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The effect of Bufferman on the disappearance of nutrients in the rumen bacteria and fungi culture

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ABSTRACT

Background and Objectives: In modern livestock production, the use of dietary buffers has become an essential strategy for improving animal health and productivity, especially in ruminants like sheep. The trend toward high-concentrate, low-fiber diets has been increasingly embraced to optimize growth rates and feed efficiency. However, these diets frequently foster an environment that promotes ruminal acidosis, a condition marked by the excessive buildup of organic acids and a subsequent drop in rumen pH. This study investigates the potential of Bufferman, an advanced biological buffer, to mitigate the adverse effects of acidosis in sheep fed varying ratios of concentrate and forage. Through examining the *in vitro* digestive response to various dietary treatments, this research aims to clarify the significance of Bufferman in reducing the risks associated with high-concentrate feeding regimens. Under normal conditions, the rumen maintains a pH range of about 6.0 to 7.0, which supports the microbial population crucial for effective digestion. However, the introduction of high levels of fermentable concentrates can significantly lower this pH, creating a hostile environment for beneficial ruminal microorganisms. Effective management of rumen pH through dietary modifications, especially the inclusion of buffering agents, is essential for preventing acidosis and promoting optimal rumen function. Dietary buffers, including sodium bicarbonate and specialized formulations like Bufferman, are crucial for improving rumen function by neutralizing excess acidity and stabilizing pH levels. Buffers function by elevating the rumen pH, thus creating an environment that supports the activity of cellulolytic bacteria and fungi, essential for the breakdown of fibrous feeds. Previous studies indicate that incorporating bicarbonate or other buffering agents can result in notable enhancements in rumen fermentation parameters, such as increased dry matter (DM) disappearance and nutrient absorption. The present study aimed to investigate the effects of Bufferman on the digestive performance of sheep *in vitro*, specifically examining its impact on nutrient disappearance across different ratios of forage to concentrate.

Materials and Methods: The experimental design involved a controlled mixture of alfalfa, wheat straw, barley grain, corn grain,

soybean meal, and other mineral supplements formulated to satisfy the nutritional needs of sheep. The experimental diets included various ratios of forage and concentrate (20:80, 30:70, 40:60, and 60:40), supplemented with different treatments such as control, 1% and 2% Bufferman, and 1% sodium bicarbonate. The study employed a completely randomized design in which the experimental diets underwent *in vitro* fermentation using rumen fluid sourced from sheep. The analysis concentrated on several key parameters, including DM disappearance, the rates of neutral detergent fiber (NDF) and acid detergent fiber (ADF) disappearance, rumen pH, and ammonia nitrogen levels over incubation periods of 24, 72, and 144 hours.

Results: The findings of the study revealed that the rate of DM disappearance was significantly influenced by the dietary treatments at all times points examined ($P<0.05$). Notably, diets supplemented with 1% Bufferman showed the highest rates of DM disappearance, NDF, and ADF compared to other treatments, suggesting that Bufferman enhances microbial activity and nutrient utilization. Furthermore, the results indicated that rumen pH levels were significantly affected by the type of diet, particularly after the 24- and 72-hour incubation periods ($P<0.05$), while the pH at 144 hours showed no significant changes. Despite these observations, the levels of rumen ammonia nitrogen were found to be unaffected by the treatments ($P<0.05$), indicating that Bufferman primarily serves to enhance fermentation processes rather than alter protein degradation rates. The consistent improvement in nutrient disappearance, particularly with the inclusion of Bufferman at 1%, underscores its potential as a biological regulator that not only stabilizes rumen pH but also supports overall ruminal health.

Conclusion: This study clearly demonstrates that incorporating the enhanced buffer, Bufferman, into sheep diets can significantly improve nutrient utilization and stabilize rumen pH under high-concentrate feeding conditions. The differing effects noted across the various forage-to-concentrate ratios underscore the significance of optimizing these dietary components to enhance healthy rumen function. By effectively mitigating the risks linked to acidosis, Bufferman positions itself as a viable nutritional strategy for livestock producers seeking to enhance productivity while safeguarding the health and well-being of their animals. Future research should concentrate on the long-term effects of Bufferman supplementation in practical feeding regimes, evaluating its impact on animal performance in real-world scenarios.

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تأثیر بافرمن بر ناپدیدشدن مواد مغذی در محیط کشت باکتری و قارچ‌های شکمبه

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چکیده

مقدمه و هدف: در دام‌پروری معاصر، ترکیب بافرهای غذایی به‌عنوان یک استراتژی حیاتی برای افزایش سلامت و بهره‌وری دام به‌ویژه در نشخوارکنندگانی مانند گوسفند مطرح‌شده است. گرایش به سمت جیره‌های با غلظت بالا و کم فیبر به طور فزاینده‌ای برای به حداکثر رساندن نرخ رشد و بهره‌وری خوراک اتخاذ شده است. با این حال، این رژیم‌ها اغلب محیطی مناسب برای اسیدوز شکمبه ایجاد می‌کنند، وضعیتی که با تجمع بیش‌ازحد اسیدهای آلی و در نتیجه کاهش pH شکمبه مشخص می‌شود. این مطالعه به بررسی پتانسیل بافرمن، یک بافر بیولوژیکی پیشرفته، به‌عنوان وسیله‌ای برای مقابله با اثرات نامطلوب اسیدوز در گوسفندان تغذیه‌شده با نسبت‌های مختلف کنسانتره و علوفه می‌پردازد. از طریق بررسی پاسخ گوارشی در شرایط آزمایشگاهی به درمان‌های غذایی مختلف، این تحقیق با هدف روشن کردن اهمیت بافرمن در کاهش خطرات مرتبط با رژیم‌های تغذیه با غلظت بالا است. اسیدوز شکمبه یک اختلال متابولیک ناشی از تخمیر سریع کربوهیدرات‌ها در شکمبه است که منجر به تولید بیش‌ازحد لاکتات و سایر اسیدهای آلی می‌شود. در شرایط عادی، شکمبه محدوده pH تقریباً ۶/۰ تا ۷/۰ را حفظ می‌کند که از جمعیت میکروبی ضروری برای هضم مؤثر حمایت می‌کند. بااین‌حال، معرفی سطوح بالایی از کنسانتره‌های قابل تخمیر می‌تواند این pH را به میزان قابل‌توجهی کاهش دهد و یک محیط خصمانه برای میکروارگانیسم‌های مفید شکمبه ایجاد کند. اثرات بعدی اسیدوز چندوجهی است. آن‌ها شامل اختلال در هضم مواد مغذی، کاهش مصرف خوراک و بدتر شدن سلامت حیوانات هستند که همگی منجر به کاهش بهره‌وری می‌شود؛ بنابراین، مدیریت مؤثر pH شکمبه از طریق اصلاح رژیم غذایی، به‌ویژه گنجاندن عوامل بافر، در جلوگیری از اسیدوز و ارتقاء عملکرد مطلوب شکمبه حیاتی است. بافرهای غذایی، مانند بی‌کربنات سدیم و سایر فرمولاسیون‌های تخصصی مانند بافرمن، با خنثی کردن اسیدیته اضافی و تثبیت سطوح pH، نقش اساسی در تقویت عملکرد شکمبه ایفا می‌کنند. بافرها با افزایش pH شکمبه کار می‌کنند و در نتیجه محیطی را برای فعالیت باکتری‌ها و قارچ‌های سلولولیتیک ایجاد می‌کنند که برای تجزیه خوراک‌های فیبری حیاتی هستند. مطالعات قبلی نشان داده‌اند که گنجاندن بی‌کربنات یا سایر عوامل بافر می‌تواند منجر به بهبود قابل‌توجهی در پارامترهای تخمیر شکمبه، ازجمله افزایش ناپدید شدن ماده خشک (DM) و جذب مواد مغذی شود. مطالعه حاضر باهدف بررسی اثرات بافرمن بر عملکرد گوارشی گوسفند در شرایط آزمایشگاهی،

به‌ویژه بررسی تأثیر آن بر ناپدید شدن مواد مغذی در نسبت‌های مختلف علوفه به کنسانتره انجام شد.

