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Effects of Compound Buffer on Milk Composition, Plasma Biochemical Parameters, and Hormone Levels in Dairy Goats (Postprint)

Authors: Li Jiaying, Zhuang Su, Fan Chengrui, Wang Yuyu, Wang Baozhe, Liu Qiang

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Abstract

This experiment was conducted to investigate the effects of supplementing a compound buffer (sodium bicarbonate, magnesium oxide, and sodium butyrate) in a long-term high-concentrate diet on milk composition, plasma biochemical indices, and hormone levels in dairy goats. Eight mid-lactation dairy goats fitted with portal and hepatic vein catheters were randomly divided into two groups and fed either a basal diet (high-concentrate diet group, HG group) or basal diet supplemented with compound buffer (high-concentrate diet + compound buffer group, BG group). The preliminary period was 7 d, and the formal experimental period was 143 d. The results showed that: compared with the HG group, the compound buffer significantly increased the mean ruminal fluid pH during 0–10 h post-feeding ($P<0.05$); significantly decreased the concentration of non-esterified fatty acids in hepatic vein plasma ($P<0.05$), while having no significant effects on glucose and β-hydroxybutyrate concentrations in portal and hepatic vein plasma ($P>0.05$), and no significant effects on triglyceride and total protein contents in liver tissue ($P>0.05$); significantly decreased insulin and glucagon concentrations in portal vein plasma ($P<0.05$), while having no significant effects on insulin-like growth factor 1 and growth hormone concentrations in portal and hepatic vein plasma ($P>0.05$); and significantly or extremely significantly increased milk yield, milk fat percentage, milk protein percentage, and milk solids-not-fat percentage ($P<0.05$ or $P<0.01$). In conclusion, under long-term high-concentrate feeding conditions, the compound buffer composed of sodium bicarbonate, magnesium oxide, and sodium butyrate can influence systemic nutrient metabolism by affecting plasma biochemical indices and hormone levels in dairy goats, ultimately effectively increasing milk production and improving milk quality.

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Full Text

Effects of Compound Buffers on Milk Composition, Plasma Biochemical Parameters, and Hormone Contents in Lactating Goats

LI Jiaying, ZHUANG Su*, FAN Chengrui, WANG Yuyu, WANG Baozhe, LIU Qiang
(College of Animal Science and Technology, Nanjing Agricultural University, Nanjing 210095, China)

Abstract

This study aimed to investigate the effects of compound buffers (sodium bicarbonate, magnesium oxide, and sodium butyrate) on milk composition, plasma biochemical parameters, and hormone contents in dairy goats fed a high-concentrate diet for an extended period. Eight mid-lactating goats fitted with permanent portal and hepatic vein catheters were randomly divided into two groups and fed either a basal diet (high-concentrate diet group, HG group) or the basal diet supplemented with compound buffers (high-concentrate diet + compound buffers group, BG group). The pre-trial period lasted 7 days, and the formal experimental period lasted 143 days. The results showed that, compared with the HG group, the compound buffers significantly increased the mean rumen fluid pH during 0–10 h post-feeding ($P<0.05$). The compound buffers significantly decreased plasma non-esterified fatty acid content in the hepatic vein ($P<0.05$), but had no significant effects on plasma glucose and β-hydroxybutyrate contents in the portal and hepatic veins ($P>0.05$), nor on triglyceride and total protein contents in liver tissue ($P>0.05$). The compound buffers significantly decreased plasma insulin and glucagon contents in the portal vein ($P<0.05$), but had no significant effects on plasma insulin-like growth factor 1 and growth hormone contents in the portal and hepatic veins ($P>0.05$). The compound buffers significantly or extremely significantly increased milk yield, milk fat percentage, milk protein percentage, and milk solids-not-fat percentage ($P<0.05$ or $P<0.01$). In conclusion, under long-term feeding of a high-concentrate diet, the compound buffer containing sodium bicarbonate, magnesium oxide, and sodium butyrate can affect nutrient metabolism by influencing plasma biochemical parameters and hormone contents, ultimately improving milk yield and milk quality in dairy goats.

Key words: compound buffers; high-concentrate diet; dairy goat; milk quality; insulin

Introduction

In China' s dairy industry, the lack of high-quality forage sources often leads producers to feed animals high-concentrate diets to achieve high yields and economic benefits. However, feeding ruminants high-concentrate diets can cause

rumen fluid pH to decrease, leading to abnormal metabolism and ultimately reduced productivity and milk quality [1]. Undoubtedly, this decline in milk performance is accompanied by changes in biochemical parameters and disruption of hormone metabolism.

