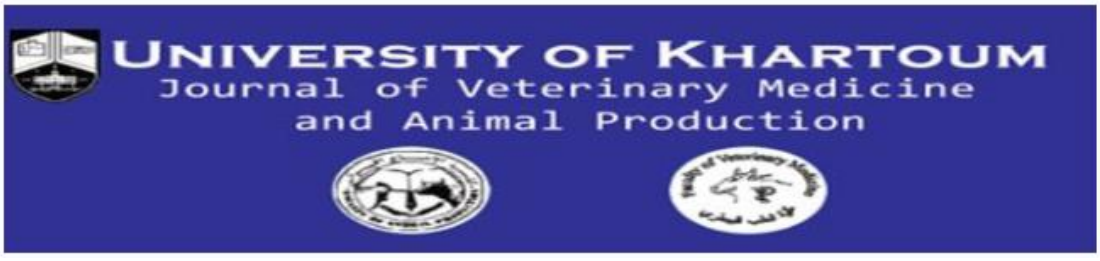
U. of K. J. Vet. Med. Anim. Prod., Vol.8, Issue 1 (2017) p 29-40
ISSN: 243456



Effect of Supplementation Different Levels of Cheese Whey to Dietary Treated Baggase on In-Vitro Nutrients Digestibility, Rumen Environment and Gas Production

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Abstract

The study aimed to assess the effect of supplementing cheese whey at levels of 0% (T0%), 5% (T5%), 10% (T10%), 15% (T15%) and 20% (T20%) to dietary urea treated baggase on *in-vitro* digestibility of dry matter (IVDMD), organic matter (IVOMD), crud protein (IVCPD), neutral detergent fiber (IVNDFD), rumen environment and gas production. The results indicated that the cheese whey supplementation to the dietary urea treated baggase significantly ($P \leq 0.05$) had positive effect on IVDMD, IVOMD, IVCPD and IVNDFD with superior effect for T5%. The pH was significantly ($P \leq 0.05$) decreased for supplementing diets and stable at the following values: 6.25, 6.32, 6.39 and 6.54 for T0%, T5%, T10%, T15% and T20% respectively. The same trend was recorded for ammonia-nitrogen concentration (5mg/100dl) and the concentration of all diets was above the recommended level (5mg/100dl). Volatile fatty acids concentration (mmol/100ml) was significantly ($P \leq 0.05$) increased in whey diets (27.57, 33.2, 32.87, 32.52 and 31.85 for T5%, T10%, T15% and T20% respectively) and decreased in control diet (27.57). The highest values of gas production and its fermentable fractions were gained by T5% diet. In conclusion supplementation of dietary urea treated baggase with different level of cheese whey had positive effects on nutrients digestibility, volatile fatty acids and gas production and its fractions with superior effect for T5% diet.

Key words: Cheese Whey, baggase, digestibility, rumen environment and gas production

المستخلص

هدفت الدراسة الي إستخدام مكونات غذائية أقل تكلفة للحيوانات المجترة (البقاس و شرش الجبن)، وذلك بإدخال مستويات مختلفة من شرش الجبن الي علائق البقاس المعامل اليوريا {0% (T0)، 5% (T5)، 10% (T10)، 15% (T15) و 20% (T20)}. تم قياس المتغيرات الأتية: الهضمية المعملية للمادة الجافة والعضوية والبروتين الخام والألياف الذائبة في المحاليل المتعادلة وبيئة الكرش وإنتاج الغاز