مواد و روش‌ها: طرح آزمایشی شامل مخلوط کنترل‌شده‌ای از یونجه، کاه گندم، دانه جو، دانه ذرت، کنجاله سویا و سایر مکمل‌های معدنی بود که برای تأمین نیازهای غذایی گوسفندان فرموله شده بود. جیره‌های آزمایشی شامل نسبت‌های مختلف علوفه و کنسانتره (۲۰:۸۰، ۳۰:۷۰، ۴۰:۶۰ و ۶۰:۴۰) همراه با تیمارهای مختلف شامل شاهد، ۱٪ و ۲٪ بافرمن و ۱٪ بی‌کربنات سدیم بود. این مطالعه از یک طرح کاملاً تصادفی استفاده کرد که در آن جیره‌های آزمایشی با استفاده از مایع شکمبه به‌دست‌آمده از گوسفند تحت تخمیر آزمایشگاهی قرار گرفتند. تجزیه‌وتحلیل بر روی چندین پارامتر کلیدی، از جمله ناپدید شدن DM، نرخ ناپدید شدن فیبر شوینده خنثی (NDF) و فیبر شوینده اسیدی (ADF)، pH شکمبه و سطوح نیتروژن آمونیاکی در طول دوره‌های انکوباسیون ۲۴، ۷۲ و ۱۴۴ ساعت متمرکز شد.

یافته‌ها: یافته‌های مطالعه نشان داد که میزان ناپدید شدن DM به‌طور معنی‌داری تحت تأثیر تیمارهای غذایی در تمام مقاطع زمانی موردبررسی قرارگرفته است (P<۰/۰۵). قابل‌توجه است که رژیم‌های مکمل با ۱٪ بافرمن بالاترین نرخ ناپدید شدن DM، NDF و ADF را در مقایسه با سایر درمان‌ها نشان می‌دهند که نشان می‌دهد بافرمن فعالیت میکروبی و استفاده از مواد مغذی را افزایش می‌دهد. علاوه بر این، نتایج نشان داد که میزان pH شکمبه به‌طور معنی‌داری تحت تأثیر نوع رژیم غذایی، به‌ویژه پس از دوره‌های انکوباسیون ۲۴ و ۷۲ ساعته بود (P<۰/۰۵)، درحالی‌که pH در ۱۴۴ ساعت تغییر معنی‌داری نشان نداد. علیرغم این مشاهدات، سطوح نیتروژن آمونیاکی شکمبه تحت تأثیر تیمارها قرار نگرفت (P<۰/۰۵) که نشان می‌دهد بافرمن در درجه اول به‌جای تغییر در نرخ تخریب پروتئین، برای تقویت فرآیندهای تخمیر عمل می‌کند. بهبود مداوم در ناپدید شدن مواد مغذی، به‌ویژه با گنجاندن بافرمن در ۱%، پتانسیل آن را به‌عنوان یک تنظیم‌کننده بیولوژیکی نشان می‌دهد که نه‌تنها pH شکمبه را تثبیت می‌کند، بلکه از سلامت کلی شکمبه نیز حمایت می‌کند.

نتیجه‌گیری: این مطالعه به‌وضوح نشان می‌دهد که ترکیب بافر تقویت‌شده، بافرمن، در جیره‌های گوسفند می‌تواند به‌طور قابل‌توجهی مصرف مواد مغذی را بهبود بخشد و pH شکمبه را در شرایط تغذیه با غلظت بالا تثبیت کند. اثرات متفاوت مشاهده‌شده در نسبت‌های مختلف علوفه به کنسانتره، اهمیت بهینه‌سازی این اجزای غذایی برای ارتقاء عملکرد سالم شکمبه را برجسته می‌کند. بافرمن با کاهش مؤثر خطرات مرتبط با اسیدوز، خود را به‌عنوان یک استراتژی تغذیه‌ای مناسب برای تولیدکنندگان دام معرفی می‌کند که با هدف افزایش بهره‌وری در عین حصول اطمینان از سلامت و رفاه دام‌هایشان انجام می‌شود. تحقیقات آینده باید بر پیامدهای درازمدت مکمل بافرمن در رژیم‌های تغذیه عملی، ارزیابی اثرات آن بر عملکرد حیوانات در محیط‌های واقعی تمرکز کند.

استناد: عالیوند، غزاله؛ محمدآبادی، طاهره. (۱۴۰۳). تأثیر بافرمن بر ناپدید شدن مواد مغذی در محیط کشت باکتری و قارچ‌های شکمبه. *پژوهش در نشخوارکنندگان*، ۱۲(۳)، ۱۵۴-۱۳۷.

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ناشر: دانشگاه علوم کشاورزی و منابع طبیعی گرگان

Introduction

Body score status and provide the required energy and protein, highly concentrated diets are used (Erickson and Kalscheur, 2020), which often reduce rumen pH and fiber digestion (Salfer *et al*., 2018) resulting in metabolic issues like low pH, elevated VFA, and increased osmolality (Davies *et al*., 2013). The high level of concentrates and fermentable seeds in the diet makes the rumen prone to acidosis (Erickson and Kalscheur, 2020). Acidosis is the result by the non-physiological accumulation of organic acids in the rumen and the subsequent decrease in rumen pH, it has significant impact on rumen microbial functions, rumen performance, and overall animal health and productivity (Elmhadi *et al.*, 2022). Since buffers have the ability to stabilize rumen pH, it will improve cellulose digestion and increase performance, leading to increased feed consumption and reduced rumen accumulation (Ramos *et al*., 2022). Therefore, adding high concentrate and low fiber to diets leads to an increase in positive response and productivity (Zali *et al*., 2019). Also, buffers prevent the reduction of protozoa in the rumen by increasing the pH and preventing acidic conditions. Dietary buffers such as bicarbonates, work to neutralize rumen acidity, raise rumen pH, and promote lactate consumption (Jafarpour Boroujeni *et al*., 2016). Higher rumen pH increases saliva production in ruminants (Castillo-Lopez *et al*., 2020). The high concentration of acids from fermentation in silage reduces palatability, it has recently been determined that neutralizing the acidity of corn silage and grass silage significantly increases their appetizing properties (Wróbel *et al*, 2023). Also, feed buffer additives help prevent the rapid decrease in rumen pH (Ramos *et al*., 2021). The aim of this study was to examine the impact of Bufferman as a biological regulator on the disappearance of nutrients by bacterial and fungi in anaerobic culture within diets with varying ratios of concentrate to fodder.

Materials and Methods

The basal ration used in the experiment and the approximate analysis them were conducted using the tables of sheep nutritional requirements (NRC, 2007). Were set as described in Table 1. Buffered compounds include: Ag=>0.5, Al=2038, Ca>10, Cd=1.3, Co=10, Cr=2, Cu=10, Fe=2709, K=7535, Mg=>2%, Mn=90, Mo=0.62, Ni=1.3 ppm.

Experimental diets include different ratios of forage to concentrate 20-80, forage to concentrate 30-70, forage to concentrate 40-60, forage to concentrate 60-40 and treatments including two levels of Bufferman 1% and 2%, one level of sodium bicarbonate 1% and one the control level (zero percent) was randomly assigned one treatment to each group of vials.

