jotaf Journal of Tekirdag Agricultural Faculty
Tekirdağ Ziraat Fakültesi Dergisi

Eylül/September 2024, 21(4)
Başvuru/Received: 14/12/23
Kabul/Accepted: 12/07/24
DOI: 10.33462/jotaf.1404962

http://dergipark.gov.tr/jotaf
http://jotaf.nku.edu.tr/

ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

The Effects of Restricted and Ad Libitum Milk Feeding on Growth and Health of Calves*

Sınırlı ve Serbest Süt İçirmenin Buzağıların Gelişimi ve Sağlığı Üzerine Etkisi

İbrahim ÖZTOP[1], Serkan ÖZKAYA[2]

Abstract

In recent years, there has been a growing concern among consumers regarding animal welfare and their requirements, leading to an increased interest in exploring new approaches to calf rearing. Therefore, the aim of this study was to investigate the effects of milk feeding levels on the growth, feed intake, and blood variables of group-raised calves during the suckling period. Ten Holstein calves, 5 in each group, were used in the study when they were five years old. The calves were acclimated to an automated feeder during the initial 19 days of the experiment, and their initial LWs were recorded after weighing them again. The study concluded upon weaning the calves when they were sixty years old, with a total duration of 36 days The calves in the first group (G1) were provided with a total of 4 L/day of milk replacer, divided into 2 L servings in the morning and evening. Conversely, calves in the second group (G2) were allowed a maximum intake of 12 L/day of milk replacer, mimicking the natural sucking behaviour, with feedings in the morning (05:00-08:00), midday (10:00-13:00), and evening (16:00-20:00). Despite no significant differences in the LW, total and daily LW gains, and body measurements averages between of the groups, calves in G1 consumed more starter feed compared to those in G2 (P<0.05). There was a non-significant improvement in feed efficiency in favor of calves in G2. Additionally, significant increases were noted in serum glucose concentrations among the biochemical variables in G2 (P<0.05). Furthermore, hematological blood variables, including haemoglobin, haematocrit, and erythrocyte values, tended to increase in calves in G2. However, there was no significant effect of milk feeding levels observed on oxidative stress, antioxidative defense mechanisms and immune response. In conclusion, the results from this study suggest that the amount of milk feeding does not exert a significant effect on the growth and health of the calves during the milk-feeding period.

Keywords: Calves, Growth, Health, Milk feeding amount

[1]İbrahim Öztop, Isparta University of Applied Sciences, Faculty of Agriculture, Department of Animal Science, Isparta, Turkey. E-mail: i.oztop94@gmail.com OrcID: 0000-0002-4865-5618
[2]**Sorumlu Yazar/Corresponding Author:** Serkan ÖZKAYA, Isparta University of Applied Sciences, Faculty of Agriculture, Department of Animal Science, İzmir, Turkey. E-mail: serkanozkaya@isparta.edu.tr OrcID: 0000-0003-3389-0188
Atıf: Öztop, İ., Özkaya, S. (2024). Sınırlı ve serbest süt içirmenin buzağıların gelişimi ve sağlığı üzerine etkisi. *Tekirdağ Ziraat Fakültesi Dergisi,* 21(4): 1033-1044.
Citation: Öztop, İ., Özkaya, S. (2024). The effects of restricted and ad libitum milk feeding on growth and health of calves. *Journal of Tekirdag Agricultural Faculty,* 21(4): 1033-1044.

*This article is summarized from the Serkan ÖZKAYA's MSc. Thesis.

Öz

Son yıllarda, hayvan refahı ve hayvan gereksinimleri ile ilgili tüketicilerdeki endişeler, buzağı yetiştirmenin yeni yollarını bulma arzusunu arttırmıştır. Bu nedenle çalışmanın amacı, süt içirme miktarının grup halinde yetiştirilen buzağıların süt içme dönemindeki büyüme, yem tüketimi ve kan değişkenleri üzerindeki etkilerinin incelenmesidir. Çalışmada 5 günlük yaşta 10 Holstein buzağı (her grupta 5 buzağı) kullanılmıştır. Denemenin ilk 19 gününde buzağılar otomatik yemleme robotuna alıştırılmıştır. Buzağılar tekrar tartılarak deneme başı canlı ağırlıkları belirlenmiştir. Buzağılar 60 günlük yaşta sütten kesilmişler ve deneme sonlandırılmıştır. Deneme toplam 36 gün sürmüştür. Birinci grupta bulunan (G1) buzağıların, sabah 2 L ve akşam 2 L olmak üzere toplam 4 L/gün süt ikame yemi tüketmelerine müsaade edilmiştir. İkinci grupta bulunan (G2) buzağıların ise doğal emzirme davranışı olan sabah (05:00-08:00), öğlen (10.00-13:00) ve akşam (16:00-20:00) olmak üzere azami 12 L/gün süt ikame yemi tüketmelerine müsaade edilmiştir. Grupların canlı ağırlıkları, toplam ve günlük canlı ağırlık artışları ve vücut ölçüleri ortalamaları arasında önemli farklılık bulunamamıştır. Ancak G1'de bulunan buzağıların, G2'de bulunan buzağılara nazaran daha fazla buzağı başlangıç yemi tükettikleri belirlenmiştir ($P<0.05$). Yemden yararlanma oranında G2'de bulunan buzağıların lehine önemli olmayan bir iyileşme elde edilmiştir. Biyokimya kan değişkenlerinden serum glikoz konsantrasyonlarında G2'de bulunan buzağılarda önemli artış elde edilmiştir ($P<0.05$). Hematolojik kan değişkenlerinden Hemoglobin, hematokrit, ve eritrosit değerleri G2'de bulunan buzağılarda artma eğilimi göstermiştir. Ancak, oksidatif stres, antioksidatif savunma mekanizması ve bağışıklık sistemi üzerine süt içirme miktarının önemli bir etkisi gözlenmemiştir. Çalışmada elde edilen sonuçlar, süt içirme miktarının buzağıların süt içme dönemindeki gelişimleri ve sağlıkları üzerine önemli etkisinin olmadığını göstermiştir.