والجدوي الإقتصادية. أشارت النتائج الي ان إضافة شرش الجبن لعليقة البقاس المعامل باليوريا لها أثر إيجابي علي الهضمية المعملية للمادة الجافة والعضوية والبروتين الخام والألياف الذائبة في المحاليل المتعادلة وكان الأثر التفوقي للعليقة T5. إنخفض الأس الهيدروجيني معنويا (P≤0.05) مع علائق شرش الجبن، وثبت عند القيم الأتية: 6.53 و 6.42 و 6.39 و 6.32 و6.26 للعلائق T0 و T5 و T10 و T15 و T20 ، علي التوالي. قل تركيز الأمونيا نيتروجين (mg/100dl) في علائق شرش الجبن لكن التركيز في كل العلائق أعلي من المستوي الموصي به (5mg/100dl). زاد تركيز الأحماض الدهنية الطيارة (mmol/100ml) معنويا (P≤0.05) في علائق شرش الجبن (33.2، 32.87، 32.52، 31.85 للعلائق T5 ، T10 ، T15 ، T20 ، علي التوالي و إنخفض في العليقة الشاهد (27.57). سجلت أعلي قيم لإنتاج الغاز ومكوناته (a+b و b) بواسطة علائق شرش الجبن. أقل تكلفة وأعلي ربحية أكتسبت بزيادة مستويات شرش الجبن في العلائق. خلصت الدراسة الي أن المستويات المختلفة من شرش الجبن المضاف الي علائق البقاس المعامل باليوريا حسنت القيمة الغذائية والربحية مقارنة بالعليقة الشاهد و لكن كان الأثر التفوقي للعليقه T5. إضافة الي ذلك لابد من إجراء المزيد من الدراسات لتقييم صحة هذه النتائج بإجراء تجربة هضمية في المجترات.

Introduction

The feeds that are available to ruminants in developing countries are fibrous and relatively high in ligno-cellulose (Dayani *et al*., 2010). Bagasse is a highly fibrous by product after sugar cane is crushed to remove sucrose. Many sugar milling factories around the world release large quantities of bagasse as a part of their byproducts, some even dispose it as a waste. It contains 60 to 70% carbohydrate, mostly in the form of polysaccharides and is a potential source of dietary energy for animal. The major limitation of bagasse as feed is its low digestibility and energy which are due to association of lignin with cellulose and hemicelluloses (Suksombat, 2004). To enhance the nutritive value of lignocelluloses materials of livestock, some form of pre-treatment is required (Fadel Elseed *et al*., 2003; Attaelmnan *et al*., 2007; 2009 and 2015). Ammonation improves the nutritive value of crop residues (Aregheore and Perera, 2004) by breaking the lignocelluloses bonds and cellulose for digestion by rumen microbes. Bagasse is used as a basal diet, it's important to give the correct supplementation in order to obtain satisfactory physical and economic responses. The supplementation must take account of the productivity of the animals (Mahala *et al*., 2007).

Cheese whey is the liquid remaining after the precipitation and removal of milk casein during cheese-making, this byproduct represents approximately 85–90% of the milk volume and retains 55% of milk nutrients. Among the most abundant of these nutrients are lactose, soluble proteins, lipids and mineral salts (Dragone *et al.*, 2009).

Cheese whey represents an important environmental problem because of the high volumes produced and its high organic matter content. Worldwide production of whey is estimated to be in the order of 160 million tonnes per year, showing a 1–2% annual growth rate (Smithers, 2008). The pressure of antipollution regulations together with cheese whey nutritional value challenges the dairy industry to face whey surplus as a resource and not only as a waste problem (Guimarães *et al.*, 2010). Cheese Whey as a nutritious by-product can effectively be fed to ruminants (Bayat *et al*., 2002).

The main objective of the present study was to find less expensive ingredients resources for ruminant feeds (bagasse and cheese whey), while the specific objective was to investigate the effect of inclusion different level of cheese whey (0%, 5%, 10%, 15% and 20%) to urea treated bagasse in a total mix ration in term of *in vitro* dry matter, organic matter, crude protein, neutral detergent fiber digestibility, rumen environment, gas production and economic feasibility.

Materials and methods

Experimental site

This experiment was carried out during the period from September to November 2014 at Department of Animal Nutrition, Faculty of Animal Production and University of Khartoum.