To prepare bacterial culture, salt solution 1 (6g of dipotassium hydrogen phosphate in 1L of distilled water) and salt solution 2 (3gr of potassium hydrogen phosphate, 6g of ammonium sulfate, 0.5g of magnesium sulfate, 0.6g of sodium chloride, 0.2g of calcium chloride in 1L of distilled water) were each mixed with 150ml. Additionally, 0.5 g of yeast extract, 2g of peptone triptychase, 70ml of 8% sodium carbonate, 10ml of VFA and 1ml of rososarin solution were included. The mixture was boiled until the volume reached one liter. Subsequently, a 0.1% reduction solution (cysteine HCL and 9% sodium sulfide) was added. The culture medium prepared under anaerobic conditions was transferred into serum jars containing the test sample and autoclaved. Then 5ml of pure ruminal bacterial inoculant and 1.5% sugar solution (glucose) were added to the amount of 3 ml. Finally, there were cultured in the incubator for 24, 48 and 72 hours (Kenters *et al*, 2011). Also, to prepare the fungi culture, salt solution 1 (3g of dipotassium hydrogen phosphate in 1L of distilled water) and salt solution 2 (3g of potassium hydrogen phosphate, 6g of ammonium sulfate, 6g of sodium chloride, 0.6g of chloride calcium in 1L of distilled water) 150ml each, 150ml of rumen fluid (centrifuged at 15,000 rpm for 30 min),

2.5g of yeast extract, 10g peptone trypticase, 0.5g glucose, 1g cellobiose 6g of sodium bicarbonate, 1g of cysteine HCL and 1ml of 0.1% resazurin were combined for each liter of culture medium (Ishaq *et al*, 2017). The culture medium was transferred to serum jars under anaerobic conditions and autoclaved. Fungus isolates were cultured as inoculants at a ratio of 1 to 9 in serum jars containing specific fungi culture medium along with 1g of test samples and 1ml of antibiotics. Finally, the samples were cultured in the incubator for 1, 3 and 6 days.

Table 1: Ingredients and chemical composition of the experimental diets

Items	Experimental diets (F:C)			
	20:80	30:70	40:60	60:40
Ingredient's composition, g/kg of DM[1]				
Alfalfa	0	201	325	600
Wheat straw	200	99	75	0
Barley grain	319	300	243	15
Corn	236	210	238	318
Soybean meal	182	123.5	106.5	51
Wheat bran	52	55	0	0
Salt	2	2.5	2.5	4
Limestone	4	4	4.5	5
Vitamins and minerals supplements [1]	5	5	5.5	7
Chemical composition	per 1000 g			
% of DM	90.77	90.88	90.94	90.74
CP[2]	14.7	14.5	14.4	14.2
EE[3]	2.95	2.63	2.51	2.4
CF[4]	12.89	14.5	16.42	20.53
ADF[5]	15.01	16.65	18.68	22.17
NDF [6]	30.30	31.55	32.80	35.32
Ash	4.86	5.41	5.41	6.82
ME[7]	4190	4193	4195	4198
GE[8] (kcal/kg of DM)	60.69	59.27	57.61	54.74
NFE[9] (%)	0	201	325	600

1. Each kg of mineral-vitamin supplement contains 500,000 IU vitamin A, 100,000 IU vitamin D 3, 100 mg vitamin E, 180 g Ca, 60,000 P, 60 g of Na, 19 g Mg, 3 mg of Fa, 19 g Mn, 300 mg Co, 1 mg Se, 100 mg I, 400 mg of antioxidants.

To measure the disappearance of experimental diets, the method of preparing a specific culture medium for rumen bacterial and fungi was used. Ruminal fluid was collected from the slaughterhouse and after filtering, it was transferred to the laboratory. To measure the desired parameters, 1g of the experimental diets was placed in 100 ml glass syringes.

[1]. Dry matter
[2] . Crude protein
[3] . Ether extract
[4] . Crude fiber
[5] . Acidic detergent fibers
[6] . Neutral detergent fiber
[7] . Metabolizable energy
[8] . Gross energy
[10]. Nitrogen-free extract

To determine the organic matter actually digested after the end of incubation, the syringe contents were boiled with neutral detergent solution for one hour. The solution was then filtered and the residue was transferred to the oven for drying. by subtracting the weights before and after drying, the really digested organic matter was calculated. this value was used to measure the partitioning factor (PF), microbial mass and microbial mass efficiency (Blumel *et al*., 1993). The pH of the culture medium was measured with a pH meter and the ammonia nitrogen concentration of the medium was measured by the phenol-hypochlorite method and using a spectrophotometer. The data were analyzed using a completely randomized design with SAS statistical software for model 2 and effects were considered significant at P<0.05.

$$1)\ Yij = \mu + Tj + \varepsilon ij$$

In this context, Yij represents the numerical value of each observation in this experiment, μ is the population mean, Tj represents the effect of the treatment used, and εij is the experimental error.

Results

Disappearance of nutrients in bacterial culture: As indicated in Table 2, the data on the disappearance of DM, NDF, ADF, ammonia nitrogen and rumen pH after 24 hours of incubation in a specific culture medium of rumen bacterial reveal that the disappearance of DM, NDF and ADF was influenced by the experimental diets (P<0.05). At all investigated diets (20:80, 30:70, 40:60, 60:40 F:C) were influenced by Bufferman, with the highest disappearance of NDF and ADF in the rumen associated with the treatments containing 1% Bufferman. Also, the level of ammonia nitrogen at 24 hours of incubation was not affected by the experimental diets (P<0.05). The pH of the rumen at 24 hours was affected by Bufferman (P<0.05) with the highest pH observed in the rumen for all experimental diets (20:80, 30:70, 40:60, 60:40 F:C) It was related to the treatment containing 1% level of Bufferman.

Table 2. The effect of experimental diets containing Bufferman on the disappearance of nutrients at 24 hours in bacterial culture

The ratio of fodder to concentrate	**Treatment**	Disappearance of DM (%)	Disappearance of NDF (%)	Disappearance of ADF (%)	Rumen ammonia nitrogen(mmol/l)	pH
20:80	Control	63.23^{ab}	40.59^{abc}	33.66^{ab}	21.07	5.25^{g}
	1% Bufferman	66.50^{a}	58.12^{a}	51.29^{a}	25.69	5.84^{efg}
	2% Bufferman	63.84^{ab}	53.11^{sbc}	46.39^{ab}	24.76	5.61^{fg}
	1% Sodium Bicarbonate	62.15^{ab}	52.99^{abc}	46.18^{ab}	21.71	5.61^{fg}
30:70	Control	57.33^{bc}	37.12^{bc}	29.72^{ab}	19.36	5.79^{efg}
	1% Buffermn	62.27^{ab}	56.31^{a}	49.23^{ab}	22.58	6.02^{bcdet}
	2% Bufferman	63.23^{ab}	52.17^{abc}	45.09^{ab}	21.35	5.86^{defg}
	1% Sodium Bicarbonate	59.28^{ab}	52.10^{abc}	45.44^{ab}	20.95	5.83^{efg}
40:60	Control	58.49^{abc}	40.99^{abc}	34.99^{ab}	19.09	5.89^{cdefg}
	1% Bufferman	62.34^{ab}	54.32^{ab}	47.62^{ab}	21.47	6.29^{abcd}
	2% Bufferman	61.02^{ab}	50.12^{abc}	43.43^{ab}	20.48	6.14^{abcde}
	1% Sodium Bicarbonate	61.75^{ab}	50.12^{abc}	43.11^{ab}	20.08	6.00^{bcdef}
60:40	Control	50.57^{c}	35.08^{c}	28.12^{b}	20.40	6.28^{abcd}
	1% Bufferman	56.84^{bc}	52.31^{abc}	45.29^{ab}	20.48	6.52^{a}
	2% Bufferman	56.74^{bc}	48.11^{abc}	41.18^{ab}	19.88	6.40^{ab}
	1% Sodium Bicarbonate	55.17^{bc}	48.02^{abc}	41.15^{ab}	21.07	6.30^{abc}
SEM		2.576	5.402	6.263	3.970	0.135
P_Value		0.010	0.015	0.031	0.999	0.0001

SEM: The standard error of the averages has a statistically significant difference in each row of numbers with different letters (P<0.05).