Anahtar Kelimeler: Buzağı, Gelişim, Sağlık, Süt içirme miktarı

1. Introduction

It is widely recognized that a significant portion of revenue in dairy farming, approximately 40%, is derived from calves, with the remaining 60% coming from milk production (Anonymous, 2016). Consequently, the successful rearing of healthy calves is paramount for the future sustainability and profitability of the herd. The primary objective in raising calves is to ensure their optimal health and performance from birth throughout their lifespan. Achieving this goal necessitates the implementation of an effective calf-rearing system characterized by attributes such as high animal performance, cost-effectiveness, and minimal disease incidence and morbidity (Anonymous, 2011).

In modern farming practice, calves are typically separated from their dams shortly after birth and are provided with restricted milk intake via bottle feeding. However, restricted milk feeding can lead to various issues including calf starvation, impaired growth, elevated calf mortality rates, and behavioural abnormalities. While understanding the impact of a*d libitum* milk feeding on calf growth, development, and health before weaning is crucial, it is equally important to consider factors such as hunger, pain and other welfare concerns. The quantity of milk intake, the method of milk delivery, and the overall rearing protocols employed significantly influence calf growth, health, and welfare outcomes. Therefore, careful consideration of these factors is essential for optimizing calf-rearing practices and ensuring the well-being of the animals (Schaff et al., 2016).

Hunger is a subjective sensation characterized by the desire to eat and is regulated by the nervous system. For example, decreased levels of blood glucose prompt the emergence of hunger symptoms, whereas adequate glycaemia indicates a feeling of fullness and the cessation of eating (Stunkard, 1975). Hedonic systems, such as odours and learned cues associated with preferred tastes, also play a role in hunger. For example, the taste of lactose can heighten dairy calves' inclination to suckle milk (de Passille and Rushen, 2006). As a result, hunger is not solely connected to nutrient deficiencies but can also be influenced by various internal and external factors, including neural pathways that are not yet fully understood (Saper et al., 2002).

Growth parameters such as live weight and body measurements provide insights into the general health status of calves (Kozaklı et al., 2022). Calves raised as replacement heifers on dairy farms are generally fed daily with 10% of their live weight, which is about half of the milk they desire (Appleby et al., 2001). These calves usually have free access to starter feed, but their intake of very little starter feed is not enough to compensate for their limited milk intake (Jasper and Weary, 2002). In fact, the traditional practice of feeding young calves limited milk does not account for the energy levels they require for growth (Diaz et al., 2001; Van Amburgh and Drackley, 2005; Bartlett et al., 2006).

Calves that were fed very high quantities of milk (Khan et al., 2007a) or provided milk and milk replacer ad libitum (Jasper and Weary, 2002) demonstrated improved growth rates. Additionally, calves fed ad libitum tended to spend more time resting (de Paula Vieira et al., 2008), implying that calves expend more energy while standing than when lying down (Schrama et al., 1995). However, it is also reported that the increased amount of milk can negatively affect calf health (Quigley et al., 2006). Contrarily, some studies show no significant increase or decrease in disease incidence with higher milk intake (Appleby et al., 2001; Jasper and Weary, 2002; Khan et al., 2007a). Furthermore, it has been reported that calves fed a high percentage of milk or milk replacers have improved live weight gain, reduced starter intake, and no increase in disease incidence (Borderas et al., 2009).

Feeding with a high rate of milk decreases feed intake and increases weight gain. Calves fed with a high rate of milk consume less starter and straw while obtaining more digestible energy, leading to greater live weight gain (de Passille et al., 2011).

When fed with high amounts of milk, calves show more growth (Hartel et al., 2002; Jensen, 2006; Huuskonen and Khalili, 2008). Increased milk intake during the pre-weaning period influenced LW gain, leading to a decreased age of puberty onset and augmented fat-corrected milk yield during the first lactation. However, it does not impact skeletal development (Shamay et al., 2005). Bar-Peled et al. (1997) compared calves suckling from their dams three times a day with restricted milk intake to calves on a different feeding regimen. They reported rapid increases in live weight and wither height in the suckling calves during the first

6 months. Additionally, nutritional status may benefit the immune response and reduce mortality (Drackley, 2005).

As stated above, the difference in milk feeding methods impacts the performance and welfare of the calves. This study contributes to the understanding the effects of the frequency of giving milk replacer to calves using computer-controlled automatic feeders on their growth and health.

The objective of this research is to investigate the effects of milk feeding quantity on the growth, feed intake and health of calves during the suckling period. The study sought to answer the following questions:

How does the amount of milk given to calves fed with a computer-controlled automatic feeder affect their growth and feed intake?

How does this affect the oxidative stress level in calves during the suckling period?

How does this affect the antioxidative defense mechanism and the immune response?

2. Material and Method

In the study, 10 Holstein male calves born at the Faculty of Agriculture, Education, Research and Practice Farm at Isparta University of Applied Sciences, were used.

Commercially available starter and milk replacer used on the farm were utilized in the study.

Calves with similar live weights, at an average of 5 days old, were divided into 2 groups. The calves were adjusted to the automatic feeder in the first 19 days of the experiment. The calves in the groups were weighed again and their initial LW were recorded. The calves were weaned at 60-day-old and the study was terminated. The experiment lasted 36 days. The first group (G1) was given 2 L of milk replacer (Pro Milk, Interchem Limited Dublin, Ireland) in the morning and evening, 4 L/day in total, with a computer-controlled automatic feeder (I-MOM, Itech Robotic Automation Medical Ltd. Isparta, Türkiye). The second group (G2) was fed a maximum of 12 L/day of milk replacer in the morning (05:00-08:00), midday (10:00-13:00), and evening (16:00-20:00) (Odde et al., 1985; Day et al., 1987).

The milk replacer was prepared in the automatic feeder during sucking according to daily limits. 125 g of powder was mixed with 1 L of water at 38-40 °C and fed to the calves.