Feed Preparation

Five iso-nitrogenous and iso-caloric concentrate rations Table 1 were formulated to meet the nutrient requirement of goat according to (NRC, 1989). Baggase treated with 5% urea then supplemented with different level of cheese whey at rate of 0% (T0%), 5% (T5%), 10% (T10%), 15% (T15%) and 20% (T20%) as shown in Table 2.

Chemical analysis

Total solid, protein, fat, ash and lactose of cheese whey sample were determined by lactoscan analyzer according to instruction of manufacture (milk kotronic LTD, Europe) after brought from dairy lab manufacturing in plastic bag Table 3.

Rations and residual samples were analyzed for dry matter (DM), crude protein (CP) and ash according to the method of AOAC (1990). Neutral detergent fiber (NDF) was determined according to Van Soest and Robertson (1980). Metabolic energy (ME) calculated according to equation of Qrskv and McDonald (1979).

ME (MJ\KgDM) = (0.018CP + 0.0315EE + 0.0163NFE + 0.0149CF).

In-vitro Dry Matter digestibility

The two steps procedure was used for *in vitro* determination of digestibility of rations and replicates four times to any treatment. Rumen liquor was collected by stomach tube from four mature goats. In the first step, dried rations are incubated in test tubes with rumen fluid. The tubes also contain buffer solution, macro-minerals, trace-minerals, nitrogen sources, and reducing agents to maintain pH and provide nutrients required for growth of rumen bacteria. Because oxygen is toxic to rumen bacteria, solutions are gassed with carbon dioxide to maintain anaerobic conditions, and temperature is held at 39 °C (body temperature) during the incubation. In the second step, after 48 hours of incubation, an enzyme solution is added to stimulate digestion in the small intestine (Tilley and Terrie, 1963).

Rumen environment study

` Rumen liquor was collected after microbial digestion in **(3-4)** by tubes, immediately the pH was measured using standard laboratory pH meter. Then the fluid strained through two layers of cheese cloth

adding drop from H_2SO_4 and stored at -20^0 C for further analysis.

Rumen ammonia nitrogen

NH_3-N was determined as described by Abdulrazak and Fujihara (1999). Three ml of 20% tricholoacetic acid and 3 ml of rumen fluid were added in test tube and centrifuged for 10 minutes. 2 ml of above solution were directly infused to distillation set up and then added 10 ml sodium borate dehydrate, distillated for about 5 minutes then titrated using 0.05 sulphuric acid.

Calculation

Rumen NH3-N (mg) as =Titrate (ml×0.0014) ÷Amount of sample

Volatile fatty acid (VFA)

The (VFAs) were determined by steam distillation as described by Abdulrazak and Fujihara (1999). 4 ml of rumen fluid were pipette and 4 ml of $MgSo_4$ and H_2SO_4 were added into semi macro kjeldhal flask. 100 ml of distillated were collected in flask and placed 1-2 drop of phenolphthalein as indicator and titrated using 0.05 NaOH solutions.

Calculation:

VFA (mmol/100ml) =Titrate (ml×NaOH×100) ÷Vol. of rumen fluid

***In vitro* gas production**

The *in vitro gas* production was carried out using the method described by (Menke and Steingass, 1988). Buffer and mineral solution were prepared and placed in water bath at 39 C under continues flushing with CO2. Both solid and liquid rumen fraction (50 % solid: 50% liquid) were collected before the morning feeding by stomach tube from four mature goats. Rumen liquid was collected into pre-warmed insulted bottles, homogenized in a laboratory blender. The well mixed and CO_2 flushed rumen fluid was added to the buffered rumen fluid solution (1:2 v/v), which was maintained in water bath at 39^0 C. Buffered rumen fluid (30 ml) was piptted into each syringes were immediately placed into the water bath at 39^0C. Two syringes with only buffered rumen fluid were incubation terminated after recording the 96 h gas volume. The gas value was corrected for the blank incubation, and report gas value is expressed in ml per 200 of DM.

Statistical analysis

Data obtained from the experiment was subjected to analysis of variance (ANOVA) according to (Steel and Torrie, 1980) for complete randomized design (CRD) using a computer programmed knows as statistix[8]. The comparison among means was separated by the least significant difference (LSD).