According to the findings in Table 3, the rate disappearance of DM after 48 hours of incubation in a specific culture medium for rumen bacterial increased significantly with the addition of Bufferman at a 1% level compared to other treatments (P<0.05). Additionally, the data indicates the effect of the diets tested on rumen pH and rumen ammonia nitrogen levels (P<0.05). The highest pH of the rumen is in the 20:80 F:C corresponding to the treatment containing 2% Bufferman and the rations (30:70, 40:60 and 60:40 F:C) corresponding to the treatment containing 1% Bufferman. According to the results related to the disappearance of insoluble fibers in neutral detergent and the disappearance of ADF in Table 3, no significant difference was observed between the tested treatments (P<0.05).

Table 3: The effect of experimental diets containing Bufferman on the disappearance of nutrients at 48 hours in bacterial culture

The ratio of fodder to concentrate	**Treatment**	Disappearance of DM (%)	Disappearance of NDF (%)	Disappearance of ADF (%)	Rumen ammonia nitrogen(mmol/l)	pH
20:80	Control	64.00^{ab}	42.09	35.18	20.35^{ab}	5.71^{c}
	1% Bufferman	67.09^{a}	60.12	53.21	22.08^{ab}	6.04^{abc}
	2% Bufferman	64.44^{ab}	54.13	47.11	21.58^{ab}	6.10^{abc}
	1% Sodium Bicarbonate	64.18^{ab}	53.97	46.67	27.59^{a}	6.06^{abc}
30:70	Control	60.76^{bc}	37.94	30.61	18.37^{b}	5.90^{bc}
	1% Bufferman	66.99^{a}	58.52	51.53	21.58^{ab}	6.28^{ab}
	2% Bufferman	64.00^{ab}	53.12	46.58	20.35^{ab}	6.12^{abc}
	1% Sodium Bicarbonate	63.28^{abc}	52.87	45.76	20.35^{ab}	5.99^{abc}
40:60	Control	59.67^{bc}	42.00	36.25	18.88^{b}	6.35^{ab}
	1% Bufferman	65.27^{ab}	55.52	48.66	20.28^{ab}	6.45^{a}
	2% Bufferman	62.41^{abc}	51.12	44.60	20.07^{ab}	6.35^{ab}
	1% Sodium Bicarbonate	61.87^{abc}	50.88	44.00	19.81^{ab}	6.34^{ab}
60:40	Control	52.57^{d}	35.96	29.09	18.49^{b}	6.21^{ab}
	1% Bufferman	62.88^{abc}	53.52	46.53	20.08^{ab}	6.50^{a}
	2% Bufferman	60.18^{bc}	49.12	42.24	19.68^{ab}	6.30^{ab}
	1% Sodium Bicarbonate	57.98^{c}	48.87	41.87	18.88^{b}	6.23^{ab}
SEM		1.767	6.313	7.212	2.481	0.148
P_Value		0.0001	0.299	0.493	0.709	0.037

SEM: The standard error of the averages has a statistically significant difference in each row of numbers with different letters (P<0.05).

By examining the results concerning the specific culture medium of rumen bacterial, it is evident that the rate of DM disappearance was significantly influenced by the test diets in all three in investigated times (P<0.05). According to the results of Table 4, it is evident that the experimental rations were influenced by Bufferman. in all the experimental rations (20:80, 30:70, 40:60, 60:40 F:C) the highest level of DM disappearance associated with the Bufferman treatment was 1%. Additionally, according to the information in Table 4, it is apparent that the disappearance rate of insoluble fibers in neutral detergent and acidic detergent after 72 hours of incubation in the specific culture medium of rumen bacterial was not significantly

impacted by the tested treatments (P<0.05). Also, based on the findings of this table, it is evident that the levels of ammonia nitrogen and rumen pH were not significantly impacted by the treatments tested (P<0.05). Upon analyzing the results of the specific culture medium of rumen bacterial, it can be inferred that rumen pH at 24 and 48 hours of incubation were significantly influenced by the experimental diets (P<0.05), whereas rumen pH at 72 hours of incubation was significantly affected by the diets (P<0.05). The concentration of rumen ammonia nitrogen, on the whole, remained unaffected by the experimental diets at any of the times examined (P<0.05).

Table 4: The effect of experimental diets containing Bufferman on the disappearance of nutrients at 72 hours in bacterial culture

The ratio of fodder to concentrate	**Treatment**	Disappearance of DM (%)	Disappearance of NDF (%)	Disappearance of ADF (%)	Rumen ammonia nitrogen(mmol/l)	pH
20:80	Control	64.52^{abc}	43.09	36.22	19.36	5.96
	1% Bufferman	71.34^{a}	61.95	54.89	27.68	6.30
	2% Bufferman	68.60^{ab}	55.12	48.19	20.59	6.21
	1% Sodium Bicarbonate	66.04^{abc}	55.00	48.07	20.35	6.18
30:70	Control	67.07^{abc}	39.22	31.84	17.38	5.96
	1% Bufferman	69.70^{ab}	59.95	52.56	18.61	6.39
	2% Bufferman	64.52^{abc}	54.07	47.36	17.97	6.04
	1% Sodium Bicarbonate	65.59^{abc}	54.57	46.96	17.38	6.03
40:60	Control	61.05^{bc}	43.90	37.90	17.69	6.10
	1% Bufferman	67.24^{abc}	57.95	50.60	20.07	6.49
	2% Bufferman	64.53^{abc}	52.07	45.25	19.08	6.38
	1% Sodium Bicarbonate	62.33^{bc}	51.57	44.57	18.69	6.35
60:40	Control	52.61^{d}	37.24	29.95	15.31	6.11
	1% Bufferman	65.10^{abc}	55.95	48.89	19.09	6.37
	2% Bufferman	61.65^{bc}	51.07	43.09	18.69	6.25
	1% Sodium Bicarbonate	58.77^{cd}	49.57	42.57	16.67	6.19
SEM		2.649	6.940	8.008	3.931	0.157
P_Value		0.002	0.409	0.622	0.945	3.384

SEM: The standard error of the averages has a statistically significant difference in each row of numbers with different letters (P<0.05).