Live weight and body measurements of the calves were taken at the beginning of the experiment and followed up on a weekly basis. The live weights of the calves were recorded with a 1 g precision scale (Jadever, Coreks Kimya Gıda San. Tic. Ltd. Şti. İstanbul, Türkiye). A measuring stick (Hauptner-Herberholz GmbH & Co. Solingen, Germany) and strip (Adevet Kilometer, Vetaş Veteriner, İstanbul, Türkiye) were used to take body measurements (Yüksel and Karaçuhalılar, 2024) of the calves (*Figure 1*)

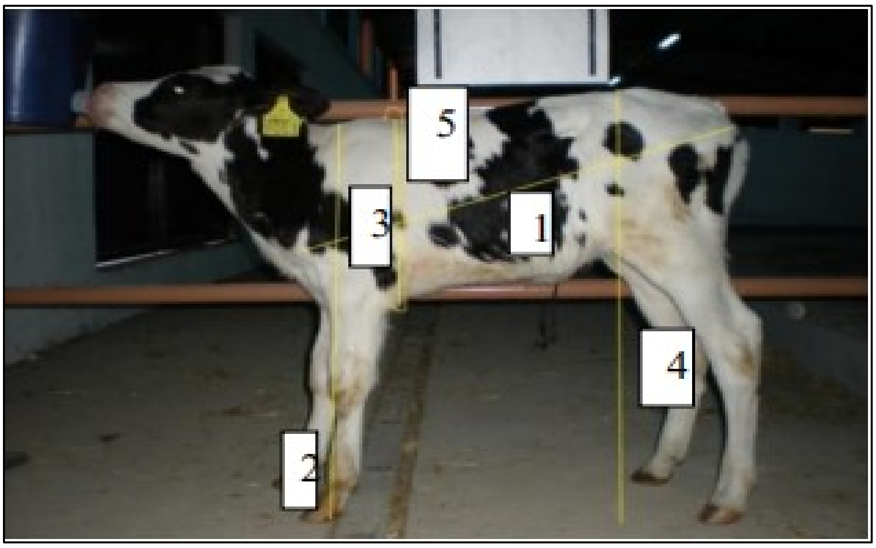

Figure 1. Body measurement parts of the calves.

Body length (BL): Measured from the shoulder to the outside angle of the seat bump using a measuring stick (cm), 2) Wither height (WH): The vertical distance from the top of the withers to the ground, measured by a measuring stick (cm), 3) Body depth (BD): The vertical depth between the highest point of the withers and the sternum measured by a measuring stick (cm), 4) Hip height (HH): The vertical distance from the highest point of the hip to the ground, measured by a measuring stick (cm), 5) Chest girth (CG): The girth (cm) measured with a measuring strip just behind the shoulder blades.

The milk replacer and starter intake of the calves were recorded daily by the computer-controlled automatic feeder's system. The percentage of starter crude protein in the starter was determined using the Kjeldahl method (AOAC, 2005; method 945.18), ether extract was determined using the Soxhlet method (AOAC, 2005; method 920.39), starch content was analyzed using method 996.11 of AOAC (2005), crude cellulose was determined using the Weende method (AOAC, 2005; method 978.01), and moisture content was analyzed using method 930.15 of AOAC (2005). Fat, protein, lactose and dry matter analyses of milk replacer were performed using HasVet Milk Test (HasVet Medikal Yazılım Sağlık Hizmetleri San. Tic. Anonim Şti., Antalya, Türkiye) milk analyser (*Table 1*).

Table 1. Chemical composition of starter, and milk replacer for calves

	Calf starter		Milk replacer
Dry matter, %	90.05	Dry matter, %	12.63
Crude Protein, %	18.17	Protein, %	2.15
Ether extract, %	2.79	Fat, %	2.88
Crude fiber, %	9.41	Fiber, %	-
Moisture, %	9.95	Moisture, %	4.5
Crude ash, %	7.52	Ash, %	0.64
Starch, %	28.25		-
Metabolic energy, kcal/kg*	2751.97		-
	-	Lactose, %	6.90

* The metabolic energy value was calculated according to the Turkish Standards Institution (TSE, 1991).

The starter intake of the calves was measured with a scale integrated into the computer-controlled automatic feeder system with a precision of 1 g. Using chips on the calves' necks and sensors in the manger, the system automatically recorded the amount of starter intake each time a calf entered the manger to feed.

Blood samples were collected from the jugular vein of calves at the beginning and end of the experiment at noon. The samples were collected in EDTA tubes and analyzed for hematological variables using the Mindray BC30 Vet V3D (Mindray Medical Int. Ltd., Shenzhen, China) blood count device.

Liver function tests (Albumin-ALB, Alkaline phosphatase-ALP, Alanine transaminase-ALT, Gamma-glutamyl transferase-GGT, Glucose-GLU, Total bilirubin-TBil, Lactate dehydrogenase-LDH), and kidney function tests (ALB, Blood urea nitrogen-BUN, Calcium-Ca, Creatine-CREA, Inorganic phosphorus-IP, Total protein-TP), as well as triglyceride and total cholesterol variables were analyzed using the Mindray BS120 (Mindray Medical Int. Ltd., Shenzhen, China) blood analyser device. The Globulin variable was calculated using the formula; Globulin = Total protein - Albumin.

Oxidative stress markers (Total oxidative capacity-TOC, Oxidative stress index-OSI, Malondialdehyde-MDA), antioxidative defense mechanism markers (Total antioxidant capacity-TAC, Paraoxonase-1-PON1, Superoxide dismutase-SOD, Glutathione peroxidase-GPx, Catalase-CAT) and immune response markers (ImmunoglobulinA-IgA, IgM and IgG) were analysed using the Mindray BS400 (Mindray Medical Int. Ltd., Shenzhen, China) blood analyser. CAT and MDA were analysed using Rel-Biochem (Rel Assay, Mega Medicine San. Tic. Ltd. Şti., Gaziantep, Türkiye) and IgG was analysed using the Bio-Tek EIX800 (BioTek Ins. Inc. Vermont, USA) Elisa reader with the Bovine Interferon Gamma (E0005BO) Elisa kit (Bioassay Technology Laboratory, Shanghai, China).

The oxidative stress index (OSI) was calculated using the following formula described by Yumru et al. (2009): OSI (Arbitrary unit) = (TOC (µmol H_2O_2 equivalent/L)) / (TAC (mmol trolox equivalent/L)x1000)x100.