Buffers are chemical substances that enhance the acid-base buffering capacity of solutions, maintaining relatively stable pH levels. In livestock production, buffers are commonly used to prevent rumen acidosis or improve ruminant performance. Zhong et al. [2] found that dietary supplementation with buffers can increase rumen buffering capacity to prevent acidosis.

Numerous studies on buffer utilization have been reported both domestically and internationally [3-5]. Commonly used buffers include sodium bicarbonate, sodium acetate, calcium carbonate, potassium carbonate, potassium bicarbonate, magnesium oxide, natural soda, and compound buffers such as sodium bicarbonate-magnesium oxide and sodium bicarbonate-potassium dihydrogen phosphate. Research has primarily focused on single buffers or sodium bicarbonate-magnesium oxide compound buffers [3,6-7].

Currently, studies on the effects of buffers in ruminants have mainly concentrated on rumen fermentation and metabolism under short-term experimental conditions, with relatively few investigations into changes in production performance, blood biochemical parameters (especially in hepatic blood), and hormone contents following long-term feeding of high-concentrate diets. Therefore, this study used Saanen dairy goats as experimental animals to investigate the effects of compound buffers (sodium bicarbonate, magnesium oxide, and sodium butyrate) on milk composition, plasma biochemical parameters, and hormone contents under long-term high-concentrate feeding conditions, aiming to provide a theoretical basis for the feeding efficacy of buffers.

Materials and Methods

1.1 Experimental Animals and Diets

Eight lactating goats at 100 days in milk, fitted with permanent portal and hepatic vein catheters [(38$±$3) kg], were randomly divided into two groups of four animals each. The goats were fed either a basal diet with a concentrate-to-forage ratio of 6:4 (high-concentrate group, HG group) (feed formulation and nutritional levels are shown in Table 1) or the basal diet supplemented with compound buffers (sodium bicarbonate, magnesium oxide, and sodium butyrate) (high-concentrate + compound buffers group, BG group). The compound buffers were mixed into the feed and fed to animals in two equal portions. Experimental animals were housed individually and fed and milked at 08:00 and 18:00 daily. The pre-trial period lasted 7 days, and the formal experimental period lasted 143 days.

Table 1 Composition and Nutritional Levels of the Basal Diet (Air-Dry Basis)

Ingredients	Content (%)	Nutritional levels2)	Content
Oat hay		Net energy for lactation/(MJ/kg)	
Alfalfa hay		Crude protein	
Corn		Digestible crude protein	
Wheat bran		Neutral detergent fiber	
Soybean meal		Acid detergent fiber	
Limestone		Calcium	
Salt			
Premix1)			
Total			

1) The premix provided the following per kg of diet: VA 8,000 IU, VD 2,500 IU, VE 20 mg, Fe (as ferrous sulfate) 62.5 mg, Cu (as copper sulfate) 7.5 mg, Mn (as manganese sulfate) 50 mg, Zn (as zinc sulfate) 62.5 mg, Se (as sodium selenite) 0.25 mg, I (as potassium iodide) 0.3 mg, Co (as cobalt sulfate) 0.15 mg, Mo (as molybdenum sulfate) 0.15 mg.

2) Net energy for lactation was a calculated value, while the others were measured values.

1.2 Sample Collection

Milk yield was recorded twice daily (morning and evening) throughout the trial. During the final 3 days of the experiment, rumen fluid was collected before feeding (0 h) and at 1, 2, 4, 6, 8, and 10 h post-feeding. The collected rumen fluid was filtered through four layers of cheesecloth, and the filtrate was used for pH measurement before being stored at -20 °C for later analysis. Blood (5 mL) was collected from the portal and hepatic veins, anticoagulated with heparin sodium (3.6 U/mL), and immediately transported to the laboratory. Plasma was prepared by centrifugation at 4,000 r/min for 10 min at 4 °C, and plasma samples were stored at -20 °C. On the final day of the experiment, goats were slaughtered and liver tissue samples were collected and stored at -70 °C.

1.3 Laboratory Analyses

1.3.1 Rumen Fluid pH Measurement Rumen fluid pH was measured using a pH meter (HI8424, HANNA, Italy).

1.3.2 Milk Composition Analysis Milk composition was analyzed using an automatic milk composition analyzer (Julie Z9, Scope, Bulgaria).

