminal parameters, blood parameters, health, and performance of dairy calves.

Regarding the milk composition, the lower percentage of lactose in WM and PWM treatment and higher values of TBC in the WM treatment are due to the presence of transition milk. Although the WM used in the experiment was obtained only from cows undergoing antimicrobial treatment (for clinical mastitis, placental retention, metritis, or foot infections), some conditions such as placental retention and metritis occurred a few days after calving, while the milk was still considered in a transition stage. In comparison to whole milk, transition milk has higher total solid, fat, and protein contents but lower lactose concentrations [20].

After the homogenization of WM, the fraction of milk required for both meals of calves in the PWM treatment was separated, pasteurized, and stored. This process was performed once per day, with the WM milked in the morning. Even though heating during pasteurization deactivates a large part of lipases, some might have remained active during the storage process, causing the lower fat content observed in PWM. In addition, the elevated SCC for the PWM treatment (1.424,67 x 103 cells/ml) may have contributed to an increase in lipase concentrations, further intensifying the lipolysis during the storage period (6 h) and decreasing the fat content in the PWM.

A recent study did not find any differences in the protein contents of BTM, WM, and PWM [1]. In another analysis, while evaluating the nutritional value of WM and PWM, it was not possible to find significant differences in protein (3.51%), fat (3.90%), or lactose (4.42%) contents [21]. In general, many different compositions of WM and PWM are found in the scientific literature [1,21,3].

These differences in composition and nutrient content are mainly related to the differences in volumes of colostrum, transition milk, and milk from cows with clinical mastitis used in the WM.

Regarding the microbial assays, a reduction of 64% in the TBC was observed immediately after pasteurization. Some studies have reported reductions of approximately 98–99% of the microbial load [10,22,23]. Other studies have reported a wider range of reductions, from 20 to 70% [24,25]. Variations in the efficiency of the pasteurization process are related to factors such as temperature, sanitation of the pasteurizer, sanitation during the pasteurization process, and training of the operator. Furthermore, because the pasteurization is not 100% efficient, the microbial quality of the milk before pasteurization is an important factor.

Even though a 64% reduction in TBC was observed directly after pasteurization, the samples obtained immediately before the second meal of animals showed reductions of TBC of 49%, indicating possible recontamination after the pasteurization process. Recontamination of pasteurized milk is common in dairy farms, mainly due to failures in the refrigeration process or a lack of sanitation in equipment and utensils [21,22,25]. Reductions in microbial load in WM from 64.71 CFU/mL to 5.87 CFU/mL have been found after pasteurization [22]. However, in this same study, recontamination also occurred, with microbial load values reaching 30.44 CFU/mL.

The higher milk DMI of calves fed BTM are related to the differences in the nutritional value of the milk used in each treatment. Even though every animal received the same volume of milk every day (6 L), the DM content presented daily fluctuations. Milk used in the BTM treatment presented higher values of DM in comparison to WM and PWM, explaining the higher DMI observed for BTM.

The higher concentrate intake observed for calves fed WM and PWM from the sixth to eighth week are possibly due to physiological mechanisms of intake regulation. To meet their nutritional demands, animals in the WM and PWM treatments increased their concentrate intake because the DMI obtained from milk was insufficient. Contrary to our findings, in another study, no differences were observed for total milk intake or total concentrate intake between calves receiving WM, PWM, BTM, and pasteurized whole milk [25]. However, the assessment of the milk DMI and other intake

parameters were not performed weekly, making full comparisons with our results difficult.

For the ruminal parameters, the higher values of acetate and propionate in the rumen fluid of calves fed PWM are related to the higher intake of concentrate. The gradual increase on each week of the acetate, propionate, and butyrate concentrations, as well as the AC:PRO ratio, are consistent with the increase in solid intake [26].

During the milk-feeding of calves, small amounts of milk pass through the rumen [27,28]. In this sense, the antibiotic residue and the microbial properties of WM and PWM may alter the rumen microbiota, directly affecting the ruminal parameters of animals in these treatments [11,12]. This subject is still not well discussed in the literature and to the best of our knowledge, only one study has assessed the effect of WM on ruminal parameters. The ruminal parameters of calves fed WM, BTM, and milk replacer have been previously evaluated; however, significant changes were observed only for the concentration of VFA, with higher values of VFA for calves fed milk replacer [12]. The rumen fluid of calves fed WM presented higher concentrations of isovalerate. Because this substance originated from leucine, the authors state that the higher concentrations of isovalerate in the rumen are related to fermentation of milk with elevated protein content.

