國立屏東科技大學動物科學與畜產系
Department of Animal Science
National Pingtung University of Science and Technology

碩士學位論文
Master Thesis

添加氧化鎂及離胺酸對攝取高精料之羊隻其瘤胃性狀之影響
Effects of supplementation of MgO and lysine in high ratio concentrate diets on rumen condition

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中華民國 102 年 01 月 19 日
January 19, 2013

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國立屏東科技大學
碩士學位論文口試委員會審定書

動物科學與畜產系 碩士班　研究生 慕 克 明 君 學號：M10026015

所提之論文　添加氧化鎂及離胺酸對攝取高精料之羊隻其瘤胃性狀之影響
Effects of supplementation of MgO and lysine in high ratio concentrate diets on rumen condition

經本委員會審定通過，特此證明。

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中　華　民　國　1　0　2　年　1　月　1　9　日

National Pingtung University of Science and Technology
Certification of the Completion of Oral Exam
by the Oral Exam Committee for
Master's Thesis

Department of Animal Science

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Title: Effects of supplementation of MgO and lysine in high ratio concentrate diets on rumen condition

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hay and 40% concentrate in DM basis. Three percentages of molasses and water were added to increase palatability and water content of the diet to a level of 60%. The feeds were offered twice a day, at 07.00 AM and 07.00 PM, at restricted feeding of 1,050 g of DM/head/day, whereas water was provided in *at libitum* free access. The results indicated that feeding high ratio concentrate diets improved rumen liquid condition as shown by the increasing of ruminal temperature, NH_4^+ concentration, population of protozoa and bacteria, total VFA and amino acids, and reduce ruminal ORP. However, the risk of ruminal acidosis was increased due to decrease ruminal pH. Improving the rumen condition was achieved by supplementation of MgO on levels 0.2, 0.4, and 0.8% and lysine on level 0.25%, but interaction between MgO and lysine has altered the pattern of feed fermentation in the rumen.

Key words: Acidosis, High concentrate diets, Lysine, MgO, Rumen condition

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5. All officials in Faculty of Animal Husbandry and Double Degree Program of University of Brawijaya, and all official in Animal Science and Office of International Affair who help me about administration for my study.
6. My laboratory mates who help me in my studies, experiments, socialize, and share about Taiwanese culture.
7. My country mates and dormitory mates who full fill my days with unforgettable sweet memories.
8. All friends and people who help me during my study which can not mentioned one by one.

Pingtung, Taiwan

January 31, 2013

Amiril Mukmin

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NE_L – Net energy for lactation

NEm – Net energy required for maintenance

NFC – Non fiber carbohydrate

NH_4^+ – Ammonium

NRC – National research council

NS – Not significant

ORP – Oxidation reduction potential

Osm – Osmolality

P – Probability

PCA – Plate count agar

pH – Power of hydrogen/acidity value

PM – Post meridiem

RDP – Rumen degradable protein

SCP – Soluble crude protein

SEM – Standard error of the mean

T – Treatment

TDN – Total digestible nutrient

TMR – Total mixed ration

UDP – Undegraded dietary protein

VFA – Volatile fatty acids

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Lysine is an essential amino acid for ruminant, not only for host animal but also for rumen microbes. Although rumen microbes, especially protozoa and bacteria can produce and provide lysine for host animal (Onodera, 1993), but in many cases, because of it production is lower than required by animal, lysine status also becomes the first limiting amino acid (Annison *et al*., 2002). Lysine supplementation is required to optimize animal performance.

Supplementation of MgO and lysine are expected to change the pattern of feed fermentation in the rumen and reduce occurrence of ruminal acidosis, especially when animal consume high amount of concentrate diet. Finally, feeding high ratio concentrate diets supplemented by MgO and lysine could increases animal performance, animal production, and profit for farmers.

1.2 Objective

This study was conducted to evaluate the effect of supplementation of MgO and lysine in high ratio concentrate diets on rumen condition, including temperature, acidity, oxidation reduction potential, conductivity, concentration of NH_4^+, population of protozoa and bacteria, and total of VFA and amino acids.

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Table 1 Typical values for the component of nutritive value in the dry matter of mixed pasture herbage as it matures from young leafy material, stage 1, to mature stemmy flowering herbage, stage 4 (data from MAFF, 1990, equations from SCA, 1990)

	Stage			
	1	2	3	4
CP (g kg^{-1})	190	150	120	84
Protein degradation [a] (h^{-1})	0.86	0.81	0.73	0.68
Neutral detergent soluble (g kg^{-1})	465	418	373	291
Neutral detergent fiber (g kg^{-1})	535	582	627	709
Acid detergent fiber (g kg^{-1})	264	301	329	400
Ether extract (g kg^{-1})	25	21	19	14
DM Digestibility [a]	0.79	0.72	0.65	0.52
ME/DM (MJ kg^{-1})	12.6	11.1	9.5	7.4
Efficiency of use of ME for:				
Maintenance, k_m	0.75	0.72	0.69	0.65
Milk production, k_l	0.65	0.62	0.59	0.55
Weight gain, k_g	0.51	0.43	0.35	0.23

Source: Coleman and Henry (2002)

[a] Measured at a feeding level adequate for maintenance only.

$k_m = 0.02M + 0.5$

$k_l = 0.02M + 0.4$

$k_g = (0.3L + 0.9)\,[0.043M + 0.01(15.4 - M)((\lambda/40)\sin(2\pi D/365) - 1.00)]$

Table 2 Chemical compositions of Napier grass and pangola grass

Forages	DM	Ash	EE	CP	DIP	SCP	NDF	Lignin	eNDF	ME	NEm	NEg
	%	%DM			%CP		%DM	%NDF		Mcal/kg		
Napier grass, fresh 30 d	20.0	9.0	3.0	8.7	83.0	46.0	70.0	14.29	41.0	1.99	1.14	0.58
Napier grass, fresh 60 d	23.0	6.0	1.0	7.8	81.0	46.0	75.0	18.67	41.0	1.92	1.07	0.52
Pangola grass, fresh	21.0	7.6	2.3	9.1	84.0	42.0	70.0	11.40	41.0	1.99	1.14	0.58

Source: NRC (2000)

DM = Dry matter
EE = Extract ether
CP = Crude protein
DIP = Degraded intake protein
SCP = Soluble crude protein
NDF = Neutral detergent fiber
eNDF = effective neutral detergent fiber
ME = Metabolizable energy
NEm = Net energy required for maintenance
NEg = Net energy required for gain

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Management practices include the feeding system of forages. Grazing, restricted grazing, tethering, and stall feeding are some of the feeding system that commonly applied for forages.

A study by Moniruzzaman *et al*. (2002) reported that feeding system has affected dry matter intake (DMI) and growth performance of female Black Bengal goats. From that study, the highest feeding intake was achieved by tethering system ($P < 0.05$). DMI of grazing, restricted grazing, tethering, and stall feeding goats were 342.88, 318.84, 356.60, and 334.36 g/day, respectively. Different with DMI, the highest average daily gain (ADG) was achieved by stall feeding system ($P < 0.05$) although this system was the second lowest in DMI. The variation of ADG values were 7.985, 9.127, 10.572, and 15.978 g/day for grazing, restricted grazing, tethering, and stall feeding, respectively.

In comparison to other feeding system, stall feeding system is more expensive because this system requires extra housing facilities and more labors for collecting and feeding forages, as well as intensive care. But, stall feeding system is better than other feeding system, especially in growth performance and feed efficiency. This feeding system is also very important in case of land scarcity (Moniruzzaman *et al*., 2002).

Related to stall feeding system, forages could be offered to the animal on fresh or dry (hay) conditions. Napier grass is the most popular grass as provider of fresh fodder for ruminant animal (Orodho, 2006[a]), whereas for hay making, pangola grass is the best (Cameron and Lemcke, 2003).

Napier grass (*Pennisetum purpureum*), is also called elephant grass (Orodho, 2006[a]), is native forage from tropical Africa, but now it is available throughout the tropical area in the world (NRC, 1993). To improve genetic quality and productivity, Napier grass has been crossed with a wild relative

(e.g., P. typhoides) to give a sterile triploid. This is said to be produce more fodder than its parents (NRC, 1993). Napier grass can grows optimally in high rainfall area (more than 1000 mm per year) and in temperature range of 25 to 40 ^{o}C. This grass also can grows in a wide range of soils. Napier grass generates best performance in fertile and well in drained soils, but it cannot tolerate in flooding or waterlogging areas (Orodho, 2006[a]).

Most of farmers prefer to cultivate Napier grass, because this grass has high productivity, which can produce biomass up to 20-30 ton of DM/ha/year with good agronomic and management practice (Orodho, 2006[b]). Not only for fresh forage purposes, hay and silage also can be made from Napier grass. But, especially for hay making, Napier grass should be cut at a young stage. In Taiwan Napier grass is widely used for the production of dehydrated grass pellets used as supplementary stock feed (Orodho, 2006[a]).

Different from Napier grass, pangola grass (*Digitaria eriantha*) is native forage from subtropical Africa. It can grows optimally in areas that receive rainfall in average 1,100 mm/year, or more. Pangola grass is adapted to a wide range of soil type, from sands to heavy clays. It can survives withstand several months of waterlogging, but will not persist under prolonged flooding (Cameron and Lemcke, 2003).

Pangola grass is an excellent grass for hay making. It can produce DM in the average of 4-6 ton/ha/year without any nitrogen fertilizer. Applications of nitrogen fertilizer in the level of 200 kg can increase DM yield up to12-15 ton/ha/year (Cameron and Lemcke, 2003).

2.2 Concentrate diets

Nutritive value of forages is relatively lower than nutritive required by ruminant animal to express their genetic potential for production. From energy point, GE value of forages is relatively low. Moreover, about 15-60%

(depend on forages quality) of energy intake is lost via the faces, and more than 18% is released during ruminal fermentation (lost as heat and combustible gases). These cases are some reasons to reveal the inefficiency of forages as single feed (Weston, 2002). To optimize animal productivity, supplementation is required for grass based diets (Dove, 2002).

Supplement can be divided into 3 types, such as energy sources, protein sources, and premix. Energy source supplements have to contain large amount of readily digestible carbohydrate, such as starch. Energy sources can be obtained from cereal grains, such as barley, wheat, oats, sorghum, and maize, or crop by-product such as bran and molasses (Dove, 2002).

In addition, protein source supplements have to be able to provide rumen degradable protein (RDP) and undegraded dietary protein (UDP) for ruminant. Availability of RDP is required to improve the efficiency of fiber digestion and microbial protein production in the rumen. Urea is the most frequently used as RDP source because of its low cost and easily converted into ammonia in the rumen. UDP should be a dietary true protein that can be easily absorbed and metabolized by host animal. Dietary true protein supplements can be derived from plant sources, such as grain legumes (e.g. lupins, vetches), pulses (e.g. peas, faba beans), oilseed and oilseed meal (e.g. whole cottonseeds, cottonseed meal, soybean meal, sunflower meal), as well as animal protein sources such as fish meal and blood. Supplementations of RDP and UDP in diets affect in increasing metabolizable protein leaving the rumen for digestion and absorption in the small intestine (Dove, 2002).

Micronutrient mix, or more familiar called premix, contains micro mineral or trace elements and vitamins (sometimes added some essential amino acids). Micronutrients were required in living tissue in low concentration as the reaction for maintaining normal cellular metabolism (Lee *et al*., 2002).

Table 3 Chemical composition of raw materials of concentrate diets

Materials	DM	TDN	DE	ME	CP	EE	Fiber	NDF	ADF	Lignin	Ash
	%		Mcal/kg		%DM						
Barley grain*	88.1	88.0	3.84	3.03	13.2	2.20	3.32	18.1	5.77	--	2.40
Barley grain rolled**	91.0	82.7	3.64	2.92	12.4	2.20	--	20.8	7.20	1.90	2.90
Blood meal*	90.5	66.0	2.91	2.49	93.8	1.69	1.35	41.6	2.81	--	2.62
Chocolate by-product**	95.2	102.7	4.46	3.77	11.9	20.5	--	23.8	15.7	3.20	2.10
Corn grain cracked*	90.0	90.0	3.92	3.25	9.80	4.06	2.29	10.8	3.30	--	1.46
Corn distillers grain**	90.2	79.5	3.72	3.03	29.7	10.0	--	38.8	19.7	4.30	5.20
Cotton seed meal*	90.2	75.0	3.31	2.71	46.1	3.15	13.2	28.9	17.9	--	7.00
Feather meal poultry*	93.3	68.0	3.00	2.46	85.5	7.21	0.90	54.9	18.3	--	3.50
Fish by-product meal, anchovy**	92.0	76.1	4.16	3.42	71.2	4.60	--	--	--	--	16.0
Meat and bone rendered**	94.0	61.9	3.19	2.54	54.2	10.4	--	--	--	--	30.4
Molasses sugarcane*	74.3	72.0	3.17	2.60	5.80	0.20	0.50	--	0.40	--	13.3
Oat grain*	89.2	77.0	3.40	2.78	13.6	5.20	12.0	29.3	14.0	--	3.30
Oat grain rolled**	90.0	78.5	3.47	2.78	13.2	5.10	--	30.0	14.6	4.90	3.30
Potato by-product **	35.4	80.7	3.51	2.84	10.5	10.8	--	22.1	16.5	2.30	12.8
Rice bran*	90.5	70.0	3.09	2.53	14.4	15.0	12.9	33.0	20.0	--	11.5
Sorghum grain*	90.0	82.0	3.62	2.96	12.6	3.03	2.76	16.1	6.38	--	1.87

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Table 3 Continued

Materials	DM	TDN	DE	ME	CP	EE	Fiber	NDF	ADF	Lignin	Ash
	%		Mcal/kg		%DM						
Soybean meal*	90.9	84.0	4.70	3.04	51.8	1.67	5.37	10.3	7.00	--	6.90
Sunflower seed meal*	92.5	65.0	2.87	2.35	26.0	2.90	12.7	40.0	30.0	--	8.10
Vegetable oil**	100	184.0	7.70	7.07	0	99.9	--	0	0	0	0
Wheat bran*	89.0	70.0	3.09	2.53	17.4	4.30	11.3	42.8	14.0	--	6.60

Sources: * NRC (2000) and ** NRC (2001)

TDN = Total digestible nutrient

DE = Digestible energy

ME = Metabolizable energy

NDF = Neutral detergent fiber

ADF = Acid detergent fiber

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Energy source supplements, protein source supplements and premix were often served together as concentrate diets. Compared to forages, concentrate diets contain higher energy density and more predictable in nutritional value (Cheng *et al*., 1998). Processing of materials of concentrate may significantly effects on the nutritive value (NRC, 2000). Chemical composition of several raw materials of concentrate diets was shown in Table 3.