1.3.3 Plasma Glucose, BHBA, and NEFA Determination Plasma glucose, BHBA, and NEFA contents were determined using commercial biochemical assay kits according to the manufacturer' s instructions.

1.3.4 Liver Tissue Total Protein and TG Determination Total protein and TG contents in liver tissue were determined using a bicinchoninic acid (BCA) protein quantification kit and a TG assay kit, respectively.

1.3.5 Plasma Hormone Determination Plasma insulin (INS), glucagon (GC), insulin-like growth factor 1 (IGF-1), and growth hormone (GH) contents were determined using enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer' s instructions.

1.4 Data Processing

Experimental data were initially processed using Excel 2007 and then subjected to one-way ANOVA using SPSS 18.0 statistical software. Diet was the main effect factor. Data are presented as means, with $P<0.05$ considered statistically significant and $P<0.01$ considered extremely significant.

Results

2.1 Effects of Compound Buffers on Rumen Fluid pH

As shown in Figure 1 [Figure 1: see original paper], rumen fluid pH gradually decreased after feeding, reached its lowest point at 4 h post-feeding, and then gradually recovered to pre-feeding levels. At 1, 2, 4, and 10 h post-feeding, rumen fluid pH in the BG group was significantly or extremely significantly higher than that in the HG group ($P<0.05$ or $P<0.01$). The mean rumen fluid pH in the BG group was significantly higher than that in the HG group (6.04 vs. 5.82, $P<0.05$). * indicates significant difference between groups ($P<0.05$); ** indicates extremely significant difference between groups ($P<0.01$). The same notation applies to the following figures.

Figure 1 Effect of compound buffers on dynamic changes in rumen fluid pH in lactating goats

2.2 Effects of Compound Buffers on Milk Yield and Composition

As shown in Table 2 , supplementation with compound buffers significantly or extremely significantly increased milk yield, milk fat percentage, milk fat yield, milk protein percentage, milk protein yield, milk solids-not-fat percentage, milk solids-not-fat yield, and lactose yield in dairy goats ($P<0.05$ or $P<0.01$).

Table 2 Effects of compound buffers on milk yield and milk composition of lactating goats

Items	HG group	BG group	P-value
Milk yield/(kg/d)			
Milk fat percentage/%			
Milk fat yield/(g/d)			

Items	HG group	BG group	P-value
Milk protein percentage/%			
Milk protein yield/(g/d)			
Lactose percentage/%			
Lactose yield/(g/d)			
Milk solid not-fat percentage/%			
Milk solid not-fat yield/(g/d)			

In the same row, values with no superscript letters indicate no significant difference (P>0.05), different lowercase letters indicate significant difference (P<0.05), and different uppercase letters indicate extremely significant difference (P<0.01). The same notation applies to the following tables.

2.3 Effects of Compound Buffers on Dynamic Changes in Milk Yield and Milk Fat Percentage

As shown in Figure 2 [Figure 2: see original paper], milk yield remained relatively stable during the first 9 weeks in both groups, then gradually declined thereafter. Milk yield in the BG group was higher than that in the HG group throughout the lactation period, reaching significant levels during weeks 1, 2, 5, 7, 10, 11, 12, 13, 14, and 15 (P<0.05).

Figure 2 Effect of compound buffers on dynamic changes in milk yield of lactating goats

As shown in Figure 3 [Figure 3: see original paper], milk fat percentage gradually increased from week 3 onward in both groups. Although intergroup comparisons revealed no statistically significant differences in weekly milk fat percentage between the two groups (P>0.05), the BG group consistently showed numerically higher values than the HG group.

Figure 3 Effect of compound buffers on dynamic changes in milk fat percentage of lactating goats

2.4 Effects of Compound Buffers on Biochemical Parameters in Plasma and Liver

As shown in Table 3 , there were no significant differences in plasma glucose and BHBA contents between the two groups in either the portal or hepatic veins (P>0.05), and no significant differences in TG and total protein contents in liver tissue (P>0.05). Compared with the HG group, plasma NEFA content decreased in the BG group, with a significant reduction observed in hepatic vein plasma (P<0.05).