A buffer is defined as a weak acid, oxide or hydroxide that neutralizes the acids in feed or those acids produced during digestion and metabolism of nutrients. Edible buffers are commonly utilized to mitigate the adverse effects of acidity in diets containing high-density materials (Mahdavirad *et al.*, 2021), however the response to buffers can be inconsistent and unpredictable. The conditions of the rumen ecosystem vary among different animals, and the types, and quantities of bacterial present in the rumen environment differ depending on the species and breed of the animals. Consentini *et al.* (2023) reported that there is a direct relationship between

Rumen pH is one of the most important factors in determining feed disappearance in ruminants. A decrease to less than 5.8 limits cellulolytic activity in the rumen and reduces fiber disappearance (Tseu *et al.,* 2022). In this experiment, the pH reported affects the disappearance of fiber-degrading microorganisms of the cell wall. One factor contributing to improved feed disappearance is the maintenance of optimal pH levels by buffers, which support the growth and activity of fiber-decomposing microbes (Aschenbach *et al*., 2019). Magnesium oxide prevents the pH drops by alkalizing the rumen environment, leading to an increase in the activity of cellulolytic bacterial (Underwood *et al.,* 2015). Effective degradability decreases as the passage rate increases because higher passage rates reduce the microorganisms access to nutrients (Firkins., 2021).

the amount of non-fiber carbohydrates in the diet and acid production, indicating that as non-fiber carbohydrates increase, the acidogenic value of the diet also increases. Their experiments suggest that ration buffers could help prevent a decrease in rumen pH. In an experiment conducted by Alhidary *et al.* (2019), it was found that adding various buffers to the ration led to a significant difference in rumen pH, with no significant variance observed between the buffers. Giger-Reverdin *et al*. (2014) reported that feed buffers helped maintain rumen pH and enhanced rumen ecology when feeding high levels of concentrates. The inclusion of sodium bicarbonate increased NDF disappearance but didn't affect DM disappearance in dairy cows consuming diets with high dense material and corn silage (Miller *et al.,* 2021). Niepes *et al*. (2023) also reported that dietary supplementation with sodium bicarbonate had no effect on rumen, post-ruminal or apparent NDF disappearance.

Table 5: The effect of experimental diets containing Bufferman on the disappearance of nutrients at 24 hours in fungi culture

The ratio of fodder to concentrate	**Treatment**	Disappearance of DM (%)	Disappearance of NDF (%)	Disappearance of ADF (%)	Rumen ammonia nitrogen(mmol/l)	pH
20:80	Control	58.13^{cd}	41.09^{bcde}	37.76	21.13	5.54^{g}
	1% Bufferman	65.09^{a}	58.63^{a}	52.97	23.77	5.85^{efg}
	2% Bufferman	61.78^{abc}	53.62^{abcd}	47.19	22.64	5.63^{fg}
	1% Sodium Bicarbonate	58.49^{bcd}	53.50^{abcd}	47.53	23.04	5.63^{fg}
30:70	Control	57.32^{cd}	37.59^{de}	30.85	19.42	5.80^{efg}
	1% Bufferman	64.15^{ab}	56.82^{ab}	50.03	23.64	6.04^{bcdef}
	2% Bufferman	62.55^{abc}	52.61^{abcd}	45.89	23.16	5.88^{defg}
	1% Sodium Bicarbonate	57.28^{cd}	52.51^{abcd}	46.78	21.01	5.84^{efg}
40:60	Control	56.84^{cd}	40.49^{cde}	35.99	19.14	5.91^{cdefg}
	1% Bufferman	59.47^{abcd}	54.81^{abc}	48.40	21.53	6.31^{abcd}
	2% Bufferman	57.28^{cd}	50.62^{abcde}	44.24	50.54	6.16^{abcde}
	1% Sodium Bicarbonate	59.63^{abcd}	50.51^{abcde}	44.46	20.14	6.02^{bcdef}
60:40	Control	54.07^{d}	35.58^{e}	29.22	20.50	6.30^{abcd}
	1% Bufferman	59.63^{abcd}	52.82^{abcd}	46.07	19.03	6.42^{a}
	2% Bufferman	56.74^{cd}	48.61^{abcde}	41.98	19.94	6.42^{ab}
	1% Sodium Bicarbonate	57.32^{cd}	48.52^{abcde}	42.49	19.15	6.32^{abc}
SEM		1.790	4.502	6.286	3.703	0.135
P_Value		0.004	0.044	0.332	0.992	0.0001

SEM: The standard error of the averages has a statistically significant difference in each row of numbers with different letters (P<0.05).

Disappearance of nutrients in fungi culture

As indicated in Table 5, the data on the disappearance of DM, NDF and ADF after 24 hours of incubation in the rumen fungi culture medium reveal that the disappearance rates were influenced by the experimental diets (P<0.05). And the highest rate of DM disappearance in diets (20:80, 30:70 and 60:40 F:C) was observed in the 1% Bufferman treatment, as well as in the diet with a 40:60 F:C in the Sodium Bicarbonate treatment. Also, the rate of disappearance of insoluble fibers in rumen neutral detergent and rumen pH in all the diets studied (20:80, 30:70, 40:60, 60:40 F:C) were influenced by Bufferman, with the highest impact observed in diets containing 1% bufferman. According to the information in Table 5, the rate of disappearance of insoluble fibers in acidic detergent and ammonia nitrogen after 24 hours of incubation in the specific rumen fungi culture medium was not significantly altered the treatments tested (P<0.05).

The results related to the rate of DM disappearance after 72 hours of incubation in the specific fungi culture medium are presented in Table 6. Across all tested rations (20:80, 30:70, 40:60, 60:40 F:C), the treatments with 1% Bufferman showed the highest level of DM disappearance (P<0.05). Additionally, the data obtained show the effect of the tested diets on rumen pH (P<0.05). The highest rumen pH was observed in the diet with a 20:80 F:C ratio in the group with 2% Bufferman, while the other diets (30:70, 40:60 and 60:40 F:C) showed a similar pH level to the group with1% Bufferman. Analysis of the disappearance of insoluble fibers in neutral detergent, disappearance of insoluble fibers in acidic detergent and ammonia nitrogen in Table 6, observed no significant differences among the tested treatments (P<0.05).

Table 6: The effect of experimental diets containing Bufferman on the disappearance of nutrients at 72 hours in fungi culture

The ratio of fodder to concentrate	Treatment	Disappearance of DM (%)	Disappearance of NDF (%)	Disappearance of ADF (%)	Rumen ammonia nitrogen(mmol/l)	pH
20:80	Control	63.23^{bc}	43.34	36.52	20.41	5.74^{c}
	1% Bufferman	69.55^{a}	61.37	54.31	22.14	6.06^{abc}
	2% Bufferman	65.02^{abc}	55.37	47.91	21.64	6.12^{abc}
	1% Sodium Bicarbonate	65.37^{abc}	55.22	47.47	21.31	6.09^{abc}
30:70	Control	61.56^{bc}	39.44	32.07	18.43	5.92^{bc}
	1% Bufferman	67.45^{ab}	59.77	52.63	21.65	6.30^{ab}
	2% Bufferman	64.80^{abc}	54.37	47.38	20.42	6.14^{abc}
	1% Sodium Bicarbonate	64.93^{abc}	54.12	46.56	20.41	6.01^{abc}
40:60	Control	61.46^{bc}	42.50	37.25	18.95	6.37^{ab}
	1% Bufferman	66.23^{ab}	56.77	49.76	20.34	6.47^{a}
	2% Bufferman	64.94^{abc}	52.37	45.40	20.14	6.37^{ab}
	1% Sodium Bicarbonate	63.23^{bc}	52.12	44.80	19.94	6.36^{ab}
60:40	Control	59.38^{c}	37.21	30.43	18.55	6.23^{ab}
	1% Bufferman	64.75^{abc}	54.77	47.63	20.14	6.52^{a}
	2% Bufferman	62.80^{bc}	50.37	43.04	19.74	6.32^{ab}
	1% Sodium Bicarbonate	61.56^{bc}	50.12	42.67	18.95	6.24^{ab}
SEM		1.811	5.613	7.253	2.383	0.148
P_Value		0.042	0.165	0.532	0.998	0.037

SEM: The standard error of the averages has a statistically significant difference in each row of numbers with different letters (P<0.05).