2.3 Effects of high concentrate diet in the rumen

Concentrate diet is the main feed supplement to increase ruminant productivity, meat or milk production, by increasing nutrient intake. Compared with forages, concentrate relative more palatable and more predictable and greater in nutrients density. Increasing nutrient intake was easier by feeding high concentrate diets than forages. Study by Commun *et al*. (2009) had shown that DMI of sheep increase by providing of concentrate diets. In this experiment, sheep consumes 1.10 kg of DM per day by feeding *at libitum* of alfalfa hay as single diet. DMI increase becomes 1.34 kg per day when wheat concentrate was provided in feed container. This condition may also because of bulky alfalfa hay, relative to wheat concentrate.

Another study, McLeod and Baldwin (2000) had also shown that lamb performance fed by high concentrate diets was better than lamb feeding by high forages. Feed efficiency (Gain:DMI) of high concentrate diet treatment (75% of concentrate and 25% of forages) was higher than high forage diet treatment (25% of concentrate and 75% of forages) by 0.00 and 0.05 in low intake group and 0.19 and 0.30 in high intake group, respectively. Franzolin and Dehority (1996) also have reported about the effect of increasing concentrate levels in the diet on ruminal protozoa population in steers. Based on their study results, total protozoa population in the rumen of steers was increased by increasing levels of concentrate in the diets. Totals ruminal

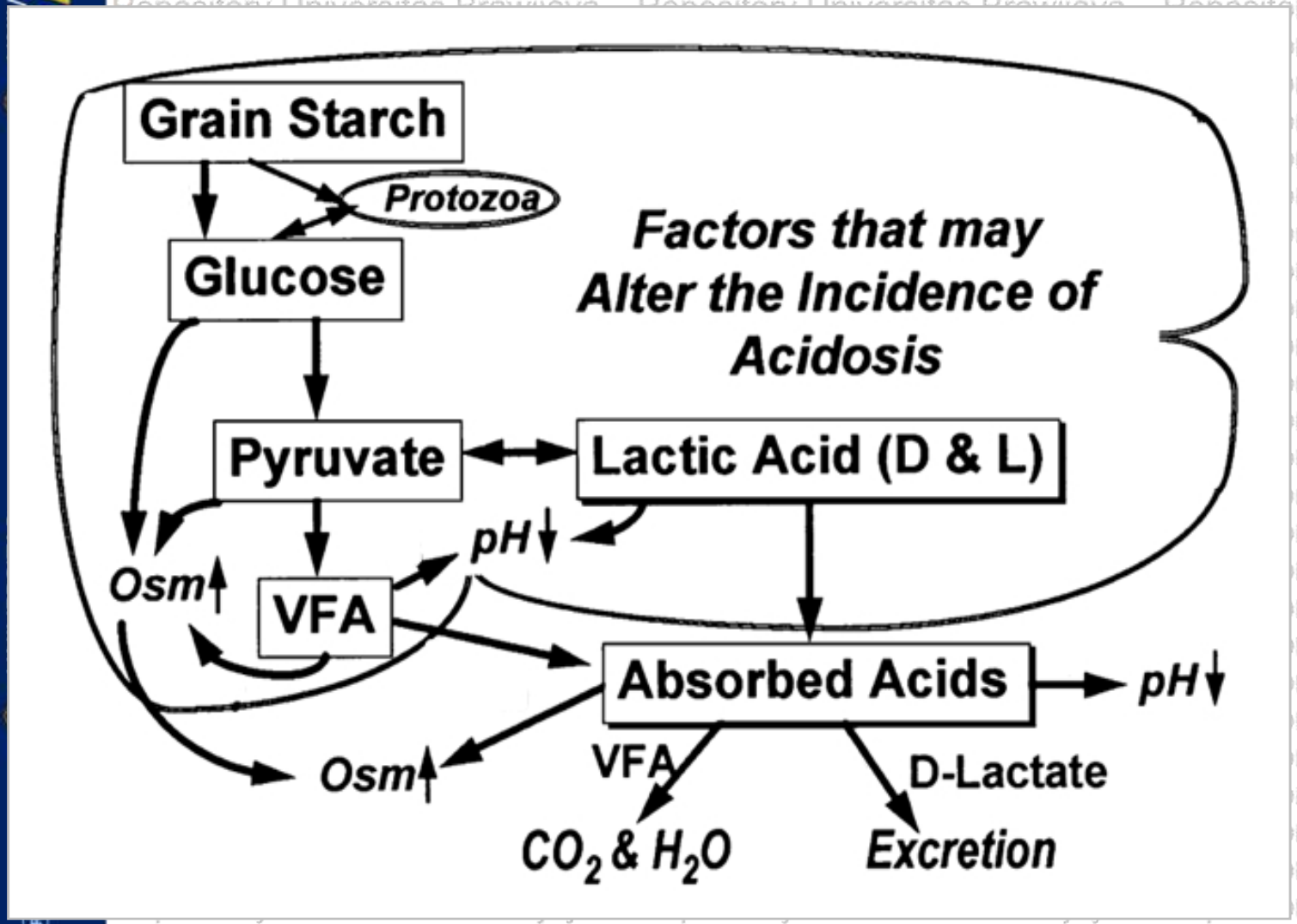
protozoa were 3.61, 6.42, and 8.03 x10^5/mL rumen liquid by levels 0%, 50%, and 75% of concentrate, respectively.

Those are several reasons why concentrate diets become very popular in most of feedlot or dairy farm as feed supplement to improve animal performance (meat or milk production). Even, in North America, finishing diets for feedlot cattle typically consist of 90% concentrate and 10% forage, based on DM (Cheng *et al*., 1998).

In contras, feeding high ratio of concentrate will decreases NDF and ADF concentration in the diets (Coleman and Henry, 2002). As a result, decreasing 1% of ADF concentration in the diets leads to declining in 0.0564 unit of rumen pH. Furthermore, when rumen pH below 6.3, ADF digestibility will declines by 3.6% per decreasing 0.1 unit of rumen pH, especially in dairy cows (Erdman, 1988). Declining of ADF digestibility in the rumen was a result of decreasing the activity of cellulolitic bacteria in the rumen by decreasing rumen pH (Martin *et al*., 2010).

High ratio of concentrate in the diets also increases amount of available energy in the rumen. An abundance energy in the rumen allows acid-tolerant bacteria (e.g., *Streptococcus bovis* and *Lactobacillus spp*.) to proliferate and produce excessive quantities of fermentation acids. As a result, rumen pH becomes exceedingly low, and this increase risk of ruminal acidosis (Cheng *et al*., 1998).

Ruminal acidosis is related to accumulation of fermentation acids in the rumen (acid production is higher than acid utilization). Terminologically, ruminal acidosis can be separated into two types, acute and chronic acidosis. Acute acidosis usually occurs when ruminal pH $\leq$ 5.2 and the animals look overt illness. Whereas chronic acidosis occurs when ruminal pH $\leq$ 5.6 and animals may not appear sick, but feed intake and performance are decreases.



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Both of acute and chronic acidosis, event the animals have been recovered, nutrients absorption may be still retarded (Owens *et al*., 1998).

Figure 1 explains about the reactions in acidosis of ruminants. This figure is cited from Owens *et al*. (1998). The principles of this figure are: when the animal consumes high amount of concentrate, an abundance of available energy will present in the rumen. Protozoa in the rumen can engulf available starch and glucose in small amount. Otherwise, bacteria ferment all available starch and glucose to produce fermentation acid (VFA and lactate). VFA and lactate are passively absorbed through the rumen epithelium. In abundance energy situation, acids fermentation accumulated in the rumen because of acids production which is higher than acids utilization or absorption. In this condition, lactate-producer bacteria (acid-tolerant bacteria such as *Streptococcus bovis* and lactobacilli) proliferate quickly but lactate-user bacteria (acid-intolerant bacteria such as *Selenomonas ruminantium* and *Megasphera alsdenii*) cannot grow well. Accumulation of mineral, glucose, VFA and lactate has increased rumen osmolality. High rumen osmolality affect on decreasing acids absorption rate through rumen epithelium. Rumen pH becomes exceedingly low because of acids production and accumulation in the rumen, especially lactic acid, which finally result in ruminal acidosis (Owens *et al*., 1998).

Another condition, in normal osmolality and high acids concentration in the rumen, rate of acids absorption to the blood is greater (Tabaru *et al*., 1990; Owens *et al*., 1998). Acids accumulation in the blood, especially D-lactate, causes a metabolic acidosis (Nocek, 1997). Both of ruminal acidosis and metabolic acidosis reduce animal performance.

Ruminal acidosis may cause reduction of cellulolytic bacteria and protozoa population, depress salivary excretion, decrease intestinal motility, reduce rumen turnover rate, and damage rumen, liver, and other tissues (Slyter, 1976). Moreover, even ruminant animal have been recovered from

acidosis, nutrients absorption may be retarded (Owens *et al*., 1998) because of permanently damage in important digestive organs.

In conclusions, acidosis can reduce the profitability of production by compromising animal performance (e.g., reduce feed intake, digestibility, and feed efficiency) and more directly by causing fatalities (Cheng *et al*., 1998). Understanding of energy metabolisms in the rumen is importance to prevent the occurrence of ruminal acidosis. Additional of MgO in the diets may be also reducing the incidence of acidosis by increases buffering capacity in the rumen.

2.4 MgO Supplementation

Buffer agents are materials when present in aqueous solution causes an effective resistance to change in pH of that solution when a strong acid or base is added. Addition of buffer agents in low forage diets are effective to increase rumen pH, rumen acetate:propionate molar ratio, and milk fat percent (Erdman, 1988).

MgO is a white hygroscopic solid mineral that occurs naturally as periclase and is a source of magnesium (Mg). MgO has potential as a buffer agent (Emery *et al*., 1986) and its role in the cellulolytic activity of rumen microorganisms is considerably important (Ammerman *et al*., 1971). MgO is very effective in raising rumen pH and milk fat percentage in dairy cow (Erdman, 1988). Addition of MgO in high concentrate diets alters rumen pH, liquid turnover, and patterns of rumen fermentation (Erdman *et al*., 1982)

Several previous studies showed that dietary MgO increased rumen pH (Erdman *et al*., 1980; Ermand *et al*., 1982; Peirce *et al*., 1983; Teh *et al*., 1985). Related to animal performances, addition of MgO in ruminant consuming high ratio concentrate diets increased DMI (Erdman *et al*., 1980; Ermand *et al*., 1982; Peirce *et al*., 1983; Teh *et al*., 1985; Martin *et al*., 2010),

digestibility of DM (Martin *et al*., 2010; Peirce *et al*., 1983), starch and NDF (Peirce *et al*., 1983), milk production (Erdman *et al*., 1980; Ermand *et al*., 1982; Teh *et al*., 1985; Martin *et al*., 2010), and milk fat (Ermand *et al*., 1982; Teh *et al*., 1985; Martin *et al*., 2010), but slightly decreased milk protein and total solid content (Martin *et al*., 2010).

Increasing milk production as a result of MgO supplementation is related to the increase of dry matter intake and digestibility. While, the increasing milk fat is related to fiber digestibility and consistently with the increment in the acetate to propionate ratio. Whereas, slightly decrease of protein and total solid content are related to high milk production (Martin *et al*., 2010).

Supplementation of MgO in the diets increases available magnesium (Mg) for microorganisms in the rumen. Rumen microorganisms require magnesium to catalyze many essential enzymes for cellular function. Feeding sheep on a semi-purified diet virtually devoid of magnesium rapidly impairs cellulolytic activity by the rumen microflora (Suttle, 2010).

Supplementation of MgO also increases level of serum Mg (concentration of Mg in the blood) (Erdman *et al*., 1980; Ermand *et al*., 1982). Serum Mg is very important for normal metabolism in ruminant animal. Serum Mg for normal cows should be about 1.7-4.0mg/100mL. Very low concentration of serum Mg in the blood causes symptom hypomagnesemic tetany such as nervousness, tremors, contractions in the face muscle, stiffness in the legs, and convulsions (McDonald *et al*., 1995). Additional Mg urgently required when serum Mg below 0.60 mmol/L, especially for lactating ewes (Treacher and Caja, 2002).

Those all potentials have shown that dietary MgO was effective to reduce risk of ruminal acidosis in ruminant consuming high ratio concentrate

diets without any compromising in animal performance and economic efficiency, even improve animal performances.

2.5 Lysine supplementation

Lysine is an essential amino acid for ruminant animal as well as leucine, isoleucine, methionine, phenylalanine, threonine, tryptophan, and valine (Annison *et al*., 2002). Although rumen protozoa can synthesize and provide amino acids for host animals (Stevenson 1978; Onodera, 1993; Schingoethe, 1994), but lysine often becomes the first-limiting amino acid in many metabolic reactions.

Lysine metabolism in the rumen was explained by Onodera (1993) through Figure 2. Rumen protozoa are not only effective to synthesize lysine from free 2,6 diaminopimelate (DAP), but also from DAP bound in rumen bacterial cell wall. DAP is effectively synthesized by rumen bacteria from aspartate and incorporated in the peptidoglycan of bacteria cell wall. This condition leads to synthesis lysine by bacteria is not quite effective, because peptidoglycans are resistant to pepsin and trypsin, hence DAP becomes undegraded by ruminant animals. In contrast, protozoa have a poor capability to synthesize DAP, but they can utilize DAP-containing peptidoglycan from bacteria cell wall to synthesis lysine effectively.