Table 3 Effects of compound buffers on biochemical parameters in plasma and liver of lactating goats

Items	HG group	BG group	P-value
Portal vein			
Glucose/(mmol/L)			
BHBA/(mmol/L)			
NEFA/(mmol/L)			
Hepatic vein			
Glucose/(mmol/L)			
BHBA/(mmol/L)			
NEFA/(mmol/L)			
Liver tissue			
TG (g/g prot)			
Total protein (mg/g prot)			

2.5 Effects of Compound Buffers on Hormone Contents in Plasma

As shown in Table 4 , compared with the HG group, plasma INS and GC contents in the portal vein of BG group goats were significantly decreased ($P<0.05$), while these parameters showed no significant differences in the hepatic vein ($P>0.05$). Although IGF-1 and GH contents in portal and hepatic vein plasma did not differ significantly between the two groups ($P>0.05$), the BG group showed numerically higher values than the HG group.

Table 4 Effects of compound buffers on hormone contents in plasma of lactating goats (ng/mL)

Items	HG group	BG group	P-value
Portal vein			
INS			
GC			
IGF-1			
GH			
Hepatic vein			
INS			
GC			
IGF-1			
GH			

Discussion

3.1 Effects of Compound Buffers on Rumen Environment

Feeding high-concentrate diets can cause rumen fluid pH to decrease in ruminants, leading to rumen acidosis [8]. This study found that rumen fluid pH decreased in dairy goats fed a high-concentrate diet, while supplementation

with compound buffers increased rumen fluid pH, indicating that compound buffers help alleviate rumen acidosis caused by decreased pH. Additionally, compound buffer supplementation significantly increased rumen fluid acetate content (51.84 mmol/L vs. 46.82 mmol/L, data from an accepted paper), likely because cellulolytic bacterial activity decreases under high-concentrate conditions, reducing acetate content, and compound buffers help mitigate this effect.

3.2 Effects of Compound Buffers on Plasma Biochemical Parameters in Dairy Goats

Glucose is a crucial energy source for ruminants. In addition to small amounts of dietary carbohydrates that escape rumen degradation and are digested and absorbed in the small intestine, most glucose in ruminants is derived from hepatic gluconeogenesis [9]. This study found that glucose content in hepatic vein plasma increased by 9.38% compared with portal vein plasma in the BG group, while the increase was limited in the HG group. We speculate that this result occurred because hepatic gluconeogenesis was enhanced in the BG group, allowing the liver to utilize more glucogenic substrates such as propionate for glucose conversion, thereby providing more precursors for lactose synthesis in the mammary gland. Therefore, this may be the fundamental reason why lactose yield was significantly higher in the BG group. Related studies have shown that high-concentrate diets can provide more hepatic gluconeogenic precursors and increase plasma glucose content [10-11]. In this experiment, since both groups received the same basal diet, rumen fluid propionate concentration showed no significant difference (17.44 mmol/L vs. 17.96 mmol/L, data from an accepted paper). The increased lactose percentage and lactose yield in the BG group are hypothesized to result from enhanced hepatic gluconeogenic capacity due to compound buffer supplementation, which increased glucose content in hepatic vein plasma and ultimately led to increased lactose yield. Additionally, studies have shown that feeding high-concentrate diets can decrease rumen fluid pH and increase production of abnormal rumen metabolites such as lipopolysaccharide (LPS), which can induce increased inflammatory factors in the liver and impair liver function [12]. After compound buffer supplementation, rumen fluid pH increased, reducing the production of abnormal rumen metabolites and their damaging effects on the liver, thereby ensuring normal liver function. This is also one reason why hepatic vein plasma glucose content was higher in the BG group than in the HG group.

The energy balance and mobilization status of the body can be indicated by NEFA and BHBA contents [13], which are affected by diet composition and physiological stage [14]. Studies have found that ruminants in negative energy balance during lactation are prone to ketosis, with increased blood NEFA content [15]. When the capacity for hepatic fatty acid oxidation or TG output in the form of very low-density lipoproteins is limited in ruminants, fatty acids exceed the liver's capacity for output or mitochondrial β-oxidation, leading to excessive accumulation of fatty acids as TG in the liver and resulting in fatty liver. This

study found that compound buffer supplementation decreased plasma NEFA content, possibly because elevated plasma glucose content reduced lipolysis, indicating improved energy utilization efficiency.

BHBA is the primary ketone body in plasma [16] and also a precursor for milk fat synthesis. Studies have found a positive correlation between serum BHBA and NEFA [17]. However, in this experiment, plasma BHBA content in the BG group did not decrease with the reduction in NEFA content. We speculate that this may be because the compound buffers contained sodium butyrate, which increased butyrate (the precursor of BHBA) in blood, thus maintaining BHBA content without significant changes.