With the overall evaluation of the results related to the test of the specific culture medium of rumen fungi, it is evident that DM disappearance was notably influenced by the test diets at the 24th and 72nd incubation times, (P<0.05), as indicated in Table 7. The rate of DM disappearance at 144 hours of incubation was not influenced by the experimental diets (P<0.05). Additionally, based on the data in Table 7, the degradation rate of insoluble fibers in neutral detergent and acidic detergent after 144 hours of incubation in the rumen fungi culture medium was not significantly influenced by the experimental treatments (P<0.05). In general, the rate of disappearance of insoluble fibers in acidic detergent was not influenced by experimental diets at any of the examined times in the specific culture medium of rumen fungi (P<0.05). The rate of disappearance of insoluble fibers in neutral detergent at the 24th incubation time was significantly impacted by the test diets (P<0.05), but at the 72nd and 144th time, it was not significantly affected by the tested diets (P<0.05). According to the findings in Table 7, it is evident that the levels of ammonia nitrogen and rumen pH were not significantly impacted by the treatments tested (P<0.05). Furthermore, analysis of the specific culture medium of rumen fungi, indicates that the pH of the rumen after 24 and 72 hours of incubation was significantly influenced by the experimental diets (P<0.05), whereas the rumen pH at 144 hours of showed no significant impact. The experimental diets didn't have a significant effect on the levels of rumen ammonia nitrogen at any of the time points examined (P<0.05).

Table 7: The effect of experimental diets containing Bufferman on the disappearance of nutrients at 144 hours in fungi culture

The ratio of fodder to concentrate	Treatment	Disappearance of DM (%)	Disappearance of NDF (%)	Disappearance of ADF (%)	Rumen ammonia nitrogen(mmol/l)	pH
20:80	Control	67.21	44.34	37.02	17.17	5.98
	1% Bufferman	76.74	63.19	56.23	22.03	6.32
	2% Bufferman	71.55	56.37	49.29	20.65	6.24
	1% Sodium Bicarbonate	69.87	56.25	48.87	20.42	6.20
30:70	Control	65.31	4.72	32.58	16.19	5.98
	1% Bufferman	71.64	61.19	53.90	20.65	6.42
	2% Bufferman	67.33	55.32	48.46	19.02	6.06
	1% Sodium Bicarbonate	66.18	54.82	47.76	16.67	6.04
40:60	Control	63.07	44.40	38.90	19.01	6.12
	1% Bufferman	68.27	59.20	51.94	19.39	6.51
	2% Bufferman	66.18	53.32	46.35	17.40	6.40
	1% Sodium Bicarbonate	63.44	52.82	45.37	18.75	6.38
60:40	Control	62.92	38.49	30.75	15.61	6.14
	1% Bufferman	66.26	57.20	50.23	19.15	6.39
	2% Bufferman	63.10	51.32	44.19	18.50	6.27
	1% Sodium Bicarbonate	62.06	50.82	43.37	17.76	6.21
SEM		3.689	6.221	7.986	3.814	0.157
P_Value		0.336	0.529	0.583	0.998	0.384

SEM: The standard error of the averages has a statistically significant difference in each row of numbers with different letters ($P<0.05$).

Since ammonia nitrogen is the main substrate source for microbial protein synthesis, the addition of buffer to the diets in this study didn't result in an increase in the concentration of ammonia nitrogen in the treatments. Therefore, the addition of thesis buffer mixtures to each of treatments didn't enhance microbial protein synthesis. Although some studies reported that sodium bicarbonate can increase the rate of ammonia utilization by rumen bacterial (Mahdavirad *et al*., 2021), other researchers conducting experiments on sheep (Khorasani *et al.*, 2021) and cattle (Khorasani *et al*., 2001) have found no significant effect of sodium bicarbonate on rumen ammonia levels. A study by Khorasani *et al*. (2020) indicated that dietary sodium bicarbonate supplementation decreased rumen ammonia nitrogen concentrations. They also reported that increasing the percentage of sodium bicarbonate in the diet led to a reduction in ammonia nitrogen concentration in the rumen. While some studies reported no impact of sodium bicarbonate on ammonia nitrogen levels (Sony *et al*., 2021), others reported either an increase (Khorasani *et al*, 2020) or a decrease (Farghaly *et al*., 2019) in ammonia nitrogen concentration. However, additional research (Kang *et al*., 2013) reported that adding sodium bicarbonate to the diet increased the efficiency of microbial protein synthesis. Effective degradability decreases as the passage rate increases because the opportunity for microorganisms to access nutrients decreases (Maheri-Sis *et al*.,

2007). In numerous studies by Erdman *et al*. (1982), Rogers *et al*. (1982), Rogers *et al*. (1983), Solorzanom *et al*. (1989), Stokes *et al.* (1985), West *et al*. (1987) and Abbas *et al.* (2019) sodium bicarbonate has shown the ability to enhance DM disappearance, which is consistent with the results of this experiment or has no effect on it. In ruminants, there is a correlation between DM intake and food digestibility (Al-Arif *et al.,* 2017). The increase in food disappearance is mainly linked to the enhancement of fiber and starch digestion, with fiber digestion being contingent on rumen pH fluctuations (Kim *et al*., 2022). The most appropriate rumen pH for fiber digestion is 6.4 to 6.8 (Li Meng *et al*., 2020). Therefore, increasing or stabilizing the rumen pH by adding sodium bicarbonate to the diet is the main factor in increasing the disappearance of fiber in the diet (Fadaee *et al.*, 2021) and consequently, it increases the digestion of food. In this test, buffer was successful in enhancing the digestibility of DM. Sodium bicarbonate, potassium bicarbonate, magnesium oxide, zeolite and most medicinal plants showed significant effects in maintaining pH, DM disappeared in external conditions and controlling the acidification index. By increasing the ratio of concentrate from 50% to 65% in the diet, the effects of buffers on pH maintaining and other external parameters became more pronounced and efficient. In essence, it is advisable to incorporate buffers in to diets containing over 60% concentrate. (Desrosiers *et al*., 2022). In other in vitro experiments, it was reported that barley digestibility increased in diets with sodium bicarbonate (Ma *et al*., 2022). Additionally, *in vivo* research has demonstrated that rumen pH was higher in lactating cows fed sodium bicarbonate and clinoptilolite (Amanzoungarene *et al*., 2022). Some studies indicated that adding sodium bicarbonate to the diets of dairy cows fed high-density feed and corn silage increased NDF disappearance but had no impact on DM disappearance (Miller *et al*., 2021). Ferraretto *et al*., (2012) also reported that adding sodium bicarbonate to the diet didn't impact ruminal, post-ruminal or apparent NDF disappearance. Livestock that use highly fermentable starch show significant fluctuations in rumen VFA concentrations and rumen pH. This decreased rumen pH may restrict microbial growth and fiber digestion, ultimately has a negative effect on the amount of microbial protein entering the small intestine (Firkins *et al*., 2021), consequently leading to an increase in ammonia production within the rumen. Which isn't used in the production of microbial protein, and this causes an increase in nitrogen losses in the rumen (Hailemariam *et al*., 2021), liver stress (Bach *et al.,* 2005), reduced fertility (Javaid *et al*., 2011) and a decrease in milk protein (Sultan *et al.,* 2009). A common strategy to decrease negative energy balance in early parturition is to increase the energy density of the diet by increasing the amount of easily digestible carbohydrates instead of forage (Erickson *et al*., 2020). The most important factors influencing protein digestibility in the rumen are: the physical and chemical properties of protein, the duration of sample retention in the rumen, the concentration, the rumen pH, and the food processing method. (Matthews *et al*., 2019). At most levels, essential oils have no effect on the concentration of ammonia nitrogen *in vitro* (Zhou *et al*., 2020). Kirwan *et al*., (2022) suggested that a high intake of protein (resulting in an increase in rumen ammonia nitrogen concentration) along with relatively low levels of soluble carbohydrates in the diet and significant amounts of forage ash may account for the stabilization of pH levels when consuming high concentrates. It prevents the reduction of rumen pH by destroying protein or non-protein nitrogen (Wang *et al*., 2018).