Lysine in the rumen is degraded by bacteria to produce 1 mol of acetate and butyrate, and 2 moles of ammonia. Acetate and butyrate are energy sources for ruminant, while the ammonia is nitrogen source for synthesize of microbial cell. Lysine degradation by protozoa also produces pipecolate. Pipecolate has importance neorophysiological role in mammalian brain. Pipecolate controls GABA (γ-aminobutyric acid) in cerebral cortex slices. GABA is known as an inhibitory neurotransmitter in central nervous system in animals (Onodera, 1993).

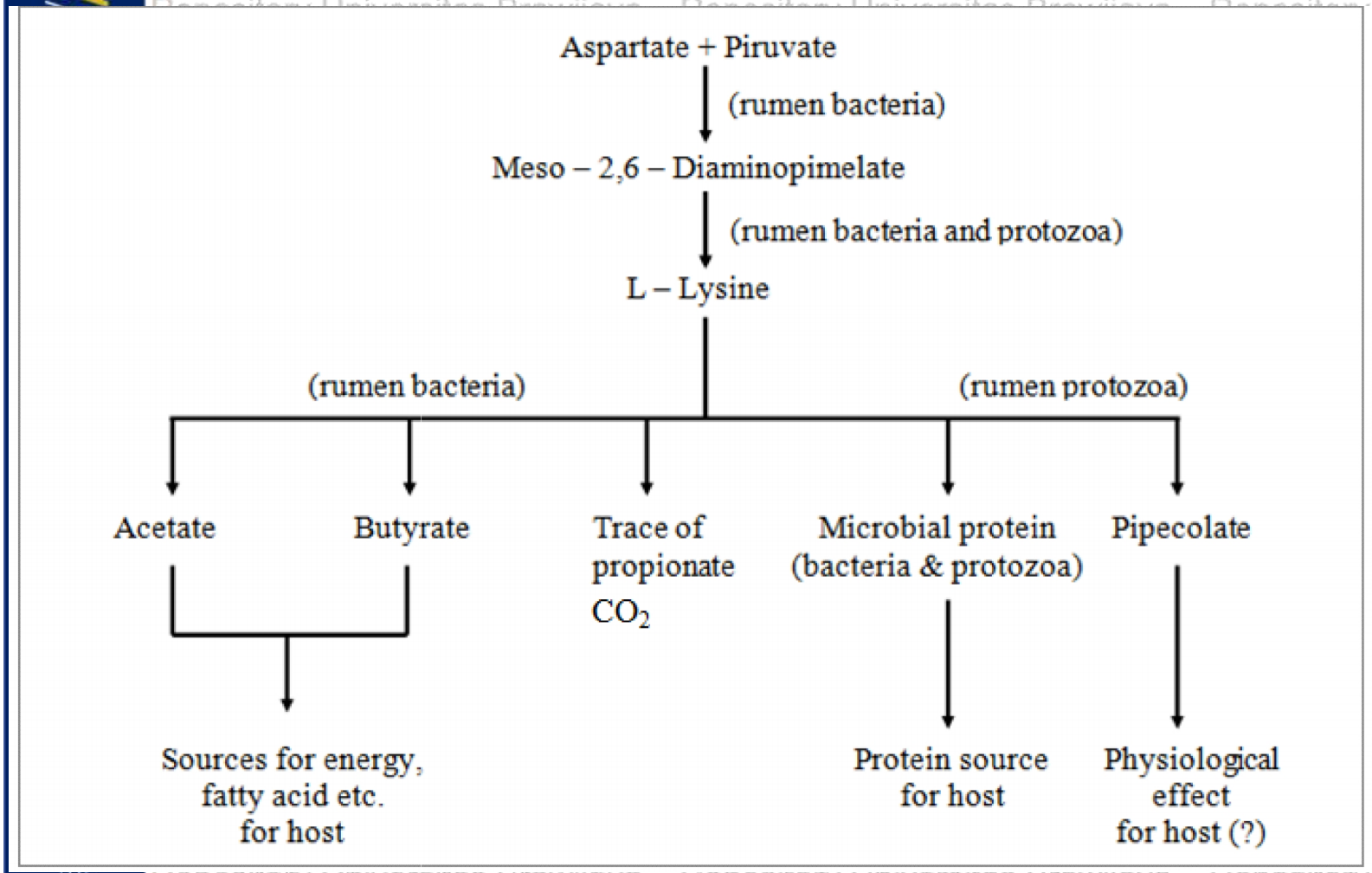

Figure 2 L-lysine metabolism in the rumen and rules of its metabolites in host animal (Onodera,1993)

Amino acids for ruminant are fulfilled from the microbial protein, UDP, and endogenous secretion. These amino acids are absorbed in the intestinal track (Schingoethe, 1994). Rumen microorganism can supply around 81.2 to 115.5 grams of lysine/kg true protein (Storm and Ørskov, 1983). Protozoa contain higher lysine than bacteria (Bergen *et al*., 1968). To optimize the utilization of dietary amino acids for ruminant, amino acids supplementation must be in the form of by-pass protein or UDP (Bandyk *et al*., 2001).

Study in lactating dairy cow by Chung *et al*. (2006) indicated that total VFA, concentration of butyrate, and NH_4^+ increased by supplementation L-lysine-HCl in the diets. It was compatible with Onodera (1993), NH_4^+, butyrate, and acetate were generated form lysine digestibility by rumen microbes. In contrast, study by Bernard *et al*. (2004) observed that there were no any differences on ruminal pH, concentrations of NH_3, total VFA, and proportions of individual VFA with supplementation of 10 g/d of L-lysine-HCL to the total mixed rations. This may be because of deamination of L-lysine-HCL becomes UDP and available for absorption in the intestines.

In conclusions, high ratio of concentrate diets and supplementation of MgO or/and lysine may affect to the pattern of feed fermentation in the rumen. Further studies need to be carried out to learn about the effects of additional MgO and lysine on rumen characteristic, related to high ratio of concentrate diets.

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Table 4 Ingredients and chemical composition of the concentrate

Item	
Ingredient, % of DM	
Corn meal	65
Soybean meal	15
Cracked corn	5
Wheat brand	10
Tallow	0.5
$CaCO_3$	2
NaCl	1
$NaHCO_3$	1
Premix	0.5
Chemical composition	
DM	89%
CP	16.25%
ME	3 Mcal/Kg
NEL	1.95 Mcal/Kg
NDF	15.80%
NFC	60%
FAT	3.75%
UDP	35%

ME Metabolizable energy
NE_L Net energy for lactation
NDF Neutral detergent fiber
NFC Non fiber carbohydrate
EE Extract ether
UDP Undegradable dietary protein

measured from rumen liquid samples that were daily collected at one hour after morning feeding.

3.1.4 Statistical analysis

The data were statistically analyzed using a general linear model (GLM) procedure of SAS for statistical analysis package program. The significant different among the data were further tested using Duncan's multiple range test, which was set at $P < 0.05$.

3.2 Experiment 2

3.2.1 Time and location

This study was carried out from June 2012 until September 2012 (in period length of 75 days). The experiment was conducted in the Innovation and Practical Training Center (IPTC), Animal Science Department, National Pingtung University of Science and Technology, Taiwan.

3.2.2 Materials and methods

The materials used were five male cannulated Black Belly sheep housed in individual pens. The sheep were fed TMR (total mixed ration) which consist of 60% pangola grass (*Digitaria eriantha*) hay and 40% concentrate, in DM basis. Three percentages of molasses and water were added to increase palatability and water content of the level approximately 60%. The ingredients and chemical compositions of concentrate were shown in Table 4.

The treatments were 5 levels of MgO (0%, 0.2%, 0.4%, 0.8%, and 1.2%) and 3 levels of lysine (0%, 0.25%, and 0.5%):

T1 = Basal feed + 0% MgO + 0% lysine

T2 = Basal feed + 0.2% MgO + 0% lysine

T3 = Basal feed + 0.4% MgO + 0% lysine

T4 = Basal feed + 0.8% MgO + 0% lysine
T5 = Basal feed + 1.2% MgO + 0% lysine
T6 = Basal feed + 0% MgO + 0.25% lysine
T7 = Basal feed + 0.2% MgO + 0.25% lysine
T8 = Basal feed + 0.4% MgO + 0.25% lysine
T9 = Basal feed + 0.8% MgO + 0.25% lysine
T10 = Basal feed + 1.2% MgO + 0.25% lysine
T11 = Basal feed + 0% MgO + 0.50% lysine
T12 = Basal feed + 0.2% MgO + 0.50% lysine
T13 = Basal feed + 0.4% MgO + 0.50% lysine
T14 = Basal feed + 0.8% MgO + 0.50% lysine
T15 = Basal feed + 1.2% MgO + 0.50% lysine

Each treatment was conducted in 5 days. The feeds were offered twice a day, at 07.00 AM and 07.00 PM, at restricted feeding of 1,050 g of DM/head/day, and drinking water was provided *at libitum* in free access.

3.2.3 Variables

Variables measured in this experiment were rumen condition, including rumen temperature, pH, conductivity, ORP, concentration of NH_4^+, VFA and amino acids, and population of protozoa and bacteria. The variables were measured from rumen liquid samples that were daily collected at one hour after morning feeding. In addition, concentration of VFA and amino acids in the rumen liquid were measured from rumen liquid samples that were collected every five days at one hour after morning feeding.

3.2.4 Statistical analysis

The data were statistically analyzed using a general linear model (GLM) procedure of SAS for statistical analysis package program. The significant different among the data were further tested using Duncan's multiple range test, which was set at $P < 0.05$.

3.3 Analysis methods

Rumen temperature was daily measured at one hour after morning feeding by reading CYFROWY digital thermometer which accuracy of ± 0.1° C where it electrode was inserted into the rumen via rumen cannula. Then, rumen liquid samples were collected via rumen cannula for pH, conductivity, ORP, concentration of NH_4^+, VFA and AA, and population of bacteria and protozoa measurement.

Rumen liquid pH was immediately measured using pH meter, WTW/315i (pH electrode SenTix 61) with accuracy of ± 0.01. Conductivity, ORP, and NH_4^+ concentration were immediately measured from the samples using OAKTON® Ion 6 Acorn series, after the samples were diluted 100 times using pure water.

Population of protozoa was measured manually using counting chamber (hemocytometer) branded Assistant and/or Marienfeld equipped by microscope "Nikon" model YS100. Sample of rumen liquid was diluted 10 times in pure water and put on counting chamber. Protozoa population was counted under a microscope with a magnification of 400 times (10 x 40).

Bacteria population was measured after inoculated 48 hours on plate count agar (PCA) media. PCA powder (23.5 g) was dissolved in 1000 mL of pure water. PCA solution was heated and sterilized by using autoclave for 15 minutes (121ºC, 1.2 kg/cm^2). PCA solution was poured on disposable petri dish 9 cm in diameter (± 20 mL/petri). Petri dish was slightly opened to allow moisture moved out and allowed to stand until PCA solution change into gel (PCA media). A hundred micro liter of rumen liquid was diluted in 0.9 mL sterile pure water = 10^{-1}, then a hundred micro liter of the diluted rumen liquid was further diluted in 0.9 mL sterile pure water = 10^{-2}, then further gradually diluting process was continued to get diluted rumen liquid of 10^{-5}, 10^{-6}, and 10^{-7} concentration. Twenty five micro liter of diluted rumen liquid

sample of 10^{-5}, 10^{-6}, and 10^{-7} concentration were inoculated in PCA media and incubated at 37.6 ^{o}C. Bacteria population was determined from the number of colony observed on PCA media after 48 hours incubation.

Amino acids content of rumen liquid samples were measured using high performance liquid chromatography (HPLC) Agilent Technologies 1200 series. For those rumen liquid samples were dried using freeze dryer (EYELA FDU-1200 Bennet®) at -50° C for 5 days. Dried rumen liquid was subjected to acid hydroysis. As much as 0.1 gram of the dried rumen liquid was mixed with 1.5 cc of DTDPA 2% and 1.0 cc of HCL Phenol in a ceramic crucibles, then it was put in the vacuum pump (model: multipose® 700 mm Hg Vac) for 3 minutes to aspirate the air out. The samples were subsequently heated 0n infrared heater plate in a wet digester (BUCHI wet Digester B-400, SUNTEX®) for 24 hours at 110 °C. The wet digester was connected to a BUCHI Scrubber B-414 for extraction and neutralizaion of acid fumes and toxic reaction gases. After acid hydrolysis, the sample was adjusted to pH 2.2 ± 0.1 by adding NaOH (4N) and/or sodium citrate and standardized to a volume of up to 50 mL by adding HPLC buffer, pH 2.2. Cooled hydrolysates were then filtered through filter paper (AVANTEC LOT, 110 mm). Then, about 1.5 cc of the filtered hydrolysates were injected using glass stringe into Agilent auto sampler vials tightly fitted with blue vial screw caps and septa. The vials were then placed in the HPLC auto sampler for the separation of the various amino acids content.

Rumen liquid samples for VFA analysis were centrifuged (30 x 100 rpm for 20 minutes) in order to obtain the supernatant. The supernatant was then analyzed for VFA concentration using gas chromatography of Hawlett Packard model 5890 Series II fitted with auto sampler and a flame ionization detector (Agilent Technologies Inc., Wilmington, DE). Individual VFA was separated by using a fuse silica capillary column (30 m x 0.53 mm ID, 1 µm film thickness). One mL of 30 mM 4-methylvaleric was used as an internal

standard and analyzed prior to the samples analysis. A split injection (51:1) of 0.5 µl was used. Injector and detector temperature were 250 °C. Initial oven temperature was set at 125 °C for 5 minutes and increased to 180 °C at a rate of 15 °C/minute, and set 6 minutes. The total run time should be 16 minutes per sample. High purity methanol was carried by gas with a flow rate of 4.2 mL/minute. Inlet pressure was kept in constant. A Chem Data Station was used for integrating and qualificating of individual VFA (Agilent Technologies Inc., Wilmington, DE).