Results

Effect of inclusion of Different Levels of Cheese Whey in Complete Diet System on *In Vitro* Dry Matter Digestibility:

The effect of inclusion different level of cheese whey (T0%, T5%, T10%, T15% and T20%) on

in vitro dry matter digestibility (IVDMD), organic matter digestibility (IVOMD), crud protein digestibility (IVCPD) and neutral detergent fiber digestibility (IVNDFD) was illustrated in Table (4). The results indicated that there were significant differences ($P \leq 0.05$) between control and experimental rations on *in vitro* nutrients digestibility of IVDMD, IVOMD, IVCPD and IVNDFD. The highest nutrients digestibility was gained by the T5% of cheese whey while the lowest recorded by T0% of cheese whey.

Table 1: Calculated chemical analysis of experiment diet (%) DM

Items	**Treatment**				
		T5%	**T10%**	**T15%**	**T20%**
CP	13.38	13.83	13.78	14.44	14.95
ME(MJ/kg)	9.86	9.33	10.14	10.32	10.63

(T0%) control diet contains 0% of cheese whey, (T5%) contains 5% of cheese whey, (T10%) contains 10% of cheese whey, (T15%) contains 15% cheese whey, (T20%) contains 20% of cheese whey (CP) crud protein and (ME) Metabolic energy.

Table 2: Ingredient composition (%) of experimental diets

	Treatment %				
Item	T0%	T5%	T10%	T15%	T20%
Sorghum	12	9	8	7	7
Groundnut cake	9	8	7	7	6
Molasses	18	14	12	9	5
Wheat bran	30	33	32	31	32
Baggase	30	30	30	30	30
Whey	0	5	10	15	20
Limestone	0.5	0.5	0.5	0.5	0.5
NaCl	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100

(T0%) control diet contains 0% of cheese whey, (T5%) contains 5% of cheese whey, (T10%) contains 10% of cheese whey, (T15%) contains 15% cheese of whey, (T20%) contains 20% of cheese whey

Table 3: Chemical Composition (%) of Cheese Whey on DM Basis

Items		**DM**	**Lactose Protein Ash Fat**
Percentage	4.4	6.2	62.12 18.26 8.68 8.71

DM= dry matter

Effect of inclusion of different levels of cheese whey in complete diet system on rumen environment

The effect of inclusion of different level of cheese whey in the rumen environment was summarized in Table (5). The results showed there were significant differences ($P \leq 0.05$) in rumen pH, ammonia nitrogen (NH_3-N)) and volatile fatty acids (VFA) between control and supplemented groups. The VFA increase in T5% treatment compared to control and other whey supplemented treatments (T10%, T15%, T20%) but NH_3-N was lower in T5% than other supplemented diets.

Effect of inclusion of different levels of cheese whey in complete diet system on gas production/hour:

Result of gas production during fermentation period was given in Figure (4-1) which extended from 0 to 96 hours. The gas produced from control sample was significant ($P \leq 0.05$) lower compared with other samples. Diet T5% at different incubation time significantly ($P \leq 0.05$) recorded the highest gas volume compared to other samples. During 3, 6, 12, 24, 48 and 72 hours of incubation the investigated T5% produced 5.1, 11.1, 19.2, 27.9 and 36.2 ml/200 mg DM respectively. Table (6) showed the results of gas fermentable from control and different level of whey rations. Gas production for readily fermentable fraction (a), slowly fermentable fraction (b), potential gas production (a+b) and rate gas production (c) was higher significant ($P \leq 0.05$) for T5% level than other different level of whey and control ration.