Conclusion

By reviewing the results of rumen bacterial and fungi culture, it was discovered that the use of 1% Bufferman significantly increased nutrient disappearance, DM intake, and rumen pH. This enhancement may be attributed to the established function of buffers in regulating and stabilizing rumen pH. The buffer has the potential to stabilize rumen pH and prevent acidosis in animals with high concentrate consumption.

Références

Abbas, G., Ahmad, F., Saeed, M., Ayasan, T., Mahmood, A., Yasmeen, R. & Kamboh, A. (2019). Effect of dietary inclusion of sodium bicarbonate on digestibility of nutrients and immune response in caged layers during the summer. *Brazilian Journal of Poultry Science*, 21, eRBCA-2018.

Al-Arif, M. A., Suwanti, L. T., Estoepangestie, A. S. & Lamid, M. (2017). The nutrients contents, DM digestibility, organic matter digestibility, total digestible nutrient, and NH3 rumen production of three kinds of cattle feeding models. *KnE Life Sciences*, 338-343.

Alhidary, I. A., Abdelrahman, M. M. & Elsabagh, M. A. (2019). comparative study of four rumen buffering agents on productive performance, rumen fermentation and meat quality in growing lambs fed a total mixed ration. *Animal*. Oct;13(10):2252-2259. doi: 10.1017/S1751731119000296. Epub 2019 Mar 1. PMID: 30819265.

Amanzougarene, Z. & Fondevila, M. (2022). Rumen Fermentation of Feed Mixtures Supplemented with Clay Minerals in a Semicontinuous *in Vitro* System. *Animals*, 12(3): 345.

Aschenbach, J. R., Zebeli, Q., Patra, A. K., Greco, G., Amasheh, S. & Penner, G. B. (2019). Symposium review: The importance of the ruminal epithelial barrier for a healthy and productive cow. *Journal of Dairy Science*, 102(2): 1866-1882.

Bach, A., Calsamiglia, S. & Stern, M. D. (2005). Nitrogen metabolism in the rumen. *Journal of Dairy Science*, Apr 1(88): E9-21.

Blu, M. & Ørskov, E. R. (1993). Comparison of *in vitro* gas production and nylon bag degradability of roughages in predicting feed intake in cattle. *Animal Feed Science and Technology*, 40(2-3): 109-119.

Castillo-Lopez, E., Petri, R. M., Ricci, S., Rivera-Chacon, R., Sener-Aydemir, A., Sharma, S. ... & Zebeli, Q. (2021). Dynamic changes in salivation, salivary composition, and rumen fermentation associated with duration of high-grain feeding in cows. *Journal of Dairy Science*, 104(4): 4875-4892.

Consentini, C. E., Souza, A. H., Sartori, R., Carvalho, P. D., Shaver, R. & Wiltbank, M. C. (2023). Relationships among total mixed ration nutritional components and reproductive performance in high-producing dairy herds. JDS Communications.

Davies, K. L., McKinnon, J. J. & Mutsvangwa, T. (2013). Effects of dietary ruminally degradable starch and ruminally degradable protein levels on urea recycling, microbial protein production, nitrogen balance, and duodenal nutrient flow in beef heifers fed low crude protein diets. *Canadian Journal of Animal Science*, 93(1): 123-136.

Desrosiers, A., Derbali, R. M., Hassine, S., Berdugo, J., Long, V., Lauzon, D. & Vallée-Bélisle, A. (2022). Programmable self-regulated molecular buffers for precise sustained drug delivery. *Nature Communications*, 13(1): 6504.

Elmhadi, M. E., Ali, D. K., Khogali, M. K. & Wang, H. (2022). Subacute ruminal acidosis in dairy herds: Microbiological and nutritional causes, consequences, and prevention strategies. *Animal Nutrition*, 10: 148-155.

Erdman, R. A., Hemken, R. W. & Bull, L. S. (1982). Dietary sodium bicarbonate and magnesium oxide for early postpartum lactating dairy cows: effects of production, acid-based metabolism, and digestion. *Journal of Dairy Science*, 65(5): 712-731.

Erickson, P. S. & Kalscheur, K. F. (2020). Nutrition and feeding of dairy cattle. *Animal Agriculture*.

Fadaee, S., Danesh Mesgaran, M. & Vakili, A. (2021). *In vitro* Effect of the Inorganic Buffers in the Diets of Holstein Dairy Cow Varying in Forage: Concentrate Ratios on the Rumen Acid Load and Methane Emission. *Iranian Journal of Applied Animal Science*, 11(3): 485-496.

Farghaly, M. M., Hassan, E. H. & Abdo, S. G. (2019). Influence of sodium bicarbonate supplementation on nutrients digestibility, milk production, rumen fermentation and some blood parameters in sheep. *Egyptian Journal of Animal Production*, 56(2): 71-77.

Ferraretto, L. F., Shaver, R. D. & Bertics, S. J. (2012). Effect of dietary supplementation with live-cell yeast at two dosages on lactation performance, ruminal fermentation, and total-tract nutrient digestibility in dairy cows. *Journal of Dairy Science*, 95(7): 4017-4028.

Firkins, J. L. (2021). Invited Review: Advances in rumen efficiency. *Applied Animal Science*, 37(4): 388-403.

Giger-Reverdin, S., Rigalma, K., Desnoyers, M., Sauvant, D. & Duvaux-Ponter, C. (2014). Effect of concentrate level on feeding behavior and rumen and blood parameters in dairy goats: Relationships between behavioral and physiological parameters and effect of between-animal variability. *Journal of Dairy Science*, 97(7): 4367-4378.

Hailemariam, S., Zhao, S., He, Y. & Wang, J. (2021). Urea transport and hydrolysis in the rumen: A review. *Animal Nutrition*, 7(4): 989-996.

Ishaq, S. L., AlZahal, O., Walker, N. & McBride, B. (2017). An investigation into rumen fungal and protozoal diversity in three rumen fractions, during high-fiber or grain-induced sub-acute ruminal acidosis conditions, with or without active dry yeast supplementation. *Frontiers in Microbiology*, 8: 1943.

Jafarpour Boroujeni, M., Danesh Mesgaran, M., Vakili, A. R. & Naserian, A. A. (2016). *In vitro* ruminal acid load and methane emission responses to supplemented lactating dairy cow diets with inorganic compounds varying in buffering capacities. *Iranian Journal of Applied Animal Science*, 6(4): 769-775.

Javaid, A., Shahzad, M. A., Nisa, M. & Sarwar, M. (2011). Ruminal dynamics of ad libitum feeding in buffalo bulls receiving different level of rumen degradable protein. *Livestock Science*, 135(1): 98-102.

Kang, S. & Wanapat, M. (2013). Using plant source as a buffering agent to manipulating rumen fermentation in an *in vitro* gas production system. *Asian-Australasian Journal of Animal Sciences*, 26(10): 1424.

Kenters, N., Henderson, G., Jeyanathan, J., Kittelmann, S. & Janssen, P. H. (2011). Isolation of previously uncultured rumen bacteria by dilution to extinction using a new liquid culture medium. *Journal of Microbiological Methods*, 84(1): 52-60.

Khorasani, G. R. & Kennelly, J. J. (2001). Influence of carbohydrate source and buffer on rumen fermentation characteristics, milk yield, and milk composition in late-lactation Holstein cows. *Journal of Dairy Science*, 84(7): 1707-1716.

Khorasani, O., Chaji, M. & Baghban, F. (2020). Comparison of the effect of sodium bicarbonate buffer with *Megasphaera elsdenii* as a rumen-consuming acid on growth performance, digestibility, rumen and blood parameters of lambs in high concentrate. *Journal of Animal Science Research*, 30(2): 85-99.