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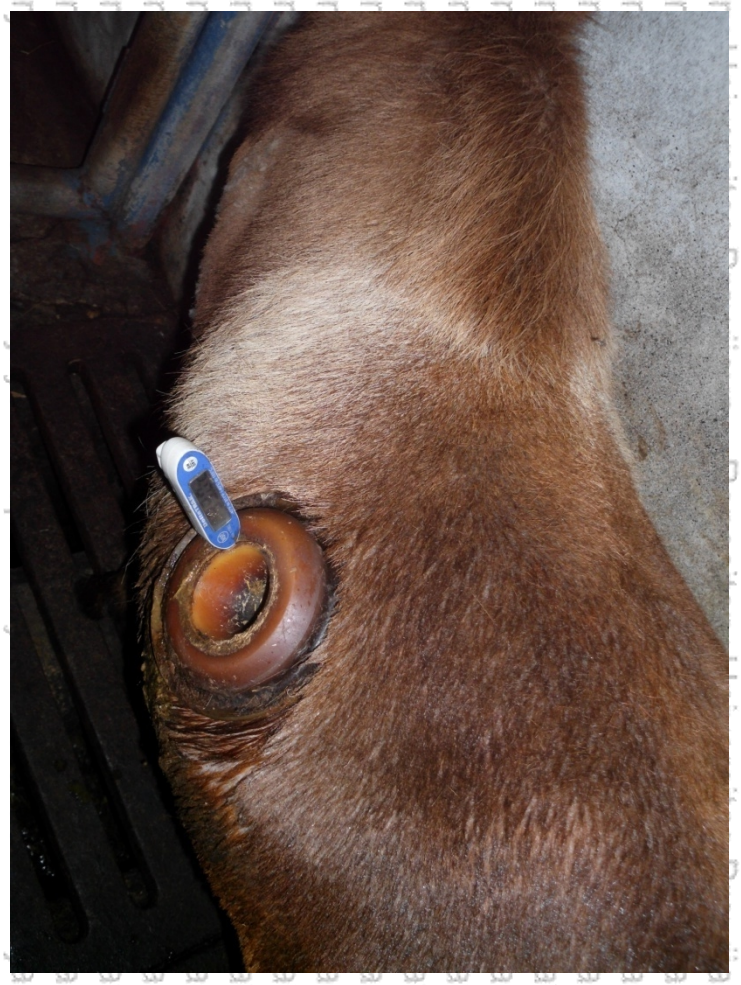

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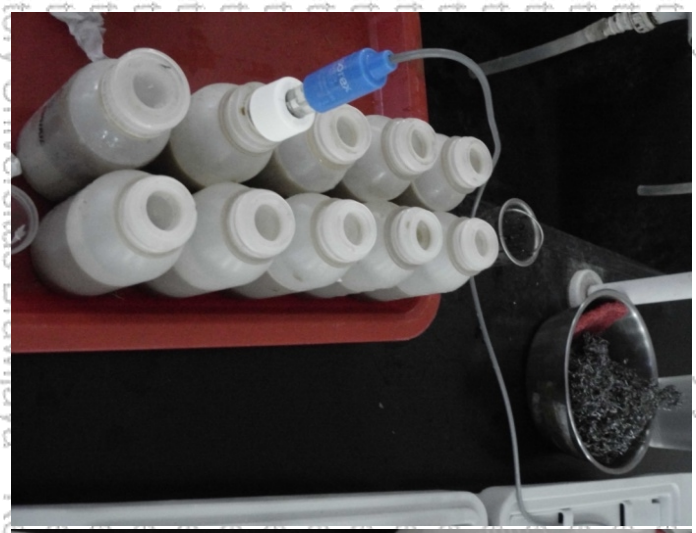

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Table 5 The effect of different level of concentrate in the diets on rumen liquid condition

Item	Diet[1]			SEM[2]	Sign
	T1	T2	T3		
Temperature (^oC)	38.32	38.44	38.65	0.11	NS
pH	7.10^a	6.96^b	6.77^c	0.03	***
Conductivity (µS/cm)	160.70^b	171.34^a	172.59^a	1.98	***
ORP (mV)	-24.90	-30.43	-36.57	7.92	NS
NH_4^+ (mg/100 mL)	22.14^b	25.81^a	27.90^a	0.08	***
Protozoa (x 10^6 cell/mL)	1.14^c	2.26^b	3.05^a	0.27	***
Bacteria (x 10^8 cfu/mL)	3.00^b	4.82^{ab}	6.49^a	0.08	*
Total VFA (mg/L)	417.38^c	494.25^b	540.14^c	15.73	***
Total amino acids (mg/ 100mL)	44.83^b	57.10^{ab}	71.25^a	5.68	**

[1] = Forage:concentrate, T1 = 100:0, T2 = 80:20, and T3 = 60:40

[2] = n=21except for temperature (n=16)

a-c = Means with different superscripts in the same row differ significantly, $P < 0.05$

NS = $P > 0.05$

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

Table 6 The effect of different level of concentrate in the diets on individual FVA of rumen liquid

Item	Diet[1]			SEM[2]	Sign
	T1	T2	T3		
Total VFA, mg/L	417.38[c]	494.25[b]	540.14[a]	15.73	***
Acetate, mg/L	262.85[b]	301.98[a]	320.73[a]	10.76	**
Propionate, mg/L	77.04[c]	89.57[b]	101.68[a]	2.97	***
Isobutyrate, mg/L	6.02[b]	7.97[a]	8.49[a]	0.34	***
Butyrate, mg/L	31.39[c]	47.32[b]	58.50[a]	1.93	***
Isovalerate, mg/L	22.30[c]	25.74[b]	27.28[a]	0.53	***
Valerate, mg/L	18.68[c]	21.67[b]	23.46[a]	0.332	***
Acetate:Propionate	3.41[a]	3.38[a]	3.15[b]	0.05	**

[1] = Forage:concentrate, T1 = 100:0, T2 = 80:20, and T3 = 60:40

[2] = n=21 except for valerate at T1 (n=20); SEM calculated using n=20

[a-c] = Means with different superscripts in the same row differ significantly, $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

Table 7 The effect of different level of concentrate in the diets on amino acids profile of rumen liquid (mg/100 mL)

Item	Diet[1]			SEM[2]	Sign
	T1	T2	T3		
Total	44.83[b]	57.10[ab]	71.25[a]	5.68	**
Asparatic acid	3.89[c]	5.36[b]	6.44[a]	0.28	***
Glutamic acid	4.26[c]	5.73[b]	7.99[a]	0.37	***
Serine	1.80[c]	2.28[b]	3.10[a]	0.15	***
Histidine	1.72[b]	2.17[ab]	2.85[a]	0.26	*
Glycine	2.10[b]	2.61[b]	3.48[a]	0.22	***
Threonine	2.28[b]	2.92[b]	3.78[a]	0.24	***
Arginine	2.33[b]	2.67[b]	3.97[a]	0.30	***
Alanine	3.25[c]	4.23[b]	5.61[a]	0.31	***
Tyrosine	2.17[b]	2.96[ab]	3.75[a]	0.43	*
Cysteine	1.46	1.82	2.18	0.33	NS
Valine	2.42	2.95	4.08	0.63	NS
Methionine	2.46	3.23	3.42	0.73	NS
Phenylalanine	2.95	3.45	3.91	0.64	NS
Isoleucine	1.89	2.50	2.83	0.34	NS
Leucine	4.46	5.63	6.65	0.75	NS
Lysine	3.77	4.39	4.91	0.58	NS
Proline	2.19	2.73	3.29	0.36	NS

[1] = Forage:concentrate, T1 = 100:0, T2 = 80:20, and T3 = 60:40

[2] = n=21 except for glutamic acid at T1 and T3, histidine and methionine at T2 and T3, and phenylalanine at T1 (n=20), SEM calculated using n=20; proline n=19

[a-c] = Means with different superscripts in the same row differ significantly, $P < 0.05$

NS = $P > 0.05$; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$

Table 8 Effect of daily feeding on rumen liquid condition

Item	Day[1]							SEM[2]	Sign[3]
	1	2	3	4	5	6	7		
Temperature (^oC)	38.63	38.53	38.45	38.22	38.34	38.55	38.75	0.17	NS
pH	6.93	6.92	6.95	7.01	6.94	6.90	6.94	0.04	NS
Conductivity (uS/cm)	163.97	166.97	166.80	169.07	167.82	170.48	172.36	3.02	NS
ORP (mV)	-27.44	-17.33	-24.22	-25.22	-34.67	-34.22	-51.33	12.09	NS
NH_4^+ (mg/100 mL)	24.33	26.22	24.67	25.00	23.78	26.00	27.00	0.12	NS
Protozoa (x 10^6 cell/mL)	1.89	1.89	1.78	2.28	2.11	2.83	2.28	0.42	NS
Bacteria (x 10^8 cfu/mL)	3.24	4.10	6.50	4.50	4.86	5.43	4.73	0.12	NS
Total VFA (mg/L)	453.49	481.69	507.98	473.16	505.47	487.45	478.24	24.03	NS
Total amino acids (mg/100 mL)	62.09	64.27	69.31	58.41	57.19	41.03	51.79	8.67	NS

[1] = The averages of periods 1, 2, and 3

[2] = n=9 except for temperature at day 1, 2, 3, 6, and 7 (n=6); SEM calculated using n=6

[3] = Level of significance $P < 0.05$

NS = $P > 0.05$

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Table 9 Effect of daily feeding on individual VFA of rumen liquid

Item	Day[1]							SEM[2]	Sign[3]
	1	2	3	4	5	6	7		
Total VFA, mg/L	453.49	481.69	507.98	473.16	505.47	487.45	478.24	24.03	NS
Acetate, mg/L	273.05	292.01	314.51	292.85	306.69	293.60	293.59	16.44	NS
Propionate, mg/L	88.69	89.18	91.93	85.11	92.86	91.41	86.81	4.53	NS
Isobutyrate, mg/L	6.63	7.13	7.71	7.66	8.31	7.87	7.14	0.52	NS
Butyrate, mg/L	40.84	48.12	48.02	43.05	48.63	47.19	44.32	2.95	NS
Isovalerate, mg/L	23.50	24.42	24.83	25.48	26.91	25.66	24.93	0.81	NS
Valerate, mg/L	20.78	20.82	20.97	21.38	22.07	21.72	21.45	0.51	NS
Acetate:Propionate	3.12	3.29	3.45	3.46	3.29	3.23	3.39	0.08	NS

[1] = The averages of periods 1, 2, and 3

[2] = n=9 except for valerate at day 4 (n=8); SEM calculated using n=8

[3] = Level of significance $P < 0.05$

NS = $P > 0.05$

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Table 10 Effect of daily feeding on amino acids profile of rumen liquid (mg/100 mL)

Item	Day[1]							SEM[2]	Sign
	1	2	3	4	5	6	7		
Total	62.09	64.27	69.31	58.41	57.19	41.03	51.79	8.67	NS
Asparatic acid	5.69^{ab}	6.05^{a}	5.59^{ab}	5.73^{ab}	5.17^{abc}	3.97^{c}	4.43^{bc}	0.43	*
Glutamic acid	6.36^{ab}	5.77^{abc}	6.88^{a}	7.15^{a}	6.28^{ab}	4.19^{c}	4.96^{bc}	0.59	*
Serine	2.50^{ab}	2.51^{ab}	2.74^{a}	2.78^{a}	2.46^{ab}	1.75^{c}	2.00^{bc}	0.23	*
Histidine	2.15	3.12	2.02	2.39	2.55	1.66	1.66	0.41	NS
Glycine	2.86	3.17	2.92	3.00	2.64	2.11	2.39	0.34	NS
Threonine	3.16^{ab}	3.22^{ab}	3.50^{a}	3.56^{a}	3.21^{ab}	2.19^{b}	2.10^{b}	0.37	*
Arginine	3.45	2.96	3.52	3.33	3.15	2.30	2.22	0.45	NS
Alanine	4.82^{ab}	4.92^{ab}	5.23^{a}	4.66^{ab}	4.27^{abc}	3.07^{c}	3.59^{bc}	0.47	*
Tyrosine	3.30	3.96	3.87	2.64	2.69	1.77	2.47	0.66	NS
Cysteine	1.98	1.98	1.85	1.51	1.79	1.37	2.23	0.50	NS
Valine	3.50	3.25	4.48	2.66	2.73	1.84	3.58	0.96	NS
Methionine	3.74	4.15	3.72	2.17	2.29	1.92	3.17	1.15	NS
Phenylalanine	3.91	4.05	4.11	2.84	2.76	2.66	3.70	1.01	NS
Isoleucine	2.54	2.48	3.02	2.40	2.54	1.62	2.25	0.53	NS
Leucine	5.70	5.77	6.94	5.23	5.91	4.58	4.93	1.14	NS

Table 10 Continued

Item	Day[1]							SEM[2]	Sign
	1	2	3	4	5	6	7		
Lysine	4.19	4.71	6.40	4.34	4.44	2.99	3.44	0.88	NS
Proline	3.97	3.32	2.52	2.02	2.30	2.21	3.40	0.64	NS

[1] = The averages of periods 1, 2, and 3

[2] = n=9 except for glutamic acid and histidine at day 6 and 7, methionine at day 1 and 6, and phenylalanine at day 6 (n=8), SEM calculated using n=8; proline at day 1 dan 2 n=6, SEM calculated using n=6

[a-b] = Means with different superscripts in the same row differ significantly, $P < 0.05$

NS = $P > 0.05$

* = $P < 0.05$

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Ruminal ORP is a measure of microbial activity in degrading feed in the an aerobic condition of rumen. In an aerobic condition of rumen, degradation of feed in the rumen take mostly place through reduction processes by rumen microbes' activity rather than oxidation process. Hence, lower ORP value indicates higher microbial activity. Different level of concentrate in the diets did not significantly affect ORP values of rumen liquid. But, because of concentrate is generally easier to be degraded by rumen microbes than forage, which then the higher proportion of concentrate in the diets, average of ruminal ORP tends to decrease doe to increasing of rumen microbes activity. Concentrate diets might provide more energy to improve microbial activity in the rumen (Misrah *et al*., 1970).

Ammonia in rumen liquid was in the proton form NH_4^+ (ammonium). Rate and amounts of ammonia production reflected the solubility and fermentability of dietary protein. NH_4^+ concentration increased significantly ($P < 0.05$) with the increasing level of concentrate in the diets. Concentrate used in this experiment contained 16.25% CP (Table 4), which was higher than forages (Napier grass contains CP < 9%, Table 2). Increment of the level of concentrate increased the concentration of CP in the diets. High available protein increases potential to release NH_4^+ in the rumen.