Repeated Measurements ANOVA was employed to analyze the data from the study. Differences between group means were considered significant when P<0.05, and a tendency when 0.06<P≤0.10. The data analysis was conducted using Minitab 20 (Minitab LLC, 2020, Penn State, USA.

3. Results and Discussion

The study did not find significant effects of milk feeding quantity on the live weight, body measurements, and feed efficiency of calves (*Table 2*). However, there was a noteworthy difference in average feed intakes (P<0.05). It is well recognized that the effect of milk feeding on calves' starter intake and growth performance depends significantly on both the quality and quantity of milk provided (Silper et al., 2014). Typically, there is an inverse relationship between milk intake and starter intake among calves (Raeth-Knight et al., 2009; Terre et al., 2009; Gelsinger et al., 2016), a relationship that strengthens with higher milk quantities offered. In this study, the feed intake of G2 was significantly lower compared to G1 (P<0.05; *Table 2*). When calves are fed higher amounts of milk rather than restricted amounts, reduced starter feed intake is observed (Hill et al., 2010; Silper et al., 2014). This reduced starter feed consumption may be attributed to increased satiety resulting from elevated blood glucose and insulin levels and increased gut fill (Khan et al., 2011).

Table 2. The effect of restricted and ad libitum milk feeding growth performance of calves

	G1 (restricted) Mean±S. E.	G2 (ad libitum) Mean±S. E.	P
Live weight (LW), kg			
Initial	58.13±8.27	57.50±8.27	0.97
Final	85.30±13.80	85.90±13.80	
Total LW Gain	27.13±6.79	28.38±6.79	
Daily LW Gain	0.75±0.19	0.79±0.19	
Body length (BL), cm			
Initial	77.50±3.70	74.50±3.70	0.76
Final	88.25±4.05	87.75±4.05	
Total BL Gain	10.75±0.83	13.25±0.83	
Daily BL Gain	0.30±0.02	0.37±0.02	
Body depth (BD), cm			
Initial	31.50±1.17	31.38±1.17	
Final	35.88±1.45	35.25±1.45	0.77
Total BD Gain	4.38±0.24	3.88±0.24	
Daily BD Gain	0.12±0.01	0.11±0.01	
Wither height (WH), cm			
Initial	82.00±2.88	80.25±2.88	0.61
Final	90.13±3.15	86.75±3.15	
Total WH Gain	8.13±0.52	6.50±0.52	
Daily WH Gain	0.23±0.01	0.18±0.01	
Hip height (HH), cm			
Initial	83.50±3.05	82.50±3.05	0.76
Final	91.25±3.43	89.75±3.3.43	
Total HH Gain	7.75±1.11	7.25±1.11	
Daily HH Gain	0.22±0.03	0.20±0.03	
Chest girth (CG), cm			
Initial	83.88±3.55	83.00±3.55	
Final	92.63±4.62	94.00±4.62	0.94
Total CG Gain	8.75±1.29	11.00±1.29	
Daily CG Gain	0.24±0.04	0.31±0.04	
Milk replacer and Feed intake and Feed efficiency			
Milk replacer DM intake, kg	13.87±0.36	31.58±1.37	0.00
Starter Feed DM Intake, kg	10.66±0.01	7.28±0.01	0.00
Total DM Intake, kg	24.53±0.35	38.86±1.37	0.00
Daily DM Intake, kg	0.681±0.01	1.08±0.03	0.00
Feed Efficiency	1.30±0.34	1.73±0.49	0.36

a,b Difference between the means in the same row is statistically significant (P<0.05)
Feed Efficiency=Daily DM Intake/Daily LW Gain

During the milk suckling period, non-significant differences in live weights were observed between the groups receiving different amounts of milk (*Table 2*). The live weights of G2 increased insignificantly compared to G1. Calves fed large amounts of milk exhibited higher live weight gains during the milk suckling period due to the intake of more digestible nutrients from milk (Dennis et al., 2018; Orellana Rivas et al., 2020; van Niekerk et al., 2020). Studies have reported non-significant differences in live weights among calves fed different amounts and frequencies of milk replacer meals (MacPherson et al., 2019). Scoley et al. (2020) found that milk feeding frequency did not affect the live weight and live weight gains of the calves. Additionally, Jafari et al. (2021) noted that calves with higher daily milk intake gained more live weight, though the milk feeding frequency insignificantly affected live weight gain and showed an increasing trend.s

The differences in body measurements between G2 and G1 were not significant (*Table 2*). Similarly, Kmicikewycz et al. (2013) and Jafari et al. (2021) reported that the milk feeding frequency and amount did not significantly affect body measurements of calves. While high milk intake tends to increase live weight, it also tends to linearly increase chest girth (Stamey et al., 2012; Rosenberger et al., 2017). Khan et al. (2011) reported that the body depth of calves fed with restricted milk tended to increase compared to calves consuming high amount of milk.

Feed efficiency remained unaffected (*Table 2*), consistent with previous studies that did not report significant improvements in feed efficiency (Hill et al., 2016; Rosenberger et al., 2017; Dennis et al., 2019; Jafari et al., 2021). While the effect of milk and milk replacer intake on feed efficiency may vary, calves consuming higher amounts of milk have been associated with higher feed efficiency in some studies (Quigley et al., 2018).

3.1. Blood variables

3.1.1. Biochemical blood variables

The biochemical blood results of the groups are presented in *Table 3*. No significant difference was found in biochemical blood variables at the beginning of the experiment, thus these values are not shown in the table. However, a significant increase in GLU concentration was observed at the end of the experiment in G2 ($P<0.05$).