3.3 Effects of Compound Buffers on Plasma Hormone Contents in Dairy Goats

In nutrient metabolism, changes in blood biochemical parameters can directly reflect metabolic processes, while changes in hormone contents not only reflect these processes but also serve as important factors regulating metabolism. GC and INS are crucial hormones for maintaining glucose homeostasis. Studies have shown that GC can enhance the liver' s ability to absorb glucose precursors such as propionate and alanine, thereby strengthening hepatic gluconeogenesis and promoting glycogenolysis and glucose production [18-19], which increases blood glucose content. In contrast, INS has opposite effects; when blood glucose content rises, INS secretion increases, inhibiting hepatic gluconeogenesis and glycogenolysis, thereby reducing the liver' s capacity to output glucose and decreasing blood glucose content [20-21]. Additionally, increased INS content can promote fatty acid and TG synthesis in adipose tissue while inhibiting lipolysis, causing a series of changes in lipid metabolism [22]. This is because INS and its receptors can inhibit lipolytic hormones and adenylate cyclase activators (forskolin), promoting increased cAMP content and NEFA release from adipocytes [1]. Conversely, GC enhances body fat decomposition [23]. Recent studies have shown that high-concentrate diets not only decrease rumen fluid pH in ruminants but also increase plasma INS content [24]. Elevated INS content can cause a significant decrease in milk fat percentage in dairy goats, primarily because INS promotes fatty acid uptake in adipose tissue and reduces their release, ultimately decreasing mammary gland absorption of blood NEFA and TG [25]. Another study showed that additives such as magnesium supplementation can increase pancreatic β-cell proliferation activity, reduce blood glucose content, decrease INS content, and thereby alleviate INS resistance [26]. This study found that compound buffer supplementation decreased plasma GC and INS contents. This may be because compound buffers affected INS resistance mechanisms, significantly reducing INS content in portal vein plasma of the BG group, thereby enhancing mammary gland absorption of blood NEFA and TG and ultimately significantly increasing milk fat percentage.

GH is a hormone secreted by the anterior pituitary that regulates the partitioning of nutrients during synthesis, increasing protein synthesis in muscle tis-

sue and reducing adipose tissue accretion [27]. GH has both physiological and pharmacological effects on lipid metabolism. Its physiological effect (anti-INS-like effect) can lead to body fat decomposition and increased NEFA content, while its pharmacological effect (INS-like effect) can promote glucose uptake and adipocyte esterification [28]. GH is an important hormone in the GH/IGF-1 axis that regulates growth and is a key factor controlling IGF-1 secretion [29]. The growth-promoting effects of GH in animals are primarily mediated by IGF-1. Studies have found a positive correlation between GH and milk yield in ruminants, with GH treatment significantly increasing milk production [30-31]. Plasma IGF-1 is mainly derived from the liver, and its synthesis is influenced by GH secretion and nutritional status; conversely, IGF-1 can negatively regulate GH at the hypothalamic and pituitary levels [32]. Additionally, IGF-1 participates in glucose metabolism and transport in adipose tissue, promoting fat and glycogen synthesis and enhancing cellular glucose utilization. This study found that compound buffer supplementation increased IGF-1 and GH numerically, suggesting that compound buffers elevated GH content in the body, thereby stimulating hepatic IGF-1 synthesis, enhancing TG synthesis and transport in hepatocytes, providing more precursors for milk fat synthesis, and ultimately significantly increasing milk fat percentage and milk yield. These results are consistent with the findings of Li Xinwei [33].

3.4 Effects of Compound Buffers on Milk Composition in Dairy Goats

Studies have found that sodium bicarbonate can increase milk yield and milk fat percentage [34]. This study found that under long-term feeding conditions with a dietary concentrate-to-forage ratio of 60:40, supplementation with compound buffers could increase milk yield, milk fat percentage, milk protein percentage, and other indices in dairy goats. These results demonstrate that compound buffer supplementation is more effective in improving milk yield and milk quality.

Conclusion

Under the conditions of this experiment, compound buffer supplementation increased rumen fluid pH, decreased plasma NEFA content, enhanced hepatic gluconeogenesis, reduced plasma INS and GC contents, and increased GH and IGF-1 contents, ultimately improving milk yield and milk quality in dairy goats.

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