The population of protozoa and bacteria in rumen liquid increased significantly ($P < 0.05$) by increasing of the level of concentrate in the diets. Compared with forages, concentrate contained more energy and CP. Increment of concentrate level in the diets increased availability of energy and nitrogen (NH_4^+) for microbial growth in the rumen. High availability of energy and nitrogen allowed the rumen microbes to proliferate well.

VFA is a main energy source for ruminant (Onodera, 1993). Anaerobic microbes in the rumen produce VFA from carbohydrate (Owens *et al*., 1998). Concentrate contains more carbohydrate than forage. Hence, increasing the

level of concentrate in diets provides more carbohydrate available in the rumen and rumen microbes will produce more VFA (Table 5). Individual VFA, such as acetate, propionate, isobutyrate, butyrate, isovalerate, and valerate (Table 6) were significantly ($P < 0.05$) increase by increasing the level of concentrate in the diets. In contrast, acetate:propionate ratio significantly ($P < 0.05$) decrease by increasing level of concentrate. Acetate produces from fiber and propionate produces from starch. Compare to forage, concentrate contains more starch and less fiber. Hence, the increasing level of concentrate in the diet increases propionate production by rumen microbes.

Total amino acid significantly ($P < 0.05$) increased by increasing level concentrate in the diets. Concentrate contains more substrate for microbial growth. Increment of concentrate level in the diets stimulates rumen microbes to proliferate well. Rumen microbes are protein sources for host animal (Onodera 1993). Rumen microbes provide high digestibility and more balance amino acids for host animal (Leng, 1991).

Table 7 shows the effect of different level of concentrate in the diet on amino acids profile of rumen liquid. Nine of seventeen individual amino acids (asparatic acid, glutamic acid, serine, histidine, glycine, threonine, arginine, alanine, and tyrosine) were significantly ($P < 0.05$) increased by increasing level of concentrate in the diets. Other amino acids (cysteine, valine, methionine, phenylalanine, isolleucine, leucine, lysine, and proline) even statistically not significant ($P > 0.05$), but their number in the rumen liquid tended to increase by increasing level of concentrate.

Based on this study, condition of rumen liquid was not influenced by daily sampling (Table 8, 9, 10). There did not different significantly ($P > 0.05$) between daily sampling on all variables, except for amino acids profile at asparatic acid, glutamic acid, serine, threonine, and alanine. Condition of rumen liquid was more affected by feed intake rather than daily sampling. In

this experiment, sheep in each treatment was given same amount and type of feed during observation (day 1 to 7) in each period.

4.2 Experiment 2

The significance effects of supplementation of MgO and lysine in the high ratio concentrate diets were shown in Table 11, 12, and 13. Related to rumen liquid condition, supplementation of MgO in high ratio concentrate diets significantly ($P < 0.05$) affected on rumen temperature, pH, conductivity, ORP, NH_4^+, number of protozoa and bacteria, and total VFA, but not significantly ($P > 0.05$) influenced on total amino acids. Lysine supplementation in high ratio concentrate diets significantly ($P < 0.05$) affected on rumen temperature, conductivity, ORP, NH_4^+, and number of protozoa and bacteria, but not significantly ($P > 0.05$) influenced on ruminal pH, and total of VFA and amino acids. In the other hand, interaction between MgO and lysine supplementation significantly ($P < 0.05$) effect on rumen temperature, pH, conductivity, ORP, NH_4^+, and bacteria number, but not significantly ($P > 0.05$) affected on population of protozoa and total of VFA and amino acids.

Supplementation of MgO and lysine in high ratio concentrate diets slightly affected individual VFA of rumen liquid. Significantly ($P < 0.05$) effects only appear on average value of acetate and propionate by MgO treatment and acetate:propionate ratio by lysine treatment. Even, interaction between MgO and lysine supplementation not significantly ($P > 0.05$) influenced on individual VFA of rumen liquid.

As well as individual VFA, amino acids profile of rumen liquid was slightly affected by supplementation of MgO and lysine. Treatment of MgO significantly ($P < 0.05$) affected lysine. Treatment of lysine significantly ($P < 0.05$) affected on histidine, lysine, and proline. Interaction between MgO and lysine supplementation significantly ($P < 0.05$) affected average of proline.

Table 11 Significance effects of supplementation of MgO and lysine in high ratio concentrate diets on rumen liquid condition

Item	Effects		
	MgO	Lysine	MgO x lysine
Temperature	***	***	***
pH	***	NS	*
Conductivity	***	***	***
ORP	**	***	***
NH_4^+	***	***	***
Protozoa	**	***	NS
Bacteria	**	***	***
Total VFA	*	NS	NS
Total amino acids	NS	NS	NS

NS = P > 0.05; * = P < 0.05; ** = P < 0.01; *** = P < 0.001

Table 12 Significance effects of supplementation of MgO and lysine in high ratio concentrate diets on individual VFA of rumen liquid

Item	Effects		
	MgO	Lysine	MgO x lysine
Total VFA	*	NS	NS
Acetate	*	NS	NS
Propionate	*	NS	NS
Isobutyrate	NS	NS	NS
Butyrate	NS	NS	NS
Isovalerate	NS	NS	NS
Valerate	NS	NS	NS
Acetate:Propionate	NS	*	NS

NS = P > 0.05; * = P < 0.05; ** = P < 0.01; *** = P < 0.001

Table 13 Significance effects of supplementation of MgO and lysine in high ratio concentrate diets on amino acids profile of rumen liquid

Item	Effects		
	MgO	Lysine	MgO x lysine
Total	NS	NS	NS
Asparatic acid	NS	NS	NS
Glutamic acid	NS	NS	NS
Serine	NS	NS	NS
Histidine	NS	***	NS
Glycine	NS	NS	NS
Threonine	NS	NS	NS
Arginine	NS	NS	NS
Alanine	NS	NS	NS
Tyrosine	NS	NS	NS
Cysteine	NS	NS	NS
Valine	NS	NS	NS
Methionine	NS	NS	NS
Phenylalanine	NS	NS	NS
Isoleucine	NS	NS	NS
Leucine	NS	NS	NS
Lysine	*	*	NS
Proline	NS	**	***

NS = P > 0.05
* = P < 0.05
** = P < 0.01
*** = P < 0.001

4.2.1 MgO supplementation

Table 14 shows the effects of MgO supplementation in high ratio concentrate diets on rumen liquid condition. Ruminal temperature was significantly ($P < 0.05$) decreased by MgO treatments, except for supplementation of MgO at level 0.4%. Compared with temperature values of 0 and 0.2% MgO, supplementation of 0.4% MgO has increased average of ruminal temperature. It indicated that fermentation activity in treatment 0.4% MgO was higher than treatments 0 and 0.2% MgO. Moreover, supplementation of MgO in levels of higher than 0.4% showed lower average of ruminal temperature. It may be because of supplementation of MgO in the diets increase mineral and osmolality in the rumen. High osmolality inhibits digestion of fiber and starch (Owens *et al*., 1998).

Ruminal pH was significantly ($P < 0.05$) increased by supplementation of MgO. Based on this study, it was dose-dependently increased by MgO supplementation until the concentration of 0.8% and was decreased afterward. MgO is a very effective buffer in rising ruminal pH (Erdman, 1988). But, decreasing ruminal pH by treatment 1.2% MgO may be because of rumen osmolality. High osmolality decreases acids absorption (Owens *et al*., 1998). In this condition, acids production and acids absorption were in imbalance condition.

As well as minerals ($CaCO_3$, NaCl, and $NaHCO_3$) which compose concentrate diet for this experiment, MgO is also a mineral salt which is capable to increase conductivity of rumen liquid. In this study, conductivity was significantly ($P < 0.05$) affected by MgO treatments. Increasing the level of MgO in the diets has increased ruminal conductivity.

ORP was significantly ($P < 0.05$) affected by MgO treatments. But, its effect was not dose-dependently. The lowest value was achieved by supplementation of 0.2% MgO, it meant that in this treatment, microbial

Table 14 The effects of MgO supplementation in high ratio concentrate diets on rumen liquid condition

Item	Level of MgO					SEM	Sign
	0%	0.2%	0.4%	0.8%	1.2%		
Temperature (°C)	39.23^{a}	39.23^{a}	39.30^{a}	39.20^{a}	39.06^{b}	0.04	***
pH	6.47^{e}	6.62^{d}	6.70^{c}	6.84^{a}	6.77^{b}	0.02	***
Conductivity (µS/cm)	165.22^{b}	167.01^{b}	167.33^{b}	172.18^{a}	173.82^{a}	1.27	***
ORP (mV)	-58.51^{bc}	-61.04^{c}	-54.25^{ab}	-52.60^{a}	-54.51^{ab}	1.79	**
NH_4^+ (mg/100 mL)	48.00^{a}	35.43^{c}	38.63^{b}	37.57^{bc}	36.55^{bc}	0.87	***
Protozoa (x 10^6 cell/mL)	5.29^{ab}	5.50^{a}	4.56^{bc}	4.30^{c}	5.03^{abc}	0.25	**
Bacteria (x 10^8 cfu/mL)	3.90^{a}	2.95^{b}	2.60^{b}	2.80^{b}	2.85^{b}	0.27	**
Total VFA (mg/L)	647.30^{ab}	607.33^{b}	603.08^{b}	570.05^{b}	698.83^{a}	28.49	*
Total amino acids (mg/100 mL)	74.19	66.45	67.57	72.32	63.46	5.31	NS

$^{a-e}$ = Means with different superscripts in the same row differ significantly, $P < 0.05$

NS = $P > 0.05$

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

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Table 15 The effects of MgO supplementation in high ratio concentrate diets on individual VFA of rumen liquid

Item	Level of MgO					SEM	Sign
	0%	0.2%	0.4%	0.8%	1.2%		
Total VFA, mg/L	647.30^{ab}	607.33^{b}	603.08^{b}	570.05^{b}	698.83^{a}	28.49	*
Acetate, mg/L	374.52^{ab}	347.16^{b}	349.02^{b}	325.54^{b}	402.86^{a}	16.55	*
Propionate, mg/L	121.67^{ab}	113.82^{b}	111.03^{b}	108.26^{b}	132.78^{a}	5.83	*
Isobutyrate, mg/L	6.53	6.66	6.36	6.19	6.90	0.38	NS
Butyrate, mg/L	93.76	88.63	86.47	81.40	104.34	5.71	NS
Isovalerate, mg/L	25.86	26.21	25.79	25.25	26.16	064	NS
Valerate, mg/L	24.96	24.85	24.41	23.42	25.79	0.67	NS
Acetate:Propionate	3.09	3.06	3.16	3.03	3.05	0.05	NS

$^{a-b}$ = Means with different superscripts in the same row differ significantly, $P < 0.05$

NS = $P > 0.05$

* = $P < 0.05$

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Table 16 The effects of MgO supplementation in high ratio concentrate diets on amino acids profile of rumen liquid (mg/100 mL)

Item	Level of MgO					SEM[1]	Sign
	0%	0.2%	0.4%	0.8%	1.2%		
Total amino acids	74.19	66.45	67.57	72.32	63.46	5.31	NS
Asparatic acid	8.64	7.49	7.30	7.89	7.45	0.61	NS
Glutamic acid	10.42	9.36	9.78	10.69	9.32	0.81	NS
Serine	3.77	3.40	3.45	3.86	3.44	0.28	NS
Histidine	3.06	3.06	2.48	2.19	2.50	0.26	NS
Glycine	4.40	3.98	4.02	4.27	4.24	0.29	NS
Threonine	3.61	3.35	3.50	3.74	3.14	0.34	NS
Arginine	4.60	4.44	4.38	4.95	4.03	0.39	NS
Alanine	5.96	5.36	5.35	5.83	5.32	0.44	NS
Tyrosine	3.24	2.93	2.94	3.20	2.80	0.25	NS
Cysteine	1.40	1.32	1.20	1.37	1.43	0.14	NS
Valine	2.54	2.31	2.69	2.77	2.10	0.24	NS
Methionine	1.91	1.80	1.87	1.84	1.67	0.15	NS
Phenylalanine	3.06	2.74	2.89	3.00	2.53	0.24	NS
Isoleucine	2.13	1.99	2.18	2.40	1.85	0.21	NS

Table 16 Continued

Item	Level of MgO					SEM	Sign
	0%	0.2%	0.4%	0.8%	1.2%		
Leucine	5.78	5.22	5.40	5.80	4.84	0.45	NS
Lysine	5.42[a]	4.71[ab]	4.92[ab]	4.65[ab]	3.80[b]	0.37	*
Proline	4.41	3.32	3.22	3.88	3.83	0.38	NS

[1] = n=15 except for histidine at level of MgO 0 and 1.2%, and alanine at level of MgO 0.2% (n=14), SEM calculated using n=14; threonine at level of MgO 1,2% (n=12), SEM calculated using n=12

[a-b] = Means with different superscripts in the same row differ significantly, $P < 0.05$

NS = $P > 0.05$

* = $P < 0.05$

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activity was the highest in treatment 0.2% MgO. Distribution of ORP values from the lowest to the highest were achieved by supplementation of MgO at levels 0.2, 0, 1.2, 0.4, and 0.8%, respectively.

As well as ORP, NH_4^+ concentration also was significantly ($P < 0.05$) affected by supplementation MgO, but it was not dose-dependently. NH_4^+ concentration reflected deamination process and rate of N-uptake by microbes. High NH_4^+ concentration indicated the high deamination process, low N-uptake rate by microbes, and low N absorption through rumen wall. In this study, all animals in each MgO treatment have received same quality and quantity of feed. It meant that deamination potential in each MgO treatment should be equal. NH_4^+ concentration in all rumen animals which received MgO (0.2, 0.4, 0.8, and 1.2%) have lower than the control treatment (0% MgO). It may be that presence of MgO has improved N utilization by rumen microbes. In addition, NH_4^+ absorption from rumen is higher in higher rumen pH. The increase of MgO supplementation increased rumen pH.