No differences were observed between treatment for pH, but pH values were reduced from the second to the eighth week. In accordance with our results, the most significant pH changes occur when animals have a higher intake of solid feed, typically between the fourth and seventh week [29].

In the first weeks of life, calves use glucose as a primary source of energy. As the rumen develops the concentration of glucose decreases, and those of VFA progressively increase. After weaning, calves complete their rumen development and, VFA produced by ruminal microbiota becomes the primary energy source, justifying BHB concentration increase, and glucose concentration decrease [15]. In our study, no glucose changes were found, possibly due to the supply of 6 L of milk / d throughout the period (60 d). Concentrations of BHB were lower in the first and second week, in comparison to the other weeks of the experiment, with the increase in BHB starting at the third week related to the steady increase in concentrate intake.

Feeding WM to calves raises many concerns due to the high microbial load the milk may contain, possibly increasing the spread of diseases. This high microbial load may be originated from infections in the mammary gland, ineffective sanitation practices, and inappropriate storage of WM [3]. Pasteurization has arisen as a possible tool for controlling the microbial contamination of milk and reducing the adverse effects on animal health. No relationship between the usage of WM and health parameters was found in our study. Even though the TBC of WM and PWM were above the recommended values [10] (TBC < 20.000 CFU/mL), and the TBC of BTM was below the recommended value, no differences were found in the assessed health parameters (diarrhea or pneumonia). It is known that diarrhea and respiratory problems are caused by a combination of factors and related to the immunity status, nutrition, type of housing, and season [19]. In accordance with those findings, research conducted with calves fed with BTM, WM, or PWM reported no differences in fecal score [1]. In another study, researchers observed that calves fed WM containing antibiotic residues showed fewer diarrhea symptoms than calves fed milk replacer; however, no direct relationship between the antibiotic residues and such an effect was reported [30]. The effects of antibiotic residues on diarrhea are small [31,32]. In our experiment, the lack of differences in the health parameters between treatments is possibly due to the intense cleaning of facilities and utensils used during animal handling, which may have reduced animal contamination and disease transmission. However, a retrospective analysis using data from the study revealed that the experimental power was 70% for fecal score, which limits the inferential capacity of the variable.

The similar values in the performance parameters (final BW, total weight gain, and ADG) are due to the similar DMI in all treatments. The ADG values were affected only by week, with lower ADG at the second week (0.44 kg/d), coinciding with the period of higher incidence of diarrhea (15 d ± 6.6) and higher fecal score (0.81). The week factor significantly affected the body measurement parameters, with steady increases of WH, RH, RW, and HG at each week. In addition, a study with calves receiving WM, PWM, BTM, or pasteurized whole milk also did not observe differences in ADG values from 0–14, 15–28, or 29–56 d [25]. On the other hand, a previous study reported higher ADG in calves receiving WM and PWM in comparison to calves fed BTM [1]. However, the

experimental period was shorter than our experiment (21 d), making direct comparisons with our data difficult.

The differences in experimental design make comparisons and conclusions regarding the effects of feeding WM or PWM on the health and performance of calves challenging. In general, the scientific literature still offers controversial results regarding the usage of WM. Due to the contribution of colostrum and transition milk, WM may present higher concentrations of total solids. In turn, higher solids can contribute to health and performance gains, masking the negative effects of microbial contamination and antibiotic residues. Regarding pasteurized WM, flaws in the pasteurization process that jeopardize its efficiency, possible spoilage of milk content, and milk recontamination are factors that may impair the beneficial effects of PWM on the health and performance of animals.

5. Conclusions

Feeding waste milk (WM) and pasteurized WM did not show significant negative effects on the intake, ruminal parameters, blood parameters, health, or performance of crossbred dairy calves.

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6. References

1. Zou, Y.; Wang, Y.; Deng, Y.; Cao, Z.; Li, S.; Wang, J. Effects of feeding untreated, pasteurized and acidified waste milk and bunk tank milk on the performance, serum metabolic profiles, immunity, and intestinal development in Holstein calves. *J. Animal Sci*. Biotech. 2017, 8, 53. https://doi.org/10.1186/s40104-017-0182-4.
2. Duse, A.; Waller, K.P.; Emanuelson, U.; Unnerstad, H.E.; Persson, Y.; Bengtsson, B. Farming practices in Sweden related to feeding milk and colostrum from cows treated with antimicrobials to dairy calves. *Acta Vet. Scand*. 2013, 55, 49-57. http://www.actavetscand.com/content/55/1/49.