Table 3. The effect of restricted and ad libitum milk feeding on biochemical blood variables of calves at the end of the experiment

	G1 (restricted) Mean±S. E.	G2 (ad libitum) Mean±S. E.	P
ALT, U/L	23.25±2,48	23.75±2.48	0.70
GGT, U/L	15.75±1,32	20.75±1.32	0,31
ALP, U/L	173.50±22,08	217.80±22,08	0.23
Total cholesterol, mg/dL	62.25±4.06	68.50±4.06	0.81
Creatine, mg/dL	0.27±0.46	0.25±0.46	0.65
Calcium, mg/dL	9.43±0.35	9.48±0.35	0.84
Inorganic phosphorus, mg/dL	4.35±0.18	5.02±0.18	0.28
Total Protein, g/dL	5.53±0.15	5.58±0.15	0.77
Albumin, g/dL	2.87±0.13	2.91±0.13	0.80
LDH, U/L	868.50±49.89	967.50±49.89	0.79
Triglyceride, mg/dL	14.00±3.10	14.25±3.10	0.65
Total Bilirubin, mg/dL	0.15±0.06	0.08±0.06	0.59
Glucose, mg/dL	70.60±5.57	98.25±5.57	0.04
Blood urea nitrogen, g/L	6.05±0.06	6.18±0.06	0.48
Globulin, g/dL	2.60±0.11	2.73±0.11	0.53

ALT: Alkaline transaminase, GGT: Gamma-glutamyl transferase, ALP: Alkaline phosphatase, LDH: Lactate dehydrogenase, a,b Difference between the means in the same row is statistically significant ($P<0.05$)

The feed intake of calves significantly affects GLU concentration. GLU derived from intestinal absorption is the primary energy substrate in calves (Khan et al., 2000b). As solid feed intake, the energy source shifts to volatile fatty acids, particularly propionate, produced through ruminal fermentation, which is converted to GLU (Vi et al., 2004). The increase in the starter intake in calves fed restricted milk may be due to a hyperphagic response triggered by reduced milk volume, leading to decreased blood glucose concentration (Khan et al., 2007b; Khan et al., 2011; Omidi-Mirzaei et al., 2015; de Paula et al., 2017). Elevated GLU concentration in calves fed higher milk amounts are consistent with findings from Terre et al. (2009), MacPherson et al. (2016), Mirzaei et al. (2018) and Jafari et al. (2021). Increased lactose intake likely contributed to higher blood GLU concentration (Palmquist et al., 1992). Conversely, studies have reported that calves fed excessive milk exhibit lower GLU concentrations compared to those fed with restricted amounts (Silper et al., 2014; de Paula et al., 2017), suggesting that lactose from milk replacer alone may not suffice to elevate glycaemia.

3.1.2. Hematological blood variables

There were no significant differences in hematological blood variables at the beginning of the experiment, which are not presented in Table. However, by the end of the experiment, there was a tendency for concentrations of haemoglobin, haematocrit, and erythrocyte to increase in G2 ($P \leq 0.10$) (*Table 4*).

Table 4. The effect of restricted and ad libitum of milk feeding on hematological blood variables of calves at the end of the experiment

	G1 (restricted) Mean±S. E.	G2 (ad libitum) Mean±S. E.	P
Leukocyte, 10^9/L	12.50±1.39	9.35±1.39	0.53
Haemoglobin, g/dL	10.13±0.20	11.10±0.20	0.10
Haematocrit, %	24.63±1.44	29.88±1.44	0.08
Platelet, 10^9/L	522.25±42.53	647.50±42.53	0.52
Erythrocyte, 10^{12}/L	6.87±0.40	8.55±0.40	0.06
MCV, fl	36.05±0.89	34.93±0.89	0.19
MCH, pg	15.10±1.15	13.00±0.15	0.17
PDW, fL	6.75±0.03	6.90±0.3	0.38
P-LCR, %	2.45±0.46	1.90±0.46	0.90
PCT, ng/mL	0.32±0.03	0.37±0.03	0.25

MCV: Mean red blood cell volume, MCH: Mean corpuscular haemoglobin, PDW: Platelet distribution width, P-LCR: Large platelet cell ratio, PCT: Procalcitonin, a,b Difference between the means in the same row is statistically significant ($P<0.05$)

Hematological variables such as leukocyte count, erythrocyte count, and haemoglobin concentration are crucial clinical indicators widely used to assess health and disease status (Kelada et al., 2012). Measurement of these variables helps evaluate the health and physiological conditions of calves (Roland et al., 2014). Although the haemoglobin levels were elevated in G2, they remained within the referenced range (8-15 g/dL) (Plumb, 2005). Similarly, the haematocrit concentrations of both groups were within the reference range (22-47%), as were the erythrocyte counts (5-10x1012/L). The PDW concentrations in both groups also fell within the reference range (12.00-17.50).

3.1.3. Oxidative stress and antioxidative defense mechanism

The differences in oxidative stress and antioxidative defense mechanism markers of calves at the beginning of the experiment were not found to be significant and therefore are not presented in the Table. Similarly, no significant differences were observed between the groups for these markers at the end of the experiment (*Table 5*). The effect of feeding a high amount of milk on oxidative stress and antioxidative defense mechanism was not significant. Wu et al. (2021) also reported that the effect of feeding excess milk on the oxidative stress and antioxidative defense mechanism of calves is not significant.

Table 5. The effect of restricted and ad libitum milk feeding on oxidative stress and antioxidant defense mechanism of calves at the end of the experiment

	G1 (restricted) Mean±S. E.	G2 (ad lbitum) Mean±S. E.	P
TAC, mmol/L	0.60±0.04	0.63±0.04	0.12
TOC, µmol/L	3.58±0.51	3.60±0.51	0.66
OSI	0.61±0.12	0.58±0.12	0.74
PON-1, U/L	332.33±45.47	264.00±45.47	0.38
SOD, U/mL	220.00±97.80	259.33±97.80	0.89
GPx, U/L	361.00±65.43	351.00±65.43	0.81
CAT, U/mL	36.24±3.52	38.84±3.52	0.50
MDA, mmol/L	29.04±10.27	39.80±10.27	0.07

TAC: Total antioxidative capacity, TOC: Total oxidative capacity, OSI: Oxidaitive stress index, PON-1: Paraoxanase-1 enzyme, SOD: Super oxide dismutase, GPx: Glutathione peroxidase, CAT: Catalase, MDA: Malondialdehyde

3.1.4. Immune responses

Initially, the difference in immune response between calves was not significant and thus is not shown in the table. Similarly, at the end of the experiment, there was no significant difference observed in the immune response of the calves (*Table 6*). Wu et al. (2021) also reported that feeding a high amount of milk did not affect the concentration of IgA and IgM in calves, but it significantly increased IgG concentration.