Population of protozoa in the rumen was significantly ($P < 0.05$) affected by MgO treatment. The highest number of protozoa was achieved by MgO supplementation at level 0.2%. Population of protozoa in the rumen is influenced by feed composition, feed level, feeding frequency, ruminal pH, and turnover rate (Franzolin and Dehority, 1996). In this study, feed composition, feed level, and frequency of feeding were set in equal condition. Even MgO treatments have affected on ruminal pH, but there was no linear correlation between ruminal pH and protozoa population. It may be because of pH values for all treatments were quite high (the lowest ruminal pH was 6.47). Number of protozoa will decrease when ruminal pH decreases below 6.0 (Franzolin and Dehority, 1996). MgO treatments may affect on number of protozoa as well as turnover rate.

Number of bacteria significantly ($P < 0.05$) decreased by supplementation of MgO. Compared to the control, supplementation of 0.2, 0.4, 0.8, and 1.2% MgO have lower average of bacteria number. It may be because of high rumen salinity and osmolality. High osmolality inhibit bacteria activity (Owens *et al*., 1998), including proliferation. But, comparison between level of 0.2, 0.4, 0.8, and 1.2% MgO, even statistically not significant different ($P > 0.05$), the average of bacteria number tended to decrease by increasing level of MgO until the concentration of 0.4% and afterward tended to increase.

Supplementation of MgO significantly ($P < 0.05$) influenced total VFA of rumen liquid. As well as ruminal pH, total VFA was dose-dependently decreased by MgO supplementation until the concentration of 0.8% and was increased afterward. Supplementation of 1.2% MgO was highest total VFA. It is may be because of MgO supplementation increase ruminal osmolality. Increase the mineral intake increase ruminal osmolality and high osmolality reduce acids absorption through ruminal wall (Owens *et al*., 1998).

As well as total VFA, individual VFA, even significantly ($P < 0.05$) influenced by MgO treatment only on acetate and propionate, each individual VFA tended to give similar response with total VFA, except on acetate:propionate ratio. Although acetate:propionate ratio was not significantly ($P > 0.05$) influenced by MgO treatment, the average of acetate:propionate ratio was highest in level of 0.4% MgO. It was indicated that fiber digestion was highest by supplementation of 0.4% MgO.

Total amino acids was not significantly ($P > 0.05$) affected by MgO supplementation. Amino acids profile of rumen liquid was also not significantly ($P > 0.05$) affected by MgO supplementation, except for lysine was significantly ($P < 0.05$) influenced by supplementation of MgO. The

number of lysine tended to decrease by increasing the levels of MgO. It was indicated that MgO supplementation increase lysine uptake.

4.2.2 Lysine supplementation

The effect of lysine supplementation in high ratio concentrate diets was shown in Table 17. Ruminal temperature was significantly ($P < 0.05$) affected by lysine. Treatments with lysine (0.25 and 0.5%) have relative higher temperature than the control treatment (0% lysine). The presence of lysine increased fermentation activity of rumen microbes (Table 17). Lysine is the limiting amino acid which is required by rumen microbes to grow. Some heat is produced during microbial activity, such as fermentation and grows. In this study, rumen temperature was highest by additional 0.25% of lysine, than 0.5%, and the lowest was 0%. It may because of lysine affected on improving efficiency of energy utilization by host animal. Heat increment indicates an inefficiency of microbial metabolic activity (Blaxter and Wainman, 1964).

Lysine supplementation did not affect ruminal pH. Even lysine has increased fermentation activity in the rumen, deamination of lysine also produce ammonia which tend to increase ruminal pH. Deamination of 1 mole lysine produces 1 mole of acetate and butyrate, and 2 moles of ammonia (Onodera, 1993). Ruminal pH primarily is affected by production of fermentation acids (Krause, *et al*., 2002). While, fermentation acids production is affected by available energy in the rumen (Franzolin and Dehority, 1996; Cheng *et al*., 1998; Owens *et al*., 1998; Martin *et al*., 2010).

Lysine supplementation also has significantly ($P < 0.05$) affected on rumen liquid conductivity and ORP, but its values were not dose-dependently. Conductivity values from the highest to the lowest were achieved by MgO supplementation at levels 0.25, 0, and 0.5%, respectively. As well as conductivity, pattern of ORP responses was similar. In this case, the values of conductivity and ORP were on the contrary.

Table 17 The effects of lysine supplementation in high ratio concentrate diets on rumen liquid condition

Item	Level of lysine			SEM	Sign
	0%	0.25%	0.5%		
Temperature (^oC)	38.94^c	39.41^a	39.26^b	0.03	***
pH	6.68	6.67	6.70	0.02	NS
Conductivity (µS/cm)	167.84^b	175.11^a	164.39^c	0.99	***
ORP (mV)	-57.33^b	-60.00^b	-51.22^a	1.38	***
NH_4^+ (mg/100 mL)	46.78^a	40.32^b	30.60^c	0.67	***
Protozoa (x 10^6 cell/mL)	4.46^b	5.75^a	4.60^b	0.20	***
Bacteria (x 10^8 cfu/mL)	3.52^b	4.50^a	1.05^c	0.21	***
Total VFA (mg/L)	655.78	611.58	608.59	22.07	NS
Total amino acids (mg/100 mL)	66.48	73.55	66.37	4.11	NS

$^{a-c}$ = Means with different superscripts in the same row differ significantly, $P < 0.05$

NS = $P > 0.05$

*** = $P < 0.001$

Table 18 The effects of lysine supplementation in high ratio concentrate diets on individual VFA of rumen liquid

Item	Level of lysine			SEM	Sign
	0%	0.25%	0.5%		
Total VFA, mg/L	655.78	611.58	608.59	22.07	NS
Acetate, mg/L	378.51	346.85	354.10	12.82	NS
Propionate, mg/L	122.36	117.27	112.90	4.51	NS
Isobutyrate, mg/L	6.49	6.35	6.74	0.30	NS
Butyrate, mg/L	98.08	90.06	84.62	4.42	NS
Isovalerate, mg/L	25.43	25.96	26.17	0.50	NS
Valerate, mg/L	24.91	25.08	24.07	0.52	NS
Acetate:Propionate	3.11^{a}	2.97^{b}	3.14^{a}	0.04	*

$^{a-b}$ = Means with different superscripts in the same row differ significantly, $P < 0.05$

NS = $P > 0.05$

* = $P < 0.05$

Table 19 The effects of lysine supplementation in high ratio concentrate diets on amino acids profile of rumen liquid (mg/100 mL)

Item	Level of lysine			SEM[1]	Sign
	0%	0.25%	0.5%		
Total amino acids	66.48	73.55	66.37	4.11	NS
Asparatic acid	6.98	8.54	7.75	0.48	NS
Glutamic acid	8.86	10.57	10.31	0.63	NS
Serine	3.31	3.72	3.72	0.21	NS
Histidine	2.91[a]	3.09[a]	1.99[b]	0.20	***
Glycine	3.77	4.34	4.43	0.23	NS
Threonine	3.36	3.70	3.35	0.25	NS
Arginine	4.35	4.76	4.32	0.30	NS
Alanine	5.17	5.97	5.56	0.33	NS
Tyrosine	2.94	3.22	2.91	0.20	NS
Cysteine	1.35	1.42	1.27	0.11	NS
Valine	2.49	2.51	2.45	0.18	NS
Methionine	1.89	1.90	1.67	0.12	NS
Phenylalanine	2.92	2.93	2.69	0.18	NS
Isoleucine	2.19	2.06	2.08	0.16	NS
Leucine	5.22	5.65	5.36	0.35	NS
Lysine	4.82[ab]	5.16[a]	4.12[b]	0.29	*
Proline	4.17[a]	4.13[a]	2.89[b]	0.29	**

[1] = n=25 except for histidine at level of lysine 0.25 and 0.5%, and alanine at level of lysine 0% (n=24), SEM calculated using n=24; threonine at level of lysine 0.5% (n=22), SEM calculated using n=22;

[a-b] = Means with different superscripts in the same row differ significantly, $P < 0.05$

NS = $P > 0.05$

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

About NH_4^+, the experiment result showed that lysine supplementation has significantly ($P < 0.05$) decreased concentration of NH_4^+ in rumen liquid. In this result, NH_4^+ decreased by increasing the levels of lysine. It was in contrast with the result of experiment conducted by Chung *et al*. (2006) which NH_4^+ was increased by increasing levels of lysine in the diets. In the other hand, lysine is the first limiting amino acids in the rumen (Annison *et al.*, 2002). Availability of lysine in the rumen increased ammonia uptake by microbes or host animal through ruminal wall.

Protozoa number was significantly ($P < 0.05$) affected by lysine treatments. Average of protozoa number in the rumen from the highest to the lowest was achieved by lysine supplementation at levels 0.25, 0.5, and 0%, respectively. In this experiment, highest lysine treatment (0.5%) should be highest lysine availability in the rumen. But, the fact has shown that it was not reflected on number of protozoa. It may because of available NH_4^+ in the rumen was the lowest, or lysine has affected on rumen turnover rate and protozoa flowed out from rumen.

Supplementation of lysine significantly ($P < 0.05$) affected on ruminal bacteria. The highest average of bacteria number was achieved by supplementation of 0.25% lysine. Supplementation of lysine in higher level (0.5%) dramatically ($P < 0.05$) decreased population of bacteria. It may be because of supplementation of 0.5% lysine in the diets is too high for optimum bacterial growth.

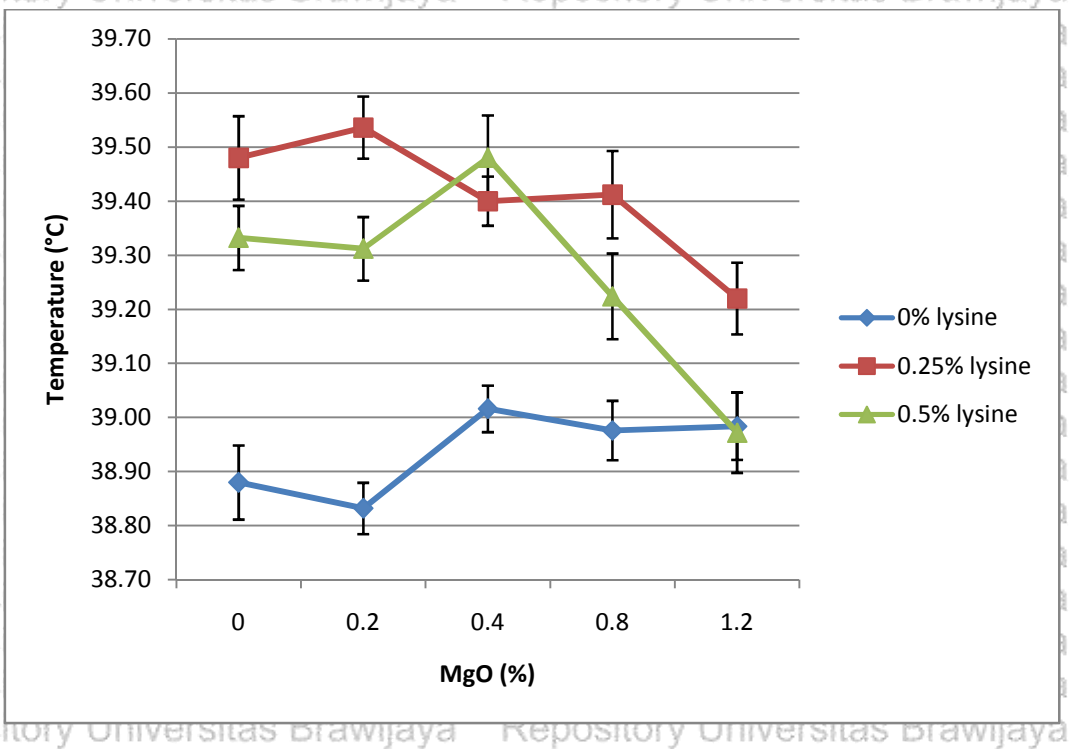
Supplementation of lysine did not significantly ($P > 0.05$) effect on total VFA in rumen liquid. But, average of total VFA tended to decrease by increasing the levels of lysine. Individual VFA, except for acetate:propionate ratio, was also not significantly ($P > 0.05$) influenced by lysine treatment. Acetate:propionate ratio was significantly ($P < 0.05$) affected by supplementation of lysine. The lowest acetate:propionate ratio was achieved

by supplementation of 0.25% lysine. It was indicated that supplementation of 0.25% lysine leaded to decrease NDF degradability by rumen microbes.

Total amino acids was not significantly ($P > 0.05$) influenced by lysine supplementation. The highest total amino acids tended to be achieved by treatment of 0.25% lysine. Amino acids profile of rumen liquid was slightly influenced by lysine treatment. Significantly effects ($P < 0.05$) only appeared on histidine, lysine, and proline. The highest histidine and lysine was in treatment of 0.25% lysine and the lowest was in treatment of 0.5% lysine. Different with histidine and lysine, the proline was decreased by increasing the level of lysine.

4.2.3 Interaction between MgO and lysine supplementation

Figure 11 to Figure 19 express the interaction between MgO and lysine supplementation on rumen liquid condition. Interaction between MgO and lysine supplementation has significantly ($P < 0.05$) affected on ruminal temperature (Figure 11). In all levels of MgO supplementation, treatment 0.25% of lysine was always higher ruminal temperature than 0% lysine, whereas treatment 0.5% lysine was often, but not always, medium ruminal temperature, except in levels 0.4 and 1.2% of MgO. When the level of MgO was 0.4%, treatment 0.5% lysine was the higher ruminal temperature than 0.25% lysine. In contrast, ruminal temperature of treatment 0.5% lysine was drop down to be the lower than 0% lysine when the MgO was in level 1.2%. For all treatments, the highest temperature was achieved by treatment 0.2% MgO + 0.25% lysine and the lowest was 0.2% MgO + 0% lysine, with average temperature 39.54 and 38.83 ^{o}C, respectively. This pattern tends to be similar with treatment of lysine more than MgO. It has indicated that lysine may play a greater role on ruminal temperature than MgO.