3. Moore, D.A.; Taylor, J.; Hartman, M.L.; Sischo, W.M. Quality assessments of waste milk at a calf ranch. *J. Dairy Sci*. 2009, 92, 3503-3509. https://doi.org/10.3168/jds.2008-1623.

4. Menezes, M.F.C.; Simeoni, C.P.; Etchepare, M.A.; Huerta, K.; Bortoluzzi, D.P.; Menezes, C.R. Microbiota e conservação do leite. *RECEN*. 2014, 18, 76-89. http://dx.doi.org/10.5902/2236117013033.

5. Butler, J.A.; Sickles, S.A.; Johanns, C.J.; Rosenbusch, R.F. Pasteurization of Discard Mycoplasma Mastitic Milk Used to Feed Calves: Thermal Effects on Various Mycoplasma. *J. Dairy Sci*. 2000, 83, 2285-2288. https://doi.org/10.3168/jds.S0022-0302(00)75114-9.

6. Chardavoyne, J.R.; Ibeawuchi, J.A.; Kesler, E.M.; Borland, K.M. Waste milk from antibiotic treated cows as feed for young calves. *J. Dairy Sci*. 1979, 62, 1285-1289. https://doi.org/10.3168/jds.S0022-0302(79)83413-X.

7. Edrington, T.S.; Dowd, S.E.; Farrow, R.F.; Callaway,T.R.; Anderson, R.C.; Nisbet, D.J. Development of colonic microflora as assessed by pyrosequencing in dairy calves fed waste Milk. *J. Dairy Sci*. 2012, 95, 4519-4525. https://doi.org/10.3168/jds.2011-5119.

8. Maynou, G.; Jones, H.C.; Bach, A.; Terré, M. Feeding pasteurized waste milk to preweaned dairy calves changes fecal and upper respiratory tract microbiota. *Front Vet. Sci*. 2019. 6, 159. https://doi.org/10.3389/fvets.2019.00159.

9. Jamaluddin, A.A.; Carpenter, T.E.; Hird, D.W.; Thurmond, M.C. Economics of feeding pasteurized colostrum and pasteurized waste milk to dairy calves. *J. Am. Vet. Med. Assoc*. 1996, 209, 751-756.

10. Godden, S.M.; Fetrow, J.P.; Feirtag, J.M.; Green, L.R.; Wells, S.J. Economic analysis of feeding pasteurized nonsaleable milk versus conventional milk replacer to dairy calves**.** *J Am Vet Med Assoc*. 2005, 226, 1547-1554. https://doi.org/10.2460/javma.2005.226.1547.

11. Li, J.H.; Yousif, M.H.; Li, Z.Q.; Wu, Z.H.; Li, S.L.; Yang, H.J.; Wang, Y.J.; Cao, Z.J. Effects of antibiotic residues in milk on growth, ruminal fermentation, and microbial

community of preweaning dairy calves. *J. Dairy Sci*. 2019, 102, 2298-2307. https://doi.org/10.3168/jds.2018-15506.

12. Zhang, R.; Zhang, W.; Bi, W.; Tu, Y.; Beckers, Y.; Du, H.; Diao, Q. Early feeding regime of waste milk, milk, and milk replacer for calves has different effects on rumen fermentation and the bacterial community. *Animals*. 2019, 9, 443. https://doi.org/10.3390/ani9070443.
13. Quigley, J.D.; Lago, A.; Chapman, C.; Erickson, P.; Polo, J. Evaluation of the Brix refractometer to estimate immunoglobulin G concentration in bovine colostrum. *J. Dairy Sci*. 2013, 96, 1148-1155. http://dx.doi.org/ 10.3168/jds.2012-5823.
14. McGuirk, S.M. 2013. University of Wisconsin, School of Veterinary Medicine. Calf health scoring chart. https://fyi.extension.wisc.edu/heifermgmt/files/2015/02/calf_health_scoring_chart.
15. Drackley, J.K. Calf Nutrition from Birth to Breeding 2008. Vet. Clin. North Am. Food Anim. Pract. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0749072008000029.
16. Van Soest, P.J.; Robertson, J.B.; Lewis, B.A. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci*. 1991, 74, 3583-3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2.
17. Chaney, A.L.; Marbach, E.P. Modified Reagents for Determination of Urea and Ammonia. *Clin. Chem*. 1962, 8, 130-132. Available from: http://clinchem.aaccjnls.org/content/8/2/130.abstract.
18. McGuirk, S.M. Disease management of dairy calves and heifers. Vet. Clin. North Am. Food. Anim. Pract. 2008, 24, 139-53. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0749072007000722.
19. Cramer, M.C.; Ollivett, T.L. Growth of preweaned, group-housed dairy calves diagnosed with respiratory disease using clinical respiratory scoring and thoracic ultrasound. A cohort study. *J. Dairy Sci*. 2019, 102, 1-10. https://doi.org/10.3168/jds.2018-15420.