Table 4: Effect of inclusion different levels of cheese whey in complete diet system on *in vitro* nutrients digestibility of experimental diets (%)

Treatment	T0%	T5%	T10%	T15%	T20%	SEM
DMD	70.04^{d}	80.50^{a}	75.05^{b}	74.60^{b}	72.75^{c}	0.18
OMD	75.40^{d}	77.80^{a}	77.10^{b}	76.80^{bc}	76.50^{c}	0.13
CPD	82.20^{c}	89.02^{a}	86.75^{b}	86.37^{b}	85.85^{b}	0.47
NDFD	40.47^{d}	58.32^{a}	57.15^{b}	56.2^{c}	55.95^{c}	0.07

(T0%) control diet contains 0% of cheese whey, (T5%) contains 5% of cheese whey, (T10%) contains 10% of cheese whey, (T15%) contains 15% of cheese whey, (T20%) contain 20% of cheeses whey. DMD= dry matter digestibility, OMD= organic matter digestibility, CPD= crud protein digestibility, NDFD= neutral detergent fiber digestibility. ^{abc} Mean with different superscripts in the same column were significant different ($P \leq 0.05$) SEM= Standard Error of mean

Table 5: Effect of inclusion different levels of cheese whey in complete diet system on pH, volatile fatty acid and ammonia nitrogen concentration

Treatment	T0%	T5%	T10%	%	T20%	SEM
pH	6.53[a]	6.42[b]	6.39[b]	6.32[c]	6.26[d]	0 .01
NH3-N(5ml /100/dl)	6.85[a]	5.0[e]	5.13[c]	5. 9[b]	5.2[b]	0.04
VFA (mmol/100ml)	27.57[e]	33.2[a]	32.87[b]	32.52[c]	31.85[d]	0.04

(T0%) control diet contains 0% of cheese whey, (T5%) contains 5% of cheese whey, (T10%) contains 10% of cheese whey, (T15%) contains 15% of cheese whey, (T20%) contains 20% of cheese whey. VFA= volatile fatty acids, [abc] Mean with different superscripts in the same column were significant different (P≤0.05) SEM= Standard Error of mean