Table 6. The effect of restricted and ad libitum milk feeding on immune response of calves at the end of the experiment

	G1 (restricted) Mean±S. E.	**G2 (ad libitum) Mean±S. E.**	**P**
Ig A, mg/dL	21.53±6.76	32.80±6.76	0.16
Ig M, mg/dL	540.37±152.71	445.37±169.00	0.58
Ig G, ug/mL	245.53±22.47	213.85±22.47	0.74

4. Conclusion

This study investigated the effects of varying amounts of milk replacer during the suckling period on growth, antioxidative defense mechanism, and immune response of calves.

The amount of milk replacer given did not affect the growth of the calves. However, calves fed with restricted milk showed an expected increase in feed intake. Conversely, calves fed with excess milk demonstrated a non-significant improvement in feed efficiency.

Biochemical variables were generally unaffected by milk feeding amount, except GGT, IP, and GLU, which significantly decreased in calves fed with restricted milk. Other hematological variables, including haemoglobin, haematocrit, erythrocyte, platelet and PDW concentration, were not significantly influenced by milk amount and remained within the referenced range. However, these concentrations were notably higher in calves fed a higher amount of milk.

Milk feeding amount showed a non-significant effect on oxidative stress and antioxidative defense mechanism. Similarly, there was no significant effect on the immune response of calves.

The study's findings may have been influenced by the decrease in starter consumption alongside the increase in milk intake. It is noteworthy that there was no adverse health issues observed in the calves. However, these results might differ if the health of group-housed calves deteriorates or disease rate increases. Increased starter consumption by calves fed milk replacer twice a day may indicate hunger, underscoring the importance of timely starter feeding for calf welfare.

Acknowlegement

This project was supported by the Isparta University of Applied Sciences Scientific Research Proejcts Coordination Unit (2020-YL1-0107). We extend our gratitude to Baran Medikal Ltd (Ankara, Türkiye) for their assistance in the analysis of oxidative stress, antioxidative defense mechanism enzymes and immunoglobulins.

Ethical Statement

This study was prepared under the permission numbered 002, dated 27.05.2021, from the Ethics Committee of Isparta University of Applied Sciences.

Conflicts of Interest

We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

Concept: Özkaya, S.; Design: Özkaya, S.; Data Collection or Processing: Öztop, İ.; Statistical Analyses: Özkaya, S., Öztop, İ.; Literature Search: Öztop, I.; Writing, Review and Editing: Özkaya, S.

References

Anonymous (2011). Calf health. https://www.thecattlesite.com/articles/2612/calf-health (Accessed Date: 29.03.2024).

Anonymous (2016). Buzağı Bakım ve Beslenmesi. https://www.tarimorman.gov.tr/HAYGEM/Belgeler/Hayvancılık/Büyükbaş%20Hayvancılık/2016%20Yılı/Buzağı%20ve%20Beslenmesi.pdf (Accessed Date: 29.03.2024) (In Turkish).

AOAC (2005). Official Metehods of Analysis. 18th edn. Association of Official Analytical Chemists; Arlington, VA, USA.

Appleby, M. C., Weary, D. M. and Chua B. (2001). Performance and feding behaviour of calves on ad libitum milk from artificial teats. *Applied Animal Behaviour Science*, 74: 191-201.

Bar-Peled, U., Robinzon, B., Maltz, E., Tagari, H., Folman, Y., Bruckental, I., Voet, H., Gacitua, H. and Lehrer A. R. (1997). Increased weight gain and effects on production parameters of Holstein heifer calves that were allowed to suckle from birth to six weeks of age. *Journal of Dairy Science*, 80: 2523-2528.

Bartlett, K. S., McKeith, F. K., VandeHaar, M. J., Dahl, G. E. and Drackley, J. K. (2006). Performance and feeding behavior of calves on ad libitum milk from artificial teats. *Applied Animal Behaviour Science*, 74: 191-201.

Borderas, T. F., de Passille, A. M. B. and Rushen, J. (2009). Feeding behaviour of calves fed small or large amounts of milk. *Journal of Dairy Science*, 92: 2843-2852.

Day, M. L., Imakawa, K., Clutter, A. C., Wolfe, P. L., Zalesky, D. D., Nielsen, M. K. and Kinder, L. E. (1987). Suckling behavior of calves with dams varying in milk production. *Journal of Animal Science*, 65: 1207-1212.

de Passille, A. M. B. and Rushen, J. (2006). What components of milk stimulate sucking in calves? *Applied Animal Behaviour Science*, 101: 243-252.

de Passille, A. M. B., Borderas, T. F. and Rushen, J. (2011). Weaning age of calves fed a high milk allowance by automated feeders: Effects on feed, water, and energy intake, behavioural signs of hunger, and weight gains. Journal of Dairy Science, 94(3): 1401-1408.

de Paula Vieira, A., Guesdon, V., de Passille, A. M. B., von Keyserlingk, M. A. G. and Weary, D. M. (2008). Behavioural indicators of hunger in dairy calves. *Applied Animal Behaviour Science*, 109(2-4): 180-189.

de Paula, M., Oltramari, C., Silva, J., Gallo, M., Mourao, G. and Bittar, C. (2017). Intensive liquid feeding of dairy calves with a medium crude protein milk replacer: effects on performance, rumen, and blood parameters. *Journal of Dairy Science*, 100(6): 4448-4456.

Dennis, T., Suarez-Mena, F., Hill, T., Quigley, J., Schlotterbeck, R. and Hulbert, L. (2018). Effect of milk replacer feeding rate, age at weaning, and method of reducing milk replacer to weaning on digestion, performance, rumination, and activity in dairy calves to 4 months of age. *Journal of Dairy Science*, 101(1): 268-278.

Dennis, T., Suarez-Mena, F., Hu, W., Hill, T., Quigley, J. and Schlotterbeck, R. (2019). 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. *Journal of Dairy Science*, 102(3): 2094-2102.