Khorasani, O., Chaji, M. & Baghban, F. (2021). Effect of chemical buffer and Megasphaera elsdenii-yeast on histomorphometry and histopathology of rumen and liver of Arabian fattening lambs fed with concentrated diets. *Animal Production*, 23(1): 47-59.

Kim, S. H. & Sung, H. G. (2022). Effects of Different Fiber Substrates on *in Vitro* Rumen Fermentation Characteristics and Rumen Microbial Community in Korean Native Goats and Hanwoo Steers. *Fermentation*, 8(11): 611.

Kirwan, S. F., Pierce, K. M., Serra, E., Gath, V., Rajauria, G. & Boland, T. M. (2022). Effect of supplementing grass silage-based diets with concentrate carbohydrate sources with different fermentation profiles on N metabolism of beef heifers fed to Maintenance. *Ruminants*, 2(2): 188-200.

Li Meng. M. & Hanigan, M. D. (2020). A revised representation of ruminal pH and digestive reparameterization of the Molly cow model. *Journal of Dairy Science*, 103(12): 11285-11299.

Ma, X., Zhou, W., Guo, T., Li, F., Li, F., Ran, T. & Guo, L. (2022). Effects of dietary barley starch contents on the performance, nutrient digestion, rumen fermentation, and bacterial community of fattening Hu sheep. *Frontiers in Nutrition*, 8: 1302.

Mahdavirad, N., Chaji, M., Bojarpour, M. & Dehghanbanadaky, M. (2021). Comparison of the effect of sodium bicarbonate, sodium sesquicarbonate, and zeolite as rumen buffers on apparent digestibility, growth performance, and rumen fermentation parameters of Arabi lambs. *Tropical Animal Health and Production*, 53(5): 465.

Maheri-Sis, N., Safaei, A. R., Mirzaei-Aghsaghali, A., Mirza-Aghazadeh, A. & Dastoori, M. R. (2007). Use of *in vitro* gas production technique to compare nutritive value of quackgrass and alfalfa for ruminants. *Journal of Animal and Veterinary Advances*, 6(12): 1351-1356.

Matthews, C., Crispie, F., Lewis, E., Reid, M., O'Toole, P. W. & Cotter, P. D. (2019). The rumen microbiome: a crucial consideration when optimising milk and meat production and nitrogen utilisation efficiency. *Gut Microbes*, 10(2): 115-132.

Miller, M. D., Kokko, C., Ballard, C. S., Dann, H. M., Fustini, M., Palmonari, A. & Grant, R. J. (2021). Influence of fiber degradability of corn silage in diets with lower and higher fiber content on lactational performance, nutrient digestibility, and ruminal characteristics in lactating Holstein cows. *Journal of Dairy Science*, 104(2): 1728-1743.

Niepes, R. A. & Bestil, L. C. (2023). Nutrient digestibility of fibrous feedstuffs in high-concentrate diet with sodium-bicarbonate ($NaHco_3$) addition in rumen-fistulated brahman bull. *Online Journal of Animal and Feed Research*, 13(4): 234-241.

Ramos, S. C., Jeong, C. D., Mamuad, L. L., Kim, S. H., Son, A. R., Miguel, M. A. & Lee, S. S. (2021). Enhanced ruminal fermentation parameters and altered rumen bacterial community composition by formulated rumen buffer agents fed to dairy cows with a high-concentrate diet. *Agriculture*, 11(6): 554.

Ramos, S. C., Kim, S. H., Jeong, C. D., Mamuad, L. L., Son, A. R., Kang, S. H. & Lee, S. S. (2022). Increasing buffering capacity enhances rumen fermentation characteristics and alters rumen microbiota composition of high-concentrate fed Hanwoo steers. *Scientific Reports*, 12(1): 20739.

Rogers, J. A. & Davis, C. L. (1982). Effects of intraruminal infusions of mineral salts on volatile fatty acid production in steers fed high-grain and high-roughage diets. *Journal of Dairy Science*, 65(6): 953-962.

Salfer, I. J., Morelli, M. C., Ying, Y., Allen, M. S. & Harvatine, K. J. (2018). The effects of source and concentration of dietary fiber, starch, and fatty acids on the daily patterns of feed intake, rumination, and rumen pH in dairy cows. *Journal of Dairy Science*, 101(12): 10911-10921.

Solorzano, L. C., Armentano, L. E., Grummer, R. R. & Dentine, M. R. (1989). Effects of sodium bicarbonate or sodium sesquicarbonate on lactating Holsteins fed a high grain diet. *Journal of Dairy Science*, 72(2): 453-461.

Sony, C., Ramos, S. C, Jeong, C. D., Mamuad, L. L., Kim, S.H., Son, A.R., Miguel, M.A., Islam, M., Cho, Y. I. & Lee, S. S. (2021). Enhanced ruminal fermentation parameters and altered rumen bacterial community composition by formulated rumen buffer agents fed to dairy cows with a high-concentrate diet. *Agriculture*, Jun 17; 11(6):554.

Stokes, M. R., Vandemark, L. L. & Bull, L. S. (1986). Effects of sodium bicarbonate, magnesium oxide, and a commercial buffer mixture in early lactation cows fed hay crop silage. *Journal of Dairy Science*, 69(6): 1595-1603.

Sultan, J. I., Javaid, A., Nadeem, M., Akhtar, M. Z. & Mustafa, M. I. (2009). Effect of varying ruminally degradable to ruminally undegradable protein ratio on nutrient intake, digestibility and N metabolism in Nili Ravi buffalo calves (*Bubalus bubalis*). *Livestock Science*, 122(2-3): 130-133.

Tseu, R. J., Paucar, L. L. C., Perna Junior, F., Carvalho, R. F., Nogueira, R. G. S., Cassiano, E. C. O. & Rodrigues, P. H. M. (2022). Effect of exogenous enzymes on nutrient digestibility and ruminal fermentation of Holstein cows. *Iranian Journal of Applied Animal Science*, 12(3): 447-459.

Underwood, W. J., Blauwiekel, R., Delano, M. L., Gillesby, R., Mischler, S. A. & Schoell, A. (2015). Biology and diseases of ruminants (sheep, goats, and cattle). In Laboratory animal medicine (pp. 623-694). Academic Press.

Wang, R., Wang, M., Ungerfeld, E. M., Zhang, X. M., Long, D. L., Mao, H. X. & Tan, Z. L. (2018). Nitrate improves ammonia incorporation into rumen microbial protein in lactating dairy cows fed a low-protein diet. *Journal of Dairy Science*, 101(11): 9789-9799.

West, J. W., Coppock, C. E., Nave, D. H., Labore, J. M., Greene, L. W. & Odom, T. W. (1987). Effects of potassium carbonate and sodium bicarbonate on rumen function in lactating Holstein cows. *Journal of Dairy Science*, 70(1): 81-90.

Wróbel, B., Nowak, J., Fabiszewska, A., Paszkiewicz-Jasińska, A. & Przystupa, W. (2023). DM Losses in Silages Resulting from Epiphytic Microbiota Activity—A Comprehensive Study. *Agronomy*, 13(2): 450.

Zali, A., Nasrollahi, S. M. & Khodabandelo, S. (2019). Effects of two new formulas of dietary buffers with a high buffering capacity containing Na or K on performance and metabolism of mid-lactation dairy cows. *Preventive Veterinary Medicine*, 163: 87-92.

Zhou, R., Wu, J., Lang, X., Liu, L., Casper, D. P., Wang, C. & Wei, S. (2020). Effects of oregano essential oil on *in vitro* ruminal fermentation, methane production, and ruminal microbial community. *Journal of Dairy Science*, 103(3): 2303-2314.