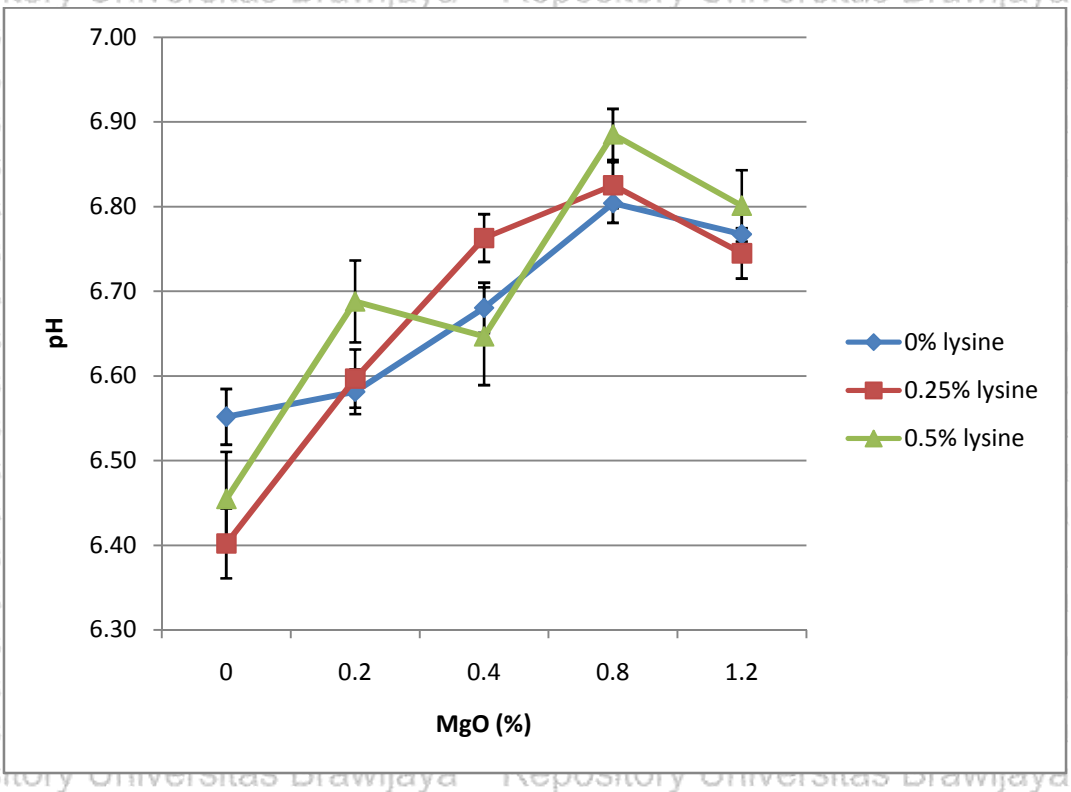


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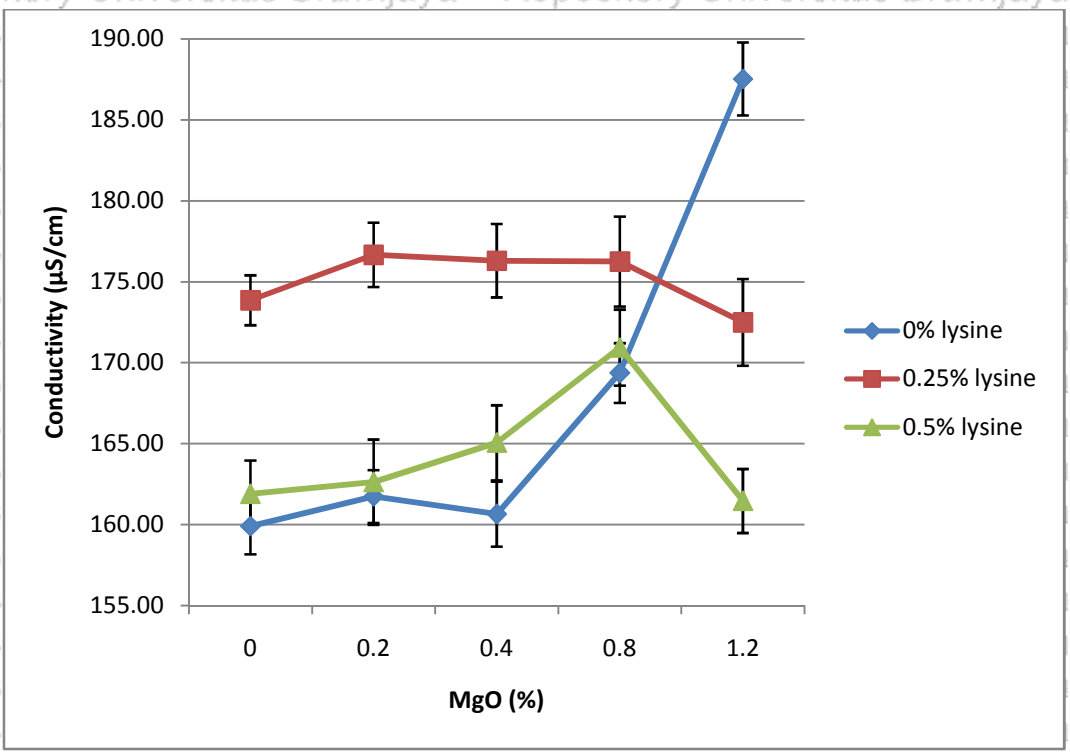
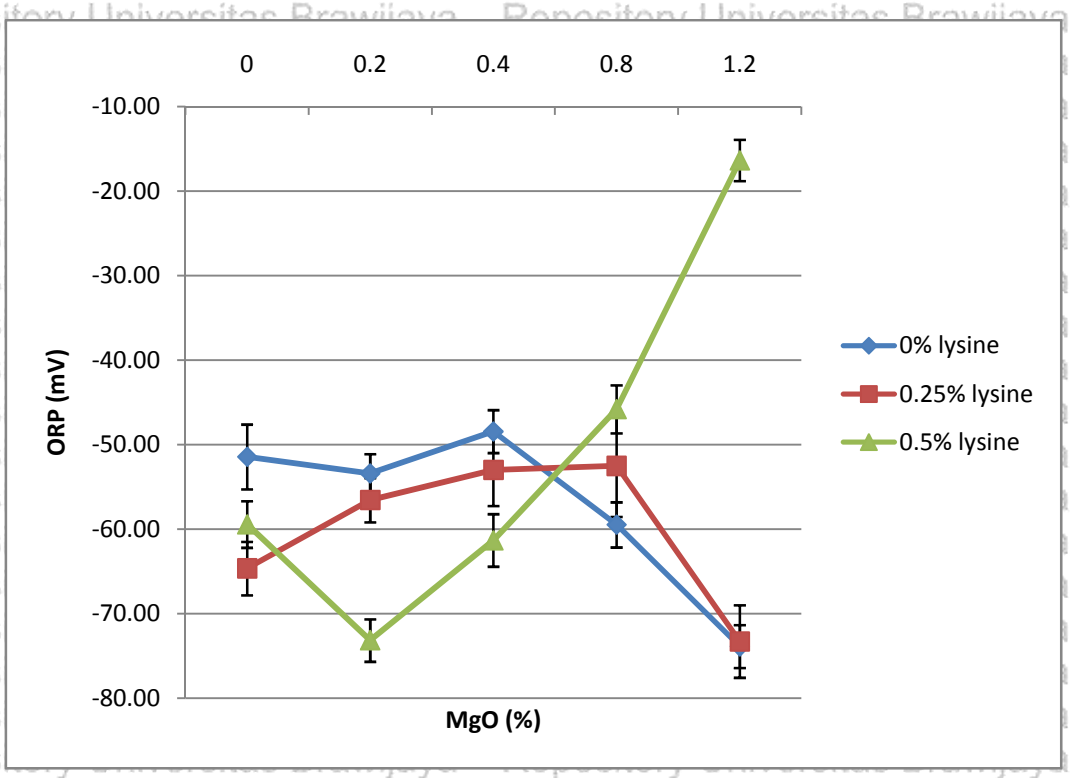
Figure 12 shows interaction between MgO and lysine supplementation on ruminal pH. Based on statistical analysis, interaction between MgO and lysine supplementation significantly ($P < 0.05$) influenced ruminal pH. In all treatments of lysine, ruminal pH constantly increased by increasing level MgO, except for 0.5% lysine at level of 0.4% MgO which slightly decreased compared 0.2% MgO. Peaks of ruminal pH were accomplished by level of 0.8% MgO for all treatments of lysine. Decreasing the average of ruminal pH was occurred for all treatment of lysine which be combined with 1.2% MgO. This case may be because supplementation MgO at level 1.2% was too much for normal rumen condition, eventually impact on acids absorption. In general, the highest and the lowest ruminal pH were achieved by treatments of 0.8% MgO + 0.5% lysine and 0% MgO + 0.25% lysine with average values 6.89 and 6.40, respectively. This achievement has been the same as in treatments MgO or lysine.

Rumen liquid conductivity was significantly ($P < 0.05$) affected by interaction between MgO and lysine supplementation. Their interaction was shown at Figure 13. Based on statistical analysis, both of MgO and lysine have markedly influenced on ruminal conductivity. Figure 13 shows that treatment 0.25% lysine was consistently higher than 0.5% lysine in all levels of MgO. In these two treatments (0.25 and 0.5% lysine), conductivity values was apparently affected by lysine more than MgO. Modification the levels of MgO have not clearly affected on ruminal conductivity. In contrast, treatment 0% lysine was clearly influenced by MgO supplementation, especially when MgO was added at level 0.8 % or more. These cases may be because presence of lysine has influenced mineral metabolism in the rumen. The highest and the lowest ruminal conductivity were accomplished by treatments 1.2% MgO + 0% lysine and 0% MgO + 0% lysine with averages value 187.52 and 159.90 µS/cm, respectively.





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Interaction between MgO and lysine supplementation has significantly ($P < 0.05$) influenced on ruminal ORP (Figure 14). Compared with the lower treatments, 0.5% lysine has seemingly shown greater influence on ruminal ORP. Moreover, it also given difference response with the other lysine treatments, especially when be observed at level MgO 1.2%. Treatments 0 and 0.25% lysine have shown declining ruminal ORP at 1.2% MgO supplementation, but treatment 0.5% lysine has greatly increased. 0% lysine was higher ruminal ORP than 0.25% when MgO levels were 0, 0.2, and 0.4%, but the condition become inverted when level MgO be modified to 0.8% or more. The lowest ruminal ORP was reached by treatment 1.2% MgO + 0% lysine with average value -73.88 mV. In contrast, the highest ruminal ORP was accomplished by treatment 1.2% MgO + 0.5% lysine with average value -16.36 mV. These achievements were totally different from achievements of single measurement of ORP or lysine treatments.

Interaction between MgO and lysine supplements on NH_4^+ was shown on Figure 15. As well as supplementation of MgO and lysine in single treatment, this interaction has significantly ($P < 0.05$) affected on NH_4^+ number in the rumen. Based on the experiment result, increment lysine in diets tends to decrease NH_4^+ concentration in rumen liquid. Theoretically, high supplementation lysine should be increase NH_4^+ available in the rumen. Degradation of lysine by bacteria in the rumen produces ammonia (Onodera, 1993). In this study, averages of NH_4^+ concentration also tended to decrease by increasing the level of MgO. It may be because presences of MgO and lysine in the rumen were influenced to increase rate of ammonia utilization by ruminal microbes or ammonia absorption by host animal through ruminal wall. In general, the highest and the lowest average of NH_4^+ concentration were reached by treatments 0% MgO + 0% lysine and 1.2% Mg0 + 0.5% lysine with averages in value 53.2 and 20.7 mg/100 mL rumen liquid, respectively.