Diaz, M. C., Van Amburgh, M. E., Smith, J. M., Kelsey, J. M. and Hutten, E. L. (2001). Composition of growth of Holstein calves fed milk replacer from birth to 105-kilogram body weight. *Journal of Dairy Science*, 84: 830-842.

Drackley, J. K. (2005). Early growth effect on subsequent health and performance of dairy heifers. In: Calf and Heifer Rearing: Principles of Rearing the Modern Dairy Heifer from Calf to Calving, Eds: Garnsworthy, P. C., Nottingham University Press, Nottingham, UK.

Gelsinger, S., Heinrichs, A. and Jones, C. (2016). A meta-analysis of the effects of preweaned calf nutrition and growth on first-lactation performance. *Journal of Dairy Science*, 99(8): 6206-6214.

Hartel, H., Hepola, H., Seppanen, N., Rauhala, R., Sankari, S., Soveri, T., Saloniemi, H. and Syrjala-Qvist, L. (2002). Pikkuvasikoiden hapanmaitojuotto. *Suomen Elainlaakarilehti*, 2: 84-89 (In Finnish).

Hill, T. M., Bateman, H. G., Aldrich, J. M. and Schlotterbeck, R. L. (2010). Effect of milk replacer program on digestion of nutrients in dairy calves. *Journal of Dairy Science*, 93(3): 1105-1115.

Hill, T., Quigley, J., Bateman, I. I. H., Suarez-Mena, F., Dennis, T. and Schlotterbeck, R. (2016). Effect of milk replacer program on calf performance and digestion of nutrients in dairy calves to 4 months of age. *Journal of Dairy Science*, 99(10): 8103-8110.

Huuskonen, A. and Khalili, H. (2008). Computer-controlled milk replacer feeding strategies for group-reared dairy calves. *Livestock Science*, 113(2-3): 302-306.

Jafari, A., Azarfar, A., Alugongo, G. M., Ghorbani, G. R., Mirzaei, M., Fadayifar, A., Omidi-Mirzaei, H., Cao, Z., Drackley, J. K. and Ghaffari, M. H. (2021). Milk feeding quantity and feeding frequency: effects growth performance, rumen fermentation and blood metabolites of Holstein dairy calves. *Italian Journal of Animal Science*, 20(1): 336-351.

Jasper, J. and Weary, D. M. (2002). Effects of ad libitum milk intake on dairy calves. *Journal of Dairy Science*, 85: 3054-3058.

Jensen, M. B. (2006). Computer-controlled milk feeding of group-housed calves: the effect of milk allowance and weaning type. *Journal of Dairy Science* ,89(1): 201-206.

Kelada, S. N. P., Aylor, D. L., Peck, B. C. E., Ryan, J. F., Tavarez, U., Buus, R. J., Miller, D. R., Chesler, E. J., Threadgill, D. W., Churchill, G. A., Vilhena, F. P. -M. and Collins, F. S. (2012). Genetic analysis of hematological parameters in incipient lines of the collaborative cross. *Genes, Genomes, Genetics*, 2: 157-165.

Khan, M. A., Lee, H. J., Lee, W. S., Kim, H. S., Kim, S. B., Ki, K. S., Ha, J. K., Lee, H. G. and Choi, Y. J. (2007a). Pre-and postweaning performance of Holstein female calves fed milk through step-down and conventional methods. *Journal of Dairy Science*, 90(2): 876-885.

Khan, M. A., Lee, H., Lee, W., Kim, H., Ki, K., Hur, T., Suh, G., Kang, S. and Choi, Y. (2007b). Structural growth, rumen development, and metabolic and immune responses of Holstein male calves fed milk through step-down and conventional methods. *Journal of Dairy Science*, 90(7): 3376-3387.

Khan, M., Weary, D. and Von Keyserlingk, M. (2011). Invited review: Effects of milk ration on solid feed intake, weaning, and performance in dairy heifers. *Journal of Dairy Science*, 94(3): 1071-1081.

Kmicikewycz, A., Da Silva, D., Linn, J. and Litherland, N. (2013). Effects of milk replacer grogram fed 2 or 4 times daily on nutrient intake and calf growth. *Journal of Dairy Science*, 96(2): 1125-1134.

Kozaklı, Ö., Ul Hassan, M. and Ceyhan, A. (2022). The prediction of Brody, Logistic and Von Bertalanffy models by using the Bayesian approach for modeling the growth curves in Holstein calves. *Journal of Tekirdag Agricultural Faculty*, 19(3): 600-609.

MacPherson, J., Berends, H., Leal, L., Cant, J., Martin-Tereso, J. and Steele, M. (2016). Effect of plane of milk replacer intake and age on glucose and insulin kinetics and abomasal emptying in female Holstein Friesian dairy calves fed twice daily. *Journal of Dairy Science*, 99(10): 8007-8017.

MacPherson, J., Meale, S. J., Macmillian, K., Haisan, J., Bench, C. J., Oba, M. and Steele, M. A. (2019). Effects of feeding frequency of an elevated plane of milk replacer and calf age on behaviour, and glucose and insulin kinetics in male Holstein calves. *Animal*, 13(7): 1385-1393.

Mirzaei, M., Dadkhah, N., Baghbanzadeh-Nobari, B., Agha-Tehrani, A., Eshraghi, M., Imani, M., Shiasi-Sardoabi, R. and Ghaffari, M. (2018). Effects of preweaning total plane of milk intake and weaning age on intake, growth performance, and blood metabolites of dairy calves. *Journal of Dairy Science*, 101(5): 4212-4220.

Odde, K. G., Kiracofe, G. H. and Schalles, R. R. (1985). Suckling behavior in range beef calves. *Journal of Animal Science*, 109: 222-236.

Omidi-Mirzaei, H., Khorvash, M., Ghorbani, G., Moshiri, B., Mirzaei, M., Pezeshki, A. and Ghaffari, M. (2015). Effects of the step-up/step-down and step-down milk feeding procedures on the performance, structural growth, and blood metabolites of Holstein dairy calves. *Journal of Dairy Science*, 98(11): 7975-7981.

Orellana Rivas, R., Komori, G., Beihling, V., Marins, T., Bernard, J. and Tao, S. (2020). Effects of milk replacer feding levels on performance and metabolism of preweaned dairy calves during summer. *Journal of Dairy Science*, 103(1): 313-324.