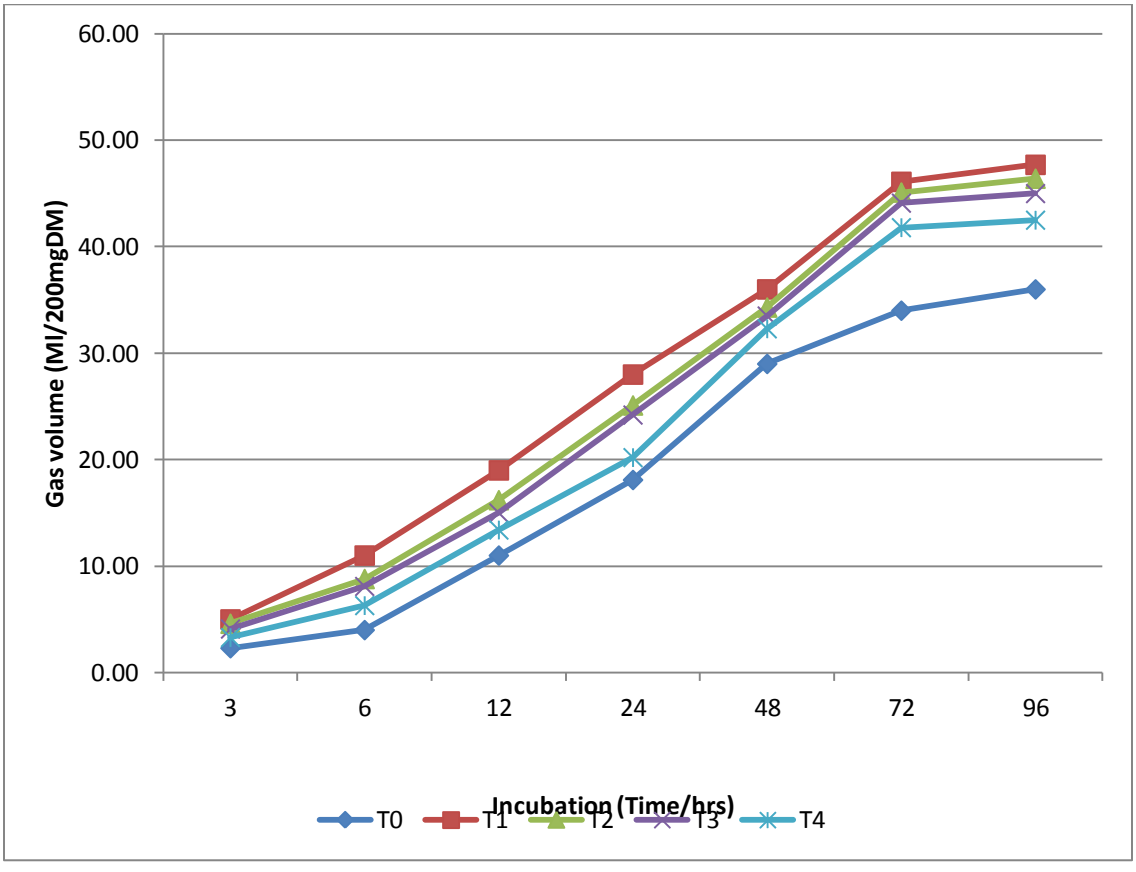

Figure (1) Effect of Inclusion Different levels of Cheese Whey in Complete Diet System on gas production (ml/200 mg DM)

Table 6: Effect of inclusion different levels of cheese whey in complete diet system on *in vitro* gas parameters for tested rations

Treatment	a	b	a+b	c
T0%	-1.41[e]	40.30[e]	38.92[e]	0.029[e]
T5%	2.58[a]	47.29[a]	49.87[a]	0.031[a]
T10%	1.70[b]	49.07[b]	50.76[b]	0.026[b]
T15%	1.03[c]	48.66[c]	49.69[c]	0.026[c]
T20%	0.0003[d]	48.28[d]	48.28[d]	0.024[d]
1	0.11	0.11	0.15	1.21

(T0%) control diet contains 0% of cheese whey, (T5%) contains 5% of cheese whey, (T10%) contains 10% of cheese whey, (T15%) contains 15% of cheese whey, (T20%) 20% of cheese whey contains. (a)= readily fermentable fraction, (b) = slowly fermentable fraction, (a+b) = potential gas production and (c)= rate gas production. [abc] Mean

Discussion

The nutrients digestibility was significantly differed between control and treated groups, which was improved with the addition of whey cheese in the diets. Most of the improvement attributed to whey additions in dry matter and energy digestibility could be accounted for by amount of highly digestible energy added in the form of whey (Schingoethe and Beardsley, 1984). Several previous results are in the same line with the results obtained from present study: Salem and Fraj (2007) fed liquid whey to dairy cow at 40 liters/cow/day and they found that the nutrients digestibility was increased in whey ration compared to control. Also Bayat *et al.* (2003) fed whole whey to 3-6 month old Holstein steers and he found when whey replaced 1/3 –2/3 of the concentrate in the diet the digestibility of nutrient was increased for whey diets compared with the control diet.

Nutrients digestibility was higher for T5% whey when compared with T10%, T15%, and T20% whey; this decrease can be justified by the following reasons: firstly may be due to the fact that whey contains high fat content which leads to negative impact on rumen microorganisms' activities. McDonald *et al.* (2010) stated that an excess of dietary lipid will inhibit the activity of rumen microorganisms. Secondly it is possible that inclusion of high levels of lactose whey in the diets stimulate utilizes to convert some portion of lactose whey to storage polysaccharide. Although this storage polysaccharide is considered part of the microbial cell mass, it represents available substrate that has been stored but not yet metabolized by the cells. Therefore, when the sugar concentration declined, they used their stores for maintenance of the microbial population (Hall and Herejk, 2001). The superiority of T5% treatment over other supplemented treatments is in same line with the result obtained by Hussain and Miller (1998), when replacing nearly 5% of starch with lactose or whey in ration consisting of dry hay and concentrates and he found that the rumen metabolism improved and consequently the nutrient digestibility enhance.