20. Foley, J.A.; Otterby, D.E. Availability, storage, treatment, composition, and feeding value of surplus colostrum: a review. *J. Dairy Sci*. 1978, 61, 1033-1060. https://doi.org/10.3168/jds.S0022-0302(78)83686-8.

21. Jorgensen, M.A.; Hoffman, P.C.; Nytes, A.J. Case study: A field survey of on farm milk pasteurization efficacy. *The Prof. Anim. Sci*. 2006, 22, 472-476.

22. Elizondo-Salazar, J.A.; Jones, C.M.; Heinrichs, A.J. Evaluation of calf milk pasteurization systems on 6 Pennsylvania dairy farms. *J. Dairy Sci*. 2010, 93, 5509-5513. https://doi.org/10.3168/jds.2010-3460.

23. Grant, I.R.; Williams, A.G.; Rowe, M.T.; Muir, D.D. Efficacy of various pasteurization time-temperature conditions in combination with homogenization on inactivation of *mycobacterium avium* ssp. *paratuberculosis* in milk. *Appl. Environ. Microbiol*. 2005, 71, 2853-2861. https://doi.org/10.1128/AEM.71.6.2853-2861.2005.

24. Ruzante, J.M.; Gardner, I.A.; Cullor, J.S.; Smith, W.L.; Kirk, J.H.; Adaska, J.M. Isolation of *Mycobacterium avium* ssp. *paratuberculosis* from waste milk delivered to California calf ranches. *Foodborne Pathog. Dis*. 2008, 5, 681-686. https://doi.org/10.1089/fpd.2008.0082.

25. Aust, V.; Knappstein, K.; Kunz, H.J.; Kaspar, H.; Wallmann, J.; Kaske, M. Feeding untreated and pasteurized waste milk and bulk milk to calves: effects on calf performance, health status and antibiotic resistance of faecal bacteria. *J. Animal Physiol Anim. Nutr.* 2012, 97, 1091-1013. https://doi.org/10.1111/jpn.12019.

26. Robinson, P.H.; Tamminga, S.; Van Vuuren, A.M. Influence of declining level of feed intake and varying the proportion of starch in the concentrate on rumen fermentation in dairy cows. *Livest. Prod. Sci*. 1986, 15, 173-189. https://doi.org/10.1016/0301-6226(86)90026-6.

27. Berends, H.; Reenen Van, C.G.; Stockhofe-Zurwieden, N.; Gerrits, W.J.J. Effects of early rumen development and solid feed composition on growth performance and abomasal health in veal calves. *J. Dairy Sci*. 2012, 95, 3190-3199. https://doi.org/10.3168/jds.2011-4643.

28. Ellingsen, K.; Mejdell, C.M.; Ottesen, N.; Larsen, S.; Grondahl, A.M. The effect of large milk meals on digestive physiology and behaviour in dairy calves. *Physiol. Behav*. 2016, 154, 169-174. https://doi.org/10.1016/j.physbeh.2015.11.025.

29. Davis, C.L.; Drackley, J.K. 1998. The development, nutrition, and management of the young calf. Iowa State University Press. Iowa. First edition. 339.

30. Brunton, L.A.; Duncan, D.; Coldham, N.G.; Snow, L.C.; Jones, J.R. A survey of antimicrobial usage on dairy farms and waste milk feeding practices in England and Wales. *Vet. Rec*. 2012, 171, 296-302. https://doi.org/10.1136/vr.100924.

31. Langford, F.M.; Weary, D.M.; Fisher, L. Antibiotic resistance in gut bacteria from dairy calves: A dose response to the level of antibiotics fed in milk. *J. Dairy Sci*. 2003, 86, 3963-3966. https://doi.org/10.3168/jds.S0022-0302(03)74006-5.

32. Thames, C.H.; Pruden, A.; James, R.E.; Ray, P.P.; Knowlton, K.F. Excretion of antibiotic resistance genes by dairy calves fed milk replacers with varying doses of antibiotics. *Front in Microbiol*. 2012, 3, 139 https://doi.org/10.3389/fmicb.2012.00139.