Palmquist, D., Doppenberg, J., Roehring, K. and Kinsey, D. (1992). Glucose and insulin metabolism in ruminating and veal calves fed high and low fat diets. *Domestic Animal Endocrinology*, 9(3): 233-241.

Plumb, D. C. (2005). Plumb's Veterinary Drug Handbook. 5th Edition. Wiley-Blackwell, Iowa, USA.

Quigley, J. D., Hill, T., Dennis, T., Suarez-Mena, F. and Schlotterbeck, R. (2018). Effects of feeding milk replacer at 2 rates with pelleted, low-starch or texturized, high-starch starters on calf performance and digestion. *Journal of Dairy Science*, 101(7): 5937-5948.

Quigley, J. D., Wolfe, T. A. and Elsasser, T. H. (2006). Effects of additional milk replacer feeding on calf health, growth, and selected blood metabolites in calves. *Journal of Dairy Science*, 89(1): 207-216.

Raeth-Knight, M., Chester-Jones, H., Hayes, S., Linn, J., Larson, R., Ziegler, D., Ziegler, B. and Broadwater, N. (2009). Impact of conventional or intensive milk replacer programs on Holstein heifer performance through six months of age and during first lactation. *Journal of Dairy Science*, 92(2): 799-809.

Roland, L., Drillich, M. and Iwersen, M., (2014). Hematology as a diagnostics tool in bovine medicine. *Journal of Veterinary Diagnostic Investigation*, 26: 592-598.

Rosenberger, K., Costa, J., Neave, H., Von Keyserlingk, M. and Weary, D. (2017). The effect of milk allowance on behaviour and weight gains in dairy calves. *Journal of Dairy Science*, 100(1): 504-512.

Saper, C. B., Chou, T. C. and Elmquist, J. K. (2002). The need to feed: homeostatic and hedonic control of eating. *Neuron*, 36: 199-211.

Schaff, C. T., Gruse, J., Maciej, J., Mielenz, M., Wirthgen, E., Hoeflich, A., Schmicke, M., Pfuhl, R., Jawor, P., Stefaniak, T. and Hammon, H. M. (2016). Effects of feeding milk replacer *ad libitum* or in restricted amounts for the first five weeks of life on the growth, metabolic adaptation, and immune status of newborn calves. *PloS One*, 11(12): e0168974.

Schrama, J. W., Roefs, J. P. A., Gorssen, J., Heetkamp, M. J. W. and Verstegen, M. W. A. (1995). Alternation of heat production in young calves in relation to posture. *Journal of Animal Science*, 73: 2254-2262.

Scoley, G., Ashfield, A., Romero Oiartzun, M., Gordon, A. and Morrison, S. J. (2020). Behavioural and physiological response of individually housed dairy calves to change in milk feding frequency at different ages. *Irish Journal of Agriculture and Food Research*, 59(1): 70-84.

Shamay, A., Werner, D., Moallem, U., Barash, H. and Bruckental, I. (2005). Effect of nursing management and skeletal size at weaning on puberty, skeletal growth rate, and milk production during first lactation of dairy heifers. *Journal of Dairy Science*, 88: 1460-1469.

Silper, B., Lana, A., Carvalho, A., Ferreira, C., Franzoni, A., Lima, J., Saturnino, H., Reis, R. and Coelho, S. (2014). Effects of milk replacer feding strategies on performance, ruminal development, and metabolism of dairy calves. *Journal of Dairy Science*, 97(2): 1016-1025.

Stamey, J., Kanovick, N., Kertz, A. and Drackley, J. (2012). Influence of starter protein content on growth of dairy calves in an enhanced early nutrition program. *Journal of Dairy Science*, 95(6): 3327-3336.

Stunkard, A. (1975). Satiety is a conditioned reflex. *Psychological Medicine*, 37(5): 383-387.

Terre, M., Tejero, C. and Bach, A. (2009). Long-term effects on heifer performance of an enhanced-growth feding programme applied during the preweaning period. *Journal of Dairy Science*, 76(3): 331-339.

TSE (1991). Method of the determination metabolic (converted) energy of animal feeds. Turkish Standard Institution. Standard No: 9610, Ankara, Türkiye.

Van Amburgh, M. and Drackley, J. (2005). Current perspectives on the energy and protein requirements of the pre-weaned calf. In: Calf and Heifers Rearing: Principles of Rearing the Modern Dairy Heifers from Calf to Calving. Eds: Garnsworthy, P.C., Nottingham University Press, Nottingham, UK.

van Niekerk, J., Fischer-Tlustos, A., Deikun, L., Quigley, J., Dennis, T., Suarez-Mena, F., Hill, T., Schlotterbeck, R., Guan, L. and Steele, M. (2020). Effect of amount of milk replacer fed and the processing of corn in starter on growth performance, nutrient digestibility, and rumen and fecal fibrolytic bacteria of dairy calves. *Journal of Dairy Science*, 103(3): 2186-2199.

Vi, R. B., McLeod, K., Klotz, J. and Heitmann, R. (2004). Rumen development, intestinal growth and hepatic metabolism in the pre- and postweaning ruminant. *Journal of Dairy Science*, 87: 55-65.

Wu, S., Li, X., Chen, X., Zhu, Y. and Yao, J. (2021). Optimizing the growth and immune system of dairy calves by subdividing the pre-weaning period and providing different milk volumes for each stage. *Animal Nutrition*, 7(4): 1296-1302.

Yüksek, S. and Karaçuhalılar, A. (2024). Defining of some morphological characteristics in order to determine the infrastructure of conservation strategies of indigenous cattle for designing sustainable methods and conservation of biodiversity: Eastern Anatolian Red example reared under ex-situ conditions in Türkiye. *Journal of Tekirdağ Agricultural Faculty*, 21(1): 233-245.

Yumru, M., Savaş, H.A., Kalenderoğlu, A., Bulut, M., Çelik, H. and Erel, O. (2009). Oxidative imbalance in bipolar disorder subtypes: a comparative study. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 33 6): 1070-1074.