As indicated by several researches the value of pH is the most important factor that influence fermentation in the rumen and affect its function. In the current study the pH values among experiment treatments were found to be ranged from (6.54-6.25) and stable at this range, which were within the range of (6.0-7.0) that considered for optimal microbial digestion of fiber and protein (Wanapat, 1985).

Ammonia nitrogen concentration of all diet was above the recommended level of NH3-N concentration (5 mg 100/dl) to support efficient use of fermentable carbohydrates for microbial growth. In cheese whey rations the lower concentrations of ruminal ammonia compared to control diet may be indicate more ammonia was being utilized for synthesis of microbial protein because cheese whey rations contained more soluble nitrogen than the control ration. Reductions in ruminal NH3-N for whey rations may suggest a more efficient utilization of the rapidly available nitrogen components in the diet and a concomitant increase in microbial growth (Windschitl and Schingoethe, 1984).

Whey rations (T5%, T10%, T15% and T20%) gained high concentration of VFA than control diet. This may suggest that lactose could supply more energy in the form of VFA to the rumen as opposed to production of microbial proteins. Secondly, it could be the result of a population shift of the micro flora resulting in an increase in species that generate more VFA in relation to microbial protein. The branched chain VFA is produced in the rumen from the deamination and decarboxylation of the branch-chained amino acids (Allison, 1970). Ruminal branched-chain amino acids may arise from feed protein or microbial protein, and differences in branched chain VFA concentrations probably reflect differences in one or both of these components. In this study rations contain whey produced more VFA than control diet may be due to amino acid in whey protein (Schingoethe, 1976). Ration T5% produces more VFA than T10%, T15%, and T20% whey rations. This could be explained by the high level of whey in latter three rations, which contain high minerals salt content. Thomson et al., (1975) stated that there is negative relation between fluid dilution rate and production of VFA in the rumen; moreover, he mentioned that the increase of cheese whey level in the ration increase the ruminal fluid rate dilution and consequently decrease the VFAs production. Total VFA concentrations often decrease, whereas ruminal fluid dilution rates increase (Roger and Davis; 1982).

The gas production volume during different times of incubation was higher for whey rations compared to control ration. Cheese Whey acidity decrease fiber content in rations by degraded cell wall than control diet. Muck and Kung, (1997) stated that cheese whey supplementation associated with reduction of fiber content which attributed to partial hydrolysis of hemicelluloses in the diet. Also Fazaeli *et al*., (2003) and Guney *et al*., (2007) reported that there was a decrease in fiber content of liquid cheese whey treated

straw silage and for molasses treated sorghum silage. Aberra, (2011) said that the CF feeds constituents are known to be less degradable than soluble carbohydrates and therefore reduce gas production. The negative effect of cell wall content on gas production could be due to the reduction of the microbial activity through increasing the adverse environmental conditions (Bakhashwain *et al*., 2010). Cheese Whey at level T10%, T15% and 20% decreased gas production compared to T5% level this may be due to increased fat content with increased cheese whey level in the latter rations. Aberra *et al*., (2009) reported high fat content which contributes to decreased gas production. Chemical constituents of the feeds correlated negatively or positively with rate of fermentation fraction (c), fraction (b) and fraction (a+b) (Kamalak *et al*., 2005). Tested diets gained high fermentable fraction (b) and potential degradable (a+b) may be due to its high content of CP. Gasmi *et al*., (2005) reported that there was a positive correlation of CP content with gas production.

The high fermentation rates in whey rations indicate high nutrient availability for ruminal microorganisms while lower fermentation rate values in T0% ration may be the resulted from the CF content which slow down fermentation speed (Mirzaei *et al*., 2011 and Fievez *et al*., 2005).

Conclusion

The present study concluded that the supplementation of different levels of whey to dietary urea treated baggase resulted in improve the IVDMD IVOMD IVCPD,IVNDFD, FVA and gas production in whey diets compare to control with superior effect for T5% diet.

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