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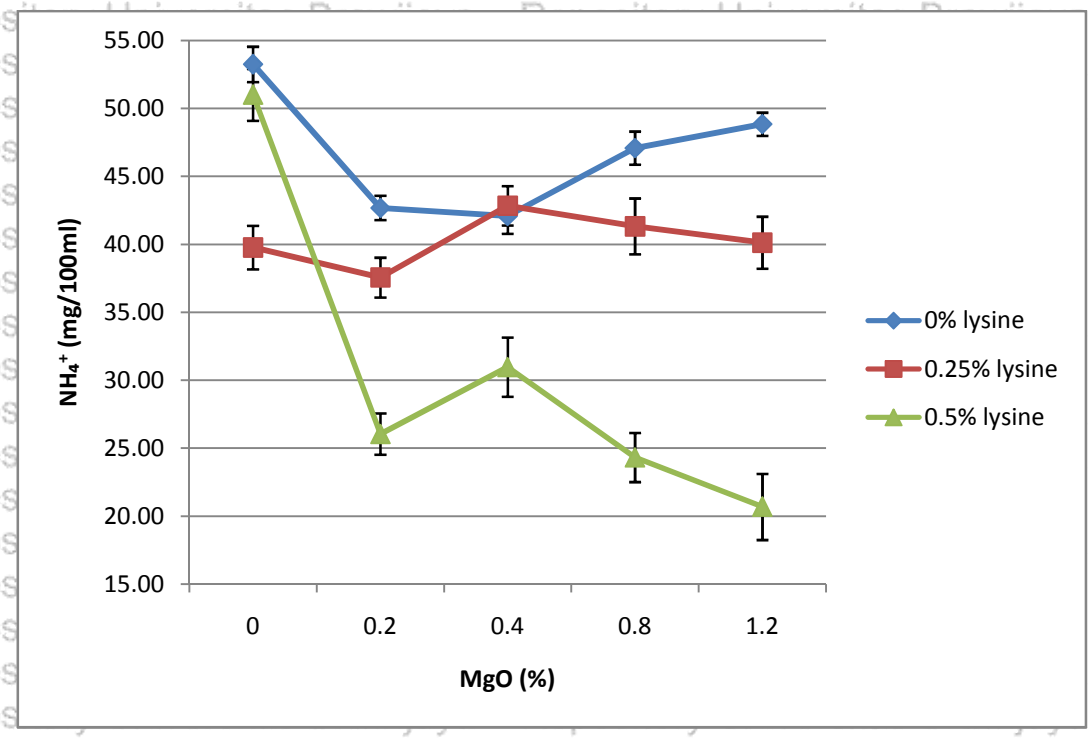
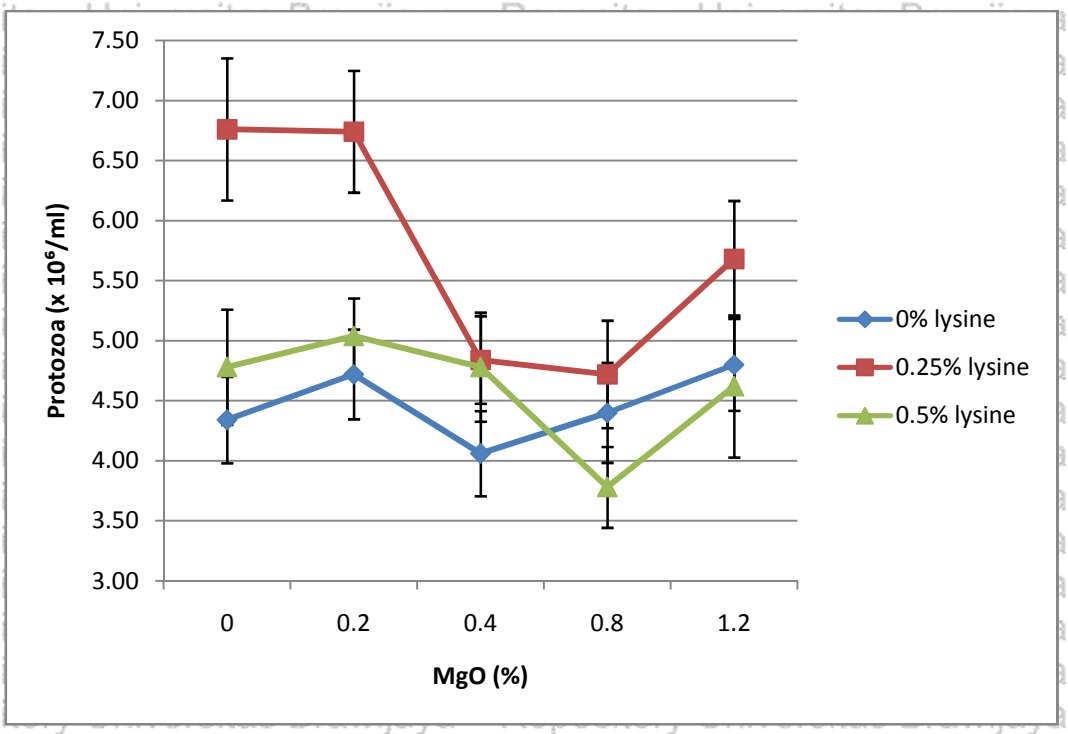
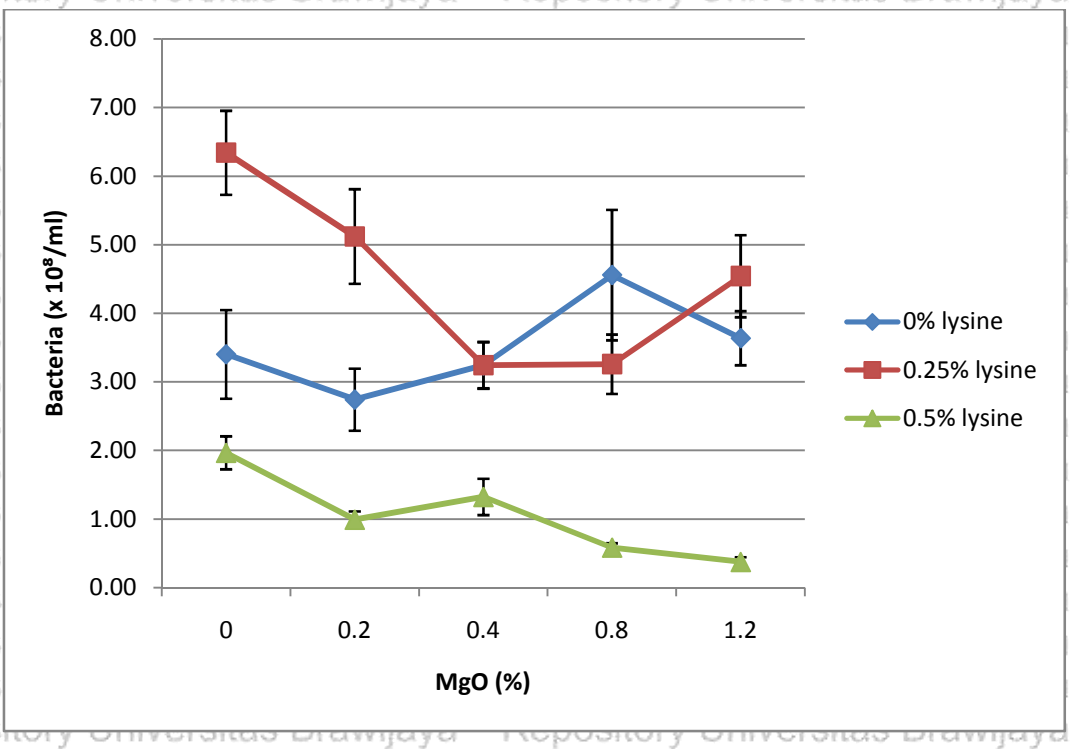
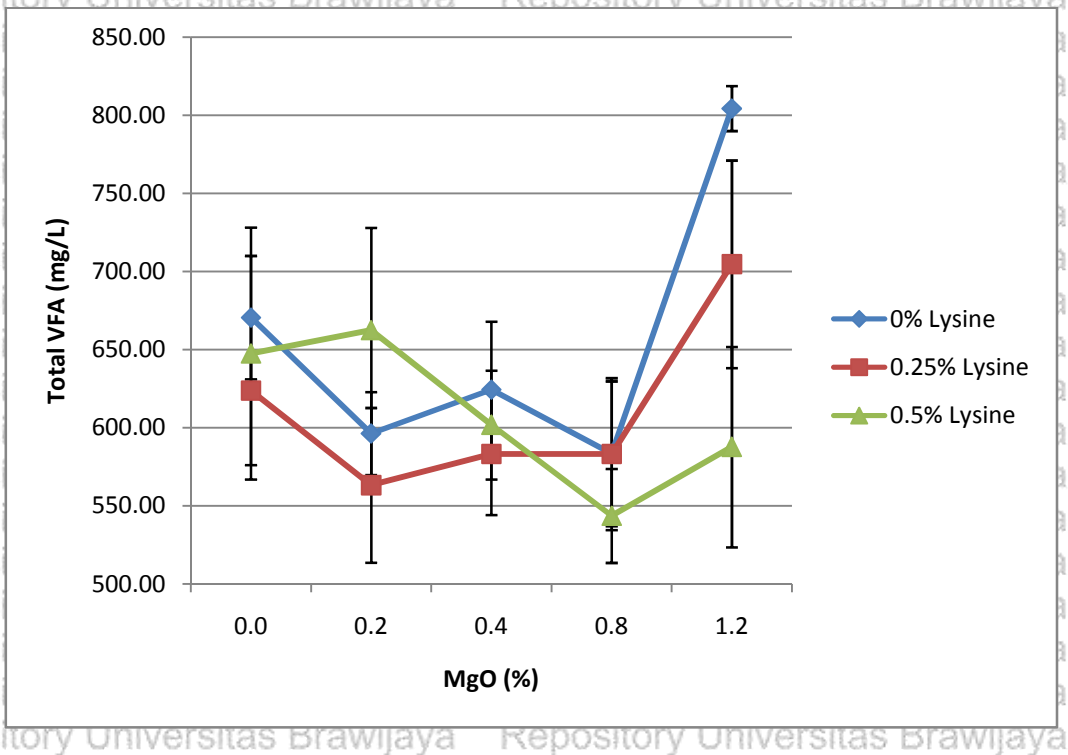




The number of protozoa was not significantly ($P > 0.05$) affected by interaction between MgO and lysine supplementation (Figure 16). Supplementation of MgO and lysine in single treatment was significantly influenced population of protozoa in rumen. Treatment of 0.25% lysine has always highest protozoa number in all levels MgO. Number of protozoa tended to decrease by increasing MgO from level of 0.2 to 0.8% and was increased afterward, except for treatment of 0% lysine was increased from the level of 0.8% MgO. The highest protozoa number was accomplished by treatment 0% MgO + 0.25% lysine with average 6.76 x 10^6/mL rumen liquid. In contrast, the lowest protozoa number was reached by treatment 0.8% MgO + 0.5% lysine with average 3.78 x 10^6/mL rumen liquid.

Figure 17 shows interaction between MgO and lysine supplementation on population of rumen bacteria. Statistically, population of bacteria in the rumen was significantly ($P < 0.05$) affected by interaction between MgO and lysine supplementation. Supplementation of low and medium levels of lysine (0 and 0.25%) related to higher bacteria number than the treatment of high level of lysine (0.5%). In high level of lysine, the number of bacteria tended to decrease by increasing the levels of MgO treatment. In medium level of lysine, the number of bacteria tended to decrease by increasing the levels of MgO supplementation until the concentration of 0.4%, was stabile until the concentration of 0.8%, and increased afterward. In contrast, bacteria number of low level of lysine increased by increasing the levels of MgO supplementation until the concentration of 0.8% and decreased afterward. The highest and the lowest averages values of rumen bacteria were achieved by treatments 0% MgO + 0.25% lysine (6.34 x 10^8 cfu/mL of rumen liquid) and 1.2% MgO + 0.5% lysine (0.38 x 10^8 cfu/mL of rumen liquid), respectively. Presence of mineral in the diets increases ruminal osmolality, and high osmolality inhibit bacteria activity (Owens *et al*., 1998).





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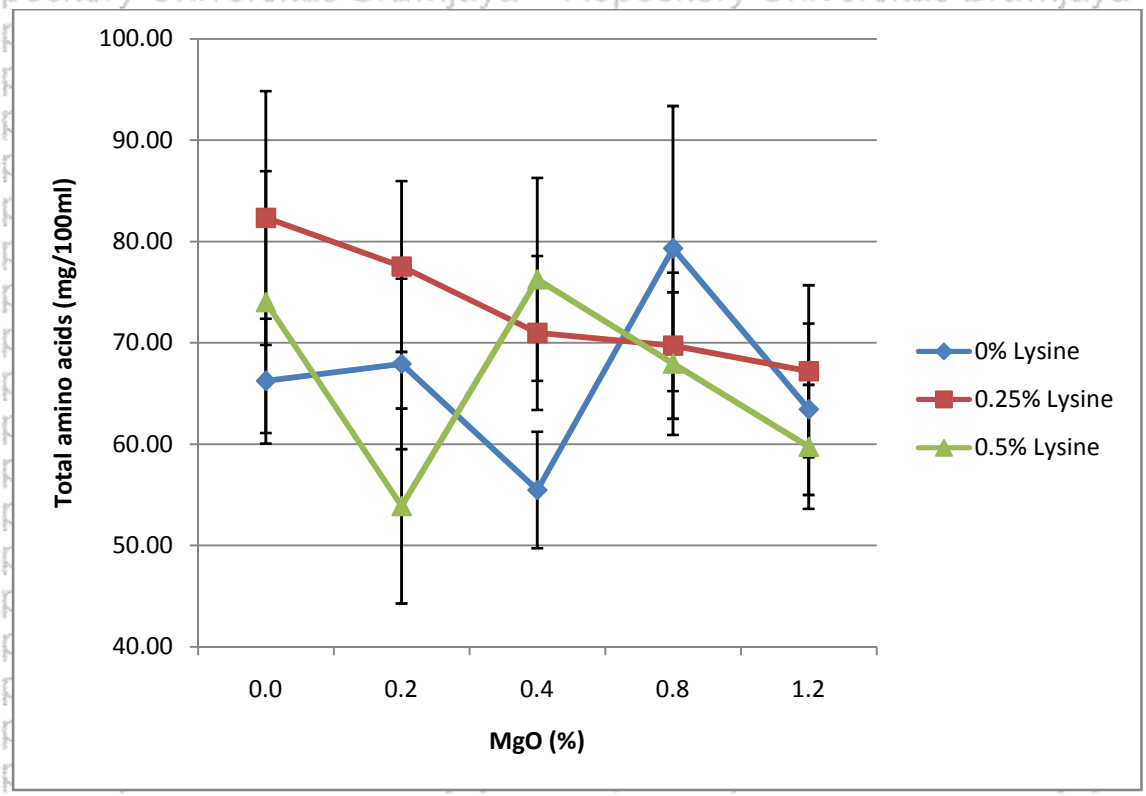
Interaction between MgO and Lysine supplementation not significantly ($P > 0.05$) affected on total VFA of rumen liquid (Figure 18). The pattern of total VFA response was similar in treatments of 0 and 0.25% lysine. Total VFA of those treatments tended to decrease by increasing the levels of MgO supplementation in the diets until the level of 0.8% and increased afterward. Treatment of 0.5% lysine has slightly different in low level of MgO (0.2%). In level of 0.2% MgO, response of treatments of 0 and 0.25% lysine was decreased total VFA values, but treatment of 0.5% lysine was increased. In the other hand, in level of 1.2% MgO, treatment of 0.5% lysine has less increment of total VFA, but other treatments of lysine have more increment. The highest total VFA was in treatment 1.2% MgO + 0% lysine with average value 804.29 mg/L of rumen liquid. While the lowest total VFA was in treatment 0.8% MgO + 0.5% lysine with average value 543.61 mg/L of rumen liquid.

Total amino acids was not significantly ($P > 0.05$) influenced by interaction of MgO and lysine supplementation in the diets. This interaction was shown in Figure 19. Total amino acids in treatment of 0.25% lysine was dose-dependently decrease by MgO supplementation. But, in treatments of 0 and 0.5% lysine the response of total amino acids was random. The highest and lowest of total amino acids were in treatments of 0% MgO + 0.25% MgO (82.32 mg/100 mL of rumen liquid) and 0.2% MgO + 0.5% lysine (53.89 mg/100 mL of rumen liquid), respectively.



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